Strategic Options in Cell-line Engineering

by

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BMLSc., University of British Columbia, 1999

PROJECTSubmitted in PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF

Master of Business Administration
MBA-MOT Program

in the Faculty

of

Business Administration

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SIMON FRASER UNIVERSITY
July 2004

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ABSTRACT

Chromos Molecular System Inc. is proposing to engineer cell-lines based on the ACE System as a service for biopharmaceutical companies and CMOs. Cell-line engineering will be part of the Company’s near term cellular protein production strategy as a source of revenue and will defray the costs of building the gene-based cell therapy business. This technology can create value by developing stable, high protein expressing cell-lines in short timeframes.

Therapeutic proteins are becoming an important component of the drug arsenal available to treat life-threatening ailments. The monoclonal antibody market is expected to be worth $16.7B by 2008 and currently, 376 antibody products are in development. There are at least 20 CMOs worldwide that manufacture proteins using mammalian cells. The majority of them do not have a technology to create high expressing cell-lines.

Among 11 identified competitors who also have technologies to create high protein expressing cell-lines, the G.S. Expression System and PER.C6™ cell-line technologies pose the strongest threat as they have been used extensively for product manufacturing and research. Other non-mammalian cell protein manufacturing technologies exist; however, they still need to prove their cost effectiveness and ability to produce regulatory approved drugs.

Chromos can engineer cell-lines that are competitive to other technologies available. However, the Company needs to complete process development and growth optimization studies before they can use the ACE System to move ahead of competitors. Chromos’ strategic options are: building the service in-house; acquiring a cell-line engineering firm; developing corporate alliances and partnerships; or maintaining the status quo.

The recommendation offered is to build the service in-house after all studies have been completed, which can take up to a year. Initial starting cost is estimated to be $200K and net income is expected to be over $1.5M after 3 years of operation assuming price per cell-line is $500K, project teams consist of 3 – 4 employees, and the service is built upon Chromos’ existing infrastructure. In addition, multiple CMO partners should be sought who could help market the service to their clients. A commercial culture needs to be developed in the Company which must be in balance with R&D.
DEDICATION

This project is written in memory of my father who has left us many years ago. I know you are watching over me as I’ve made it thus far.

Thank you, love you, and I will always remember you.
ACKNOWLEDGMENTS

I would like to thank all the staff at Chromos Molecular Systems Inc. for their support and input throughout the composition of this project. I would like to especially thank Drs. S. Fidai and J. Zendegui who have been instrumental in providing me guidance and insight into the Company's practices and vision; and also H. Zeitler for proofreading the document prior to release. Lastly, I would like to thank A. Duncan for his on-going support and inspiration to pursue a business career.

I would also like to thank my family and friends who have been extremely supportive and tolerant of me during my studies. At times it may have seemed that I was in my own little world and ignoring you all at times, let me assure you it wasn’t without good reason. I really appreciate all that you have done.

Finally, I would like to thank Dr. J. Shepherd for her supervision and suggestions in the composition of this project and Dr. E. Maine for her thoughts on making this project successful.
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<td>ACE Integrase:</td>
<td>A site-specific unidirectional integrase enzyme that catalyses the recombination of the ACE Targeting Vector and the Platform ACE.</td>
</tr>
<tr>
<td>ACE System:</td>
<td>Chromos’ proprietary gene expression platform technology that is composed of the Platform ACE, ACE Targeting Vector, and ACE Integrase. It is vehicle for transporting target genes into target cells.</td>
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<tr>
<td>ACE Targeting Vector:</td>
<td>A proprietary integrase-mediated site-specific recombination targeting vector containing a Platform ACE specific “donor” site. Also called “ACE Vector”.</td>
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<td>ACes:</td>
<td>Chromos’ first generation artificial chromosome.</td>
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<td>Adherent cell-line:</td>
<td>Cell-lines that require attachment to a scaffold or matrix to grow, such as on a culture dish.</td>
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<td>Amino acid:</td>
<td>One of twenty different molecules that combine to form proteins. The sequence of amino acids in a protein determines the protein’s structure and function.</td>
</tr>
<tr>
<td>Amplify:</td>
<td>To magnify or multiply. To increase the effect of.</td>
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<tr>
<td>Antibody:</td>
<td>Protein produced by white blood cells in response to a foreign molecule or invading organism. Often binds to the foreign molecule or cell which will either inactivate it or mark it for destruction.</td>
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<tr>
<td>Anti-codon:</td>
<td>Sequence of three nucleotides on a tRNA molecule that is complementary to the codon on an mRNA molecule.</td>
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<td>Artificial chromosome:</td>
<td>A DNA-based construct which stably replicates and expresses genes, carried alongside the nature chromosomes in a cell. It provides extragenomic specific integration sites for the introduction and expression of target genes which have been inserted. It is often referred to as a mammalian artificial chromosome (MAC) because it includes an active mammalian centromere, mammalian telomeres and is engineered from naturally occurring neutral DNA sequences.</td>
</tr>
<tr>
<td>Biogeneric:</td>
<td>A generic biologic drug which is the same as a brand name drug in dosage, safety, strength, how it is taken, quality, performance, and intended use.</td>
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<tr>
<td>Biologic:</td>
<td>Natural-based therapeutic products or therapies such as vaccines, blood and blood components, allergens, somatic cells, gene therapy, tissues, and recombinant proteins (i.e. hormones, monoclonal antibodies, enzymes, interferons, etc.) As defined by the FDA, biologic products are a subset of &quot;drug products&quot; distinguished by their manufacturing processes (biological process vs. chemical process).</td>
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<tr>
<td>Biopharmaceutical:</td>
<td>A biologic drug. Also known as biotherapeutic.</td>
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<tr>
<td>BLA:</td>
<td>Biologics License Application – the documentation submitted to the FDA for the marketing approval of a biologic therapeutic.</td>
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<tr>
<td>BOD:</td>
<td>Board of Directors</td>
</tr>
<tr>
<td>CDN:</td>
<td>Canadian Dollar</td>
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<td>eGMP:</td>
<td>Current Good Manufacturing Practice</td>
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<td>Cell therapy:</td>
<td>An approach to the treatment of genetic and acquired diseases that is based on the administration of cells that have been altered outside the body.</td>
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<td>Cell-line development:</td>
<td>The process of optimizing cell-lines for maximal protein expression and stability.</td>
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<td>Cell-line engineering:</td>
<td>The process of creating cell-lines that express genes of interest (or target gene).</td>
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<tr>
<td>Cell-line:</td>
<td>Cells which grow and replicate continuously outside the living organism.</td>
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<tr>
<td>Centromere:</td>
<td>An important structure at the centre of the chromosome, whose functions are linked to the management and proper division of the chromosome.</td>
</tr>
<tr>
<td>CHO:</td>
<td>Chinese Hamster Ovary – a common cell-line used for the manufacture of therapeutic proteins.</td>
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<tr>
<td>Chromosomes:</td>
<td>Microscopic rod shaped thread-like structures which carry the genes and DNA that convey hereditary characteristics and are constant in number for each species. The DNA contains the genetic information that controls the growth and function of single-cell and multi-cell organisms.</td>
</tr>
<tr>
<td>CMO:</td>
<td>Contract Manufacturing Organization – an entity who manufactures chemical or biological compounds for clients.</td>
</tr>
<tr>
<td>Codon:</td>
<td>Sequence of three nucleotides in a DNA or mRNA molecule that represents the instructions for incorporating a specific amino acid molecule into a growing polypeptide (protein) chain.</td>
</tr>
<tr>
<td>CPP:</td>
<td>Cellular Protein Production – the production of proteins in mammalian cellular systems.</td>
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<tr>
<td>DNA sequence:</td>
<td>The order of nucleotide bases in the DNA molecule.</td>
</tr>
<tr>
<td>DNA:</td>
<td>Deoxyribonucleic Acid - The chemical compound present in all cells of the body that is the carrier of genetic information.</td>
</tr>
<tr>
<td>DTC:</td>
<td>Direct to Consumer.</td>
</tr>
<tr>
<td>Electroporation:</td>
<td>A method of transferring DNA to target cells by application of an electric field, usually in a short pulse or pulses, which creates temporary holes in the cell membrane for the nucleic acids to pass through.</td>
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<tr>
<td>ELISA:</td>
<td>Enzyme Linked Immunosorbent Assay – A technique used to determine the quantity of a particular compound, such as an antibody.</td>
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<td>EMEA:</td>
<td>European Medicines Evaluation Agency – the drug regulatory agency of the European Union.</td>
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<tr>
<td>Enzyme:</td>
<td>A protein catalyst that facilitates specific chemical or metabolic reactions necessary for cell growth and reproduction.</td>
</tr>
<tr>
<td>Expression:</td>
<td>The process of transcription and translation in a cell that results in the production of protein that is required by the cell to properly function.</td>
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<tr>
<td>FDA:</td>
<td>Food and Drug Administration – the United States government agency which regulates the manufacture, use and sale of human diagnostic and therapeutic products in the United States.</td>
</tr>
<tr>
<td>FISH:</td>
<td>Fluorescent In-Situ Hybridization – A visual technique used for determining the presence of a specific sequence on a chromosome.</td>
</tr>
<tr>
<td>Flow cytometry:</td>
<td>Technique for separating or characterizing beads or cells based on their relative fluorescence. Chromos uses flow cytometry to separate chromosomes.</td>
</tr>
<tr>
<td>FTE:</td>
<td>Full-time Equivalent</td>
</tr>
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<td>Functional genomics:</td>
<td>The study of the function of genes and their interrelationships.</td>
</tr>
<tr>
<td>Gene therapy:</td>
<td>An approach to the treatment and prevention of genetic and acquired diseases that involves the insertion of new genetic information into target cells to produce specific proteins needed to correct or modulate disease conditions.</td>
</tr>
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<td>Gene:</td>
<td>The unit by which inheritable characteristics are transmitted to succeeding generations in all living organisms. Genes are contained by, and are arranged along the length of, the chromosome. The gene is composed of deoxyribonucleic acid (DNA) arranged in a definite sequence.</td>
</tr>
<tr>
<td>Genome:</td>
<td>An organism’s set of genes.</td>
</tr>
<tr>
<td>Genomics:</td>
<td>The study of the entire DNA in a cell, both chromosomal and extrachromosomal.</td>
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<td>Germ line:</td>
<td>The biological pathway by which genetic material is passed from one generation to the next.</td>
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<td>Glycosylation:</td>
<td>The process of adding sugar molecules to proteins. Glycosylation patterns can affect the rate of a protein’s clearance from the body, or cause immunogenic effects.</td>
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<td>Heterochromatin:</td>
<td>The portion of the chromosome that contains few or no genes and is thought to be inactive. Highly repetitive DNA sequences (satellite DNA) are located in regions of centromeric heterochromatin.</td>
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<td>HPLC:</td>
<td>High Performance Liquid Chromatography – Liquid chromatographic technique that is characterized by high inlet pressures, speed and sensitivity.</td>
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<tr>
<td>Humanized:</td>
<td>A protein from an animal or plant source that has been modified to replace the sequence of amino acids with human sequences of amino acids.</td>
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<td>IND:</td>
<td>Investigational New Drug Application – the documentation submitted to the FDA to obtain approval to test drugs in patients.</td>
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<td>Integration:</td>
<td>DNA sequences chemically recombining with the DNA sequences of the target cell chromosomal DNA.</td>
</tr>
<tr>
<td>IP:</td>
<td>Intellectual Property</td>
</tr>
<tr>
<td>MAb:</td>
<td>Monoclonal Antibody – An antibody secreted by a hybridoma clone. All the antibody molecules that the clone makes are all identical. In the case of cell-line engineering, all the antibody molecules that a cell makes derived from a target gene are all identical.</td>
</tr>
<tr>
<td>MAC:</td>
<td>Mammalian Artificial Chromosome – see artificial chromosome.</td>
</tr>
<tr>
<td>Marker gene:</td>
<td>A gene that encodes for an easily detectable protein. A selection marker is a gene that encodes for the resistance of a drug used in cell culture to distinguish transfected cells from non-transfected cells.</td>
</tr>
<tr>
<td>Microbial:</td>
<td>Organisms such as yeast and bacteria.</td>
</tr>
<tr>
<td>mRNA:</td>
<td>Messenger Ribonucleic Acid - A template for the synthesis of proteins.</td>
</tr>
<tr>
<td>NCE:</td>
<td>New Chemical Entity – a compound that has never been described in literature. With respect to drug approvals, NCEs are drugs that are approved for the first time.</td>
</tr>
<tr>
<td>Nuclear transfer:</td>
<td>The transfer of a cell nucleus from a donor cell into another cell that has had its original nucleus removed.</td>
</tr>
<tr>
<td>Nucleus:</td>
<td>A membrane-bound structure in a cell which contains the chromosomes.</td>
</tr>
<tr>
<td>OTC:</td>
<td>Over the Counter.</td>
</tr>
<tr>
<td>PCR:</td>
<td>Polymerase Chain Reaction – A technique used for amplifying a specific region of DNA.</td>
</tr>
<tr>
<td>Pharmacokinetics:</td>
<td>The study of the adsorption, distribution, metabolism, and excretion of drugs with the body.</td>
</tr>
<tr>
<td>PhRMA:</td>
<td>Pharmaceutical Research and Manufacturers of America.</td>
</tr>
<tr>
<td>Plasmid:</td>
<td>A small circular form of DNA that carries certain genes and is capable of replicating independently in a host cell.</td>
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<td>Term</td>
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<tr>
<td>Platform ACE:</td>
<td>A pre-engineered platform chromosome containing multiple sequence-specific recombination “acceptor” sites and expression enhancing sequences. It is Chromos’ second generation mammalian artificial chromosome.</td>
</tr>
<tr>
<td>Platform-Line:</td>
<td>A cell-line housing Chromos’ Platform ACE.</td>
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<tr>
<td>Polypeptide:</td>
<td>A linear molecule composed of linked amino acids. A large polypeptide constitutes a protein.</td>
</tr>
<tr>
<td>Product ACE Vector:</td>
<td>An ACE Vector incorporated with a product gene (or target gene).</td>
</tr>
<tr>
<td>Product ACE:</td>
<td>A Platform ACE that has been modified by adding one or more protein-specific genes that encode a specific product(s), to make the chromosome ready for use in production of such product(s). Essentially, the integration of target or product genes onto the Platform ACE creates a Product ACE.</td>
</tr>
<tr>
<td>Production cell-line:</td>
<td>A mammalian cell-line containing a product gene which will be used for manufacturing.</td>
</tr>
<tr>
<td>Product-Line:</td>
<td>A mammalian cell-line that houses a Product ACE and expresses a product protein. A Product ACE housed in a proprietary Chromos cell-line is a ‘Chromos Product-Line’. A Product ACE housed in a non-Chromos cell-line is a ‘Client Product-Line’. This Product-Line term distinction is used to distinguish cell-lines produced by Chromos’ proposed cell-line engineering service.</td>
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<tr>
<td>Protein:</td>
<td>A biological molecule which consists of many amino acids chained together by peptide bonds. Proteins are required for the structure, function and regulation of the body’s cells, tissues and organs.</td>
</tr>
<tr>
<td>Proteomics:</td>
<td>The study of all the proteins produced from all the genes of a genome.</td>
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<tr>
<td>Recombinant protein:</td>
<td>Proteins derived from genetic engineering.</td>
</tr>
<tr>
<td>REM:</td>
<td>Rapid Expansion Method – a technology that allows users to expand/grow cytotoxic T-cell lines that do not normally expand at large quantities.</td>
</tr>
<tr>
<td>Ribosomes:</td>
<td>A cellular particle that associates with mRNA and catalyzes the translation of mRNA sequences to synthesize proteins.</td>
</tr>
<tr>
<td>RNA polymerase:</td>
<td>An enzyme that catalyzes the synthesis of an RNA molecule on a DNA template.</td>
</tr>
<tr>
<td>RNA:</td>
<td>Ribonucleic Acid - A nucleic acid found in both the nucleus and the cytoplasm of all cells. It carries genetic information from the nucleus to the cytoplasm, where it also acts to assemble proteins. See mRNA.</td>
</tr>
<tr>
<td>rRNA:</td>
<td>Ribosomal RNA – a specific RNA molecule that forms a part of the structure of a ribosome, and participates in protein synthesis.</td>
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<td><strong>Small molecule drug:</strong></td>
<td>Drugs of low molecular weight (compared to large molecules such as proteins or peptides) that can easily pass through cell membranes and the blood-brain barrier. They are usually synthesized chemically and can be taken orally or intravenously.</td>
</tr>
<tr>
<td><strong>Stem cells:</strong></td>
<td>A relatively undifferentiated cell that can continually divide indefinitely to produce daughter cells that can undergo terminal differentiation into specific cell types.</td>
</tr>
<tr>
<td><strong>Suspension cell-line:</strong></td>
<td>Cell-lines that can be grown in suspension, such as in bioreactors or shake flasks. An advantage of suspension cell-lines is the ability to grow in large densities. They are usually grown in serum-free media.</td>
</tr>
<tr>
<td><strong>Target cell:</strong></td>
<td>The cell in which a vector or target gene(s) is being introduced.</td>
</tr>
<tr>
<td><strong>Target gene(s):</strong></td>
<td>The specific therapeutic or commercially relevant gene(s) and/or regulatory sequences that are being introduced into an artificial chromosome or target cell. Also called ‘gene of interest’.</td>
</tr>
<tr>
<td><strong>Telomere:</strong></td>
<td>Structural elements found at the end of the chromosome, thought to give stability to chromosomes and prevent the loss of genetic material during replication.</td>
</tr>
<tr>
<td><strong>Therapeutic proteins:</strong></td>
<td>Proteins that confer a therapeutic effect when introduced into humans or animals.</td>
</tr>
<tr>
<td><strong>Transcription:</strong></td>
<td>Copying of one strand of DNA molecule into a complementary RNA sequence that is catalyzed by RNA polymerase.</td>
</tr>
<tr>
<td><strong>Transfection:</strong></td>
<td>Introduction of DNA into a cell, usually followed by expression of one or more genes of the newly introduced DNA.</td>
</tr>
<tr>
<td><strong>Transgenic animals:</strong></td>
<td>Animals that have stably incorporated one or more genes from another cell or organism.</td>
</tr>
<tr>
<td><strong>Translation:</strong></td>
<td>The process by which an mRNA molecule is used to direct the incorporation of amino acids into the creation of a protein.</td>
</tr>
<tr>
<td><strong>tRNA:</strong></td>
<td>A set of RNA molecules that is used in protein synthesis as in interface between mRNA and amino acids. Each type of tRNA molecule is attached to an amino acid that is released during protein translation.</td>
</tr>
<tr>
<td><strong>USD:</strong></td>
<td>United States Dollar</td>
</tr>
<tr>
<td><strong>Vector:</strong></td>
<td>The agent (e.g., plasmid, virus, or chromosome) used to carry target genes into a target cell either for expression or replication of the target gene(s).</td>
</tr>
<tr>
<td><strong>Xenotransplantation:</strong></td>
<td>The transplantation of an organ from one species to another.</td>
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</table>
CHROMOS MOLECULAR SYSTEMS INCORPORATED

Chromos Molecular Systems Inc. (‘Chromos’, the ‘Company’) is a public Canadian biotechnology company located in Burnaby, British Columbia, founded in 1995 and made public in 2000. The Company owns intellectual property (IP) necessary to develop mammalian artificial chromosomes. These have commercial potential in the enabling of complex cellular protein production and gene-based cell therapy to treat disease. Both of these markets are growing and the technology has advantages over alternative approaches. This chapter introduces the start-up strategy, discusses its implementation and impact so far, reveals the longer term strategy and details the mid-term strategy. It is the mid-term strategy on which this project focuses.

1.1 Corporate Background

Chromos began operations in 1996 based on the scientific findings of Dr. Gyula Hadlaczky from the Institute of Genetics Biological Research Center of the Hungarian Academy of Sciences, who showed that the mouse genome can be manipulated in such a manner that a derivative of a chromosome is created. The chromosome derivative, or Mammalian Artificial Chromosome (MAC), is much smaller than its parent chromosome; however it still contains centromeres, heterochromatin, and telomeres, much like its predecessor. The derivative could be isolated from the host chromosomes, transferred into different mammalian cell types, and manipulated to express foreign genes, thus establishing its utility as a vector for gene transfer and gene expression. The Company had created two versions of the MAC: the first generation ‘ACes’ platform; and the second generation ‘ACE System’. Since the introduction of the ACE System in 2001, the ACes platform has no longer been further developed.

Commercially, the ACE System has the potential to displace plasmids and viruses as vectors for gene transfer and expression, and is better than the competition because of its non-integrating characteristics and large payload capacity. The technology has been used to create protein expressing cell-lines such as antibodies, and also has been shown to be effective in stem cell therapy in animal models. In addition, this technology has the potential to be used in gene-based cell therapy, a type of treatment to correct genetic diseases.
1.1.1 Key Features and Advantages of the ACE System

The Platform ACE coupled with ACE Targeting Vector (‘ACE Vector’) and ACE Integrase make up the ‘ACE System’. The Platform ACE features multiple site specific integration “acceptor” sites in addition to the characteristics found on the original ACes platform. ACE Vector and ACE Integrase are required to assist insertion of a gene of interest onto the Platform ACE. The reader is referred to Appendix 1 for details on how the ACE System works. There are many features and advantages of the ACE System which make it a more attractive system for protein manufacturing over conventional industry cell-lines. Some of which include the following (Chromos Molecular Systems Inc [Chromos], 2003, p. 4):

- **Speed to cell-line development**: The ACE System permits rapid, efficient, and predictable insertion of genes of interest (‘target gene’) onto a stable artificial chromosome contained in a mammalian cell-line expressing the target gene product at a high level. Multiple target sites (up to 50 sites) on the Platform ACE allows a high copy number of target genes to be integrated, alleviating the need of traditional gene amplification methods which can take six months or longer to develop a high expressing cell-line. The ACE System can be used to isolate candidate cell-lines expressing the integrated gene of interest in as little as 6 weeks, and identification of the most appropriate cell-lines for scale-up in an additional 6 weeks. A Platform ACE with integrated genes of interest, otherwise known as a Product ACE, can be transferred into different cell-lines enabling cell-line auditioning. In cell-line auditioning, a variety of different cell-lines are tested to determine which give the best protein expression characteristics. Protein quality is dependent on cell-line used. A cell-line that will be used for manufacturing is called a Production-Line.

- **Stable expression**: The Platform ACE contains no active genes and has been shown to remain stable and intact within the cell nucleus through long periods of time (at least 120 days) allowing for long-term stable expression. Traditional cell engineering methods, explained in Chapter 2, use plasmid or viral vectors which randomly integrate into the host chromosome resulting in variegated expression and other unknown genetic effects.

- **High predictable expression**: The ACE System is engineered to include regulatory elements that provide optimal and reproducible expression of the integrated gene. Integrating the gene at precisely known locations on the Platform ACE using the
proprietary ACE Vector allows for predictable, high levels of gene expression. Multiple gene expression enhancing components can be tested on the ACE System to optimize gene expression for a particular target gene. Typically, monoclonal antibody (MAb) expression in Chinese Hamster Ovary (CHO) cell-lines using the ACE System yields 0.5 – 1.0 g/L and a specific productivity level of 10 – 30 pg/cell/day. These levels are comparable to industry accepted production standards. Under certain conditions, the ACE System can produce cell-lines that express proteins exceeding industry standards.

- **Rational, tractable, predictable engineering:** The ACE System can be engineered with a large payload, including one or more large genes or multiple copies of the same gene, and associated regulatory elements onto multiple “acceptor” sites. Having multiple “acceptor” sites eliminates the need for unpredictable traditional gene amplification steps to increase protein expression levels. In addition, multiple rounds of integration with ACE Vectors carrying different target genes allows for the integration and expression of different target genes on the same Platform ACE.

- **Versatility:** The ACE System can be used to produce a range of therapeutic proteins from therapeutic antibodies and proteins to vaccines and industrial enzymes. Proteins that are encoded by more than one gene can also be expressed.

- **Non-integrating:** The Platform ACE is a self-replicating artificial chromosome with specific “acceptor” sites to the ACE Vector’s “donor” sites. Thus ACE Vector integrating events are directed at the Platform ACE rather than random integration into host chromosomes, unlike traditional gene delivery and expression systems. The chances of non-specific integration of the ACE Vector into host chromosomes are reduced in the ACE System.

- **Quality and Safety:** The cell-line engineering process developed by Chromos should result in less cell to cell variation leading to consistency in the quality of the product and greater purity. The ACE System can be extensively tested for safety and stability in a variety of host cells.

- **Serum-free medium:** Platform ACE containing cells can be grown in serum free cell culture medium thereby simplifying the purification of biopharmaceuticals produced by the ACE System and facilitating regulatory approval.
Initially, value was obtained from this technology through out-licensing and research collaborations with industry players and academia. Because the technology is still fairly young, Chromos had to prove its applicability in industrial applications. The majority of the deals that the Company has entered into were research collaborations that did not provide large sums of cash revenues or research funding. However, the collaborations did result in positive scientific findings and generated interest within the biotechnology and investment communities. Only recently had Chromos been out-licensing the ACE System to other biopharmaceutical developers, but, again, only low licensing fees could be demanded up-front because of the technology’s infancy. Low licensing fees however were offset with higher milestone and royalty payments on marketed products produced by the ACE System. The types of companies that Chromos wanted to engage include product development, protein manufacturing, and transgenic companies. Product development companies were approached because Chromos believes its technology can be used to create complex proteins in stable, high expressing cell-lines. Creating high protein expressing cell-lines using traditional methods is a long process which the ACE System could reduce by many months, thus decreasing R&D timelines. Protein manufacturing and transgenic companies were approached because of the potential of the ACE System in protein manufacturing applications. High protein expressing cell-lines leads to an overall cost savings as higher product yields could be made per cell.

1.1.2 Licensing Schemes

Chromos’ current non-exclusive licensing schemes are outlined in Table 1-1 and described below:

- **CMO Manufacturing License:** In this scenario, a Contract Manufacturing Organization (CMO) would license non-exclusively the ACE System to create for their clients production cell-lines only. In return, Chromos receives up-front and annual licensing fees, a percentage of the CMO’s revenues using the ACE System to manufacture a product, and diligence in marketing the ACE System. The CMO client would still need to separately negotiate a product development research or manufacturing license with Chromos. There is potential to exclusively license the ACE System to CMOs, however, this is not the preferred route unless the terms are extremely favourable to the Company. If the ACE System was exclusively licensed to a CMO, Chromos would receive up-front and annual licensing fees, a percentage
of the CMO’s revenues using the ACE System to manufacture products, and milestone and royalty payments on net sales of the manufactured product.

➢ Product Development Research License: In this scenario, a product development company would license the ACE System to create cell-lines for research use only. The licensee can create an unlimited number of cell-lines; however, products made using these cell-lines cannot be used for clinical trials or be marketed. Revenues come in the form of up-front and annual licensing fees.

➢ Product Development Manufacturing License: In this scenario, a product development company would license the ACE System to create cell-lines for research, development, and manufacturing of their own products. Products made using these cells can be used for clinical trials or commercialization. Revenues come in the form of up-front and annual licensing fees, and milestone and royalty payments on marketed product.

*Table 1-1: Current licensing schemes*

<table>
<thead>
<tr>
<th>Type of license</th>
<th>Rights conveyed</th>
<th>Compensation to Chromos</th>
</tr>
</thead>
</table>
| CMO manufacture              | • Licensee can use ACE System to create cell-line for client only for manufacturing client’s products  
• License is non-exclusive  
• CMO client has the right to take Product Line to any CMO for manufacturing, or manufacture on its own | • Up-front and annual license fees  
• % of CMO revenues using ACE System to manufacture product  
• Diligence in marketing ACE System |
| Product development research | • Licensee can use ACE System to create an unlimited number of cell-lines for own product research and development  
• Products derived from ACE System cannot be used beyond pre-clinical research | • Up-front and annual licensing fees |
| Product development manufacture | • Licensee can use ACE System for research, development, and manufacturing of their own products  
• Product derived from ACE System can be used beyond pre-clinical research | • Up-front and annual license fees, milestone and royalty payments on marketed product |
1.2 Licensing, Partnerships, and Collaborations

To date, Chromos has entered into 11 corporate partnerships and numerous other academic collaborations to validate the artificial chromosome technology in a variety of applications, including large scale protein manufacturing, drug development and delivery, and transgenic and academic research. Chromos has developed R&D collaborations with corporate partners to test the feasibility of the artificial chromosome technology in product development and protein manufacturing environments. Academic collaborations were established to optimize and further characterize the ACE System, and to test the system in animal models for theoretical applications such as stem cell therapy. Section 1.2.1 provides an overview of Chromos’ corporate and academic research collaborations, while section 1.2.2 provides an overview of their licensing deals to date.

1.2.1 Research Collaborations

Research collaborations with corporate partners were integral to the development of the ACes platform and ACE System, proving its applicability under a variety of usages. Some of the collaborations assisted Chromos with research funding to develop the ACE System in cellular protein production (‘CPP’) and product development, while others explored the use of artificial chromosomes in novel applications such as animal transgenics and stem cell therapy. Results from these studies verified the claims Chromos has made for the ACE System such as being able to create a stable, high expressing cell-line in a short time frame and the versatility of the system in a variety of cell types. Chromos used the scientific findings from these collaborations to market the ACE System to other product development companies, as well as leverage for negotiating with investors. Undoubtedly, these collaborations validated the ACE System technology and Chromos as a player in the biotechnology industry.

1.2.1.1 Boehringer Ingelheim Pharma KG (‘Boehringer Ingelheim’)

The Boehringer Ingelheim group of companies, headquartered in Germany, is one of the leading top 20 pharmaceutical companies with over 150 affiliated companies worldwide. Their research focus on human healthcare is in the areas of cardiovascular, central nervous system, and respiratory disorders. In addition, they are developing products to treat HIV/AIDS, arthritis, and urological disorders. Boehringer Ingelheim also has research interests in animal healthcare. Net sales were €7.4B in 2003, and they had spent €1.2B in R&D (Boehringer-Ingelheim, 2004).
In August 1998, Chromos entered into a feasibility study with Boehringer Ingelheim to engineer ACes incorporating one of Boehringer Ingelheim's proprietary genes and evaluate its expression in a production cell-line. The deal was amended to include a second study where the expression of one of Boehringer Ingelheim's monoclonal antibodies would be evaluated in another cell-line (Chromos, 2000, p. 23). The deal, which expired in April 2001, consisted of milestone payments to Chromos as well as R&D funding. Chromos was successful in demonstrating the ability to produce a MAb expressing cell-line, leading to milestone payments and extension of the deal to 2003.

1.2.1.2 Chiron Corporation (‘Chiron’)

Chiron, based in California, is a biopharmaceutical company (a biotechnology company that develops biopharmaceuticals for therapeutic usage) with research interests and over 50 marketed products worldwide in the areas of oncology, bacterial and viral vaccines, and blood testing. Net sales were $1.7B in 2003 (Chiron Corporation, 2004).

In April 1998, Chromos entered into a two-year agreement to evaluate the stability of ACes in combination with Chiron's proprietary lipid technology and to determine ACes marker gene expression in Chiron's proprietary cell-lines and animal models (Chromos, 2000, p. 23). Chromos was able to demonstrate that delivery of ACes into mammalian cells could be achieved using Chiron’s lipid formulation. The deal was not extended.

1.2.1.3 Genetronics Inc. (‘Genetronics ’)

Genetronics, based in California, is a world leader in electroporation technology for the delivery of genes, proteins, and drugs into mammalian cells. Electroporation makes cell membranes more permeable, thus allowing the passage of foreign molecules into the cell. Net revenues in 2003 were $81M; however, they have been unprofitable for the last 8 years (Genetronics, 2004, p. 31).

In August 1998, Chromos entered a deal to evaluate the use of Genetronics’ electroporation technology and accessories for the delivery of ACes into targeted cell-lines (Chromos, 2000, p. 24). The deal was terminated after a few years.
1.2.1.4  *Infigen Inc. ('Infigen')*

Infigen, a private company based in Wisconsin, is a biotechnology company focusing its research in nuclear transfer and cloning technologies to produce transgenic animals suitable for protein production, xenotransplantation, and disease models. The company is still in the R&D phase, however, in January 2004, the company announced major R&D cutbacks and are essentially defunct.

In April 1998, Chromos entered into a feasibility study agreement to demonstrate ACes transfer into bovine embryos using Infigen’s proprietary nuclear transfer technology to produce blastocysts carrying ACes (Chromos, 2000, p. 24). The agreement ended after a few years.

1.2.1.5  *Pharming Technologies BV ('Pharming Technologies')*

Pharming Technologies is a subsidiary of Pharming, a leading developer and producer of human medicines from the milk of animals. The animals used in specific are cattle and rabbits. Pharming is developing a range of products to treat genetic disorders, infectious diseases, tissue and bone damage, cardiovascular diseases, surgery and trauma. The company does not have any products in the market.

In December 1998, Chromos entered into an Amended and Restated Collaborative Research and Licence Agreement with Pharming Technologies to conduct a study to assess the feasibility of producing proteins using ACes technology in the milk of transgenic animals. Pharming Technologies was granted exclusive rights to use ACes technology for the production of pharmaceutical and nutraceutical products in the milk of transgenic cattle, pigs, and rabbits. In addition, Pharming Technologies was granted an exclusive license to use ACes technology for the development of certain products derived from the milk of any transgenic animal (Chromos, 2000, p. 24). In return, Chromos received milestone and royalty payments. The deal was terminated in 2001 as Pharming Technologies went into receivership which enacted a default clause under the terms of the collaboration.

1.2.1.6  *Cobra Therapeutics Ltd. ('Cobra')*

Cobra Therapeutics (now owned by ML Laboratories, England) is a biotechnology company with proprietary gene expression technology. Specifically, Cobra has developed the UCOE (Ubiquitous Chromatin Opening Elements) system, which supports the expression of protein products in mammalian cells.
In February 2002, Chromos entered into an evaluation agreement where Chromos evaluated Cobra’s gene expression technology to enhance gene expression of the ACE System. The deal ended later the same year.

1.2.1.7 MorphoGen Pharmaceuticals ('MorphoGen')

MorphoGen, based in California, is a development stage biotechnology company focused on adult-derived stem cell technology to develop products in the healthcare industry.

In April 2002, Chromos entered into a collaborative research agreement where Chromos expanded the application of their ACE System employing MorphoGen’s adult-derived stem cells for gene-based cell therapy (Chromos, 2002b). IP derived from this collaboration would be jointly owned by both parties. The collaboration lasted for one year. Successful results demonstrated the feasibility of using the ACE System in stem cell therapy.

1.2.1.8 Lonza Biologics PLC ('Lonza Biologics')

Lonza Biologics, part of the Lonza Group headquartered in Switzerland, is a world leader in the contract manufacturing of monoclonal antibodies and recombinant proteins. Services they offer include vector construction and cell-line development to full scale manufacturing. Lonza Biologics has developed a highly efficient mammalian cell expression system, known as the G.S. Gene Expression System (‘G.S. System’), based on glutamine metabolism and strong viral promoters. Lonza Biologics has over 20 years of manufacturing experience and over 60 clients in both the pharmaceutical and biotechnology industries, including world leaders such as Pfizer, Eli Lilly, and Genentech. Revenue derived from the manufacturing business was CHF 835M in 2003.

In June 2002, Chromos entered into an evaluation agreement whereby Chromos’ ACE System was evaluated in Lonza Biologics’ proprietary mammalian cell-line for improved expression of proteins (Chromos, 2002b). The term of the agreement was for one year with an option to license the ACE System for use in their cell-lines in cellular protein production. Positive results from the collaboration have led to a contract extension in March 2004. According to the new agreement, Lonza Biologics will have non-exclusive access to Chromos’ ACE System for evaluation in their contract manufacturing business (Chromos, 2004a). The contract was extended until Q4 2004, at which time Lonza Biologics will have the option to license the ACE
System. This deal is significant to Chromos as it will demonstrate the ability of the ACE System to be utilized in large scale manufacturing processes.

In July 2004, Chromos entered into a non-exclusive licensing deal with Lonza Biologics to in-license their CHOK1SV cell-line. The CHOK1SV cell-line can be used in conjunction with the ACE System to engineer production quality cell-lines to third parties. The CHOK1SV cell-line is a well established, cGMP quality, suspension production cell-line used in the manufacturing of biotherapeutics. The cell-line has been 'pre-adapted' to the desired suspension culture condition making adaptation easier (See Section 2.1.4). The deal is significant to Chromos in that the Company can now offer a more attractive protein production system to their partners. Chromos will forfeit some of their sub-licensing revenues to Lonza Biologics when the Company sub-licenses the CHOK1SV cell-line to their partners.

In addition to value being created by entering research collaborations with corporate partners, steps were taken by Chromos to continue the production of IP. This involved academic collaborations with institutions worldwide that resulted in demonstrating the applicability of the ACE System in somatic and stem cell therapy applications. Data from these collaborations were presented in prestigious conferences such as at the annual meeting of the American Society for Gene Therapy, and published in peer reviewed journals such as Chromosome Research.

1.2.1.9 Academic Collaborations

Chromos has entered into numerous academic collaborations with institutes worldwide to further study the utility of the ACes technology and the ACE System in animal models and to optimize its functionality. Collaboration with the University of British Columbia had resulted in the successful generation of transgenic mice carrying ACes in their germ line (Chromos, 2000, p. 25). In collaboration with the Academic Medical Center in the University of Amsterdam, researchers there have shown successful delivery of cells containing the ACes platform carrying a target gene into the joints of an animal model with rheumatoid arthritis, where the chromosome remained stable and expressed a therapeutic protein over a prolonged period of time (Chromos, 2003b, p. 6). Dr. Hadlaczky, from the Institute of Genetics and Biological Research Center at the Hungarian Academy of Sciences and the inventor of the ACes technology, has maintained a research collaboration with Chromos since January 1999 for the purpose of studying the molecular structure and optimizing the function of the ACE System.
The Company is currently involved in two other academic collaborations. The first is with Ohio State University, whose researchers engineering human haematopoietic stem cells are using the ACE System to create an artificial immune system for vaccine testing and therapies against biological agents. The second, with another major US university, is studying pre-clinical gene-based cell therapy in an animal disease model. In this proof of principle study, the Platform ACE housed in a stem cell is targeted with a gene to demonstrate therapeutic improvement or the slow down of progression of a disease.

The Company has been able to use the data from research collaborations to negotiate licensing agreements on both an exclusive and non-exclusive level. The ACE System technology is still young; hence up-front revenues from these agreements are low. However, these are off-set with higher milestone and royalty payments on marketed products. These deals demonstrate the industry’s acceptance of the ACE System and help to promote the product.

1.2.2 Technology Licensing

To date, the Company has engaged in technology licensing deals with product development and transgenic animal companies. These Companies are using the ACE System to develop monoclonal antibodies and protein manufacturing, respectively.

1.2.2.1 Cambridge Antibody Technology ('CAT')

Cambridge Antibody Technology (LSE:CAT, NASDAQ: CATG), based in the UK, is a world leading biotechnology company in the discovery and development of human therapeutic antibodies using their proprietary phage display technology. CAT has an extensive antibody library consisting of more than 100B distinct antibodies. Their leading product is Humira®, which was developed in collaboration with Abbott Laboratories. CAT is developing products in the areas of rheumatoid arthritis, scarring and inflammation, and autoimmune and neoplastic disorders. Net revenues in 2003 were £8.7M, however, overall accumulated losses amounts to £122.4M.

In May 2003, Chromos entered into its first commercial, non-exclusive license agreement with CAT to develop protein expressing cell-lines for the commercial manufacture of antibodies and biologics using the ACE System (Chromos, 2003c). CAT retains developing, manufacturing, and marking rights in exchange for an upfront payment and annual maintenance fees as well as milestone and royalty payments from the net sales of resulting products. This deal was significant
to Chromos as it validated the ACE System as a powerful tool for the generation of high antibody expressing cell-lines and commercial manufacturing. The deal is currently on-going.

1.2.2.2 Xencor

Xencor, a privately held biopharmaceutical company located in California, discovers and develops protein therapeutics using its proprietary rational protein design platform called Protein Design Automation (PDA®). Disease areas in which Xencor focuses their research includes autoimmune and bone diseases, thrombocytopenia, multiple sclerosis, and oncology. Xencor does not have any marketed products.

In September 2003, Chromos entered into a non-exclusive research license agreement to develop cell-lines that express Xencor’s monoclonal antibodies and other recombinant protein product candidates (Chromos, 2003d). The terms of the agreement allows Xencor to use the ACE System to generate cell-lines expressing proteins derived from their PDA® technology, and in addition, an option to negotiate a commercial license for manufacturing its clinical products. In return, Chromos received an upfront payment and will continue to receive annual maintenance fees. The deal is currently on-going.

1.2.2.3 AviGenics Inc.

AviGenics, headquartered in Georgia, is a development stage biotechnology company focused on the development of therapeutics in the areas of oncology, infectious and autoimmune disease conditions using their proprietary transgenic avian egg technology (Avian Transgenesis System). This technology enables the production of bioactive therapeutic proteins in chicken eggs, which is believed to be a cost effective method.

In February 2004, Chromos entered into an exclusive licensing agreement whereby AviGenics has acquired exclusive rights to the ACE System to generate transgenic chickens for the development of protein therapeutics and other applications in the avian transgenic field (Chromos, 2004b). Under the terms of the agreement, AviGenics will combine the ACE System with their Avian Transgenesis System to develop new protein therapeutics. In return, Chromos received an upfront payment and will receive annual license fees, milestone payments, and royalties upon commercialized products by AviGenics or from services provided by AviGenics to third parties utilizing the ACE System. Successful results will demonstrate the broad use of the ACE System in novel transgenic platforms. The deal is currently on-going.
1.2.3 Summary

Overall, the partnerships and collaborations demonstrated the utility of the ACE System in a variety of applications. In the area of CPP, successful collaborations with Lonza Biologics and Cambridge Antibody Technology will validate the technology in this area and increase Chromos’ profile in the biologic manufacturing sector. The deal with Xencor was significant to the Company as it was the first non-exclusive research license for the ACE System, validating its utility in cell-line engineering. Collaborations with Pharming Technologies and Infigen was aimed at demonstrating the use of ACes in the area of transgenics, however, the Company felt at the time that transgenics was not the direction to pursue thus the collaborations were not extended. In addition, Pharming Technologies went into receivership. The deal with AviGenics renewed interests in the transgenic arena. The longest collaboration to date is with the Institute of Genetics and Biological Research Center at the Hungarian Academy of Sciences. Scientists here, including the inventor of the artificial chromosome platform, are currently characterizing the ACE System and discovering ways to optimize it. The Company has initiated studies in the area of gene-based cell therapy through collaborations with the academic community and MorphoGen Pharmaceuticals. The objectives of the academic collaborations were to work with high calibre institutions to improve the visibility of the ACE System in the scientific community and generate business opportunities.

In summary, the initial strategy of Chromos was:

1. generating cash through out-licensing; and
2. continuing to develop IP

1.3 Research & Development

Chromos’ research and development (‘R&D’) achievements to date have been focused on developing mammalian artificial chromosomes for protein manufacturing and cell therapy applications. The ACE System has been shown to develop a variety of cell-lines expressing monoclonal antibodies comparable to industry standards, however in a shorter period of time (2 – 4 months compared to >6 months for the industry).

In the area of transgenics, Chromos researchers purposely engineered an artificial chromosome to express a particular protein from the mammary gland of a mouse model. The protein was found in the model’s milk in quantifiable levels. The artificial chromosome was also shown to be present intact in subsequent mouse generations through germ-line transmission.
In the area of gene based cell therapy, stem cells using the ACE System had been shown to express the same protein pre–and post–differentiation; in other words, the ACE System remained independent of the host chromosomes which were undergoing functional changes. Proof of principle studies showed that a gene expressed from the ACE System could demonstrate a therapeutic response in an animal models, such as in the Company’s erythropoietin studies.

Current R&D efforts are aimed at optimizing expression levels of targeted genes in various mammalian cell-lines such as CHO derived cells and other undisclosed stem cell-lines by methods such as incorporating and modifying expression enhancing components, varying transfection conditions and testing candidate cell-lines in different environmental conditions. Efforts are also expended in process development with the goal of creating cell-lines suitable for batch production that express target genes at industry relevant levels in less then 14 weeks post-transfection, which currently are produced in 14 – 16 weeks. Process development studies should be completed in about 1 year. Chromos is also developing cell-lines for their corporate partners using the ACE System. In addition, the Company has research collaborations with academic stem cell scientists exploring the suitability of the ACE System for stem cell related therapies. The Company has developed a strong patent portfolio worldwide surrounding the ACE System, 24 patents have been issued and another 59 are pending globally.

Chromos, by leveraging its ACE System technology, entered into the agricultural biotechnology sector in early 2001 through the creation of a subsidiary, Agrisoma Biosciences Inc. Agrisoma Biosciences is focused on utilizing the ACE System in plants to produce therapeutic proteins and enable the development of high value traits in crops.

Chromos’ R&D efforts were successful to the extent that they are now in a decent position to further exploit the ACE System in CPP and gene-based cell therapy. The following describes the financial position and organizational structure at Chromos.

1.4 Financial Position

Prior to Chromos becoming publicly traded on the Toronto Stock Exchange (TSX:CHR) in July 2000, the Company had completed three rounds of private equity financing, raising approximately $24M CDN (Chromos, 2000, p. 30). The third financing round, completed in November 1999 raised $12M (Chromos, 2000, p. 42). Other sources of revenues included research grants, interest, and other short-term cash investments. Going into the IPO in 2000, Chromos had incurred a net loss of approximately $25.2M, mostly due to R&D and general and
administrative ('G&A') expenses (Chromos, 2000, p. 36). The number of common shares issued for the IPO was 4.3M at $8.00 per share for a total of $34.5M, including the exercising of the underwriter’s over-allotment option (Chromos, 2001, back-cover). In 2001, another $1.15M was raised through the issuance of common shares due to the exercising of existing warrants (Chromos, 2002c, p. 17). Since inception, the Company has raised $60M CDN in total. 17.1M common shares are currently outstanding including all the converted preferred shares at IPO (Chromos, 2004c).

At May 1, 2004, the 52-week trading range was between $0.46 and $0.95, and the share price was $0.70 (TSX, 2004). Market valuation of the Company at the same date was approximately $12M. Much of the decrease in valuation is attributed to the poor market environment and global economic downturn. In addition, questionable corporate practices in the biotech industry have led to the loss of investor confidence and thus are more cautious of their investments. Questionable practices such as Elan Corporation’s inflated earnings and off-balance sheet entities (Capell, 2003), or ImClone Systems’ insider trading debacle has demonstrated that the biotechnology industry is not immune to corporate scandal. Chromos’ total loss to December 31, 2003 was $52.6M, and the current burn rate is approximately $0.5M per month. At the end of Q1 2004, the Company reported a cash reserve and equivalents of $6.6M which management believes is sufficient to maintain operations until Q2 2005 (Chromos, 2004d, p. 8). The Company’s recent signing of a definitive agreement to acquire a biotechnology firm in Seattle, WA, will further impact the Company’s financial position if and when the deal is closed. The acquisition of CellExSys Inc. ('CellExSys') will be completed though a share purchase where Chromos will issue 1,500,000 of their shares to CellExSys’ share holders and a convertible debenture in the principle of $3,375,000 CDN.

Current revenue sources are from licensing and milestone payments, and other short-term investment instruments. These sources of revenue do not cover the expenses the company commits on an annual basis. The Company needs to find other sources of revenue until the goal of becoming a gene-based cell therapy company is met. It is typical for an early stage biotechnology company to remain unprofitable for many years until products are marketed to offset losses. The challenge that companies face is to become profitable before their cash reserves are depleted.
1.5 Organization

Chromos’ current organizational structure reflects its financial position and corporate goals and strategies. The Company has an effective streamlined R&D and management group. Chromos employs a wide range of professionals ranging from scientific researchers to business personnel (See Figure 1-1). Of the 28 full time employees, 18 are committed to the R&D efforts of the company. The research arm is a mix of 4 PhD scientists, 5 MSc scientists, and 9 other researchers of various levels. The Director of Cellular Protein Production and Director of Projects, alongside the VP of R&D, oversee the research direction of the company. The Team Leader in Process Development Group oversees the research in optimizing the ACE System in whole, whereas the Team Leader in the Core Group oversees the research in optimizing each individual component of the ACE System. The Director of Cellular Protein Production is responsible for Process Development and Core Groups. The Director of Projects and the Gene Based Cell Therapy Group are responsible for the academic collaborations the Company is engaged in. There is also a Flow Cytometry Group whose functions includes isolating the Platform ACE, assisting in protein expression assays, and supporting the research of the other groups where flow cytometry is required. Approximately 2/3 of the R&D group work under the Director of Cellular Protein Production, and the remainder under the Director of Projects. The size of the Cellular Protein Production group reflects Chromos’ commitment to meeting its short and medium term goals, which is to expand the CPP business. However, efforts already have been afforded in developing the long term goal of becoming a gene based cell therapy company.

The executive management team consists of the CEO, CFO, VP of R&D, and VP of Business Development. The CEO is responsible to the Board of Directors. Both the VP of R&D and VP of Business Development have PhDs. Other high level managers include the Director of Investor Relations, Director of Human Resources, Director of Finance, and Director of Business Development. The management team, in general, are responsible for financing, partnership and licensing, promotion, strategic direction, and other corporate development activities.
1.6 Corporate Strategy

The Company has 3 distinct strategies that they want to pursue, each building upon one another. The strategies are categorized as long, medium, and near term. The long term strategy is focused around gene-based cell therapy, the medium term around immuno-based cell therapy, and the near term around CPP including cell-line engineering.

1.6.1 Long Term Strategy

Most applications of the ACE System are geared towards CPP because entering this market is the Company’s near term goals. However, there is potential for the ACE technology to be used in other areas such as gene-based cell therapy, genomics and gene identification, transgenics, and agricultural biotechnology. In the area of genomics and gene identification, the ACE System is a valuable tool because of its large carrying capacity, thereby the analysis of large

\[1 \text{ Note: This figure shows a simplified organizational chart at Chromos.}\]
gene constructs are more feasible. In the field of transgenics, Chromos has been able to create a transgenic mouse that secreted milk containing protein derived from a targeted gene on the ACes platform. Chromos is exploring the application of the ACE System in agricultural biotechnology though its subsidiary Agrisoma Biosciences Inc., which came into operation in 2001.

1.6.1.1 Gene Based Cell Therapy

The long term strategy that the Company is pursuing is gene based cell therapy. Jain PharmaBiotech (2004) predicts that the cell therapy market will be $35.7B by 2007, and $81.3B by the year 2012. Gene therapy is broadly defined as the introduction of genetic material (genes, or DNA) into the body for gene regulation, whereas cell therapy involves the administration of cells that have been altered outside the body (ex vivo). Cell therapy requires cells to be removed from the host, then the introduction of a gene to these cells, and finally re-implantation of the modified cells into the host. Only those cells that harbour the therapeutic gene and produce the gene product at therapeutic levels will be re-implanted. Chromos is currently exploring opportunities in this field where the ACE System would be applicable. The ACE System is advantageous as it provides: 1) stable, long term, and high expression of introduced genes; 2) co-expression of multiple genes; 3) safety due to non-integration of therapeutic genes into the host genome; and 4) a large genes and genomic sequence payload capacity. Collaborations with stem cell researchers and data from animal models have demonstrated that a Platform ACE holding a foreign gene is stably maintained during differentiation and subsequent generations, respectively. Chromos’ gene-based cell therapy is non-viral, therefore the likelihood of inciting an immunogenic response is decreased and the road to regulatory approval would be shorter compared to viral gene therapy methods.

In the business of gene-based cell therapy, a pipeline of cell therapies to treat a variety of debilitating genetic diseases will be created through either acquiring or in-licensing therapeutic genes. Several options to develop the pipeline are available and will be reviewed at such a time. Revenue streams will depend on the strategy chosen to develop products. Revenues may be generated though licensing and maintenance fees, milestone and royalty payments, or through product sales.

In June 2004, the Company took a giant step into becoming a cell therapy company by announcing that a definitive agreement is in place to acquire CellExSys Inc (‘CellExSys’).
CellExSys has programs in the discovery and pre-clinical phase, which will be developed in the near and medium term.

1.6.2 Medium Term Strategy

Chromos’ medium term strategy is focused around immuno-based cell therapy which was made possible by the acquisition of CellExSys. The Company’s executives recognized a gap was present between the long and near term strategies of the Company. CellExSys’ immuno-based cell therapy technology was considered ideal for bridging the two strategies and bringing cell therapy technologies in-house.

1.6.2.1 CellExSys Acquisition

CellExSys, based in Seattle, Washington, is a cell therapy company with a proprietary T-cell therapy technology to treat cancer and other infectious diseases. The technology, called Rapid Expansion Method (REM), allows users to rapidly generate autologous antigen-specific cytotoxic T lymphocyte (CTL) isolates for use in adoptive immunotherapy. Antigen specificity of the T-cell is unchanged during REM; therefore a large arsenal of cells can be grown to treat specific cancers or infectious diseases. Chromos anticipates entering a Phase I/II study in mid-2005 for the treatment of Hepatitis B. The acquisition of CellExSys should not have a significant impact on the R&D strategy of Chromos as immuno-based cell therapy R&D will remain in Seattle. The rationale for acquisition includes: filling Chromos’ medium term strategy; enrichment of Chromos’ gene-based cell therapy program with a technology and a patented process allowing the Company approach cell therapy in new ways; accelerated entry into the clinic with the Hepatitis B program; increased partnering potential with companies in immunotherapy and genetic diseases; addition of personnel experienced in product development; and gives Chromos the opportunity to be the leader in non-genetically modified cell therapies in North America.

The Company’s medium term strategy is to initiate pre-clinical trials in the programs obtained through the CellExSys acquisition. In addition, the Company will continue to build a pipeline of products and also out-license the ACE System for CPP applications.
1.6.3 Near Term Strategy

Chromos is focused on generating scientific data from R&D efforts, particularly in cellular protein production, and leveraging the data to enter into strategic partnerships and collaborations. The main reasons for entering into new relationships are to earn revenues, market the ACE System, gather data on the ACE System in industrial and other research settings, and providing solutions to the Company’s partners. The ACE System is currently being applied in CPP to leverage opportunities in protein manufacturing and product development where there is a demand for the generation of high protein expressing cell-lines that can be developed in short time frames.

Revenues in the CPP program are earned by the Company through out-licensing the ACE System technology to CMOs and product development firms; however, there is an opportunity to expand the CPP program and garner more revenues by initiating a cell-line engineering service, where Chromos’ scientists will create ACE System derived cell-lines for clients rather than out-licensing the ACE System to clients and having them create cell-lines on their own. Revenues through the cell-line engineering service will be realized through a service and annual licensing fees, and milestone and royalty payments on the net sales of marketed products. The revenue will be used to support the development of the immuno – and gene-based cell therapy businesses.

Overall, Chromos’ near term strategy is to expand the CPP business, develop relationships in the areas of CPP and gene-based cell therapy, and advance the programs obtained through the CellExSys acquisition.

1.6.3.1 Cellular Protein Production ('CPP')

Cellular protein production systems, which typically are mammalian cells that have been implanted with human genes, offer a method of producing therapeutic proteins in large quantities. Examples of therapeutic proteins include erythropoietin (i.e. Epogen® by Amgen) to treat anemia, and monoclonal antibodies (i.e. Herceptin ® by Genentech) to treat cancer. Other protein production systems exist that are microbial, fungal, animal, or plant based; however, mammalian cells can accurately assemble and add post-translational modifications to complex proteins (such as monoclonal antibodies) unlike the other systems. Demand on existing manufacturing capacity worldwide requires new technology to shorten production timelines to speed up the manufacturing process and to increase the yield of protein product per cell. The ACE System that Chromos has developed addresses both these issues by creating stable, high expressing cell-lines
at unprecedented rates. Chromos has the opportunity to provide manufacturing solutions to CMOs and product development companies by out-licensing the ACE System as a platform for cellular protein production.

Chromos is applying the ACE System in CPP to leverage the opportunity in manufacturing demand for near- and mid-term revenue streams, technology validation and to increase shareholder value. In addition, the ACE System is being used to leverage opportunities in product development, where companies require stable expressing cell-lines in short time frames to conduct their research. The Company’s current licensing schemes are outlined in Table 1-1. Strategic licensing deals with distinguished CMOs and product development companies will assist in marketing the ACE System as a viable alternative to traditional cell-line engineering.

Currently, a part of Chromos’ revenue stream is derived from licensing fees and milestone payments from product development companies. Having been reasonably successful to date but knowing the big prize of gene-based cell therapy is distant, another near term strategy has been developed which not only involves licensing out the technology for others to create cell-lines, but also creating the cell-lines (‘cell-line engineering’) as a service for others in order to generate more revenues. Revenues from cell-line engineering operations will be used to support the development of the immuno- and gene-based cell therapy businesses.

1.6.3.2 Cell-Line Engineering

Cell-line engineering, as part of the CPP strategy, entails Chromos generating a cell-line producing a protein of interest for a client based on the ACE System. The client will enter into a Cell-Line Engineering License, which will permit them to use the ACE System based cell-line carrying the client’s gene of interest engineered by Chromos’ scientists for research, development, and manufacturing. The client will not receive any components of the ACE System and cannot create an ACE System based cell-line themselves. They only receive from Chromos an engineered cell-line that produces the client’s protein of interest. The advantages for the client include a savings in time and resources as they do not need to create cell-lines themselves, and owning cell-lines that have been optimized for protein expression. In return for providing the service, Chromos receives a service and annual licensing fees if the cell-line is only used in a research setting up to and including the pre-clinical stage. However, if the same cell-line is subsequently used for clinical trials and product manufacturing, additional milestone and royalty payments on marketed products will be required. If Chromos engineers a cell-line strictly for the
production of a protein in a manufacturing environment, Chromos receives a service and annual licensing fees, and milestone and royalty payments on marketed products. Every cell-line that Chromos creates for a client requires a separate license.

The cell-line engineering business is expected to be self sustainable, create a steady profit stream for near – and mid – term, and will be expected to continue as long as it remains profitable. When Chromos is closer to realizing their long term goals, the cell-line engineering business will be re-evaluated in terms of how it still fits within the Company’s strategy.

Near term strategies for Chromos in the area of CPP are to continue out-licensing the ACE System to multiple corporate partners for the research and commercial manufacturing of biopharmaceuticals and to initiate the cell-line engineering business. Near term strategies in the area of gene-based cell therapy are to build a drug pipeline, and also expand existing and establish new academic collaborations in gene-based cell therapy programs. In the area of immuno-based cell therapy, near term strategies are to advance the Hepatitis B program for entry into clinical trials and further develop the other programs obtained through the CellExSys acquisition.

The realization of the overall corporate strategy will be dependent on the availability of cash. Strategic alliances with CMOs will provide licensing revenues and also technology validation, which in turn can be used to attract more CMO partners, and hence more cash. Partnerships and collaborations with product development companies will provide revenues through research funding, licensing and maintenance fees, and milestone and royalty payments on marketed products. Both research and manufacturing licensing deals will be sought. Another source of revenue is through short term financial investment instruments. Failure to meet partnership goals may require Chromos to seek additional financial support from investors, re-evaluate corporate strategies, or restructure the Company.

The announcement of the definitive agreement concerning the acquisition of CellExSys occurred during the composition of this report. The deal is expected to be officially finalized after this report is complete. How the near – and mid – term strategies will be affected remains to be seen as Chromos’ long term strategy of becoming a gene-based cell therapy company has come to the forefront. That being said, the executive management and Board of Directors of the Company still have to lay out a clear strategy for the combined entity. Chromos will likely stay on its current strategic course, however include CellExSys. In the near term, immune-based cell therapy development will be based in Seattle and CPP and gene-based cell therapy will remain in Burnaby. The time that Chromos will truly become a gene-based cell therapy company will still
take many years as products are still in the research phase. The impact the acquisition may have on the Burnaby operations include a lack of resources to conduct R&D and hire more employees as cash reserves may be allocated more towards supporting cell therapy product development. Based on Chromos’ current financial position and the recent acquisition announcement, the Company will likely need to raise additional financing to support the current and new R&D programs of the combined entity.

1.7 Project Scope/Objectives

The strategic direction Chromos is pursuing utilizing the ACE System are in the areas of CPP in the near term and gene-based cell therapy in the long term. Immuno-based cell therapy is being developed for both the near - and mid - term. Cell therapy still has many technical challenges and regulatory hurdles which the biotechnology industry has not really addressed yet. In the meantime, Chromos has recognized the value of the ACE System as a platform for cellular protein production, which can be leveraged for near - to medium-term revenues and defray the costs of developing products to enter into the cell therapy markets when it becomes more mature and accepted as a standard medical practice.

Chromos is proposing to enter the mammalian cell-line engineering business based on the ACE System. The Company envisions clients will require mammalian cell-lines to produce in high yields proteins for research, clinical, and commercialization purposes. The ACE System can offer such cell-lines that are comparable to industry standards and sometimes exceeding it, but at a fraction of the timeframe of traditional cell-line engineering methods. Through past and current collaborations, Chromos has proven the applicability of the ACE System in CPP at industry relevant levels. The cell-line engineering business will provide near- and mid - term revenues and promote the ACE System as the technology of choice in the areas of protein therapy and research.

The objective of this project is to provide a recommendation on how Chromos should strategically enter the cell-line engineering business using the ACE System as a platform for gene expression. The next chapter looks at the scientific aspects of cell-line engineering and its applications in research and biologic manufacturing, the pharmaceutical value chain and therapeutic protein drug development and manufacturing process, followed by the value added services Chromos intends to provide to the research and biotherapeutic manufacturing communities.
The subsequent chapter explores the market for cell-line engineering beginning with an analysis using Porter’s Five Forces model to examine the issues surrounding this market. The market analysis continues by looking at the market size, current unmet needs, and other technologies that also have the potential to produce biotherapeutics economically. The market analysis section ends with an examination of potential customers, and direct and indirect competitors.

Following the discussion on market analysis is a discussion on strategies that Chromos could pursue to build the cell-line engineering business based on an internal analysis of the Company such as building in-house, acquiring a cell-line engineering firm, or entering into an alliance or partnership. The financial implications, and the pros and cons of each strategy are explored. A simplified marketing strategy is also suggested.

The last chapter recommends a course of action based on the market analysis, realistic strategic options available, and corporate goals. The project concludes with an examination of the challenges and risks associated with the recommendation and possible exit strategies.
2 CELL-LINE ENGINEERING

This chapter begins with the scientific aspects of mammalian cell-line engineering and the process of creating an engineered cell-line. Next, the pharmaceutical value chain and where Chromos and the application of the ACE System fit within the chain are explored. The exploration of the value chain includes a discussion on the R&D process of creating a biopharmaceutical drug, clinical drug trials, pharmaceutical sales and marketing, retail, and protein manufacturing. In addition, how biologic drugs have affected the industry dynamics of the pharmaceutical industry is reviewed. Finally, the services that Chromos intends to offer in the cell-line engineering business are introduced. This chapter is intended to give the reader a holistic view of the value of Chromos’ cell-line engineering business and how it may add value to its clients.

Cell-line engineering is the process by which a mammalian cell is modified genetically by the insertion of genes to produce a protein product of interest, such as monoclonal antibodies, hormones, enzymes, growth factors etc. Cell-lines that express the desired product are expanded and stored, or grown in fermentation units for small or large scale protein production. There are many types of mammalian cell from different species that could be modified to express foreign genes such as mouse, human, chicken and cow cells.

2.1 Scientific Brief

The scientific brief describes the natural process of protein expression and cell-line engineering in a mammalian cell.

2.1.1 Mammalian Cells

All living creatures are made of cells, which are small membrane bound compartments filled with an aqueous mixture of chemicals that supports its viability and functions. Mammalian cells differ from bacterial cells in many ways such as the requirement for oxygen, and presence of a nucleus and other membrane bound organelles. The nucleus, a membrane bound organelle, is where the full set of the cell’s chromosomes are contained. The full set of chromosomes is referred to as ‘genomic DNA’ or ‘genome’. Chromosomes are composed of deoxyribonucleic acids (DNA), which in turn, are the basic units that comprise genes. Other membrane bound organelles are responsible for creating energy and proteins, breaking down molecules and waste,
and importing and exporting products from the cell. Cells working in conjunction give rise to tissues, which in turn gives rise to organs.

2.1.2 How Mammalian Proteins are Made

Proteins are the major macromolecular components of the cell. They control cellular and bodily structure and functions. Some examples of proteins include enzymes, antibodies, hormones, and cell surface markers. Proteins are derived from linked amino acids, of which there are 20 types. Genes, which are composed of specific DNA sequences, are the blueprint of proteins defining their structure and function. See Figure 2-1 for a diagram of how proteins are made.

The protein molecule produced by the cell undergoes folding, based upon the intramolecular attraction of the amino acids present and post-translational glycosylation, which is the process of adding simple sugar molecules. The amino acid sequence, folding and glycosylation pattern together give rise to the protein’s final structure and function. Depending on the protein’s function, it may remain in the cell or be exported.

2.1.3 Plasmid Gene Transfer

Gene transfer is the introduction of foreign DNA gene sequences into a cell. Foreign DNA may come from similar or dissimilar species. The advent of recombinant DNA technology and DNA cloning has made gene transfer possible. In DNA cloning, the gene of interest is inserted into the genome of a self-replicating genetic element, usually a plasmid or viral vector, through a series of reactions in a test-tube involving DNA cutting and repairing enzymes. A plasmid vector is a circular DNA molecule found in bacterial cells which could be separated from the bacterial genomic DNA in a series of chemical reactions. A viral vector is a virus that has been modified to accept foreign DNA into its genome. Most DNA cloning experiments performed at Chromos utilize plasmid vectors, therefore the process of using viral vectors is not explained. The ACE Vector is a proprietary plasmid that could have foreign genes of interest cloned into it.
Figure 2-1: Mammalian cell protein expression

Figure 2-1 shows the process whereby DNA encodes for the production of amino acids and proteins. This process can be divided into two parts: (1) Transcription: Before the synthesis of a protein begins, the corresponding RNA molecule is produced by RNA transcription. One strand of the DNA double helix is used as a template by the RNA polymerase to synthesize a messenger RNA (mRNA). This mRNA migrates from the nucleus to the cytoplasm. During this step, mRNA goes through different types of maturation including one called splicing when the non-coding sequences are eliminated. The coding mRNA sequence can be described as a unit of three nucleotides called a codon. (2) Translation: The ribosome binds to the mRNA at the start codon (AUG) that is recognized only by the initiator tRNA. The ribosome proceeds to the elongation phase of protein synthesis. During this stage, complexes, composed of an amino acid linked to tRNA, sequentially bind to the appropriate codon in mRNA by forming complementary base pairs with the tRNA anticodon. The ribosome moves from codon to codon along the mRNA. Amino acids are added one by one, translated into polypeptidic sequences dictated by DNA and represented by mRNA. At the end, a release factor binds to the stop codon, terminating translation and releasing the complete polypeptide from the ribosome. One specific amino acid can correspond to more than one codon. The genetic code is said to be degenerate.

Protein synthesis

2 Figure and caption used with permission by the Access Excellence @ the National Health Museum <http://www.accessexcellence.org/AB/GG/protein_synthesis.html> [cited June 28, 2004]
The typical plasmid gene transfer process begins with inserting genes of interest into a purified plasmid vector creating a ‘cloned plasmid’. The cloned plasmid is then re-introduced into a bacterial host cell devoid of plasmids and the host is propagated in bacterial culture media. During this process, bacterial cells containing the plasmid duplicate logarithmically. Each daughter cell from the duplication process contains the same genetic information as the parent, including the cloned plasmid DNA. Therefore, as the number of bacterial cells increase, so do the number of plasmid DNA molecules. When the culture reaches a certain density of bacterial cells, the cells are processed to isolate the plasmid DNA, which are in milli – or microgram amounts (depending on the culture volume). The purpose of growing up a large amount of plasmid is to provide enough DNA for subsequent experiments and analysis.

Proteins encoded by the gene of interest are produced by transferring the cloned plasmid into a mammalian cell host. Mammalian cells are used for protein expression because bacterial cells do not have the cellular components required to produce complex protein structures such as monoclonal antibodies. Cloned plasmid is introduced into a population of mammalian cells on a cell culture dish by chemical means (‘transfection’) which are commercially available. The foreign plasmid migrates into the nucleus of the cell and integrates randomly into the host’s genome. Once integrated, the host’s cellular machinery treats the plasmid DNA the same as the host’s genomic DNA and expresses the protein encoded on the cloned plasmid. Chromos’ ACE System is unique in that the ACE Vector specifically targets the Platform ACE for integration due to the proprietary integrase system.

Cloned plasmids usually have a gene encoding drug resistance (or other selection marker/agent) alongside the gene of interest. The purpose of the selection marker is to isolate cells containing the gene of interest from the remaining, untransfected population of cells. In the case of a drug selection marker, cells after the transfection treatment are exposed to a drug that will kill the cells. However, cloned plasmid carrying the drug resistant gene that has integrated into the host genome will express the protein required for drug resistance. Therefore, cells that are drug resistant will remain viable and form colonies on the tissue culture dish, inferring that these resistant cells are also expressing the gene of interest. Drug resistant colonies expressing the gene of interest can be isolated, transferred, and grown independently on a separate culture dish creating an ‘engineered cell-line’. This process of transferring cells from one dish onto a new dish is known as sub-culturing. The expressed protein from the cloned DNA can be analysed for expression levels, functionality, structure, and other characteristics.
2.1.4 Mammalian Cell Culture

Mammalian cell culture refers to the propagation of mammalian cells in media and in a defined environment. Like bacterial cells, growth is logarithmic; however doubling time is much longer. Mammalian cells are usually grown in media containing essential nutrients, vitamins, cofactors, metabolic substrates, amino acids, inorganic ions, trace elements, and growth factors necessary to support cellular functions and the synthesis of new cells (McAteer & Davis, 1994, p. 98). Environmental or physiochemical factors include pH, osmolality, CO$_2$ levels, and temperature. Different cell types require different growth conditions for optimal functionality and growth characteristics.

Mammalian cells can grow as a monolayer (adherent cell-line) adhering to a scaffold matrix, or the bottom of a tissue culture flask or dish. Mammalian cells can also grow as a suspension (suspension cell-line) in stirrer flasks, shaker incubators, or large bio-reactors. Most commercial manufacturing cell culture applications require suspension cell-lines because they can grow at a higher density compared to adherent lines, although adherent lines are simpler and cheaper to work with. In addition, plasmid transfection occurs at a higher rate with adherent cells. However, more protein product can be harvested from a suspension cell-line compared to an adherent line that is expressing proteins at the same rate because of higher growth densities. Fortunately, there are methods that can convert an adherent cell-line into a suspension cell-line. Cell-lines are maintained by sub-culturing, which is the transfer of a portion of cells from its current environment to a new one thereby giving the cells sufficient space to divide and grow in population. Otherwise cells will die because they cannot be sustained at high densities, nutrients in the media will be depleted, and cellular waste will build up in the surrounding environment.

2.2 Pharmaceuticals vs. Biologics

Pharmaceuticals in general can be divided into two classes – small molecule and biologic compounds. Small molecule compounds generally refer to drugs that are chemical based and are produced synthetically, such as Tylenol® or Lipitor®. The active ingredients in these compounds have a chemical formulation with a known structure. Biologicals (or biopharmaceuticals), on the other hand, are defined by the Food and Drug Administration (FDA, the federal watchdog of foods and drugs marketed in the United States) as wide range of natural-based products such as vaccines, blood and blood components, allergenics, somatic cells, gene therapy, tissues, and recombinant proteins. Biologics are composed of sugars, proteins, nucleic acids, or a combination
of these and they are much more complex compared to small molecules. Recombinant proteins are proteins that have been genetically modified by scientists to increase efficacy or specificity and are usually used to treat a certain disease. The time to bring a small molecule drug from research to market may take between 10 to 15 years. Research and development alone takes several years to complete.

In most cases, pharmaceutical companies develop small molecule drugs and biotechnology companies develop both small molecule and biologic drugs. The means of developing small molecules are different between pharmaceutical and biotechnology companies. However, partnerships, licensing deals, and acquisitions have blurred what were traditionally known as a pharmaceutical and a biotechnology company. Distinguishing the two in terms of technologies and product types is no longer reliable.

The traditional small molecule pharmaceutical market is significantly larger than the emerging biologics market. According to research conducted by IMS Global, in 2003, the size of the global pharmaceutical market was about $492B (IMS Global, 2004a) of which $37B (IMS Global, 2004b) were biotechnology based products. Theoretically, biotechnology based drugs can precisely modify a patient’s physiology, often with greater success and fewer side effects than traditional small molecules drugs (Dove, 2002, p. 777), although recent biologic drug failures in clinical trials suggests otherwise. Factors such as molecular size, stability, and absorption require that biologic drugs be delivered to the body through intravenous methods at a clinic, rather than orally like small molecules drugs. The cost of manufacturing biologics is also greater than small molecules, thus contributing to their high price. For example, a vial of Neupogen®, a protein used to stimulate white blood cell production made by Amgen, costs about $2,800 (Powell, 2003, p. 965). Some biopharmaceutical regimes can cost patients tens of thousands of dollars yearly. The cost of materials for biologic drugs can be 20 – 100 times more than materials for small molecules (Gorman, 2004). Unlike small molecules which are manufactured by chemical synthesis, biologic drugs are produced in living organisms and require complicated extraction and purification processes, therefore their structure and composition are less predictable than small molecules.

Biologic drugs are derived from the analysis of the human genome, rather than the trial and error approach used by small molecule drugs, therefore the length of development and clinical trials are generally shorter. However, this is not always the case and is left to debate. Recent studies have shown that the time required to develop a biologic could be just as long as small molecules (Ashton, 2001, p. 310).
2.3 Pharmaceutical Industry Value Chain

Figure 2-2 depicts the pharmaceutical industry value chain. The chain is composed of 5 components – R&D, clinical trials, manufacturing, sales and marketing, and retail (distribution). Traditionally, the greater the number of components a company owns of the value chain or the further along a company’s product is developed on the value chain, the higher the company or product is valuated, respectively. However, research enabling companies (or tool companies) can also extract high value if their technology is used extensively in R&D applications.

Figure 2-2: Pharmaceutical value chain

The left axis represents a value of a firm as they incorporate more functions of the chain. The right axis represents the level of product manufacturing required to support various stages of the value chain. The stages and timing of the drug development process are shown on the horizontal axis. Chromos’ ACE System can enter the value to the chain at two points, early in R&D or early in manufacturing – depending on the requirements of the client. The value added by Chromos’ ACE System affects the entire chain by decreasing the time for drug development, decreasing manufacturing costs, and increasing manufacturing yields.

Companies that possess a number of elements of the value chain are said to be vertically integrated. Fully integrated pharmaceutical companies are known as FIPCOs. Large
pharmaceutical companies known as Big Pharma are usually FIPCOs and have yearly revenues in the billions of dollars. In general, R&D companies want to forward integrate and build clinical development, manufacturing, and sales and marketing functions, thus essentially transform into a FIPCO. That being said, it is rarely done as these functions are very expensive to build and operate, and incorporating them is very risky. These functions are sometimes best left to the large players in the industry, to which smaller R&D companies look to partner with when their products reach a certain stage of development. The larger players will clinically develop, manufacture, and market the product, while the smaller R&D companies in return will receive licensing fees, milestone, and royalty payments or some other profit/market sharing scheme. Big Pharma are similarly dependent on the smaller biotechnology firms, although for R&D resources. However more recently, some biotech companies have stopped relying on Big Pharma for later stage product development activities. Biotechnology companies have been able to outsource many of the later stage activities thus retaining ownership of their IP and value. Investors are more educated in the biotechnology industry and are more willing to invest, thereby supporting a smaller company’s growth. As biotechnology firms become more independent, they are also becoming competitors of pharmaceutical companies.

Sustaining growth in the traditional value chain model will later prove to be difficult for Big Pharma companies as waning pipelines and a decrease in approved blockbusters will lead to a slowdown in their growth. In a study conducted by Gilbert, Preston, & Singh (2003, p. 2) for Bain & Company, they feel that the pharmaceutical companies’ blockbuster drug model will deliver only a 5% return on investment (ROI). The blockbuster model is basically focusing investments on creating blockbuster product franchises or brands that achieve global sales of more than $1B. The low ROI is attributed to declining R&D, rising costs of commercialization, increasing payor influence (i.e. health management organizations and insurers), and shorter market exclusivity periods (due to patent expiry and competitive products). Big Pharma companies that rely on the blockbuster model will have to find another model to sustain growth, which could include shifting away from the FIPCO model and establishing partnerships with companies who specialize in certain portions of the value chain to share risk and rewards.

Bell (2003, p. 3) from the Arthur D. Little consulting firm feels that the traditional pharmaceutical value chain that is practiced by Big Pharma and other large biotechnology corporations is unravelling due to legislative pressures, the shift to genomics and proteomics in R&D combined with high drug development costs, increase in manufacturing costs, and alterations in customer behaviour. Drug approval today is much more difficult than the past as
regulatory authorities require greater detail in clinical data leading to an increase in the number of patients enrolled in clinical trials and clinical development costs. In addition, drug manufacturing processes must meet strict federal guidelines leading to an increase in manufacturing costs. Alterations in customer behaviour are due to numerous reasons such as the specificity of drugs that are developed today to treat certain patient sub-populations - thereby segmenting markets further, governments enacting measures to curtail healthcare costs, and patients taking an active role in their treatment and unwillingness to take new drugs. All these factors combined lead to increased costs, decreased revenues, and overall decreased profit margins.

The pharmaceutical and biotechnology industry are fragmented with companies specializing in specific areas of the value chain and finding niche markets within them. Finding a niche market or developing a superior technology could lead to high rents within a value chain segment, thus generating substantial revenues for specialist players. For instance, there are numerous companies that specialize exclusively in R&D (product development or research enabling technologies), drug delivery technologies, clinical trials (clinical research organizations [CROs]), manufacturing (contract manufacturing organizations [CMOs]), sales and marketing, and distribution. Rather than building these functions in-house, which lead to increasing costs and risks, product development companies who do not have critical mass in their pipeline could simply outsource these activities to specialized firms. Outsourcing may cost the product development companies more money in the short term; however, development risks are lower. In addition, companies do not have to build in-house knowledge, experience, or infrastructure in these areas, thus leading to shorter development times.

Chromos does not currently want to transform into a FIPCO, but rather extract high rents by occupying certain niches in the value chain as a technology provider – particularly in R&D and manufacturing (Refer to Figure 2-2). The ACE System technology can be exploited in these functions to provide an advantage to companies competing within these segments of the chain. The current strategy of remaining as a technology provider for certain niches may change as the Company gets closer to achieving their long term goal in becoming a gene-based cell therapy company. The revenues incurred by the Company by occupying certain niches will enable them to move forward and explore other areas of the chain.
2.3.1 Research and Development

Research and development is the stage where companies discover compounds to enter into clinical trials. Globally, there are over 4,000 specialized biotechnology companies (Kermani & Bonacossa, 2003). The majority are located in the U.S. and Europe. In 2000, biotechnology companies spent over $11B on R&D. Based on an estimate by DiMasi, J. et al. (2003, p. 166), the R&D stage for an approved compound is 5 years with a capitalized cost of $335M USD. The general steps for bringing a protein therapeutic forward into clinical trials are as follows:

1. Target Identification: Identification of genes or proteins thought to contribute to disease processes.
2. Target Validation: Identifying the significance of a target in contributing to a disease process.
3. Library Construction: Creating therapeutic protein(s) that would alter the function of the target.
5. Screening: Evaluating the therapeutic against the target to determine which compounds in the library are the most efficacious.
6. Production: Exploring if manufacturing the protein at a small and large scale is feasible.

At the end of the R&D process, the most optimized and promising candidate enters into drug trials. Other types of drugs may have a different R&D process, and many of the steps above will have to be reiterated. Companies must evaluate time, logistics, expense and expertise it can allocate at every stage. In addition, companies must be careful where money is spent as spending more money on R&D does not guarantee that their products will reach the market. The cumulative R&D activities leading to the creation of IP for a company significantly contributes to the value of the company.

The loss of R&D productivity and patent protection for blockbuster drugs threaten to erode enormous revenues long enjoyed by Big Pharma. Nearly 40% of blockbuster drugs in 2002 will lose patent protection by 2008 (Coe, 2004, p. 18). Large investments in R&D by Big Pharma had not produced drugs on a consistent annual basis; hence the turn to biotechnology companies to provide focused research tools and early stage pipeline products. In addition, R&D is becoming more expensive, driven by complex clinical studies and expensive enabling
technologies. Big Pharma companies can spend anywhere between 12 - 19% of revenues on R&D, and Big Biotech even more with 20 - 30% of revenues (Jackson, 2003, p. 40). Some large biotechnology companies may find themselves in the same position as their Big Pharma cohort – a lack of R&D productivity, after which they then turn to smaller biotech companies also for R&D and technology resources. Big companies lacking a continuous pipeline are willing to spend large amounts of money to obtain products from other firms in order to fill gaps. The large number of biotechnology companies suggests that the R&D landscape is fragmented, making it difficult for larger firms to access, assess and select technologies or products for licensing or acquisition.

Chromos' ACE System can help companies in the R&D stage by providing engineered cell-lines for research purposes, in particular at the library construction and production stages. In particular, Chromos will be targeting companies developing protein therapeutics such as monoclonal antibodies. Long term cost and time savings by using the ACE System will have to be clearly emphasized by the Company to their clients.

2.3.1.1 Research Cell-lines

Cell-lines can be engineered to produce foreign proteins such as monoclonal antibodies, blood factors, insulin, interferons, growth hormones and factors, and vaccines. Prior to entering biotherapeutic drugs into clinical trials, extensive research is performed to find the most optimal candidate to test. The ability to create high expressing protein cell-lines in a short period of time will allow scientists to scale back development times, analyse more cell-lines in a given period, and decrease research costs. Every month a product is unready for the market adds significant extra costs such as lost revenues, increased cost of capital, and increased R&D spending.

Traditional methods of creating high expressing cell-lines using gene amplification techniques takes at least 6 months, and the resulting cell-line may still be unstable with random integrations in the host genome. Chromos' ACE System only requires 2 to 4 months to produce a stable, long term expressing cell-line with gene insertion in multiple known 'acceptor' sites. In addition, a Product ACE can be isolated and transferred from one cell type to another, such as from a mouse cell-line to a stem cell-line. The ability to transfer a Product ACE allows different cell types to be auditioned for a desired performance (such as expression, growth, secretion, or quality of product) negating the need to start the engineering process from the very beginning since the Product ACE already carries the target gene. Cell-lines used in research usually differ
from cell-lines used in manufacturing, thus transferability of the Platform ACE provides an advantage as the Platform ACE can be moved from a research cell-line to a manufacturing cell-line. To be successful in the R&D area, Chromos must be able to deliver on the claims made against the ACE System, especially short development times, transferability of Product ACES, non-integration into the host genome, cell-line stability, and large payload capacity. Not all product development companies are concerned with high expression levels at the R&D stage; they are only concerned that their gene of interest could be expressed stably in a research cell-line.

2.3.2 Drug Trials

The value of a company increases if they have products participating in drug trials. The further the stage a product is in the trials, the more value the product has. Investors typically like to see a range of products in many different stages, thus a continual line of products which have the potential to enter the market. Milestone payments are often triggered when a product meets a certain goal during the drug trials. The trials for one product can take place in hospitals and research centers across different countries.

Drug trials begin with pre-clinical studies on animal models, usually a rodent and non-rodent. The purposes of these studies are to determine whether or not a candidate drug is safe for use in humans and to measure pharmacological activity. These are formalized studies for the purpose of generating data for an Investigational New Drug (IND) application, and not generating general R&D data. If a compound is determined to be safe for use in humans, an IND is filed with FDA. Other jurisdictions will have different processes. Only when an IND application is approved could trials in human subjects begin. For many young drug development companies, success in clinical trials will determine the fate of the company. In addition, success in clinical trials can broaden strategic routes that a company may pursue. Companies are encouraged to work closely with the FDA for guidance during clinical trials. There are three trial phases for human testing ('clinical trials') before marketing is approved by the FDA: Phase I; Phase II; and Phase III. According to the Pharmaceutical Research and Manufacturers of America (Pharmaceutical Research and Manufacturers of America [PhRMA], 2004, p. 16), 5 of 5000 compounds that enter pre-clinical trials make it to human testing, and of the 5, only one gets marketing approval. The capitalized cost of conducting clinical trials is estimated to be $467M
USD (DeMasi et al., 2003, p. 165). Others estimate the cost of clinical development may be more than 40% of total drug development costs (Kermani & Bonacossa, 2003, p 156).

Phase I trials are designed to: 1) determine the metabolic and pharmacologic action of the drug in humans; 2) determine the side effects associated with increasing doses; 3) potentially gather evidence of efficacy; 4) evaluate the drug's mechanism of action; and 5) collect information on pharmakinetics and pharmacologic action to design further clinical studies. About 80% of drugs that enter Phase I continue to Phase II. Usually 20 – 100 healthy volunteers (if the drug permits) are used in this study. Phase I trials can be completed in about 1.5 years.

Phase II studies are conducted to: 1) evaluate the effectiveness of a drug 2) determine the short term side effects and risks; 3) determine dosing response and ranges for Phase III studies; 4) evaluate potential study endpoints; and 5) target populations for further studies. About 30% of drugs that enter Phase II continue to Phase III. From 100 to 500 diseased volunteer patients may be used in this study and takes about 2 years to complete.

The Phase III trial, also known as the pivotal trial, is a large scale study to determine the overall effectiveness of a drug at a specific dose based on data found in the previous trials. The candidate drug must demonstrate benefit against a control (placebo, which does not exhibit any therapeutic effect), in combination with other treatments, or versus existing treatments in order to be considered for approval. Phase III trials must be controlled, randomized, have a blinded design, and be of adequate size to show statistical significance that a candidate drug is more efficacious or safe than existing treatments. About 80% of drugs entering Phase III pass. These studies usually involve 1,000 to 5,000 patient volunteers and require about 3.5 years to complete. At the end of Phase III, a Biological License Application (BLA) is filed with the FDA for marketing approval. For non-biological drugs, a New Drug Application (NDA) is filed. The approval process may take a few to several months, depending on whether an unmet need will be fulfilled with the drug. In 2003, the average time to approve a biologic was 34.7 months. However, some biologics such as Genentech's Raptiva® required only 10 months.

Some drugs require Phase IV studies, which are after market studies. Patients are monitored long term, up to several years. The purpose is to expose long term side effects, strengthening data for marketing purposes, and adding more data to the drug label.
2.3.3 Sales and Marketing ('S&M')

Enough cannot be said about the importance S&M plays in the success of a drug. With good marketing and a strong sales force, an inferior drug can surpass sales of a superior drug. Marketing capabilities can be the differentiating factor in mature markets, where clinical outcomes of each drug in the market are relatively the same. Generic competition makes this task even more difficult. In addition, marketing is important in immature markets where physicians need to be taught about the therapeutic benefits of a drug. S&M plays an enormous role in making a drug a blockbuster and helps pharmaceutical companies survive when their pipeline is low or when product approval has slowed. A pharmaceutical company should not begin thinking about marketing strategies after the approval of a BLA, but rather at the R&D stage. Understanding who the target market is and what needs are not being met influences R&D direction and clinical trial designs. Clinical trials should be designed to ensure the approved indication is for the targeted market. Without a strategic clinical trial plan, the drug may be approved for another indication as the wrong dataset might be collected, the wrong patient population may have been used, the wrong surrogate endpoints have been measured, or enhanced clinical efficacy over existing therapies may not have been demonstrated. Clinical data plays an important role in marketing strategy. The data can then be used to influence clinicians, opinion leaders, and lobbyist groups.

Competition is getting extremely difficult as more drugs are entering the market. Pharmaceutical companies are no longer enjoying long term market exclusivity for a product, which now usually lasts for a few years. Exclusivity is challenged by factors such as patent disputes, more effective drugs of the same class by competitors, and regulatory stringency and surveillance leading to product withdrawal. Sales and product reputation must be maximized as soon as a drug is approved for marketing. Seven of the top ten pharmaceutical companies spend more than 30% of gross sales on S&M activities, which is on par with companies such as Coca-Cola (Jackson, 2003, p. 10). Pharmaceutical companies spend almost twice as much on S&M than R&D. Launching a blockbuster drug can cost between $300M to $500M USD five years before launch to three years after, therefore it is not uncommon for a smaller biotechnology company to partner with a larger pharmaceutical company who have S&M expertise as well as deep pockets to support a product’s launch (Jackson, 2003, p. 119). In some cases, promotional costs in the first year can be as high as $1B.
The size of a pharmaceutical company's sales force is usually in the thousands. Sales forces target their audience by using electronic territory management information systems and physician profiling. Electronic territory management allows companies to co-ordinate the movements of sales representatives to ensure each physician meets with a representative that best suits their needs. Data from the meetings are then fed back to a central computer, which is then used to create optimized promotional material and future strategies. Even a physician's prescribing habit is used to identify the most favourable target audience.

Television commercials, radio, magazines, and the Internet are also popular forms of advertising media. These types of advertising are also known as direct to consumer (DTC) advertising. In 2002, DTC spending was estimated to be $2.5B ("DTC marketing outlook", 2003, p. 8). Marketing is becoming more challenging as consumers are more informed about their drug choices. Lifestyle drugs such as Viagra® or Claritin® are effectively advertised by this method. The goals of DTC advertising are to establish brand awareness and have patients influence prescribers by asking for specific drugs. The amount spent on DTC advertising generally depends on the dynamics of the therapeutic area in question, the prevalence of disease or potential patient population, the lifecycle stage of the drug, and the company's portfolio strength ("DTC marketing outlook", 2003, p. 27).

Data from Phase IV trials also play a role in the marketing effort as it is used to capture a greater consumer base, build relationships with certain prescriber groups, counter threats from new drug entrants, and support current and new drug indications or labelling.

2.3.4 Retail

The drug retail industry is comprised of all retailers selling over-the-counter (OTC) and prescription drugs. OTC drugs are normally derived from prescription medications but have lower dosage forms and generally safer toxicology profiles. Biologics are not included in the OTC drug category. Retailers include drug store chains, pharmacies, supermarkets, or convenience stores. More recently, mail order and the Internet has become a retail channel for pharmaceutical distribution. The global drug retail market in 2002 was $385B, and is expected to grow to $467B by 2007 (DataMonitor, 2003a, p. 3). Drug store chains account for 42.2% of industry sales, and independent drug stores account for 28.9% in 2002. Most biologic drugs would not be distributed through retail channels as they are administered by professional clinicians.
2.3.5 Manufacturing

The manufacturing level of protein therapeutics must be taken into consideration throughout product development and marketing. Protein therapeutic manufacturing can be divided into two categories, small scale and large scale manufacturing. In small scale manufacturing, only enough product to meet pre-clinical and Phase I requirements are produced. In large scale manufacturing, the production process is scaled up to meet the requirements of Phase III and beyond including commercialization. The further along the development stage a product is on, the greater the quantity of product needed (See Figure 2-2). The FDA has very strict guidelines on manufacturing. A drug may not be approved for commercialization if manufacturing guidelines are not met, and after approval, manufacturing processes cannot be changed without tests being conducted. New clinical trials or other bridging studies may be required if a protein is produced by a different method than the original used in the drug’s initial clinical trials. Changes in production processes can cause physical changes to the protein, such as folding and glycosylation patterns, thus rendering them less effective than the original. The manufacturer must prove that the product from the old process is exactly the same as the product from the new process. Hence the manufacturing of a therapeutic protein is very risky from developer’s standpoint. There is no point in developing a candidate drug for market if its manufacturing is not economically viable.

Large scale manufacturing is not limited to just mammalian cells. Other manufacturing methods include bacterial and yeast based systems, insect cells, transgenic animals, and plant based methods. The choice of system is determined by the complexity of the protein drug and cost advantages. Mammalian cells are inefficient protein producers compared to other manufacturing methods, and require costly infrastructure at commercialization levels. Small, simple proteins are usually produced in *E. coli* bacterial cells, and more complex proteins (requiring folding and post-translational glycosylation) are produced in mammalian cells – in particular, Chinese-Hamster Ovary (CHO) cells. To date, only mammalian cells are known to replicate the proper glycosylation pattern required of human antibody therapeutics. Other systems lack the ability to produce a recognizable pattern. Improper glycosylation can lead to rapid clearance of therapeutic antibodies or cause an immune response in the patient. Of the 19 commercial monoclonal antibodies and antibody fusion proteins synthesized by mammalian cells, approximately two-thirds are produced by CHO cells (Morrow, 2004, p. 17). Other common mammalian cells used for manufacturing include non-engineered Hybridomas, NS0, Baby
Hamster Kidney (BHK), PER.C6™, and SP2/0. A hybridoma cell is a fusion of an antibody secreting B-cell and a lymphocyte tumor (or myeloma) cell. Hybridoma cells have been modified such that endogenous antibodies are not secreted. NS0 cells are derived from myeloma cells, and do not produce endogenous antibodies. PER.C6™ is a proprietary human cell-line owned by Crucell. SP2/0 is another type of modified myeloma cell.

2.3.5.1 Protein Manufacturing

Over the last few years, there has been an on-going debate as to whether or not there will be a shortage of capacity for protein production. Monoclonal antibodies in companies’ pipelines, if approved, will use a large portion of the world’s current production capacity. Current worldwide capacity is estimated at about 800,650L, with an additional 992,000L expected to come on-line by 2006 (Chovav, Wales, De Bruin, Samimy, Meacham, Kim, & Farhadi, 2003, p. 16). A typical 12,000L bioreactor can produce 30 – 35kg of protein per year, with monoclonal antibodies produced in higher quantities than non-antibody proteins (Chovav et al., 2003, p. 16). Dosing requirements for monoclonal antibodies are much higher than that required for simpler proteins. Much of the current shortage in protein production capacity is due to the production of marketed antibodies, which require longer production runs, and complex machinery and processing to produce the amount demanded. Manufacturers were not prepared for the success that antibodies brought.

On the other hand, some feel that the decrease in monoclonal antibody approvals has alleviated the pressure on capacity temporarily. In the next few years, more production capacity will be on-line as drug companies and CMOs will complete the building or expansion of their manufacturing facilities. The timing of new antibody approvals and production capacity will be in-sync and no production pressures will be felt.

The decision to build or outsource manufacturing is not simple. Analysts estimate the cost of producing a new commercial scale facility for mammalian cellular protein production can range from $300 to $500M and requires up to 6 years to complete (Chovav et al., 2003, p. 5). Industry experts estimate that it costs $50M to build a 15,000L bioreactor, including downstream processing and quality control (Bernstein, 2004, A5). The decision to build must come early in clinical development in order to maximize market penetration early after marketing approval, even as early as Phase II trials. In addition, the manufacturing process must also be chosen early as changes in manufacturing processes during product testing may require additional studies to
prove that the products made from two separate processes are exactly the same. Companies are taking enormous risks when deciding to build manufacturing facilities during the clinical trial phase as the product still has a chance to fail, thus leaving the company with an under-utilized facility. Building a pilot facility for the production of products for early clinical trials is not cheap either. The cost of a 2,000L pilot facility may range from $60 - $80M (Bernstein, 2004, A5), which can usually only produce enough material economically up to but not including Phase III.

Production runs and process development activities are expensive, thus CMOs prefer to deal with large companies who can guarantee contracts of 4 – 5 years (Bernstein, 2004, A2). CMOs are even demanding deposit money from clients to ensure capacity will be set aside. Small companies are paralysed because they only want a small amount of product and do not want to pay too much for it.

Monoclonal antibodies will be the driver of therapeutic protein market growth, therefore its production process will be described (however, the production of other proteins is very similar). The manufacturing process described by Chovav (2003, p. 30 – 34), typically begins with the CMO receiving from the client a seed culture of a mammalian cell-line that produces the antibody of interest at a consistent yield. The seed culture is then scaled-up (expanded) in a small batch, which is then used to seed a large bioreactor (i.e. a 10 – 30kL tank) containing media that optimizes cell growth. After 10 – 14 days of growth, the culture media containing the therapeutic antibody is separated from the cells by centrifugation and/or filtration. The media is subsequently pumped into a chromatography column, where the antibody selectively binds to the chromatography matrix and other biological wastes are eluted away. The bound antibody is further washed in the column and then eluted from the binding matrix. The binding, washing, and eluting cycle is repeated a few more times to purify the antibody in a homogenous solution. The antibody solution is subsequently pumped through a porous membrane that removes bacterial and viral particulates. The purified antibody solution is stabilized in a buffer reagent containing serum albumin or other preservatives. The final antibody solution is subjected to a battery of quality control/quality assurance (QC/QA) tests to ensure the quality of the final product. After completion of product analysis, the purified antibody undergoes the fill and finish stage, where the antibody is split into vials or lyophilized and made ready for therapeutic usage.
2.3.5.2 Production Cell-lines

Creating a stable production cell-line with high productivity is vital for long term cost savings. Low productivity cell-lines require longer run times to produce enough protein to meet market demands compared to high productivity lines. Longer run times lead to increased manufacturing costs and delays in bringing the product to market. Cell-lines must also be stable in order to maintain product consistency during batch runs. Unstable cell-lines lead to increased costs as more batch runs will be required to meet market demands. Proteins must be produced economically or else they may never reach the market. If the costs of production are too high, the cost of therapy for patients may not be economically viable and attractive.

Analysts estimate that current manufacturing cell-lines can produce on average 0.5g/L of protein (Chovav et al., 2003, p. 16). The target rate is 1.0g/L, with most achieving 0.75 – 1.0g/L. Some experience producers can raise this level to 1.5 – 2.0g/L. Chromos’ ACE System can make cell-lines that range from 0.5 – 1.0g/L, with further optimization, this range can be made even higher.

Chromos believes the ACE System is a valuable platform for protein manufacturing because stable, high protein expressing cell-lines can be created in a short period of time amongst other advantages. These characteristics of the ACE System are the success factors that Chromos needs to achieve to be reputable in the manufacturing arena. A billion dollar drug cannot afford to waste time in production delays, otherwise significant revenues and market share will be lost. The earlier that Chromos’ ACE System is used for manufacturing, such as at pre-clinical trials, the better for the client as long term manufacturing processes could be developed around the ACE System. If a client changes their manufacturing process later in the development stage to utilize the ACE System, additional tests will be required to show product similarity between the different processes. These additional tests can be costly and lengthy.

2.3.6 Value Chain Summary

The pharmaceutical value chain can be broadly categorized into 5 components: research and development; drug trials; sales and marketing; retail; and manufacturing. Drug trials are divided in three Phases – I, II, and III. The further along the value chain a product is developed, the greater its value. In addition, the more components of the value chain a company possesses, the greater the value of the firm. Product manufacturing must be taken into consideration at all
stages of product development since the further along a product is developed, the greater the amount of product is required to support demand.

Pharmaceuticals could be divided into two classes, small molecules and biologics. Small molecules are synthetically made with chemicals, whereas biologics are derived from natural biological processes. The capital cost of developing a drug from R&D to commercialization could cost close to $1B and takes about 14 years, and the probability of a drug entering the market is extremely low. Product launch could cost almost as much as the drug’s entire development. Chromos’ ACE System could be used to decrease R&D as well as manufacturing costs by creating high protein expressing cell-lines in a short period of time. Saving development and manufacturing times allows products to enter the market in a shorter period of time, thereby increasing a product’s profit potential before its patent expires or competitors take over.

2.4 Proposed Chromos Services

To reiterate, Chromos is evaluating the opportunity to put into operation a cell-line engineering service business based on the ACE System. Cell-lines will be engineered by Chromos scientists for clients in the product development business for research and/or manufacturing purposes. The ACE System can affect all areas of the pharmaceutical value chain except retail as biologic drugs are not available through retail channels. Specifically, the services that Chromos is considering to offer include cloning and integration, cell-line expansion and assaying, and various other customer support services.

2.4.1 Cloning and Integration

The client has a choice as to what cell-line they want to use for protein expression, Chromos’ Platform ACE cell-line ('Platform-Line’) or the clients’ own cell-line. In addition, clients have the option of sub-licensing the CHOK1SV cell-line. The client will provide Chromos with their gene of interest (the ‘product gene’) cloned in a plasmid and Chromos will then clone the gene onto the ACE Vector. If the client chooses to use Chromos’ Platform-Line for expression, Chromos will transfected the Product ACE Vector along with ACE Integrase into Chromos’ Platform-Line – thereby creating a Chromos Product-Line (Figure 2-3 path A, B, and C). If the client chooses to use their own cell-line, an isolated Platform ACE will be transferred from Platform-Line into the client’s cell-line prior to ACE Vector targeting – thereby creating a Client Product-Line (Figure 2-3 path B, D, F, and G). If the client requires multiple cell-lines
expressing the same gene of interest, an isolated Product ACE will be transferred to the client’s other cell-lines – again creating additional Client Product-Line (Figure 2-3 path A, B, C, E, F, and G; and see Section 2.4.2).

Figure 2-3: Proposed cloning and integration service
This figure shows the possible paths how Chromos could engineer cell-lines for clients.

2.4.2 Cell Expansion and Assay

Colonies of cells that contain the Product ACE will be sub-cultured and its population will be expanded, thereby creating the ‘Product-Line’; or ‘Production-Line’ if the cell-line is to be used for product manufacturing rather than research. A selectable marker will be used to distinguish Product-Lines from the rest of the cell population. The cells from the expanded population will be used for analysis. Analytical methods to determine expression levels include a flow cytometry based technique for monoclonal antibodies and enzyme linked immunosorbent assay (ELISA) for both monoclonal antibodies and other proteins. The client is expected to provide biological materials for the ELISA as it would be specific for their protein. In addition, the client will provide Chromos other biological material required to make comparison studies
such as control cell-lines. Analytical methods that will be performed to demonstrate proper integration of the product gene on the Platform ACE include polymerase chain reaction (PCR), fluorescent in-situ hybridization (FISH), and Southern Blotting. PCR is performed to determine if the gene integrated properly on the Platform ACE ‘acceptor’ sites. FISH, a microscopic visualization method, is performed to determine if gene integration only occurred on the Platform ACE and not into the host’s genomic DNA. A Southern Blot will be performed to determine the number of genes integrated (copy number) onto the Platform ACE. Theoretically, the higher the copy number, the higher the expression level. In addition, scientists would also know approximately how many ‘acceptor’ sites are still available on the Platform ACE for a second round of gene integration with the same or another gene. Protein expression levels, purity, and glycosylation patterns will be analyzed through High Performance Liquid Chromatography (HPLC).

After Chromos has created an initial Product-Line, the client will have the choice creating more Product-Lines based on different cell types. The Product ACE from a Product-Line will be transferred into the client’s cell-line of choice, and the cells will be subsequently be analyzed for protein expression levels and purity (See Section 2.4.1). Different cell-lines have different characteristics such as stability, protein expression, growth characteristics, or function. For example, a stem cell-line used for research would not be used for large scale protein manufacturing. Chromos can also adapt adherent cell-lines into suspension cell-lines at the client’s request.

Product-Lines that meet the client’s specifications will be expanded to a population the client requires, tested for mycoplasma (a type of bacteria that can kill cultured cells) contamination, and delivered. Chromos will offer the option of overseeing tests that can be contracted out, such as mycoplasma testing. Cell banking services, or the storage of cells for long term, would also be contracted out.

2.4.3 Customer Support

Each cell-line produced would be performed by a dedicated team of scientists, headed by a project manager whose role is to maintain relationships with clients and oversee the engineering progress. Chromos will maintain records appropriate to regulatory filing and archive biological material at the client’s request. Clients will receive bi-weekly progress reports and will be expected to work closely with the project manager. In addition, clients will be given a full
2.5 Chapter Summary

This chapter began with the general explanation of how proteins are made naturally in the mammalian cell and through the cell-line engineering process. With cell-line engineering, genes of interest could be made to express in virtually any type of cell-line, however, some are more receptive to being altered than others. In addition, protein expression is affected by other factors such as the cell culture environment. Following the discussion of engineering cell-lines, the pharmaceutical value chain was introduced. The chain is composed of R&D, drug trials, sales and marketing, retail, and manufacturing. Chromos' ACE System has the potential of adding value across the value chain, especially in R&D and manufacturing. Chromos could engineer a variety of Product-Lines in a short period of time for R&D purposes, thereby allowing scientists to shorten development times and be more productive in their research efforts. In the area of manufacturing, Chromos' ability to produce stable, high protein expressing Production-Lines decreases manufacturing run times thereby increasing the number of runs that a manufacturer could perform in a certain period of time. The services that Chromos proposes to offer to for clients include cloning and targeting product genes onto the Platform ACE, cell-line expanding and assaying, and providing customer support for the engineered cell-lines including the outsourcing of other services. The analysis of the external environment with respect to cell-line engineering will be discussed in the next chapter.
3 MARKET ANALYSIS

Chromos has the opportunity to occupy a small niche in the pharmaceutical value chain as a cell-line engineering firm, or expand its position in the chain by incorporating more upstream and downstream functions in protein manufacturing (essentially become a CMO). The more upstream and downstream functions a company offers, the higher its value. Typically, cell-line engineering is on the lower end of the value chain since most CMOs are capable of offering it. Functions such as small and large scale manufacturing and downstream processing are more valued since they are more specialized. However, value can be extracted from cell-line engineering if Chromos can develop superior expressing cell-lines at lower costs in a shorter period of time compared to their clients and occupy a niche space. In addition, value can be extracted if Chromos could engineer ‘difficult to express’ Product-Lines as not all proteins can be expressed using currently available technologies.

This chapter analyzes the market potential of mammalian cell-line engineering for the purpose of protein production. Porter’s (1979) Five Forces Model is used to examine the competitive forces that influence the industry. Subsequently, market size, potential customers, and the unmet needs of the industry are assessed, followed by a description of other technologies which may compete with mammalian cell expression systems. Finally, direct and indirect competitors are identified and described.

3.1 Porter’s Five Forces Analysis of the Cell-line Engineering Service Industry

Competitive forces in an industry are not just limited to competition from rival companies. Knowing the forces in play helps companies develop strategies to defend or influence the forces and remain in the forefront of competitors. As described by Porter (1979), players in the industry must consider the activities of rivals, suppliers, customers, potential entrants into the industry, and substitute products or services that could take market share. The sum of the forces determines the ultimate profit potential of an industry. Understanding the forces reveals the strengths and weaknesses of a company, helps companies visualize where they are positioned in the industry, identify strategies that would yield the greatest return, and discover areas of opportunities and threats. Porter’s model is applied in the analysis of the mammalian cell-line engineering service industry for the purposes of protein manufacturing and is summarized in Figure 3-1.
3.1.1 Threat of New Entrants

New entrants into an industry bring new capacity, desire to gain market share, and often substantial resources (Porter, 1979, p. 3). The significance of the threat of entry depends on barriers to entry and the response of industry incumbents.

The capitalized cost of entry is low if the new entrant has the infrastructure required to enter already in place. A laboratory with existing tissue culture capabilities can pose as a threat. A company that performs tissue culture on a routine basis will most likely use basic transfection procedures to create Product-Lines for research. The engineering of cell-lines for large scale manufacturing, though, can be more challenging as issues such as poor stability and inadequate expression levels have to be contended with. Most biopharmaceutical companies have tissue culture capabilities, and thus the basic infrastructure in place.

On the other hand, the capitalized costs for starting up a company and building infrastructure solely to perform cell-line engineering is high as labour, equipment, and operational set-up are expensive resulting in a high entry barrier.
Although the cost of entry is low for existing biotechnology companies, other high barriers of entry are demonstrating that their product offering is technologically superior to the incumbents, cost effectiveness, patent landscape, allocation of resources, and regulatory compliance. Demonstrating technological superiority is difficult as most common transfection systems produce similar results in terms of rate of protein expression. Rates of expression vary, but for the most part are low, thus offering no cost advantages. Technologies or methods that produce high expressing cell-lines will most likely be patented, which new entrants will have to avoid infringing, again, making the barrier to entry high. Early stage product development companies focused on research will not likely invest resources on developing cell-line expression optimization technology as most of their resources will be used to support core R&D. Also, new expression technologies must meet regulatory standards if the product produced is to be used as a therapeutic, which will require additional resources in order to become compliant.

In summary, new entrants must demonstrate a differentiation factor that is attractive to the industry; otherwise they will not attract any customer attention. The overall threat of new entrants is low to moderate in the near to medium term (~8 years). However, the threat of new entrants in the long term is high as new technologies such as fungal, animal, or plant based systems will be developed enough to produce protein therapeutics in a cost effective manner. These systems, theoretically, can produce many of the same proteins as mammalian systems; their advantage is cost effectiveness.

### 3.1.2 Threat of Substitutes

Substitute products can limit the profit potential of an industry if they can demonstrate an attractive price-performance trade-off. Mammalian cell-lines are not the only expression system that can produce approved therapeutic proteins at commercialization levels. Microbial systems based on *E. coli* are very efficient producers of simple therapeutic proteins that have been approved by the FDA. Their drawback, however, is that *E. coli* is limited by the types of therapeutic proteins it can produce. Mammalian cells are inefficient producers of therapeutic proteins and require complex, expensive equipment for production and processing; but their advantage is the ability to fold and glycosylate proteins, whereas *E. coli* cannot. The inefficiency of mammalian cells has led researchers to look for other cost effective protein manufacturing systems. These other systems include insect cell, fungal, plant, and transgenic animal systems (these are discussed in detail in Section 3.5). The threat of substitutes hinge upon whether or not
new production systems can produce a product that is safe for human consumption, which is determined by regulatory authorities. This may take several years as clinical trials will need to be performed. If products produced by alternative methods are deemed safe, then the threat of substitutes are high as most of these substitutes are more cost effective and biopharmaceutical companies will exploit this advantage. Recently, GTC Biotherapeutics has developed an European Medicines Evaluation Agency (EMEA, the European Union drug regulatory agency) approved product produced in the milk of transgenic goats; however, the long term cost savings still have to be determined.

Gene therapy and cell therapy are substituting technologies that could potentially eliminate the need for protein manufacturing altogether as the human body would be used as the protein production factory. These treatment methods have been used in some clinical settings and have shown some promising results, however, they are still at their infancy and many challenges still have to be overcome including technical and regulatory issues. Overall, the threat of substitutes is low to moderate in the short to medium term, but high in the long term as the new technologies overcome their current challenges.

3.1.3 Bargaining Power of Suppliers

Suppliers can exert bargaining power on industry participants by raising prices or reducing the quality of purchased goods and services (Porter, 1979, p. 5). There are a number of equipment suppliers who can provide the general equipment and consumables for tissue culture and other general laboratory processes. However, there are only a few suppliers of tissue culture media which is required for cell-line maintenance. Once a development process is chosen and optimized, changing culture media may alter the optimized conditions. Thus if supply for a certain media is low, companies are affected as they are dependent on the media supplier. In addition, specialized media for a specific cell-line may be made by only one company, thus increasing a company’s dependence. However, media suppliers are too dependent on the industry to buy their products as the media they produce is only useful for biotechnology companies; therefore it is unlikely that suppliers will exercise power over the industry.

Suppliers of technology may threaten the industry by forward integration or licensing technology to competitors or new entrants. This scenario is plausible if a company discovers a new technology that increases cellular protein production, but the technology is not part of their corporate strategy or core competency. The value of this said technology is extracted through
out-licensing. Whether or not this will happen is difficult to say, however, suppliers do have plasmids, cell-lines, genes, chemicals, or other modification techniques that claim to produce cell-lines that are superior to others already in the market place. If the supplier chooses to forward integrate into cell-line engineering, then they will become a potential competitor. Overall, the bargaining power of suppliers is low since they are dependent on the industry for survival.

3.1.4 Bargaining Power of Customers

Customers that require engineered cell-lines include biotechnology firms whose research, pipeline, or product offering include therapeutic proteins, and CMOs whose clients require therapeutic protein manufacturing.

Product development companies that have ample resources could easily backward integrate and develop their own protein expression technology, or at least optimize product cell-lines themselves. However, if the company cannot obtain an expression level to their satisfaction, they will turn to cell-line engineering companies. Product development companies can outsource activities to a wide range of cell-line engineering service providers (mostly CMOs). Thus depending on the customer’s needs, cell-line engineering can be considered a homogenous service. Customers will be price sensitive if they are not profitable and must be prudent on R&D spending, which pertain to the majority of product development companies. Price sensitivity will influence which cell-line engineering service provider they outsource activities to.

The switching cost of changing expression cell-line technologies can be low, depending on the customer’s stage of product development and if the new technology gives the client substantial cost savings. Customers will also be influenced by the service provider’s track record, if they have regulatory compliant facilities, and if they have manufactured products that were approved by regulatory agencies. Those providers that have had regulatory success will more likely be chosen. Overall, the bargaining power of customers is high.

3.1.5 Threat of Rivals

The threat of rivalry depends on factors that can change an incumbents strategic positioning within an industry. Rivals can be divided into two types: 1) those who have a competing technology to the ACE System that can increase cellular protein expression over conventional technologies; and 2) those who do not have a competing technology but perform
cell-line engineering services as part of a menu of others. There are several different cell based

technologies in the cell-line engineering business; however none have shown complete
dominance except for maybe Lonza’s G.S. System. Rivals who are threatening are those who
have number of service offerings in terms of upstream and downstream processes in addition to
cell-line engineering. These rivals, however, usually do not have a differentiated cell engineering
technology. Regulatory agencies require that manufacturing processes remain the same for a
product throughout development; therefore a customer is more likely to choose a service provider
who also provides some upstream and downstream capabilities that have been optimized with
their cell-line engineering process for process consistency and to avoid the headaches of process
transfer to other firms.

Incumbents may continue to make improvements to their technology to surpass the
competition. A company’s reputation and clientele will influence a customer’s choice of
provider. If a competitor’s technology has been validated by Big Pharma or key biotechnology
players, then new customers are likely to follow. The overall threat of rivals is high.

3.1.6 Conclusion

The strongest forces that influence the industry are customers and rivals. Most customers
will be price sensitive because they will be reluctant to allocate capital towards cell-line
engineering at the expense of compromising R&D. Cell-line engineering itself is a homogenous
service, rivals are differentiated by reputation, experience, and the types of services and cell
expression technology offered. If customers are not satisfied by any of the services offered, they
could easily backward integrate and optimize their own product cell-lines. Mid- and large-tier
companies are more likely to backward integrate than smaller companies.

The threat of new entrants and substitutes are low in the short term, but high in the long
term. New entrants must show they are technologically superior to incumbents, and that their
manufacturing methods have economic value. The biopharmaceutical industry is concerned with
the high costs of product manufacturing, especially with mammalian cells. However, mammalian
cells are currently the only system that can fold and glycosylate complex proteins the closest to
the human form. Although researchers are discovering ways of solving the problem of
glycosylation when using other manufacturing systems, they are still a few years away from
demonstrating consistency and cost effectiveness. Substituting technologies must prove that they
can produce regulatory approved products; otherwise its value in the industry will not be high. A
product development company incurs more risk when manufacturing with a method that has not been proven to be effective or approved by a regulatory body.

3.2 Market Size of Cell-line Engineering Services

Market size is based upon the number of potential clients, which include protein therapeutic product development companies, biogeneric developers, and CMOs who utilize mammalian cells as a production system. Each of these potential client groups is discussed in the following sections.

3.2.1 Product Development Companies

The therapeutic protein market will be driven by factors such as profit potential, ease of manufacturing, and acceptance by physicians, patients, and payors as a therapeutic option. According to Walsh (2003), from 2000 to June 2003, about 114 new chemical entities (NCE) were approved in the European Union and United States (Walsh, 2003, p. 865). Excluding duplicates (the same drug approved in both regions) the number is reduced to 80. During the same period, 64 biopharmaceuticals were approved in both regions. This number can be reduced to 30 when factoring that many of the active ingredients are the same in different approved products and when products were approved for the first time in one region after 2000 while already approved in another before 2000. This data suggests that over a quarter of genuinely new drug approvals were biopharmaceuticals since 2000. In 2003, 35 new drugs were approved by the FDA, of which 14 were biologics (PhRMA, 2004, p. 1).

The annual global market for biopharmaceuticals is estimated to be $30B in 2003 compared to $12B in 2000 (Walsh, 2003, p. 865). By 2010, the therapeutic protein market is expected to reach $57B (DataMonitor, 2002). Sales in monoclonal antibody products increased 38% from 2001 to 2002 totalling $5.4B (Pavlou, 2004, p. 274). Monoclonal antibodies such as Centocor’s Remicade®, and Genentech’s and Roche’s Rituxan® and MabThera® have attained blockbuster status with over $1B in worldwide sales. Pavlou (2004, p. 274) identified 376 therapeutic antibody development programs of which 132 are currently in clinical development across 95 key companies. From 2003 to 2008, 21 new antibodies for therapeutic treatment are predicted to be launched (DataMonitor, 2003b). However, over the last few years, there has been a downward trend in pharmaceutical approvals overall (see Figure 3-2) (Kermani & Bonacossa, 2003, p. 154). It remains to be seen whether or not monoclonal antibodies are unaffected by this
trend. By 2008, the global therapeutic antibody market is forecasted to be worth $16.7B. The antibody market is expected to be focused on oncology, arthritis, immune, and inflammatory disorders.

Figure 3-2: Global NMEs introduced

This figure shows the number of new molecular entities (NMEs) introduced worldwide and total monoclonal antibodies (MAb) (CMR, 2004 & Walsh, 2003). The 2003 figure is in conflict with PhRMA’s 2003 estimates. Different organizations and analysts will have different estimates depending on their definition of what is considered an NME.

The number of companies that Chromos could cater their cell-line engineering services to is high. Product development companies usually use traditional means of cell-line engineering and expression optimization such as random gene integration, gene amplification, auditioning different promoters, adding non-proprietary gene based expression enhancing elements, and media optimization. These methods of cell-line development are very time consuming and does not guarantee high expression yields. The earlier in the R&D stage a product development company is approached by Chromos, the greater the chance that the company will consider using the ACE System and the Company’s cell-line engineering service. If companies are too far into
the development stage and are starting to manufacture products for clinical trials or market, the likelihood of changing their cell expression system to the ACE System is lower since significant resources and time have already been committed. However, they could still be potential clients if their production cell-line is inefficient and cost ineffective.

Although the potential size of the market may be large, the likelihood of biopharmaceutical companies outsourcing cell-line and gene expression development at all is questionable. According to a marketing report by HighTech Business Decisions (2001, p. 4-46), the likelihood of outsourcing cell-line and gene expression development was 1.63 out of 5.0 in 2001 (where 5.0 indicates highly likely), and was also projected to be 1.64 out of 5 in 2004. The number of companies and product classification were not mentioned in the report which could influence the results. The low score could possibly be attributed to companies denying that they are having protein expression difficulties, a lack of resources to justify outsourcing, are still in the research stage where expression yield is not a large concern, or would rather in-license protein expression technology than risk outsourcing their proprietary drug molecule. As an aside, fill and finish packaging and analytical testing were the most likely functions to be outsourced in 2004 with scores of 4.05 and 3.72 out of 5, respectively.

Overall, it appears that monoclonal antibodies are being accepted as a form of therapeutic and the market is definitely growing as more products are being approved. The large number of products in the R&D and clinical stages shows that there are a large number of product development companies that could potentially use Chromos’ cell-line engineering services. Chromos’ cell-line engineering service adds value to the R&D portion of the pharmaceutical value chain, especially if they can secure deals with companies early in the R&D stage before significant investments are made in creating a sub-optimal therapeutic protein expressing cell-line and manufacturing. Multiple cell-lines could be engineered for the client concurrently, thereby giving the client the ability to study a multitude of proteins at the same time for comparison analysis. Among other benefits of the ACE System, the client saves time, and resources could be directed to other projects since cell-line engineering will be Chromos’ responsibility.

3.2.2 Biogenerics

Whether or not biogenerics will be a lucrative market is debateable. Traditional small molecule generics have been shown successfully to compete with incumbents in the marketplace; however, creating a generic version of a biologic is not as straight forward as small molecules.
Many biological factors come into play when producing a biologic compound, some of which are difficult to emulate. For example, changes in protein expression systems, manufacturing environment, or processing can change the structure or efficacy of a product. Proving a generic product is bioequivalent to the original is far more complicated than small molecule compounds. Biogeneric producers theoretically should use the same protein expression system, purification protocol, and delivery technology as the original compound. Producers must perform analytical and biological comparisons, in-vivo animal studies (pharmacokinetics, toxicity, and efficacy), human pharmacokinetics studies, human safety/immunogenecity studies, and pharmacovigilance (Hughes, 2003, p. 19). In addition, clinical trials may be required in order to prove ‘sameness’, which is costly for the biogeneric developer. The FDA has no clear guidance or standard on how to prove biogeneric equivalence. Analysts predict though that an approval pathway will be developed by 2006 (Robinson, 2003, p. 24).

Despite the regulatory hurdles, some companies have already invested in developing biogenerics. At present and over the next few years, many blockbuster biopharmaceuticals will be off patent, such as Eli Lilly’s Humulin® and Amgen’s Epogen®. Robinson (2003, p. 24) predicted that the biogeneric market could be as large as $13.5B by 2005. Today, only a handful of companies are involved in biogeneric development, however, by 2010, the market is expected to be fully established (Robinson, 2003, p. 24).

Overall, entering this market is risky as regulatory agencies do not have clear guidance on how they will address approving biogeneric compounds. When these issues have been resolved, the market will open up. The risk, however, is placed upon product development companies creating the biogeneric compounds, not Chromos. When the market opens up, Chromos has a new segment of customers to approach therefore expanding the cell-line engineering market.

### 3.2.3 Contract Manufacturing Organizations

CMOs come in a wide variety of sizes and provide a wide variety of services. Size is usually measured by the total volume of the bioreactors one owns. The level of manufacturing services one can perform is limited by the size of bioreactors. Smaller bioreactors, which can be up to several hundred litres, usually can produce enough material to complete studies up to and including Phase III. Larger bioreactors, which are at least several thousand litres, can produce enough material to meet commercialization requirements. CMOs can typically be divided into
two types – ones that could provide material up to complete clinical trials, and ones that could supply material beyond Phase III. CMOs that provide material for commercialization must also invest significantly in downstream processing activities. Upstream activities that product development companies could outsource to CMOs include: creating plasmids and engineering cell-lines; screening engineered cell-lines for production yield and stability; cell banking; process and media optimization; pilot scale studies (can use product for pre-clinical studies); product recovery (clarification and concentration); and process transfer and scale-up. Downstream activities that are usually outsourced include: separation via chromatography; product analysis; QC/QA; stability studies and formulation; and fill and finish. The actual manufacturing functions are between the upstream and downstream activities (see Figure 3-3). Experienced and reputable CMOs who have manufactured products for clinical studies and commercialization usually offer regulatory support and consultation, and have facilities approved by regulatory agencies that are cGMP compliant. In addition, a CMO will usually assist with process transfer if they cannot provide services that others could. The more services a CMO could provide, in addition to a proven track record, the higher they are valued in the pharmaceutical value chain. Their value is increased even further if they can manufacture at commercialization levels because the cost of bringing this type of infrastructure in-house in a biopharmaceutical company is very high and incurs significant risk.

Therapeutic protein contract manufacturing organizations are under constant pressure to fill underutilized capacity. Over the past few years, CMOs have expanded mammalian cell culture facilities to meet forecasted projections of a capacity shortage to produce monoclonal antibodies and other therapeutic proteins. However, recent product failures during clinical trials have left CMOs void of products to manufacture, thus leaving excess capacity. The success of large scale CMOs depends largely on a client’s product approval from regulatory agencies which in turn provide long term manufacturing contracts. Bernstein (2004, p. A2) states that CMOs prefer to work with large companies who need long term manufacturing contracts, rather than small companies who only need small amount of products and do not want to pay a lot of money for it. Overhead, process development and completing runs are very expensive. In addition, their success is also dependent on the failure of new economical manufacturing systems that could produce complex proteins such as monoclonal antibodies. The manufacturing outlook though, still remains positive as the number of antibodies in development is numerous and many of the product developers do not have full manufacturing capabilities. The rise of biogenerics will also utilize unused capacity.
Typically CMOs offer two levels of agreement: 1) a process development and upstream services agreement which provides enough material for Phase I and II trials; and 2) a long term production agreement which provides material for Phase III and commercialization, and large scale downstream services (Chovav, 2003, p. 22). The first type of agreement is typical of small scale CMOs, whereas the second type is typical of large scale CMOs who can deal with clients requiring commercialization levels.

In 2003, CMOs accounted only for 14% (115,200L) of the world’s total manufacturing capacity, which is expected to increase to 19% (339,200L) by 2006 (Chovav, 2003, p. 22). In actuality, total capacity is probably slightly higher as many CMOs are not listed in Chovav’s (2003) biomanufacturing report. The contract biopharma market is worth about $750M per year (Scott & Wood, 2003, p. 15). CMOs are facing competition with pharmaceutical and biotechnology companies who have idle manufacturing capacity due to product failure or over-
forecasted product demand. These competitors are expanding their facilities in anticipation of future product approvals and increased demand for current products. There are at least 20 CMO facilities worldwide who manufacture proteins with mammalian cells, however only a handful has capacities over 10,000L. The larger CMOs include Boehringer Ingelheim GmbH, Lonza Biologics, Cambrex Bio Science Inc, Avecia Biotechnology, and DSM Biologics. These companies are in the process of expanding further thereby increasing their dominance in the CMO market. About 1/3 of the market offers both upstream and downstream functions with manufacturing capabilities ranging from research levels to full commercialization. The rest of the market offers up to clinical development.

A small fraction of large biopharmaceutical companies have built in-house manufacturing capabilities despite the large risks associated with producing in-house and the substantial expenses. Langer (2004, p. 14) believes reasons to build in-house include: negative personal experiences with outsourcing; the desire to retain control over resources, time, and production schedules; the opportunity to gain institutional knowledge in building manufacturing capacity; IP protection; quality issues; and whether the market outlook of manufactured compounds are high (i.e. near or blockbuster status). Biopharmaceutical companies who have blockbuster compounds will likely have manufacturing in-house. Chovav et al. (2003, p. 15) in the State of Biomanufacturing report lists 27 such companies, including big players like Amgen Inc., Genentech Inc., Biogen IDEC, Wyeth, and ImClone Systems. These product development companies will also offer CMO like services to clients when their capacity is underutilized by their own products, thereby stealing market share from CMOs. They must continually run at full capacity in order to maximize the return on investment (ROI) in facilities and capital. The current capacity held by biopharmaceutical companies is 685,450L, however by 2006; total capacity is expected to increase to 1,453,450L (Chovav et al., 2003, p. 15). A number of smaller sized product development companies are shifting towards building smaller manufacturing suites to make material for clinical trials, but are contracting out larger scale manufacturing runs. These types of companies will usually have multiple products in their pipeline where bringing manufacturing in-house makes sense. The decisions product development companies have to make is whether to build, and if so, how big?

In a survey study out of 100 biopharmaceutical companies and CMOs, Langer (2004, p. 1) discovered that currently, 35% of contract out at least some of their biologics production in mammalian cell, bacterial, yeast, plant, or insect systems. This is expected to increase to 47% by 2008. Langer (2004, p. 14) believes that part of the increase is due to the fact that smaller
companies are realizing that manufacturing, process development, and regulatory affairs are not part of their core competencies. Their resources and time are better spent in R&D functions. Building expertise in cGMP such as regulated process validation, viral clearance studies, and other regulated activities is difficult and therefore are more likely to be outsourced. In addition, they may have a limited pipeline with only a small number of products, which does not justify building in-house manufacturing. Also, the limited amount of financing that small companies have cannot support manufacturing expansion. The cost of producing a small manufacturing facility with capacity in the hundreds of litres could cost tens of millions of dollars, depending on level of standards, and takes 1 – 2 years to build. 79% of the survey respondents who manufacture therapeutics in mammalian cells performed all the manufacturing in-house, whereas 21% outsource their production. By 2008, 44% of the respondents that currently perform mammalian cell manufacturing in-house will outsource some of their activities. The reason cited why biopharmaceutical producers are reluctant to outsource at the present time is because the technology is relatively immature (compared to bacterial systems) and producers want better control of overseeing projects (Langer, 2004, p. 18).

Despite the needs of small and medium sized companies for outsourcing manufacturing capabilities, some feel that the market size for small scale CMOs is dwindling. Langer (2004, p. 18) quotes Andrew Sinclair, managing director at Biopharma Services (Chesham, UK), who said that:

"The majority of the smaller companies’ Phase I and II projects will be outsourced. Their dynamic is driven by the investment cycle…recent lack of investment in biological R&D projects has had a direct impact on small-scale contract manufacturing. As investments dried up, the small- and medium-scale biotech companies slowed their R&D programs and focused on their drug candidates of greatest opportunity…the number of drug candidates going into clinical trials have not kept up with recent historical trends."

The result is that small CMOs have not seen the level of contract work as expected, and that there is overcapacity in this space. In addition, a number of larger CMOs have expanded their smaller scale capabilities. Based on a survey conducted by HighTech Business Decisions (2001, p. 5-14) of 25 CMOs, cell-line development averaged 3% of total revenues.

In the next few years, total biopharmaceutical manufacturing capacity in the industry will increase due to additions made by CMOs and biopharmaceutical companies. Expansion in capacity is likely to slow thereafter. Biopharmaceutical companies will still own the majority of
the capacity (70 – 80%). Biopharmaceutical companies with excess or idle capacity will move into the CMO business as they also have expertise in both manufacturing and regulatory affairs. That being said, a biopharmaceutical company’s main priority will still be manufacturing their own products rather than clients, therefore their interests will be in short term manufacturing contracts or clients risk having their projects put aside. The tightening of the CMO market will make it difficult for new entrants as incumbents have secured their market position. Although some small and medium sized companies still require CMO services at the small scale levels, others are beginning to build pilot scale manufacturing suites to supply their clinical trial needs. Larger, incumbent CMOs are beginning to encroach on the small CMO market by expanding their small scale services and leveraging their expertise and reputation to gain market share. In addition, current manufacturers are discovering ways of increasing production efficiency, capacity utilization, and protein yields. Indirectly, these research activities increases capacity as more runs could be performed than what is currently available. The ability to perform more runs per year means that CMOs could take on more clients, or product development companies who have built in-house do not need to resort to CMOs for extra production capacity – as what is presently being done. In addition, protein therapeutics are also becoming more potent, therefore less dosings are required, which means that less of the therapeutic has to be manufactured to meet market demand. In effect, this means that run times will be shorter and more capacity will be available for other projects.

Overall, the future of the biopharmaceutical manufacturing will be tight as capacity is being increased by both CMOs and biopharmaceutical companies. Companies with a multiple products in their pipeline are likely to build small scale manufacturing capabilities in order to retain control of projects and develop expertise; however, those with a limited pipeline will outsource their manufacturing needs. Large CMOs will be competing for contracts of all sizes, although they prefer long term commercial contracts to secure their capacity utilization. The ability to secure long term commercial contracts is difficult as large biopharmaceutical companies are manufacturing blockbuster and other high market value compounds in-house, and are also expanding their own manufacturing facilities. CMOs’ small scale capabilities are being expanded in case they cannot secure the long term production contracts. Since these large CMOs have experience, reputation, and a proven track record - encroaching and stealing market share from the small scale CMOs will not be difficult. Competition amongst CMOs and biopharmaceutical companies increase as biopharmaceutical companies are moving towards the CMO business.
because of their excess and idle capacity. Increasing manufacturing efficiency also indirectly affects competition.

Becoming a large scale CMO is not a feasible option for Chromos since construction costs or acquisition price will be extremely high, and being one is not in Chromos’ current interest. However, one has to be a big CMO to compete effectively in the marketplace. Chromos’ alternative is to become a small scale CMO. If Chromos chooses to build or acquire a small scale CMO facility to provide more than just cell-line engineering, the competition will be extremely difficult. Most biopharmaceutical companies will consider small scale manufacturing on the lower end of the pharmaceutical value chain, and will include cell-line engineering in that classification. Chromos must be able to carve itself a niche in the chain and educate the industry in the value of its technology in order to extract more value. Chromos can offer its clients a high expressing cell-line which adds value to the manufacturing portion of the value chain by shortening run times and increasing production yields. It is also difficult to assess whether capacity will be filled if Chromos enters the small scale CMO business, given the tightening of competition and potential lack of products. Entering the CMO market incurs significant risk as the market is still immature and its dynamics are unstable.

3.3 Customers

Ideally, Chromos’ customers would be Big Pharma or Big Biotech who have blockbuster monoclonal antibodies in the market, thereby allowing Chromos to collect large royalty payments. In addition, having these types of companies validate the ACE System as the cell-line engineering system of choice for production would greatly increase the ACE System’s reputation and profile. However, the likelihood of obtaining one of these big companies as a client is low since it would be difficult for them to change manufacturing process if their current processes already meet their needs. If a company changes their production cell-line of a product, a battery of tests will need to be performed to ensure that the products produced from both the old and new system are exactly the same (i.e. have the same potency and pharmacokinetic profile). This may require physical and analytical test, animal testing, and clinical studies, thereby utilizing valuable resources and time. Also, a new production cell-line must meet certain performance criteria before being considered for large scale manufacturing.

Less sophisticated players may have lower standard, therefore, likely customers would be small to medium sized product development companies that are early in the R&D stage who have
monoclonal antibodies or other complex proteins in their pipeline. Capturing clients early in the R&D stage is key, or else they may be too far into development where changing production cell-lines would not be feasible.

Not all product development biotechnology companies are experts in cell-line engineering. Most firms use traditional gene amplification methods to create high expressing cell-lines which give variable results and are time consuming. Traditional gene amplification methods are cheap and companies avoid paying exorbitant licensing fees. However, when companies have discovered a monoclonal antibody to develop clinically, the cell-line that produces that therapeutic might not be economically suitable for manufacturing. Companies that have low expressing, high value cell-lines are the types of customers that would require Chromos’ service. In addition, customers who do not have cell-line engineering expertise; need cell-lines expressing a desired product in a short period of time; want to audition several cell-lines for research or manufacturing; want to outsource cell-line engineering in order to focus on other projects; want to audition the ACE System before fully in-licensing the technology; only need to make one cell-line but do want to hire expertise; or are interested in utilizing the ACE System but do not want to bother with technology transfer agreements or learn how to use the system would also need Chromos’ services.

Low expressing cell-lines can have a profound economic impact on the company. Low expression leads to an increase in the cost of goods sold due to longer production runs. Increased manufacturing costs will increase selling price, however, there will be downward pressure on prices due to competitors, generics (although there are no biogenerics on the market yet), and government regulations. These forces combined decrease profit margins. In addition, low expressing cell-lines can lead to market erosion as the supply produced may not meet market demand, therefore consumers will look to other products to fulfill their needs. The company will lose potential revenue if demand cannot be supplied.

CMOs are also potential clients who may need Chromos’ services. Although CMOs have expertise in process development and protein expression optimization by varying culture conditions, sometimes these are not enough to optimize cellular expression if the cell itself is not productive. CMOs can use Chromos’ expertise in cell-line engineering with the ACE System to create a high expressing cell-line that would carry their client’s gene of interest, which would then be used in the CMO’s manufacturing process. The ACE System could be used to differentiate a CMO’s service offering from their competitors thereby gaining a competitive advantage. Some CMOs do not have cell-line engineering services as part of their service
offering, therefore identifying these potential clients is important. In addition, since cell-line development averages about 3% of a CMOs' total revenue, CMOs may want to outsource this activity as it does not contribute significantly to total revenues.

3.4 Unmet Needs

Therapeutic protein production by mammalian cells are not the most efficient production methods, however, they are the only system that has been approved by regulatory bodies to produce monoclonal antibodies. There is currently an ongoing debate as to whether there is over or under capacity in mammalian cellular protein manufacturing. Nonetheless, protein manufacturers must find ways to make their processes more efficient with the current capacity available, upstream and downstream. Chromos offers a rational method of engineering high expressing cell-lines to increase production efficiency.

The normal time required to produce a high expressing cell-line using conventional methods from transfection to characterization can take 6 – 12 months, or longer. The longer it takes to produce a cell-line, the shorter the time a company can capitalize on patent exclusivity leading to a loss of profit potential. Also, research milestones will be achieved later which will impact the company in terms of financing, or the ability to seek financing. Chromos' ACE System can produce high expressing cell-lines within 2 – 4 months, which is faster than industry standards and could potentially reverse the aforementioned problems with delayed cell-line development.

The ideal cell-line, upon introduction of a foreign gene, would be able to express high levels of proteins consistently throughout initial screening and large volume scale-up. Introducing foreign genes into the genome of a host cell is such a random process that it is difficult to assess how the genome, and hence the host, is affected. Problems with a transfected cell may not be seen initially, but become exposed further in development. A number of factors affect the protein yield, such as: level of mRNA transcribed and translated into proteins; proper protein folding, post-translational modification, and secretion from the cell; culture cell density and stability; culture media formulation including gases and nutrients; and the presence of proteases (enzymes that destroy proteins that are usually released after a cell dies) or toxins in the media. When cells are transferred from screening and identification to isolation and sub-culturing, followed by small scale and large scale production, factors such as those listed above are very important and must be taken into consideration during process development in order to maintain expression yields
similar or make higher to what was found during the initial screen. There is no cell-line that exists which can retain consistent protein expression levels throughout all stages of characterization and development without constant tweaking. Different cell-lines have different protein expression and growth characteristics, which in part is dependent on the gene of interest inserted. Promising cell-lines identified during screening must go through rigorous process development in order to maintain optimal protein expression.

Chromos ACE System is differentiated from other mammalian cell technologies and can address some of the unmet needs of the industry. Chromos’ on-going research is aimed at optimizing the ACE System to rapidly create efficiently the ideal cell-line.

3.5 Other Technologies

The protein expression system used to manufacture proteins is dependent on the complexity of the protein and the economic advantages gained. Factors to consider when choosing the manufacturing system for the protein in question include scalability, total annual production yields, speed of production set up, post-translational modifications, and regulatory issues (Dyck, Lacroix, Pothier, & Sirard, 2003, p. 394). All the systems discussed in this section have been used to generate proteins for R&D purposes, but not all have had successful commercial products associated with it. These protein manufacturing methods are compared against mammalian cells. Features between systems are summarized in Table 3-1.

Table 3-1: Comparison of production systems for human therapeutic proteins

<table>
<thead>
<tr>
<th>Factor</th>
<th>Mammalian cell culture</th>
<th>Bacterial cell culture</th>
<th>Insect cell culture</th>
<th>Yeast</th>
<th>Transgenic plants</th>
<th>Transgenic animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall cost</td>
<td>High</td>
<td>Low</td>
<td>High</td>
<td>Medium</td>
<td>Very Low</td>
<td>High</td>
</tr>
<tr>
<td>Production timescale</td>
<td>Long</td>
<td>Short</td>
<td>Long</td>
<td>Medium</td>
<td>Long</td>
<td>Very long</td>
</tr>
<tr>
<td>Scale-up capacity</td>
<td>Low</td>
<td>High</td>
<td>Low</td>
<td>High</td>
<td>Very high</td>
<td>Low</td>
</tr>
<tr>
<td>Glycosylation</td>
<td>Correct</td>
<td>None</td>
<td>Incorrect</td>
<td>Incorrect</td>
<td>Minor difference</td>
<td>Minor difference</td>
</tr>
<tr>
<td>Contamination risk</td>
<td>Viruses and prions</td>
<td>Endotoxins</td>
<td>Viruses</td>
<td>Low risk</td>
<td>Low risk</td>
<td>Viruses and prions</td>
</tr>
<tr>
<td>Productivity</td>
<td>Medium</td>
<td>Medium</td>
<td>Medium</td>
<td>Medium</td>
<td>High</td>
<td>High</td>
</tr>
</tbody>
</table>
3.5.1 Bacterial Systems

The very first therapeutic protein approved for human consumption was produced in a bacterial system, specifically in *E. coli*. Eli Lilly’s product Humulin®, a recombinant form of insulin, was approved in the U.S. in 1982. Since then, a number of other protein products were approved by this manufacturing method such as Amgen’s Neupogen®, Genzyme’s Cerezyme®, and Schering-Plough’s Intron® A (Walsh, 2003). The advantage of bacterial systems is the ability to produce proteins in large quantities economically. *E. coli* grows at a much faster rate than mammalian cells, thus a given amount of protein can be produced in a much shorter period of time. The cost of producing a therapeutic protein is estimated to be $1/g, compared to mammalian systems which are estimated to be $300/g (Hood, Woodard, & Horn, 2002, p. 631). The types of proteins that *E. coli* can produce are highly active compared to monoclonal antibodies; therefore dosing requirements for a patient are lower. Since dosing requirements are lower, manufacturing facilities are smaller as production volumes are smaller and cheaper compared to mammalian production facilities. Microbial manufacturing plants are estimated to cost around $250M (Bernstein, 2004, p. A5).

The drawback to bacterial systems, however, is the inability to glycosylate proteins. The inability to glycosylate limits their usefulness to only producing simple proteins that do not require glycosylation, such as hormones, antibody fragments, interferons, and interleukins. Current research in enhancing the system are aimed at improving acetate metabolism, optimizing folding conditions, and increasing expression levels through genetic manipulation.

3.5.2 Insect Cell Systems

Of late, researchers have become more interested in using insect cell systems for the production of recombinant proteins. Some of cell-lines used include Sf-9, Tn5, and High Five. Their advantages over mammalian systems include ease of culture, higher tolerance to osmolality and by-product concentration, and higher expression levels when infected with a recombinant baculovirus (Ikonomou, Schneider, & Agathos, 2002, p. 1). Gene transfer using baculovirus is the method of choice when creating recombinant insect cell-lines. Up to 0.5g/L of produced recombinant protein have been reported (Verma, Boleti, & George, 1998, p. 173). Insect cells can glycosylate proteins at the same sites as mammalian cells, however, the nature of the sugar chains appear to be different (Verma et al., 1998, p. 173). The difference in sugar chains can lead to immunogenicity and the protein’s rapid clearance from the body. There is on-going research to
solve this problem. Another problem with insect systems is proteolysis, which is the degradation of proteins by the host cell’s enzymes. Again, there is on-going research and possible solutions to solve this problem, such as the addition of protease inhibitors to the growth media, removing protease genes from the baculovirus vector, or optimizing culture conditions (Ikonomou et al., 2003, p. 7, 8, 10).

The types of cell culture vessels required for large scale insect cell production systems are similar to that of mammalian cells. Although there are no actual large scale insect cell manufacturing facilities in operation, conceivably, the cost of building one would be similar to that of mammalian cells. There are also no marketed products made by this system, most likely due to the inability to glycosylate proteins in a human-like fashion. Advances in medium design, proteolytic control, and culturing environment will make this system more attractive for commercial use.

3.5.3 Fungal Systems

Fungal systems, in particular yeast, have the combined advantages of both microbial and mammalian cells. Like microbial cells, they are easily genetically manipulated, grow rapidly in simple growth media and express high amounts of protein; and like mammalian cells, they can fold, glycosylate, and secrete complex proteins such as antibodies. However, the glycosylation pattern is limited and cannot reproduce exactly the same patterns required for the human body. The glycosylation pattern that yeasts produce can be immunogenic, thus rendering products useless for human therapies. A biotechnology company, GlycoFi Inc., claims to have solved the problem of limited glycosylation in a type of yeast called *Pichia pastoris* by genetic manipulation. In a few years, they predict they will have a number of different yeast strains which can properly glycosylate a customer’s protein of interest, including human antibodies. Proteins manufactured by fungal systems are safe because their growth media cannot harbour viruses or prions, nor can these pathogens exist in fungal cells.

Yeast strains such as *Pichia pastoris* and *Saccharomyces cerevisiae*, have both been used in research applications, and the latter also in manufacturing applications. *P. pastoris* can produce insulin precursor at yields of 1.5g/L (Anderson & Krummen, 2002, p. 120). They have been used to manufacture simple proteins for hormone replacement, vaccines, and other therapies. Companies such as Novo Nordisk A/S uses *S. cerevisiae* to produce Novolog® (an insulin
analog) and Glucagen® (a glucagon hormone), and Merck & Co. Inc. uses it to produce Recombivax®, a vaccine against the hepatitis B virus.

3.5.4 Plant Based Systems

Plant based systems are receiving serious attention from commercial manufacturers as an alternative to mammalian cell based systems. The advantages plant based systems offer are low cost of production, ease of scaling up or down, lower cost of capital compared to mammalian and transgenic systems, freedom from animal derived pathogens such as prions and viruses, and seeds carrying the protein of interest could be stored, sown, and processed when required (Hood et al., 2002, p. 630). Hood et al. (2002, p. 631) estimates the cost of producing therapeutic proteins can be as low as $0.10/g, based on using corn crops. Powell (2003, p. 967) estimates that the production of 100kg of a protein would require 450 acres of corn. The first relevant protein produced in a plant system, in particular tobacco, was human growth hormone. Since then, other proteins have been expressed in other crops including monoclonal antibodies. Researchers are now experimenting in maize, rice, soybean, potatoes and the tobacco chloroplast system. A drawback of using plant based production systems compared to mammalian cells is long development times. In general, members of the pharmaceutical industry believe that the time to produce milligram quantities of protein for utilization in animal studies requires at least a year. Mammalian cells can produce gram quantities in 3 – 4 months using a stable cell-line.

Recombinant antibodies can be produced in plants, in fact, 6 recombinant antibodies are currently in clinical trials. The protein synthesis pathway between plants and animals are very similar. In plant systems, proteins are folded correctly, however, protein glycosylation patterns can be different. Unlike yeast systems, the different glycosylation pattern do not seem to illicit an immune response even though antibodies against the residues are present in the host’s bloodstream. The glycan residues found are similar to those found in the vegetables that humans consume. The problems of functional change, biodistribution, and body clearance remain however. There is on-going research to develop strategies of ‘humanizing’ antibodies produced by plants. Another problem posed by plant systems is post-transcriptional gene silencing (Larrick & Thomas, 2001, p. 412). Scientists are developing methods to alleviate this problem.

Creating plant cell suspension cultures provides an alternative to whole-plant expression systems. The advantages over mammalian cell cultures are inexpensive media and improved safety as human and animal pathogens are not present (Schillberg, Fischer, & Emans, 2003, p.
Plant cells can be modified to secrete therapeutic proteins into the suspension culture, or be retained in the cell for later processing (the former is the more common method). Tobacco suspension cells have been shown to secrete up to 30mg/L of a simple protein (Schillberg et al., 2003, p. 439).

Currently, there are no proteins that have been approved as a human therapeutic made by plants. This may soon change as a number of plant derived therapeutic proteins are in clinical trials. Meristem Therapeutics has a gastric lipase against steatorrhea in Phase IIa and Prodigene has an oral vaccine for a bacterial toxin that causes traveller’s diarrhea in Phase I (Powell, 2003, p. 966). Some of the other companies investing in transgenic plant production include Agrisoma Biosciences, Dow Agrosciences, Monsanto Company, SemBioSys Genetics, and Ventria Biosciences.

Although plant manufactured products have entered clinical trials, regulatory hurdles still remain large. Hood et al. (2002, p. 632) has identified four criteria that have to be met no matter which plant species is used: 1) isolation of other crops to prevent out-crossing or co-mingling; 2) preservation of the protein throughout the planting of transgenic seeds to final formulation; 3) compliance with the United States Department of Agriculture (USDA) regulations (if the product is to be marketed in the U.S.); and 4) QC/QA programs with standard operating procedures to ensure that the final product is of highest quality.

3.5.5 Transgenic Animal Systems

The use of transgenic animals as bioreactors for the production of therapeutic proteins is another low cost alternative to mammalian cell production. The creation of a transgenic animal can be a long, complex process, but the potential cost savings in the long run could be great. The estimated cost of a building a transgenic farm with a single purification facility should not cost more than $80M USD., which is much lower compared to mammalian cell facilities (Dyck et al., 2003, p. 394). The cost of downstream processing, however, is similar to that of mammalian cells. The cost of producing 50kg/year of protein using transgenic animal systems is estimated to be $679 – $703/g (raw materials ~$1 – 2/g) (Dyck et al., 2003, p. 395), whereas for mammalian cell cultures, it is estimated to be $942/g (raw material ~$150/g) (Dove, 2002, p. 778).

Choosing the appropriate animal for protein production depends on factors such as amount required, ease of creating the transgenic animal, growth period of the animal before relevant expression levels begin, scale-up and maintenance costs, ease of raw material
processing, post-translational modification patterns, and regulatory, legal, ethical, and social issues. Protein expression from the mammary glands of ‘dairy’ animals such as cows, goats, and sheep, traditionally has been the production method of choice as the milk produced from these animals can be collected in large volumes. A goat can produce as much as 4kg of a therapeutic protein per year; other animals can be even higher (Larrick & Thomas, 2001, p. 414). The drawbacks to using these animals, however, are low transgenic efficiency (about 1.0%) (Dyck et al., 2003, p. 396), the long interval between birth and first lactation (lactation begins for a goat and cow is approximately 1.5 years and 3 years, respectively), and raw material processing. Processing is hampered by the presence of micelles and fat globules.

Transgenic pigs and rabbits have been produced to circumvent some of the problems associated with dairy animals; however the low rate of milk production and the number of animals needed to produce adequate amounts of product is restrictive. Despite the problems of mammary gland protein manufacturing, companies such as GTC Biotherapeutics and Pharming Group have products in clinical trials produced by this method. In fact, data from GTC Biotherapeutic’s clinical trials on ATryn® human antithrombin protein has been accepted by the EMEA for the final review of marketing approval. ATryn® is a simple protein produced in goat milk. Other companies involved in transgenic animal manufacturing space include Nexia Biotechnologies and BioProtein Technology.

Protein manufacturing in transgenic animals is not limited to mammary glands, other systems in development include pig blood, urine, and sperm, and hen eggs. Only hen eggs have been taken seriously for commercial manufacturing. A hen can produce up to 330 eggs per year, and egg white naturally contains about 4g of proteins (Dyck et al., 2003, p. 395). Hens take about 6 months to be mature enough to lay eggs or reproduce. A flock of 4,000 hens can produce about 100kg of protein per year (Dove, 2002, p. 778). The drawback of using eggs as a manufacturing method is the difficulty of producing transgenic chickens; however, AviGenics, a company developing transgenic chicken technology, has discovered a process to produce transgenic chickens efficiently. Currently there are no products in clinical trials using this method.

Transgenic animals systems have great potential to provide product development companies a low cost alternative to mammalian cell protein manufacturing. Some of the issues hampering its wide scale usage include low transgenic efficiency, speed to manufacturing commercial products, potential of introducing infectious organisms into a product or herd, and incorrect post-translational modifications. The level of post translational modification varies from protein to protein and tissue to tissue.
3.5.6 Conclusion

Bacterial systems are the only protein manufacturing technology that has fully matured and gives consistent and predictable results. However, a drawback to the system is its inability to glycosylate and produce complex proteins; therefore its usage is limited to producing simple proteins such as Humulin®. Other technologies are slowly maturing; however, they are hampered by glycosylation and regulatory issues. Companies using these other technologies will need to demonstrate that a regulatory approved compound could be made. In order for these other systems to revolutionize therapeutic protein manufacturing, companies must solve glycosylation issues and demonstrate the economic benefits compared to mammalian cell systems. Plant and transgenic animal systems have produced monoclonal antibody products that are currently in clinical trials thereby threatening the mammalian cell manufacturing market as viable production alternatives.

3.6 Competitors

Chromos’ competitors are divided into two classifications, direct and indirect. Direct competitors are defined as those who have competing mammalian cell technologies with the ACE System that could produce recombinant proteins at both research and manufacturing levels. Competing cell-lines have been modified in such a manner that protein expression is improved over conventional cell-lines. Indirect competitors are defined as companies who have manufacturing technologies other than mammalian cells. These competitors have maturing technologies that have not been proven to produce a regulatory approved product. Competitors not included in this section are CMOs who offer cell-line engineering as a service. These CMOs are excluded because the cell-line engineering methods they use are standard in the industry and do not give them any competitive advantages.

3.6.1 Direct Competitors

The competitors listed in this section are divided into three classification, high threat, medium threat, and low threat. High threat competitors have mammalian cell technology that has been used to produce approved products, or have created cell-lines that have greater expression yields than Chromos’. Medium threat competitors have technology that is used to produce compounds in clinical trials, or have created cells-lines that have expression yields comparable to
Chromos'. Low threat competitors have technology that has been used to produce research stage compounds, or have generated cell-lines with expression levels inferior to Chromos'.

3.6.1.1 High Threat Competitors

3.6.1.1.1 Lonza Biologics Inc

Lonza Biologics, a CMO with locations in Slough, England, and Portsmouth, New Hampshire, is a subsidiary of Lonza Group, a publicly traded (SWX:LONN) life sciences chemical manufacturing company. Lonza Biologics is a full scale CMO offering services in mammalian cell manufacturing of antibodies and recombinant proteins. Some of their services include vector construction, cell-line development, process development, small and large scale manufacturing, product purification and analysis, cell banking, and regulatory support. Some of the cell-lines they use for manufacturing are CHO and NS0 which have been modified to express glutamine synthetase (G.S.) and contain strong viral promoters for elevating gene expression. Glutamine synthetase expression is a method of cell selection – cells that do not express G.S. will senesce in glutamine-free media. Lonza has also developed a CHO cell-line named CHOK1SV which has been “pre-adapted” to desired culture conditions allowing quicker adaptation to suspension conditions. From the time of transfection, production cell-lines for large scale manufacturing can be created in 6 months. The CHOK1SV cell-line could be grown in chemically defined, protein and animal component free media, thereby decreasing purification times and increasing product safety.

According to Lonza Biologics, the advantages of the G.S. System are increased product yields, rapid cell selection, cell stability, industry familiarity, and reproducibility (Lonza Biologics, 2003). Expression levels as high as 4.3g/L were achieved with the G.S. System when used with their CHOK1SV cell-line under optimized conditions (Vernon, 2004, p. 2). Protein yields could be further increased by using traditional gene amplification methods, and media and process optimization techniques. Cell-lines expressing high protein yields can be selected after the initial post-transfection screen, thereby decreasing the screening process time compared to traditional methods. Selection agents that are traditionally used for cell selection are not required for the G.S. System, leading to a simple and well defined process. However, some cell-lines do require the addition of methionine sulphotimine (MSX), which is a well defined chemical compound that does not show mutagenic or carcinogenic properties, unlike other selection
agents. Regulatory authorities are familiar with the G.S. System since it has been used to manufacture three licensed monoclonal antibody compounds including Zenapax® by Roche and Synagis® by MedImmune. Many other companies have auditioned the G.S. System using a wide range of products and consistently were able to create high expressing cell-lines, demonstrating the system’s reproducibility. The G.S. System has been used in research applications for over 10 years and has been licensed to over 75 biotechnology and pharmaceutical companies. Chromos is benchmarking the ACE System against the G.S. System.

Lonza Group recorded sales of CHF 2.24B in 2003, of which CHF 835M (down 14.6% from the previous year) were attributed to the Exclusive Synthesis and Biotechnology Division. The Exclusive Synthesis and Biotechnology Division are responsible for chemical custom manufacturing for the pharmaceutical industry and mammalian cell culture for the biotechnology industry. The Division’s net income was CHF 147M in 2003, a decrease of 30% from the previous year. The decrease is attributed to client product failures and delays at the late stages of clinical development, an overall decrease in drug approvals, client de-stocking of inventory, and overcapacity in custom manufacturing and in the pharmaceutical industry itself (Lonza Group, 2004, p. 14). The new 60,000L expanded production capacity in Portsmouth is expected to be fully utilized in 2005 as two long term supply agreements has been negotiated with Genentech for the production of Rituxan® (a monoclonal antibody for the treatment of Non-Hodgekin’s lymphoma), and another top-ten pharmaceutical company product. The R&D portion of the mammalian cell culture group currently has over 35 projects ranging from cell-line development to regulatory support with other clients. Approximately 2,300 of Lonza Group’s employees are in the Exclusive Synthesis and Biotechnology Division.

Lonza Group as a whole is undergoing restructuring by decommissioning the fine chemicals business and decreasing employee numbers, however, the biologics production unit appears to be strong despite concerns in overcapacity and product failures. There is on-going expansion of facilities in this area, particularly in Portsmouth where 60,000L will be added along with additional purification capacity. Some of Lonza Biologics clientele include Eli Lilly, Pfizer, Cambridge Antibody Technology Ltd., ImClone Systems Inc., and Xcyte Therapies Inc.
3.6.1.2 Medium Threat Competitors

3.6.1.2.1 Crucell N.V. (‘Crucell’)

Crucell N.V., a public biotechnology company (NASDAQ:CRXL, EURONEXT:CRXL) in the Netherlands, was formed in October 2000 through the merger between IntroGene B.V. and U-BiSys B.V. The technologies the company has are in the areas of mammalian cell-line manufacturing, vaccine development, and antibody discovery. Their product pipeline consists of vaccines in the pre-clinical phase, however, the area of concern for Chromos is their mammalian cell-line technology named PER.C6™. PER.C6™ is an immortalized human retina cell-line designed for functional genomics research and the research, development, and manufacturing of therapeutic antibodies, vaccines, and gene therapy products. Immortalized cell-lines have the unique property of dividing indefinitely. The cell-line has been adapted to grow in suspension in serum free media, which allows the cells to grow at a greater density and eases downstream purification. Other advantages of the PER.C6™ cell-line are high yields of protein expression and ‘humanized’ glycosylation patterns on recombinant antibodies. In addition, a cell substrate biologics master file (BMF) has been filed with the FDA. The BMF describes the technology, its establishment, development, and potential product uses – thus subsequent product submission for approval from the FDA using the cell-line will be easier.

Jones et al. (2003, p. 165) found that under non-fed batch growth conditions of PER.C6™ expressing a recombinant IgG antibody in suspension, the production rate after 12 days was 0.525g/L (Jones, Kroos, Anema, van Montfort, Vooy, van der Kraats, et al., 2003, p. 165). In a hollow fibre bioreactor, after about 2 months, production rate was 0.94g/L, however it declined thereafter. The gene copy number ranges from 1 – 10 copies per cell, thereby demonstrating that high expression levels could be achieved without the need of gene amplification.

Crucell has many partners using the PER.C6™ technology for the purposes of manufacturing recombinant proteins, although it is mainly used to manufacture therapeutic vaccines. They also have an exclusive license with DSM Biologics, a CMO, to use the PER.C6™ cell-line in antibody production and other therapeutic proteins for their clients. The deal was signed in 2002 and expires in 2006. Other companies licensing the PER.C6™ cell-line for recombinant protein production include Applied Molecular Evolution (owned by Eli Lilly, Oct 2002), Biogen Idec (Jan 2004), Centocor Inc. (owned by Johnson & Johnson, Dec 2002), Merck...
& Co. Inc. (May 2003), and Millipore Corporation (Mar 2003) (Crucell N.V., 2004a, p. 23). All the products utilizing the PER.C6™ system for therapeutic protein production are in the pre-clinical phase and no products produced by PER.C6™ have been approved by the FDA or EMEA. Crucell has licensing arrangements with numerous other product development companies and CMOs; however, they are not in the area of therapeutic protein production and therefore not listed. Of interesting note, Crucell has signed a cell-line development service agreement in December, 2003, with Progenics Pharmaceuticals Inc. for the production of a PER.C6 cell-line expressing a recombinant antibody.

In June 2003, Crucell had approximately $107M USD in cash and a market cap of about $281M with 36.1M shares outstanding (Crucell N.V., 2004b). Revenues for 2003 were $9.3M. The company is not profitable as they have no marketed products and much of their resources are allocated towards vaccine development. Crucell has 182 employees, of which 151 have R&D roles (Crucell, 2004a, p. 41). The exclusive license granted to DSM Biologics precludes Crucell from entering any other licensing agreements with other CMOs. However, the company will still pursue licensing the PER.C6 system to product development companies for the production of therapeutic antibodies. They do not have an in-house cell-line engineering business (at least not advertised in their website or financial reports).

3.6.1.3 Low Threat

3.6.1.3.1 Amgen Inc. ('Amgen')

Amgen is a publicly traded (NASDAQ:AMGN) biopharmaceutical company headquartered in Thousand Oaks, CA, who has multiple recombinant proteins in the marketplace including Epogen®, Enbrel®, and Neupogen® to name a few. They also have a technology named the Expression Augmenting Sequence Element (EASE), which is an expression technology platform composed of a genomic sequence that facilitates high expression of proteins in mammalian cell-lines. Amgen claims that high expressing cell-lines using this system can be made in a short timeframe. Antibody expression levels achieved using the system in a serum free CHO cell-line were 0.35 – 0.40g/L under certain conditions (Aldrich, Viaje, & Morris, 2003, p. 1435). Whether or not this system is used to manufacture marketed proteins is unknown. Other companies licensed to use the EASE technology are also unknown.
Amgen is considered to be the largest biotechnology company in the world with a market capitalization of $70.5B as of June 15, 2004. Revenues in 2003 were $8.36B, and net income for the same year was $2.26B (Amgen Inc., 2004a, p. 28). At the end of Q1 2004, the company had about $4.5B in cash and equivalents (Amgen Inc., 2004b, p. 7), and about 13,000 employees. The company currently has about 1.28B shares outstanding.

3.6.1.3.2 Cobra Biomanufacturing Plc (‘Cobra’)

Cobra is a publicly traded (FTSE-AIM:CBF.L) CMO located in Keele, UK, who has proprietary technology, the UCOE system, to develop high protein expressing mammalian cells rapidly since no gene amplification steps are needed. In addition to protein manufacturing, the company can also produce viral, plasmid, and live cell-based therapeutic products. Another technology the company possesses is the ORT (operator repressor titration) vector which enables the production of recombinant proteins without the use of antibiotics. Other services besides biopharmaceutical manufacturing and cell-line engineering include process development, scale-up, product analysis, stability testing, formulation, and regulatory and consultancy support. Benton, T., Chen, T., McEntee, M., Fox, B., King, D., Crombie, R., et al. (2002, p. 45) claimed they can produce a cell-line expressing an antibody at 0.2g/L under 5 weeks in a pre-adapted cell-line in serum-free conditions using the UCOE system.

For the fiscal year 2003, the company’s revenue from contract manufacturing was £6.0M and net income was £1.04M. 44% of the revenue was due to protein and viral manufacturing contracts (Cobra Biomanufacturing, 2004a, p. 9). On March 31, 2004, the company had £3.8M (Cobra Biomanufacturing, 2004b) in cash and 66 employees, of which 41 are involved in manufacturing. The company also purchased land and buildings in order to expand their capabilities. On June 3, 2004, the company’s market cap was £6.93M (Reuters, 2004).

3.6.1.3.3 Cytos Biotechnology GmbH (‘Cytos’)

Cytos is a publicly traded (SWX:CYTN) product development company whose main focus is developing their propriety Immunodrugs™ product portfolio. Immunodrugs™ are unique in that they are designed to instruct the patient’s immune system to produce a desired therapeutic antibody or cytotoxic T-cell response to reverse or prevent disease progression (Cytos Biotechnology, n.d.). Disease areas the company is concentrating on include inflammation, immunology, cancer, addiction, and infectious, cardiovascular, metabolic, and nervous system
diseases. In addition, the company has developed a mammalian cell expression system named pCytTSTM, which is a temperature-inducible expression system. The advantages of the pCytTSTM system include: up to 10,000 fold increased expression of a recombinant protein under 35°C; little expression of a recombinant protein at 37°C; allows for expression of toxic or 'difficult to express' proteins for structural analysis within 3 months; and generates stable expressing cell-lines. Increased expression is achieved through amplification of the mRNA of the protein of interest by a feedback loop mechanism at temperatures below 35°C. This process can generate protein expression levels of milligrams per litre to several hundred milligrams per litre. The drawback is at too low of temperatures, cell viability cannot be sustained for long periods. Lonza was granted an exclusive license for the system to manufacture therapeutic proteins for pre-clinical, clinical, and market supply, and in-addition, the right to sublicense the system to their clients (Cytos Biotechnology, 2004a, p. 2).

In 2003, the company incurred revenues of CHF 3.7M and net losses of CHF 20.5M (Cytos Biotechnology, 2004b, p. 6). As of March 31, 2004, the company had approximately CHF 65M in cash and cash equivalents. The company’s market cap on June 1, 2004 was approximately CHF 171M with about 4.62M shares issued. At year end 2003, the company had 109 employees.

3.6.1.3.4 Gala Biotech ('Gala')

Gala Biotech, a subsidiary of Cardinal Health located in Middleton, Wisconsin, is a biotechnology company with expertise in mammalian cell engineering and small scale cGMP protein manufacturing. The company’s proprietary technology, known as GPEx™ (Gene Product Expression), is a pseudotyped, high-titre retrovector that ensures stable transduction (the introduction of foreign genes into target cells by means of a viral vector) of genes of interest in virtually 100% of target cells, such as CHO (Gala Biotech, n.d.a). Since nearly 100% of the target cells will be introduced with the gene of interest, the need for cell selection is eliminated thus saving time and costs. Almost any type of DNA sequence can be packaged into the GPEx™ vector. The time from cellular transduction to small scale manufacturing can be completed in 6 months, and the time to create a stable cell-line can be completed in 4 months. GPEx™ technology also produces cell-lines with high protein expression yields (including monoclonal antibodies) by specifically targeting high expressing sites and inserting multiple copies of the
desired gene in the host genome through an iterative process. Co-expression of different genes is also made possible by the GPEx™ system.

In addition to cell-line engineering with the GPEx™ system, other services Gala offers include process development, small-scale manufacturing of products for Phase I and II studies, and process transfer to the Biotechnology and Sterile Life Sciences division of Cardinal Health who can manufacture and purify proteins in a larger scale. The company has also developed the Transgami-T™ Process which can create transgenic cattle using GPEx™ technology. Transgenic cattle can be created in less than a year.

Gala Biotech was acquired by Cardinal Heath in October 2003 for approximately $27.5M total (Cardinal Health, 2003). Total manufacturing capacity is approximately 300L. In 2003, the company had 37 employees. The company is currently working towards being FDA compliant.

3.6.1.3.5 ICOS Corporation (‘ICOS’)

ICOS is a publicly traded (NASDAQ:ICOS) biotechnology company located in Bothell, Washington, with both R&D and mammalian cell protein manufacturing capabilities. The company’s major product is Cialis®, a drug used to treat erectile dysfunction. In addition, several other compounds are in the clinical trial and R&D stage in the areas of chronic obstruction pulmonary disease (COPD), cancer, psoriasis, and inflammatory and infectious diseases. The company is also a CMO whose services include manufacturing recombinant protein products by both microbial (1,600L) and mammalian cell (3,000L) methods for clinical trial Phases I through III, process development, product purification, QA/QC, formulation development, analytical studies, and engineering and regulatory support. ICOS also engineers cell-lines using their CHEF1 system, which is a proprietary plasmid vector that works in conjunction with CHO cells.

The CHEF1 expression system is a patented CHO line gene expression system based on the CHO EF-1α promoter. The CHEF1 system does not require gene amplification to engineer cells with high expression (6 – 25 fold greater than by using other traditional promoter systems), thereby decreasing development times. Monoclonal antibody expression levels of 0.425g/L have been achieved with the CHEF1 system (Allison, Brandenstein, Davis, Running Deer, Shah, & Ziegler, 2003, p. 38). In addition, the CHEF1 system can be used with a variety of selection markers allowing high level co-expression of protein subunits. The time required to get from initial DNA transfer to cGMP manufacturing takes between 10 – 13 months using the CHEF1 Expression System (ICOS Corp., 2003).
Total revenues were $75.1M in 2003, of which $12.2M were from contract manufacturing services (ICOS Corp., 2004a, p. 27). Net losses for the same year were $125.5M. At the end of the first quarter of 2004, the company had about $398M in cash and equivalents (ICOS Corp., 2004b, p. 2). On June 2, 2004, the company’s market cap was $1.89B with 62.9M shares outstanding. Currently, the company has 675 employees in various U.S. locations.

3.6.1.3.6 Morphotek Inc. (‘Morphotek’)

Morphotek is a private product development biotechnology company located in Exton, Pennsylvania, who has proprietary technology to accelerate the in-vivo evolution of a host’s genome by regulating the host cell’s mismatch repair function. This is achieved through one of two ways: the introduction of Morphotek’s proprietary gene, morphogene, into the cell of interest; or by the introduction of their proprietary small molecule, Morphocene™. Modulation of mismatch repair functions result in genome-wide mutations that are passed onto daughter cells. Such mutations result in cell-lines with higher expression of a desired gene (including antibodies), antibodies or protein therapeutics with enhanced activity, or organisms that show an altered response to a drug (Morphotek Inc., 2003). When the cells with the desired characteristics are isolated after screening, the morphogene is removed to stabilize the cell. The process has been shown to work in bacterial, yeast, mammalian, and plant cells. The level of protein expression achieved by this system is not publicly available. Some of their corporate partners who use the technology to enhance mammalian cell expression include Abgenix Inc., Centocor Inc., Novo Nordisk A/S, and Baxter Healthcare Corporation (see news webpage at http://www.morphotek.com).

3.6.1.3.7 Sangamo BioSciences Inc. (‘Sangamo’)

Sangamo BioSciences Inc. is a public biotechnology company (NASDAQ NM:SGMO) developing therapeutics in the areas of cardiovascular disease, cancer, neuropathic pain, and sickle-cell anaemia. The therapeutics are developed using their Zinc Finger DNA-binding Protein Transcription Factor (ZFP TF), which can be used to control protein expression. ZFP TFs are composed of two subunits, one to recognize DNA sequence, and the other to direct the protein’s function (functional domain). ZFP TFs can be modified to recognize and bind to any DNA sequence, and activate or repress its expression with a small molecule drug, or cleave its sequence. The technology is being applied in the areas of human therapeutics, targeted gene
correction, discovery and validation of gene targets, small molecule drug discovery, monoclonal antibody development, and mammalian cell-line engineering for the manufacturing of protein therapeutics (Sangamo BioSciences Inc., n.d.). Chromos' concerns are in the latter two application areas.

The advantages of ZFP TFs in the area of drug discovery and monoclonal antibody development include gene expression regulation (up or down regulation including switching genes “on” and “off”), timing and controlling the amount of gene expression, and the regulation of endogenous genes in the host cell – thereby bypassing patenting issues. Patents cannot be issued to genes that exist in their own natural cells. Normally, genes that are isolated from the original host cell for research purposes such as cloning are patented by the researchers who have discovered it. The ZFP TF system bypasses patenting issues as genes of interest do not have to be isolated from the original host cell (the cell where the gene naturally exists) and transferred to other cell-lines for study.

In the area of cellular protein production, the ZFP TF system is being exploited to over-express desired genes in a cell-line thereby increasing production yield. Current partners who are using the ZFP TF system for this purpose include R.W. Johnson Pharmaceutical Research Institute, Pharmacia Corporation, Icagen Inc, and Medarex Inc. There is no public information regarding protein expression yields based on the ZFP TF system.

In 2003, Sangamo incurred revenues of $2.58M and net losses of $10.43M. Over two years, the deal with Medarex contributed $1.2M, or $600K per year. Total losses incurred to the end of 2003 were $83.3M (Sangamo BioSciences Inc., 2004a, p. 23). As of June 1, 2004, Sangamo’s market cap was $157M with 25.16M shares outstanding. Cash and equivalents at the end of the first quarter in 2004 amounted to $42.1M (Sangamo BioSciences Inc., 2004b, p. 3). Currently, the company has 57 full time employees.

3.6.1.3.8 UniTargetingResearch (‘UTR’)

UniTargetingResearch, a private biotechnology company located in Bergen, Norway, has discovered mRNA signal peptides which could be used to increase protein secretion from cells. The technology exploits the cell’s secretory pathway to enhance secretion, rather than increasing the scale of production through using genetic elements (such as promoters, enhancers, etc.), high-expressing vectors, or modulating cellular growth conditions (Savage, 2004, p. 13). Inserting signal peptides and other required elements onto the mRNA encoding the protein of interest
directs the mRNA to the protein secretory pathway, where the mRNA is translated into a protein and secreted from the cell. This technology is useful for secreting proteins that are not normally secreted and those that are secreted at low levels. Secretion can be enhanced by more than 10 fold with UTR’s method (Savage, 2004, p. 13). The process of creating high secreting cell-line takes about 6 – 9 months. Information in terms of cellular expression yields or the financial situation of the company has not been publicly released.

3.6.1.3.9 Xcellerex

Xcellerex, a privately held biotechnology company located in Marlborough, Massachusetts, is a contract service bioprocess development and manufacturing company. They have two technology platforms, BioMax™ and FlexMax™. The BioMax™ platform process is composed of cell-line development, clone selection, medium development, bioreactor process optimization, purification and formulation (Xcellerex, n.d.a). Cell-line development features their proprietary Supercell™ system, which is composed of a CHO DG44 cell-line coupled with a proprietary vector which, when cloned with a gene of interest, it targets specific ‘hotspot’ sites on the CHO genome (Xcellerex, n.d.b). The vector eliminates the randomness usually associated with gene transfection, thus yielding higher levels of gene expression. The Supercell™ system has been adapted for serum-free suspension growth. The medium development, purification, and formulation components of the BioMax™ System are high-throughput, thus decreasing product development times by many months compared to normal methods. There is no public information on protein expression yields using the Supercell™ system.

FlexMax™ is a modular manufacturing platform which can be rapidly reconfigured as new manufacturing processes are developed or processes need to be changed (Xcellerex, n.d.a).

Xcellerex was established in 2003, and there is no public information on their financial situation or other corporate intelligence.

3.6.2 Indirect Competitors

The ability to produce properly folded and glycosylated complex proteins in manufacturing systems other than mammalian cells is difficult and no complex protein products have been made to date that were approved by any regulatory authority. Much research has been conducted in other manufacturing systems; however, there is no dominating technology. The
threat from indirect competitors is relatively low, but as their technologies mature, the protein manufacturing landscape can quickly change.

3.6.2.1 GlycoFi Inc. ('GlycoFi')

GlycoFi is a private biotechnology company located in Lebanon, New Hampshire, with technology to produce fully humanized glycosylated therapeutic proteins in yeast and other fungal cells. This ability increases the efficiency, fidelity, and scalability of protein manufacturing compared to mammalian cells. Using their technology, they have developed many yeast strains which can be used for creating specific glycosylation patterns on complex proteins. The fungal cells the company has developed can produce proteins at high yields and with great homogeneity. The company claims that their system can typically produce protein yields of $0.1 - 2.6\text{g/L}$ in un-optimized conditions with fermentation times of 72hrs (GlycoFi Inc., 2004a). Currently, the company has a research collaboration with Baxter Healthcare to develop a platform for the production of human antibodies based on their yeast system. In addition, the company has a research collaboration with Biogen IDEC to produce an undisclosed protein. To date, GlycoFi has raised about $17.6\text{M}$ through venture capital financing rounds (GlycoFi Inc., 2004b).

3.6.2.2 Neose Technologies Inc. ('Neose')

Neose is a publicly traded (NASDAQ:NTEC) product development company whose proprietary technologies, GlycoAdvance™, GlycoPEGylation™, and GlycoConjugation™, are used to modify therapeutic proteins to improve their efficacy. The company has locations in Horsham, Pennsylvania and San Diego, California. Neose's strategy, aside from developing corporate collaborations, is to use their technology on high-value protein therapeutics that are coming off patent, which could then be modified and made more effective, such as erythropoietin.

GlycoAdvance™ technology uses enzymes to complete sugar chains of proteins that are created by mammalian cells. According to Neose, the glycosylation patterns on therapeutic proteins derived from mammalian cells do not always resemble the humanized form, and are therefore less effective (i.e. rapid body clearance). GlycoAdvance™ technology can be used to fill missing sugar molecules, thus making proteins more humanized and increasing their half-life.
in the body (Neose Technologies Inc., 2002a). Overall, it represents a savings in time and costs for a patient as less dosing would be required.

GlycoAdvance™ could also potentially be used to modify the glycosylation patterns of proteins produced by bacterial, fungal, plant, insect cell, and transgenic animal systems. Traditionally, the problem with proteins manufactured by these systems is a lack of, or incomplete glycosylation. The ability to humanize the glycosylation pattern of these proteins can significantly affect mammalian cell culture manufacturing businesses because it is the most expensive method of production. The ability to manufacture raw proteins at low cost, then humanizing its structure downstream before final packaging, can be an attractive alternative to manufacturing with mammalian cells as the cost savings for the product development company can be significant. This attractiveness can be dangerous for Chromos.

GlycoPEGylation™ technology is used to add polyethylene glycol (PEG) residues to sugar chains attached to proteins, thereby increasing protein solubility, stability and half-life, and decreasing degradation and immunogenicity (Neose Technologies Inc., 2002b). GlycoConjugation™ technology, like GlycoPEGylation™, gives users the ability to add other types of compounds to the sugar chains of proteins, such as radionucleotides or cytotoxins. Attaching radionucleotides or cytotoxins to antibodies make them more potent cell killers.

Neose’s current partners who are using their technologies include Novo Nordisk A/S, MacroGenics Inc., and Sandoz Inc. To date, there are no marketed products that have used GlycoAdvance™ technology, nor has there been any indication as to whether GlycoAdvance™ has been used to modify proteins produced by means other than mammalian cells.

In 2003, Neose incurred revenues of $1.44M; however, net losses were $37.61M (Neose Technologies Inc., 2004a, p. 12). Total deficit since inception to year end 2003 were $145.74M. The company’s cash position at the end of Q1 2004 was $39.92M, which increased by $32M in May 2004, through a financing round (Neose Technologies, 2004b). Neose’s market cap on June 4, 2004 was approximately $196.63M with 24.7M shares outstanding (NASDAQ, 2004). The company employs around 125 people.

3.6.2.3 ‘Other’ CMOs

There are a number of CMOs that are manufacturing therapeutic proteins by other means besides mammalian cells, which include bacterial and yeast cells, and plant and transgenic animal systems. Although these other means are limited to what types of proteins they can produce
because of glycosylation issues, they can take business away from mammalian cell systems if glycosylation is not an issue or if the protein is a simple structure. There are no marketed products produced by plant or transgenic animal systems, therefore these methods of production have not been truly tested in the marketplace as a cost saver. However, when a product manufactured by these methods becomes marketed and demonstrates a lower cost of production compared to mammalian cells, they may be more widely accepted by the industry and shift manufacturing away from mammalian cells. Many CMOs who manufacture with mammalian cells also have microbial cell protein manufacturing capabilities such as Cambrex BioSciences Corp., Goodwin Biotechnology Inc., and DSM Biologics. CMOs focused on using plant manufacturing include Dow Chemical Company (corn), Meristem Therapeutics (tobacco, corn), and Biolex (*Lemna* plant species) among others. Some of the CMOs using transgenic animals include AviGenics Inc. (chicken), GTC Biotherapeutics Inc. (goats), and Pharming Group N.V. (cattle and rabbits).

### 3.6.3 Conclusion

Significant technological competitors include Lonza Biologic’s G.S. Expression system and Crucell’s PER.C6™ System. They are significant because other companies have validated these systems as a tool for protein manufacturing and are taking the risk of using these systems for the production of clinical development and commercialization material, which are significant milestones. The other mammalian cell systems are not as threatening because many of them are being evaluated at the research stage. In addition, the competitors are not releasing too much information about their systems in terms of expression levels or who is licensing their technology for manufacturing proteins at any developmental stage. One would think that if the technology is truly valuable, there would be greater exposure in terms of website advertising, publications, and other marketing means. Technologies from indirect competitors are still maturing and its value needs to be proven to the industry in terms of product approval and economic gains. Until that happens, their threat is relatively low.

### 3.7 Market Summary

Chromos is looking to expand its business into offering cell-line engineering services based on the ACE System. The Company can remain at their current size and use their existing competencies to engineer cell-lines based on the ACE System for clients, thereby existing in a
smaller niche in the pharmaceutical value chain; or they can expand their service offering and essentially become a small sized CMO, thereby capturing a larger portion of the value chain.

The two main sectors of the biotechnology industry that Chromos could approach with the ACE System are protein therapeutic biopharmaceutical companies and CMOs because they both use mammalian cells as a system for manufacturing complex proteins, such as monoclonal antibodies. The size of the therapeutic monoclonal antibody market is large and growing. By 2010, the biotherapeutic market in whole is expected to reach $57B, whereas the monoclonal antibody market is expected to reach $16.7B by 2008. The contract biopharma market is currently estimated to be worth $750M per year. Competition in the CMO market will be tightening in the future as CMOs will be losing market share to biopharmaceutical companies that are constructing and expanding their own manufacturing facilities. If Chromos was to expand its services and essentially become a small scale CMO, competition would be very difficult and the Company would incur significant risks. To compete effectively in this space, Chromos would have to be a large scale CMO. Biogeneric developers could also be another market to approach, however, that sector has many regulatory hurdles to overcome and their value to the pharmaceutical industry has yet to be proven. If biogenerics do become approved by drug regulatory authorities, then a whole new segment of clients would become available.

The client base that could potentially require cell-line engineering services that Chromos could provide is large. The types of clients that would require cell-line engineering services are small to medium sized product development companies who do not have expertise, resources, or time in creating high monoclonal antibody expressing cell-lines. In addition, CMOs who require high expressing cell-lines for manufacturing a client’s product could also be potential customers because the production cell-lines their clients provide may be sub-optimal. There are a few CMOs who do not have cell-line engineering capabilities at all who may want to offer it as part of their services, again which Chromos can provide.

Mammalian cells, at the moment, are the only manufacturing systems which could properly fold and glycosylate complex proteins that most closely resemble those found in the human body. Mammalian cell production is the most expensive and inefficient compared to other systems available such as insect cell, microbial, plant, or transgenic animals. There is on-going research on how to make humanized complex proteins using these other systems, which could have the effect of making mammalian cells an outmoded technology, provided that regulatory issues are met.
According to a Porter’s Five Forces analysis of the global cell engineering services industry, the highest areas of concern are the threat of rivals and the bargaining power of customers. Rivals who offer both upstream and downstream services will be sought because it makes process transfer much easier and consumers do not need to look for multiple service providers. Chromos is limited in the services they could provide compared to rivals who perform cell-line engineering regularly. In addition, rivals are continually improving their technology which could eventually be superior to Chromos’. Customers have high bargaining power since all competitors in cell-line engineering in effect offers the same basic product - an engineered cell-line. Most product development companies do not have any revenues or profits; therefore they will be highly price sensitive, even at the expense of sub-optimal cellular expression.

The threat of new entrants and substitutes, and the bargaining power of suppliers range from low to moderate. However, in the long term, the threat of new entrants and substitutes are high as new, more economical protein manufacturing technology come into fruition. The most threatening technology is fungal based systems as it combines the best of both the microbial and mammalian cell characteristics. If the company, GlycoFi succeeds in making humanized complex proteins in their yeast systems, then there may be a shift towards using yeast systems as the manufacturing method of choice.

There are a number of competitors in the protein manufacturing space, however, only a few have technology that increases mammalian cell protein expression compared to conventional methods. Competitors that have technologies which compete strongly with Chromos’ ACE System include Crucell’s PER.C6™ cell-line and Lonza Biologics’ G.S. System. The other competitors mentioned in this chapter do not all indicate specifically what levels of expression they could regularly achieve with their technology on their websites, nor are their systems used to manufacture marketed products. In addition, many of the competitors are product development companies and are using their resources to strengthen products in their pipeline, rather than on enhancing their protein expression technologies. Indirect competitors do not pose as a serious threat now; however, this may change in the future as their technologies mature and are accepted by regulatory agencies as a safe alternative method to mammalian cell-lines.

Overall, Chromos needs to take advantage of the protein therapeutic market soon, specifically monoclonal antibodies, before other manufacturing technologies mature. Customers are price sensitive and will pay what they could afford. The ACE System meets some of the needs of the industry such as increased manufacturing efficiency and decreased time required to engineer high expressing cell-lines. With further, value added improvements such as adding gene
elements to enhance furthermore protein expression levels or refining protocols to decrease engineering times, the ACE System has the potential to be the production cell-line of choice.
4 STRATEGY

This chapter analyzes the internal aspects of the company and outlines options which the company could pursue to engage the cell-line engineering business. The internal aspects include the Company’s core competencies, current management, risk analysis of entering the cell-line engineering business, and access to capital to support the business. The options available to Chromos on initiating the business include building in-house, acquiring a cell-line engineering firm, or entering an alliance or partnership with another service provider. The chapter concludes by suggesting an approach to marketing the service.

4.1 Internal Analysis

The internal analysis begins by looking at the Company’s core competencies, which are defined as the activities Chromos does to drive value. Secondly, an overview of Chromos’ management is reviewed. Thirdly, the risks involved in entering the cell-line engineering business are discussed. Lastly, the ease of access to capital and how this will affect cell-line engineering is examined.

4.1.1 Core Competencies

The industry gold standard is achieving expression levels in mammalian cell culture of 1.0g/L. Some of the difficulties in creating a high expressing cell-lines encountered using the ACE System are not a function of the ACE System itself, but are also due to growth conditions, and characteristics of the gene of interest and expression cell-line. Obtaining high expressing cell-lines requires an optimal balance of many factors which are not under the control of scientists, such as gene copy number, cellular response to culture environment, cell density, mRNA translation and protein secretion rates, protein structure, plasmid constructs, and sites of gene integration to name a few. In general, the choice of host cell and media optimization plays a large role in obtaining high rates of expression. Modifying physiochemical factors (such as pH, dissolved oxygen, temperature) in bioreactors and improving media feeding strategies can improve expression yields (Vernon, 2004, p. 2).

Traditional methods of gene amplification to obtain high expressing cell-lines is a random, hit-and-miss approach that takes about 6 – 12 months to identify a few clones out of thousands screened, a high expressing clone with expression levels that ranges from 0.4 - 0.6g/L
under optimized conditions. Industrial cell-lines such as Lonza’s G.S. System and Crucell’s PER.C6™ have been successful at achieving high expression because they have been used and studied by numerous companies in the industry for many years and their methods of use and optimal growth conditions has been shared within the scientific community. These cell-lines have also been used to bring products into the clinic (products made by PER.C6™ are in areas other than monoclonal antibodies). Chromos’ ACE System, on the other hand, is relatively new and needs further validation. Optimal growth conditions for cell-lines engineered by the ACE System have to be determined. Chromos also needs to demonstrate that the ACE System can make products suitable for use in clinical trials and full scale commercialization in order to enhance its reputation. The Company has the proper documents in place; however, the FDA will not look at them until an IND is filed either by Chromos, their partners, or clients who are manufacturing with an ACE System production cell-line. The addition of the CHOK1SV cell-line to Chromos’ collection of Platform-Lines should enhance the ACE System’s attractiveness as the CHOK1SV cell-line is widely known in the industry.

Users need to have extensive experience in cell culture and molecular biology expertise or they may encounter difficulties which can make the ACE System, or any other traditional approach in that matter, frustrating to use. Working with the ACE System requires a lot of tacit knowledge that Chromos’ scientists have developed since the conception of the technology. Transferring this knowledge to licensees is difficult, especially if their expertise in cell culture is limited. In addition, the ACE System is not in a form that is readily transferable as a package to license to customers because the ACE System components are not yet fully optimized, which can take up to a year. The ACE System as a package will not be exploited until it has been fully optimized. Difficulties with propagating the ACE Vector containing a product gene and obtaining reproducible protein expression results can occur. At times, product genes are found on the host chromosomes in addition to the Platform ACE, thus contributing to variable results. On average, there are 5 copies of a targeted gene on the platform ACE after transfection, the range is from 2 to 10 copies.

Chromos’ researchers are focused on process development to decrease the speed required to isolate and characterize high expressing cell-lines. In addition, they are optimizing protocols to increase ACE Vector targeting specificity, and create high, reproducible protein expression using different cell-lines. The researchers are also determining optimal growth conditions to maximize expression yields. Ideally, only one protocol would be required to produce a high expressing cell-line consistently irrespective of protein type, cell-line used, and culture conditions.
The ACE System technology is still premature compared to their competitor’s G.S. System and PER.C6™ cell-lines. To be competitive, Chromos must be able to produce cell-lines that express in the range of 0.5g/L un-optimized, which could increase 2 - 3 fold under optimized media conditions. Since the ACE System components are not yet fully optimized, the expression levels that Chromos typically achieves cannot be disclosed. What Chromos can guarantee to their clients is a cell-line that produces proteins, however, the Company cannot guarantee at what levels since there are many factors that affect expression beyond the Company’s control. That being said, the same is true for both the G.S. System and PER.C6™ cell-line as their advantages are entirely due to optimized growth conditions. Cell-lines produced using the ACE System will be modified for optimal expression in a certain culture environment, however, that may still not be enough for clients. It may be difficult for Chromos to achieve a level of expression that a client specifically demands, which can affect the attractiveness of the ACE System. Clients need expression levels similar (in un-optimized conditions), or higher than what they could achieve on their own and they would expect Chromos to deliver it in a short timeframe.

The Company’s core competencies fall into the following areas: research; experienced management; and a tightly knit culture. Chromos’ research function is composed of research scientists with a wide variety of experience. Researchers have knowledge in cellular and molecular biology (aseptic cell culture techniques, recombinant DNA technologies, PCR, FISH analysis, microbiology, etc...), chromosome biology, assay and process development, downstream processing, and flow cytometry. The expertise in flow cytometry is important because only Chromos can isolate a Product- or Platform-ACE and transfer it into different cell-lines. The core scientific skills are applied in the areas of cellular protein production, gene-based cell therapy, and animal transgenics. The scientists also have extensive research experience in a number of therapeutic areas. The Company’s research efforts have culminated in a number of corporate and academic collaborations, presentations in world renowned conferences, publications in peer reviewed journals, and patent filings (24 issued, 59 pending).

Chromos’ resources on an employee level are currently strained. Project leaders are frequently ‘borrowing’ technicians from different research groups to complete tasks. The line between the process development team and ACE System optimization is becoming less defined. Starting a cell-line engineering service will require the hiring of additional resources who will be dedicated to cell-line engineering as current staffing levels cannot support the service alone.

Chromos’ management is driven by business professionals, scientists, and those who have a knowledge combination of both. They are strong at raising financing, establishing and
maintaining corporate and investor relationships, promoting the Company and technology, and developing business strategies. The scientists at the senior and executive levels all have experience in industry in addition to academia. The business executives have held positions in large, multinational firms. The Company’s management is discussed further in the next section.

The corporate culture in the company is very strong, which is driven by the Company’s visions and values. Employees are dedicated, supportive of each other, and work strongly as a team. They are empowered by managers and given responsibility with accountability. Annually, the Company celebrates its achievements at an off-site retreat and holds numerous social gatherings to foster relationships internally and externally. The corporate executives believe in rewarding hard work with bonuses and promotional opportunities. Aside from competitive salary, researchers are driven by stock options, employee benefits, educational assistance, professional development opportunities, and recognition in the industry.

Areas of specialization where the Company needs to acquire expertise lie in the areas of clinical development, protein manufacturing, and downstream processing capabilities. In particular, the Company does not have any expertise in regulatory affairs, clinical trial management and strategy, QC/QA, and manufacturing, however, the pending acquisition of CellExSys will bring in some expertise. Many of these clinical development functions could be contracted out by the Company when required. Quality control and quality assurance procedures would also need to be established when the Company makes products that are entering clinical trials. In addition, QC/QA procedures need to be in place when engineered cell-lines are to be returned to clients. Written procedures and other documents need to be in place for regulatory filing of products. In-house clinical experience is limited. The lack of clinical experience will need to be addressed as the Company’s drug pipeline becomes more developed.

Chromos does not have any manufacturing or other downstream processing capabilities. The lack of these services could potentially be unattractive to potential clients as they would have to find these services elsewhere. In addition, the ACE System cell-line would have to be adapted to the protein manufacturers’ processes, which could delay development time as process development and process transfer can be time consuming. Having manufacturing and other capabilities in-house would ease process transfer, thereby speeding drug development for the client. However, creating a manufacturing suite or facility incurs significant risk, and product manufacturing is not a part of the Company’s current strategy.


4.1.2 Management

Chromos is led by 4 executive managers: CEO; VP Finance; VP R&D; and VP Business Development. The CEO and VP Finance, both Chartered Accountants, have extensive history working for a multinational accounting firm. The CEO has over 10 years of experience in the biotechnology industry. Both the CEO and VP Finance have extensive knowledge in corporate financing, business strategy, and corporate accounting.

The VP R&D and VP Business Development both come from scientific backgrounds with a PhD, and have experience in both academic and industry environments. In addition, they are well versed in business practices such as negotiation, financing, and maintaining corporate relations. They both have had previous experience in product development companies, and have expertise in establishing corporate collaborations and alliances, corporate strategy, and managing teams.

The executive management team is governed by a Board of Directors (BOD), as required by a public company. The BOD is composed of 7 members of the scientific and investment community across North America.

The cellular protein production effort of the company is led by the Director of Cellular Protein Production (CPP), who has experience from both academic and industry environments. In particular, the Director of CPP brings expertise in mammalian cell protein production, process development and scale-up, small and large scale manufacturing, and downstream processing and protein purification.

The gene-based cell therapy group is led by the Director of Projects, who also brings experience from both academic and industry environments. The Director of Projects has developed expertise in molecular biology, animal transgenics, and stem cell therapeutics.

Although the management team has expertise in a wide variety of areas, they do not have experience in managing a service based company. The cell-line engineering business is essentially a service based function.

4.1.3 Risk Analysis

There are numerous risks associated with implementing the cell-line engineering business, some of which are dependent on how Chromos performs as a whole and the investment climate. The risks are classified as business and scientific risk.
4.1.3.1 Business Risks

- **Might not be able to raise additional financing, if required:** The ability to raise money can be difficult if the investor climate is not receptive, or the terms are not favourable to Chromos. Without financing, cell-line engineering operations may have to be scaled back or ceased. Scaling back will limit the number of clients we can accommodate at any one time.

- **Fluctuation in timing of services and revenues:** Chromos cannot control the timing of the clientele requiring services. Revenues from services per period may be irregular and clients may have to be turned away if capacity is full. Turning away clients will result in lost revenues. In addition, Chromos also depends on revenues from milestones and royalty payments, which are realized when a client’s project reaches certain development stages. There is no guarantee if or when clients will meet the agreed upon milestones or if their product will be marketed.

- **May have disputes with clients thereby resulting in no payment:** Clients may not be satisfied with the final product delivered resulting in no payment. Clients may choose not to license the engineered cell-line, resulting in lost revenues. In addition, Chromos has to depend on the client to inform them of when milestones are met and the amount of royalties owed. Poor economic conditions may cause clients difficulty in making payments. Any disputes with clients may cause negative relationships, which could have an effect on current and potential clients.

- **Third party media suppliers may disrupt shipment:** Mammalian cell-lines are dependent on media in order to survive. High valued cell-lines will senesce if they are not grown in proper media, thus resulting in a loss of revenues, and wasted development time. These cell-lines may not be recoverable. If media suppliers are changed, time may be required to optimize protein expression as processes and technology will have to be adapted to that new medium.

- **May have patent disputes in the future:** Technology in the biotechnology industry is rapidly evolving and it is difficult to analyze who may be infringing Chromos’ patents and vice versa. Patent disputes concerning the ACE System, artificial chromosome technology, methods or processes, or other items may arise which can have a negative effect on Chromos’ revenues, cash position, and reputation. Legal
counsel may need to be engaged and the Company may need to settle disputes in court, which can result in significant expenses.

- The cell-line engineering business is not the long term goal of the Company: The long term goal of the Company is gene-based cell therapy, therefore resources and research projects will be directed towards achieving that goal. Cell-line engineering may not receive requested resources, which may hamper its development. It is unknown how Chromos will exit the cell-line engineering business in the future.

4.1.3.2 Scientific Risks

- Will require manpower, capital and equipment, thereby taking away resources from other projects: Starting the cell-line engineering business will require greater infrastructure than what already is present. Chromos will need to spend cash on additional employees and equipment, thus current and future projects may be negatively affected. Current employees, including senior managers, may have their roles changed or focus temporarily shifted from their regular projects to support cell-line engineering efforts.

- ACE System may be superseded by competitor’s technology: There are numerous competitors and competing technologies in the biotechnology industry of which Chromos is aware of. Competitors with superior technology may take away current or future clients, which could significantly affect revenue streams.

- Does not have regulatory approval for products used by ACE System: There are no products either marketed or in clinical development that have been produced by the ACE System. This may deter potential clients who have high valued products from committing to the ACE System. Chromos and their clients may experience difficulties with regulatory authorities if the ACE System is deemed unsafe for protein manufacturing, thus delaying the product’s time to market and lost revenues. Significant value in the ACE System will be lost if regulatory authorities do not allow it to be used for manufacturing therapeutic proteins. Clientele would then be limited to research stage companies, thus Chromos may lose certain higher valued milestone and royalty payments.

- Might not obtain the employees or expertise required to support the business: Competition within in the industry make it difficult to attract, retain, or motivate
qualified employees. If Chromos fails to attract or retain qualified individuals for the cell-line engineering business, it may be hampered in development or the Company may not fulfill their obligations to clients.

4.1.4 Access to Capital

Chromos’ current cash position is at $6.6M CDN, which is expected to last until Q2 2005. These estimates are based on current revenue and burn rates. Starting a cell-line engineering business is expected to be self sustainable; however, up-front funding from Chromos’ cash reserves will be required. To access funding, cell-line engineering must prove to have a very good return-on-investment (ROI), otherwise access to funding will be difficult as the cash resources will be dedicated to R&D and regular expenses. The executive management team and BOD must be convinced that the cash used to initially fund the engineering service has minimal investment risk.

4.2 Strategic Options

Based on the external analysis of the cell-line engineering service market and internal analysis of the company, Chromos has 4 strategic options concerning the implementation of the cell-line engineering service: 1) build the service in-house; 2) acquire a cell-line engineering firm and incorporate the ACE System into their processes; 3) alliance or partnership with another firm; and 4) maintain the status quo and do not offer the service at all. These options are analyzed below.

4.2.1 Build In-House

Building in-house is in reference to performing the cell-line engineering service at Chromos’ research facility.

4.2.1.1 Required Resources

Chromos has all of the equipment and consumables required to perform cell-line engineering, assaying, and cell expansion, since the research teams perform these functions regularly. The Company’s concerns are a current lack of designated space for client cell-line engineering and in-house human resources. The building has room for further construction if needed. The Company estimates that its current workload capacity could support the production
of 10 cell-lines at any one time, and 20 cell-lines per year. The majority of the cell-lines are
developed by the process development team. The laboratory has 4 separate cell culture suites
which are divided amongst 4 research groups, namely: process development; ACE System
optimization; stem cell research; and core maintenance. Strict cleaning, usage, and maintenance
protocols are required for client cell-line engineering because Chromos cannot risk contamination
of a client’s cell-line. Over the next few years as the process development team completes their
studies, more space will be available to support client’s projects.

Given the Company’s current financial situation, they should be careful where money is
spent. Constructing a designated suite is not an option due to a lack of financial resources. The
R&D team will have to re-organize their work areas and share certain equipment such as bio-
containment hoods, cell counters, and assay plate readers. The research areas will have to be
consolidated.

Additional equipment that may be required including shaker incubators, free standing
incubators, cell counters, biocontainment hoods, electrophoresis equipment and centrifuges.

4.2.1.2 Financial Projections

The reader is referred to Appendix 2 for pro-forma financial statements and its
derivation. The cell-line engineering business will be profitable in their first year (much of this is
attributed to the use of Chromos’ facilities and equipment which would otherwise be significant
purchases). The starting costs are low and the time required for operational set-up is short
because of the existing infrastructure and equipment. The net income present value of
implementing the service is $3.65M CDN, based on a 30% discount rate. The net present value
taking into consideration start-up costs is about $3.45M. The present value of the service is
significantly greater than the start-up costs, which is approximately $200K based on new
equipment purchases, salaries, rent and other miscellaneous expenses. The business will reach
another cell culture room will be required which, for the most part, will be already equipped with
tissue culture equipment. Capital asset amortization costs are low because many of Chromos’
equipment that will be used are near or fully depreciated, according to Chromos’ 5 year capital
asset depreciation rule.
Figure 4-1: Build in-house net income projection

Shown is the net income projection of building in-house. The net income is based on a conservative client estimate.

4.2.1.3 Pros

The pros of building in-house are related to low initial set up costs, and low fixed and overhead costs since the cell-line engineering service will be conducted at Chromos’ facilities where resources such as capital equipment could be shared.

- **Little capital expenditure is needed initially:** Chromos already has the infrastructure and equipment in place to support cell-line engineering functions. Some capital may be required to expand facilities, add additional equipment, and higher more personnel. The amount of capital required is quite low compared to starting the business from the very beginning. Many of the major equipment required for cell-line engineering could be shared with Chromos’ research teams.

- **NPV is high:** The projected NPV using a 30% discount rate is approximated $3.45M.

- **Fixed and overhead costs are low:** The fixed costs for the cell-line engineering business would be low as much of the fixed costs would be absorbed by Chromos’
main research business. Additional fixed costs from cell-line engineering would come from salaries and equipment costs. Basically, the cell-line engineering business would be “renting” space from Chromos.

- **Expertise is in-house:** Chromos’ scientists who are familiar with the ACE System and have developed tacit knowledge are already in-house and could support technicians who are engineering cell-lines for clients without delay. No technology transfer would be required and the learning curve is low.

- **Current research projects will be completed in the near future:** As current research projects conclude, there will be more capacity available to support cell-line engineering services.

### 4.2.1.4 Cons

The cons of building in-house are related to compromised R&D functions as many of the resources will be shared including capital assets and cash, although the cell-line engineering business is expected to be self-sufficient. Other cons include risk and limited services offered.

- **R&D may be compromised:** The addition of equipment and personnel to support cell-line engineering may take away physical space that could be used for R&D. In addition, the sharing of equipment may result in usage conflicts.

- **Personnel conflict between functions:** Personnel from cell-line engineering may be in conflict with personnel from R&D when using equipment or other resources. Conflict can have a negative effect on productivity and company morale. Prioritizing equipment usage may be difficult. Buying more equipment could solve some problems, but this solution would take away lab space and valuable cash resources.

- **Will need to spend more money if cell-line engineering business expands:** The cell-line engineering business may need to expand in order to meet demand, which could require cash spending. This would take resources away from Chromos’ R&D functions, slow productivity, and hamper goals of becoming a gene-based cell therapy company.

- **Chromos is absorbing all the risk on its own:** Chromos will be absorbing all the risk associated with starting a cell-line engineering business if they build the service alone in-house.
- **Limited services offered:** Chromos is limited by the number of services they can offer to clients. Most competitors have at least small scale manufacturing capabilities or other upstream services which could make them more attractive. Chromos' goal is not therapeutic protein manufacturing, therefore it is highly unlikely that any investments in manufacturing or other upstream functions will be made, thereby limiting services to only engineering cell-lines.

### 4.2.1.5 Conclusion

Building in-house is a viable option since the existing infrastructure easily supports cell-line engineering with little initial costs required. More capacity for cell-line engineering for clients will be made available when the process development team decreases their activities in the following year. The immediate risk of building in-house is negatively affecting the R&D function, which may have resources taken away from them. Despite the risk to R&D, the financial outlook is positive and Chromos will receive steady revenues in the near to mid-term as long as they attract and retain more clients. Although the cons may seem to outweigh the pros, given that the potential returns are high at a relatively low cost, this strategy is justified.

### 4.2.2 Acquire Cell-Line Engineering Firm

Acquiring a cell-line engineering firm alone may not be possible because such organizations are not likely to exist. Cell-line engineering is usually a part of the services offered by a CMO, therefore, acquiring a cell-line engineering services of a firm would require acquiring the CMO. Chromos is clear that they do not want to enter protein manufacturing, even at the pilot or research scale level. Although the number of services offered by Chromos will increase with an acquisition of a CMO facility, protein manufacturing is not currently in any of the goals of the Company.

Cell-line engineering itself is a standard process that can be practiced by all product development companies. In order for a firm to survive that only performs cell-line engineering, they must have a technology that would make their services superior to standard technologies, or else have other upstream capabilities that would make their services valuable, such as antibody purification, assay development, media evaluation, and others. If Chromos was to acquire such a firm to just have an off-site facility in place for cell-line engineering, Chromos would also be required to acquire the target firm’s IP and other extraneous equipment not relevant to Chromos'
business. This would increase the target firm’s acquisition price and Chromos would have extra IP to deal with. The IP could be shelved if it competes with Chromos’ technology, complement Chromos’ service offering, or sold to other companies.

In terms of capturing market share, suppliers of small scale contract manufacturing have not seen the level of contract work that had been expected. CMOs, with better reputation, have expanded their smaller-scale capabilities, thereby contributing to the excess capacity seen in this space (Langer, 2004, p. 16). Competition in this area is tightening and Chromos has not built the competencies required to compete effectively.

4.2.2.1 Potential Acquisitions

There are no stand alone cell-line engineering companies that the Company could acquire. Most organizations with cell-line engineering functions have other upstream/downstream capabilities. Taking on extraneous activities not related to cell-line engineering are currently not in Chromos’ best interests.

4.2.2.2 Financial Projection

The cost of building a manufacturing plant varies depending on the type of expression vector used and the volume of manufacturing performed. The cost for building mammalian cell manufacturing facility producing Phase I or II products ranges from $10M – $20M USD (Bernstein, 2004, p. A5). However, when acquiring a company, other factors such as cost of capital equipment not directly related to manufacturing, personnel, IP and other intangible assets, earnings potential, attractiveness of technology compared to what is available in market, market size and market share need to be taken into consideration in the asking price and could drive up the price substantially. Additional outside financing will be required as Chromos’ current cash position would not be able to support such an acquisition. Another option is to acquire the company by offering Chromos’ shares, however, the target company may not be interested in owning Chromos’ shares or Chromos’ shareholders may not be supportive of such an option.
4.2.2.3 Pros

The pros of acquiring a cell-line engineering company are related to offering more services, gaining expertise and others as follows:

- **More services offered to client:** Depending on the firm acquired, more services may be offered that are attractive to the potential customers. This would make Chromos more competitive in the cell-line engineering business.

- **May retain valuable IP:** The acquisition of a firm may also include the acquisition of valuable IP, which could be used to complement Chromos' service offering or sold to other companies.

- **May retain required personnel:** In addition to acquiring capital equipment, Chromos would be acquiring experts who have direct experience in the cell-line engineering industry. These personnel could fill the knowledge gaps with respect to cell-line engineering and manufacturing in Chromos' scientific and management teams.

- **Cell-line engineering functions performed off-site from Chromos:** If the cell-line engineering functions are performed off-site from Chromos' R&D lab, then there will be no possibility of conflict in sharing physical resources.

- **Acquired firm could be a source of revenue if they are already successful:** The acquired firm could be a source of cash if they are already profitable, which could then be used to feed Chromos' R&D functions and goal to become a gene-based cell therapy company. Chromos would not have to spend time cultivating the business if they are already successful.

4.2.2.4 Cons

The cons of acquiring a cell-line engineering firm are related to the financial impact of an acquisition and are identified in the following:

- **Cost of acquisition may be substantial:** Depending on the firm acquired, the number of services it offers, and the level of infrastructure, the cost of acquisition could be substantial. Financing rounds to support the acquisition may be required, which could have an effect on share prices and ownership dilution. Or the transaction may be completed by a share transaction, which would also dilute ownership.
> **Chromos is absorbing all the risk on its own**: Chromos would be incurring all the risk if they acquire and operate a firm on their own. The more services that the cell-line engineering business offers, the more risks the company is taking.

> **Can be a time consuming process**: Finding the right firm for acquisition and negotiating an offer can be a long process, which could detrimental for capturing a large market share. The process could detract executives and managers from their regular duties.

> **Share value can be negatively affected**: It is uncertain how the market will react to an acquisition of a cell-line engineering firm. The reaction may be negative causing share value to decrease.

> **Client may not need all of the services**: If clients do not use many of the services offered, fixed costs may not be recovered and there will be excess capacity. This will affect the profitability of the business.

> **Overhead and fixed costs will increase**: Chromos will be required to absorb the fixed costs of the acquired business. The amount of fixed costs incurred will depend on the type of services acquired in the purchase. Fixed costs are usually high for mammalian cell protein manufacturing firms.

> **Knowledge of the ACE System would have to be transferred to new site**: Assuming that none of Chromos’ current scientists will work at the acquired cell-line engineering site, the ability to reproduce the success achieved at Chromos may be lengthy and difficult. However, the technicians that remain from the acquired company would be expected to be well-versed in tissue culture applications.

### 4.2.2.5 Conclusion

The lack of companies who solely perform cell-line engineering will make acquiring a cell-line engineering company difficult. Most companies who offer cell-line engineering as a service also have other capabilities which Chromos does not want to market because of increased risks, costs, and other similar factors. Chromos’ competitive environment will change after an acquisition. These other services will increase the asking price and Chromos would need additional rounds of financing as their current financial situation cannot support this strategy. The cons outweigh the pros of this option given that the potential costs and risks are extremely high.
4.2.3 **Alliance or Partnership**

In an alliance or partnership scenario, likely with a CMO, the partner would provide services other than cell-line engineering, screening and cell expansion, which would be Chromos’ responsibility. Chromos, along with the other party, would need to develop transfer protocols, and undergo process development and optimization with the ACE System production cell-line. Chromos would need to build a designated suite for cell-line engineering, much like the set-up discussed in Section 4.2.1. Building an alliance will help clients because they do not need to seek for a CMO to provide protein manufacturing, thereby saving them time. If they sought for a CMO other than Chromos’ partner, that CMO would have to perform process development and optimization steps with the ACE System cell-line which would cost the client time and money. If the client works with Chromos’ partner, process development and optimization would have already been worked out.

Basically, the partner would refer their clients who require cell-line engineering or subcontract the service to Chromos. Chromos, in turn, would pay a kickback or discount the price of the engineering service, respectively. In the case of Chromos referring their partner to a cell-line engineering client, Chromos could charge a finder’s fee to their partner.

### 4.2.3.1 Potential Partners

Ideal partners should have capabilities other than cell-line engineering, to which Chromos’ engineering abilities could complement the partner’s other services. Such CMOs include Biosynergy Ltd., Cangene Corp., Celltrion Inc., CMC Biotech A/S, Formatech Inc., Diosynth RTP Inc., Goodwin Biotechnology Inc., InVivo BioTech GmbH, Lampire Biological Laboratories Inc., Q-One Biotech, Sandoz Inc., and Strategic Biosolutions. There are also CMOs with cell-line development services, but do not have a cell expression technology to differentiate themselves from competitors that Chromos could complement. Such companies include Avecia Biotechnology, BioInvent AB, Biovest International Inc., Biovitrum AB, Cambrex, IBA Biologics GmbH, KBI BioPharma Inc., Kemp BioTechnologies Inc., Laureate Pharma LP., ORPEGEN Pharma GmbH, and Vista Biologicals Corp.

However, this is only a partial list of potential partners Chromos could pursue. The partner chosen should be cGMP compliant, have regulatory approved facilities, and a respectable track record.
The alliance could be exclusive or non-exclusive. The advantage of an exclusive relationship includes the ability for Chromos to demand a greater percentage kickback. The disadvantage of an exclusive relationship is decreased exposure of the ACE System.

4.2.3.2 Financial Projection

Partnering with a company may require expenditure of cash, depending on the structure of the deal. Akin to building in-house, partnering will require the purchase of additional equipment and personnel as the cell-line engineering will be performed on Chromos’ facilities. Future revenues will be varied and depends on the partnership deal structure as well as the success of the partner.

There are four scenarios how Chromos would incur revenues from the partnership. In the first scenario, the partner could refer a product development company to Chromos for cell-line engineering. Chromos, in turn, would pay a kickback to the partner for the referral. The product development company would have to license the engineered cell-line directly from Chromos. In the second scenario, the partner would sub-contract the cell-line engineering service for their client to Chromos. Chromos would provide the service to their partner at a discounted rate, who could then mark up the cost to their client. The product development company would then have to license the cell-line from Chromos. In the scenarios aforementioned, the product development company would have the choice of manufacturing with the partner or another company. In the third scenario, Chromos could license the ACE System to the CMO, who would be responsible for engineering cell-lines for their clients. Lastly, Chromos could refer their cell-line engineering clients to the partner if they require more upstream development. In this scenario, Chromos would receive a finder’s fee from the partner and the client would license the engineered cell-line from Chromos. The complexities of the relationships make it difficult to reasonably assess financial potential.

4.2.3.3 Pros

The pros of entering an alliance or partnership with another firm are a combination of the pros of building in-house and acquisition. They are as follows:

- Increased marketing for Chromos and ACE System: Partnering with a large firm will increase Chromos’ profile and validate the ACE System as an option for cellular
protein production. However, there is no guarantee that the partner is diligently marketing the ACE System to their clients.

- **More services offered to client:** Depending on the firm partnered with, more services may be offered that are attractive to the potential customers. This would make Chromos and its partner more competitive in the cell-line engineering business.

- **Seamless transfer process for clients:** By partnering with a CMO, clients essentially have a “one-stop shop” for all the services they require – from cell-line engineering to manufacturing. Transfer protocols will be developed such that the process is efficient and tailored for ACE System cell-lines, thus the customer saves development time.

- **No significant capital investments:** The partner will provide the majority of the upstream and downstream services, whereas Chromos will just provide an engineered cell-line. Chromos already has much of the infrastructure to provide engineered cell-lines in-house. They may be required to buy some more equipment or hire additional personnel in order to support cell-line engineering functions, but these are relative insignificant compared to purchasing equipment for other upstream and downstream functions.

- **Researchers from partnering company would contribute scientific expertise to enhance ACE System process development:** As the partnering company has more upstream and downstream capabilities than Chromos, they would be knowledgeable in how to best incorporate the ACE System into their manufacturing processes and optimizing the ACE System for client usage. They could also provide insights on how to develop the ACE System to make it more amenable to manufacturing processes.

### 4.2.3.4 Cons

Some of the cons of entering into relationships with partners are as follows:

- **May not get desired agreement terms:** The ACE System is a novel technology for manufacturing therapeutic proteins, therefore the partner may demand terms in their favour. No marketed products have been manufactured by the ACE System, which partners could perceive as a significant risk when entering into a relationship with Chromos.
Process of finding suitable partner may be time consuming: Finding the right partner with complementary skill sets may be time consuming as research, due diligence, and negotiations are long processes. The longer the process takes, the greater the potential for lost profits.

Process development could be time consuming: The time required for technology transfer, process transfer, and cell-line and media optimization can take many months. Potential clients may be lost if the time required for process optimization is too long.

May limit who can license the ACE System: Depending on the terms of the agreement with a certain partner, Chromos may be precluded from entering into relations with other potential partners. This may be detrimental to the Company if the relationship with the current partner is not strong.

4.2.3.5 Conclusion

Entering an alliance or partnership is a viable option because similar to building in-house, little capital expenditure is required. If Chromos decides to build the cell-line engineering business in house, the infrastructure will already be in place to support clients from the partnership. The partnership is another way of attracting more clients and utilizing Chromos’ available capacity. In a partnering situation, the pros outweigh the cons since most relationships are designed such that both parties are satisfied. Given the number of potential partners, finding a few should not be difficult.

4.2.4 Maintain Status-quo

Chromos has the option of not offering the cell-line engineering service at all. The advantages of not offering the service include: 1) corporate focus remains in R&D, ACE System development, product development from the CellExSys acquisition, and achieving goals; 2) expenditure of cash will not be required to hire more personnel or buy more equipment; and 3) the business development team can focus on out-licensing the ACE System. On the other hand, disadvantages include: 1) will have to look for other sources of revenues to support company growth; 2) may have excess capacity in Chromos’ research facility after process development studies and collaborations with partners are completed; 3) the recognition of the ACE System as a platform for protein production may take longer to develop; and 4) companies who license the
ACE System may not have the knowledge required to use the ACE System properly and miss the value the ACE System could provide, and consequently recommend to their peers to not use the ACE System for research or manufacturing purposes. Despite the cash savings from not entering the cell-line engineering service, Chromos will still need to raise additional financing as the cash reserve is expected to last until Q2 2005.

4.3 Marketing

The target audience for the cell-line engineering business are product development companies and CMOs. In the biotechnology industry, companies learn about each others developments in scientific, financial, partnering, and industry trade conventions. Attending these conferences is one strategy that Chromos should pursue. Another marketing strategy is to advertise on Chromos’ website, emphasizing the advantages of the ACE System and collaborations with other partners who Chromos has engineered cell-lines for. Chromos does not have a budget for advertising; therefore advertising in trade magazines would not be feasible. Partners are also expected to diligently market the ACE System to their clients, and Chromos’ clients could be asked to recommend Chromos’ services to their associates.

The Company must be careful not to over promise clients on the expression levels of their cell-lines as the final optimized expression levels delivered may still be sub-optimal to clients as the biology of the cell-line cannot be predicted. However, the Company can ensure clients minimal expression levels which are comparable to what researchers in the industry are achieving. Features of the ACE System that should be emphasized are the speed to obtaining a protein expressing cell-line that is comparable to industry standards, the ability to target different genes, stable protein expression, and transferability of the Platform ACE between different mammalian cell types. In addition, the fact that the CHOK1SV cell-line is available through Chromos for sub-licensing should be advertised as it demonstrates that Lonza Biologics has validated the ACE System technology and are willing to associate their products with the System, and that high expressing cell-lines based on a known process could be created. The ability to create production quality cell-lines using CHOK1SV should make Chromos’ engineering service offering and the ACE System as a production system in whole more attractive. The names of partners who have used the ACE System should be stressed as validation by other parties enhances the reputation of the System.
4.4 Chapter Summary

This chapter was focused on evaluating the alternatives that Chromos could pursue in developing the cell-line engineering business taking into consideration issues found in the internal analysis of the company. The alternatives include: 1) building the cell-line engineering service in-house; 2) acquiring a cell-line engineering business and incorporating the ACE System into the engineering process; 3) develop an alliance or partnership with another company such as a CMO; and 4) maintaining the status-quo.

The process development team of the Company is optimizing the ACE System by developing protocols to ensure the reproducibility of high expression yields. Access to capital to support the initial start up of the engineering service may be difficult given Chromos' current financial position. The executive management and BOD must be convinced that the cell-line engineering business is a low risk investment that will provide returns in the near to mid term. If the Company builds the service in-house, an approximate yearly return can be anticipated to be in the range of $1M - $3M after around two years of commencing operations, based on a discounted cash flow model, a conservative estimate of client number, and a number of other assumptions listed in Appendix 2. The financial outlook of the other alternatives is difficult to predict as they are more complex. The pros and cons of each option are summarized in Table 4-1 with the main recommended strategic option shown in bold.
Table 4-1: Pros and cons of suggested options

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<thead>
<tr>
<th>Option</th>
<th>Pros</th>
<th>Cons</th>
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<tbody>
<tr>
<td>Build in-house</td>
<td>• Little capital expenditure</td>
<td>• Compromised R&amp;D</td>
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<td></td>
<td>• NPV is high</td>
<td>• Personnel conflict</td>
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<td></td>
<td>• Low fixed and overhead costs</td>
<td>• Will need to spend more money if cell-line engineering business expands</td>
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<tr>
<td></td>
<td>• Expertise already in-house</td>
<td>• Chromos is absorbing all the risk alone</td>
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<td></td>
<td>• Current research projects will be completed in the near future</td>
<td>• Limited services offered</td>
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<tr>
<td>Acquire cell-line</td>
<td>• More services offered</td>
<td>• Substantial cost of acquisition</td>
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<tr>
<td>engineering firm</td>
<td>• Retain valuable IP</td>
<td>• Chromos is absorbing all the risk alone</td>
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<td></td>
<td>• Retain required personnel</td>
<td>• Time consuming process</td>
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<tr>
<td></td>
<td>• Cell-line engineering functions performed off-site from Chromos</td>
<td>• Share value can be negatively affected</td>
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<td></td>
<td>• Source of revenue if acquired firm is already successful</td>
<td>• Too many services offered</td>
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<tr>
<td></td>
<td></td>
<td>• Increased overhead and fixed costs</td>
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<td>Alliance or</td>
<td>• Increased marketing</td>
<td>• Knowledge of the ACE System would have to be transferred to a new site.</td>
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<tr>
<td>partnership</td>
<td>• More services offered</td>
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<tr>
<td></td>
<td>• Seamless transfer process</td>
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<td></td>
<td>• Low capital investments</td>
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<td></td>
<td>• Contribution of expertise</td>
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<tr>
<td>Maintain status-quo</td>
<td>• R&amp;D remains focused</td>
<td>• Undesirable agreement terms</td>
</tr>
<tr>
<td></td>
<td>• No expenditure of cash</td>
<td>• Finding suitable partner may be time consuming</td>
</tr>
<tr>
<td></td>
<td>• Business Development team can focus strictly on out-licensing</td>
<td>• Process development may be time consuming</td>
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<td></td>
<td></td>
<td>• May limit ACE System out-licensing opportunities</td>
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5 RECOMMENDATION

The recommended strategy provided is based on the information gathered from the external and internal analysis of the market and Chromos, respectively, and the options generated from the analyses. This chapter begins by offering a recommended strategy, followed by the challenges and risks involved with the strategy. Finally, possible exit strategies are presented.

5.1 Recommended Course of Action

The cell-line engineering business can be valuable to the Chromos in the near-, mid-, and long-term. Based on the external analysis of protein manufacturing in the biotherapeutic industry, there is a need for a cell-line that can produce high levels of protein that could be made in a short period of time. Chromos' ACE System has the potential to deliver on these needs, and the value of the ACE System could be offered to all product development companies through Chromos' cell-line engineering business, aside from licensing.

The internal analysis has shown that the Company does have the capability and capacity to support such a business in-house, however, producing cell-lines that express protein yields that are comparable to the industry average does not make the ACE System more advantageous than other cell-lines, at least at the un-optimized level. Optimizing the cell-line through modifying the culture environment, adding expression enhancing gene elements, and developing better engineering processes can increase expression levels at least 2–3 fold, which would be superior to most cell-lines currently available.

The recommendation offered is to build the cell-line engineering service in-house, which would be supplemented by entering alliances or partnerships with a multiple number of CMOs. The reason why a mix of these two strategies is offered is because of the synergy that both these strategies alone offer. Regardless whether or not Chromos enters into a partnership, they would have to build in-house because of their current financial situation and building in-house is low cost. If only the partnering strategy is chosen, Chromos would still have to build in-house because of the nature of the partnership (Chromos will do all the cell-line engineering except if the partner licenses the ACE System) in anticipation of providing the engineering service for their partner's clients. By combining the building in-house and partnering strategy, Chromos could increase clientele, and thus also increase revenues and profits.
The timing of the implementation of the recommendation, however, is an important factor that needs to be taken into consideration. Process development needs to be closer to completion and the characteristics of the ACE System still need to be further studied such that reproducibility and expression predictability are more regular. Product development companies will expect the ACE System to be superior to other technologies available, thus failing to meet their needs will give the ACE System a poor reputation, especially since the technology is novel. Given that the number of other cell-line technologies is low, other product development companies will know about failures fairly quickly. Early failures with biopharmaceutical developing clients can be detrimental to cell-line engineering service in the long run. It is anticipated that Chromos’ process development studies will be completed in about one year. This is also the time when the cell-line engineering service should begin. The availability of the CHOK1SV cell-line for protein production should be pushed as this cell-line is well known to the industry.

From Porter’s Five Forces cell-line engineering industry analysis, the threat from customers is high. Switching costs can be low for a potential client therefore Chromos must stress good customer relations and build customer loyalty. Chromos’ main competitor is Lonza, however, as the ACE System is relatively novel to the G.S. Expression System, the ACE System can be priced lower which could make the system more attractive. It will still be a few more years before other manufacturing systems will take significant market share away from mammalian cell systems and the Company should keep up to date with their progression.

Another factor in implementation timing that should be taken into consideration is Chromos’ current financial situation. Chromos has enough cash to last until Q2 2005. A round of financing or other funded research collaborations will be required in order for the Company to remain on-going. The cell-line engineering will be profitable in the first year; however, the profits will not be enough to sustain the R&D needs of the Company over the long run. The majority of the cash currently available is dedicated to R&D functions; therefore it may be difficult to convince executive management and the BOD that the time to start the cell-line engineering business is now. Some of the pressure for financing the cell-line engineering service may be alleviated if Chromos could charge their clients a portion of the service fee upfront, which could then be used to purchase required equipment. Persuading clients to pay upfront for an ‘unproven’ cell-line will be difficult as most other cell-line engineering services are fee for service and payment occurs after the client is satisfied with the final product.
The recommendations provided will have some inherent challenges associated with them that may affect the success of the cell-line engineering business. These are discussed in the next section.

### 5.2 Challenges and Risks

The challenges of entering the cell-line engineering business include the following: garnering executive management support; building commercialisation competencies in the Company; balancing the needs of the R&D function with that of cell-line engineering; developing a positive reputation for the ACE System; and finding suitable partners who will promote the ACE System.

Garnering executive management support is important for accessing capital and steering the Company away from pure R&D. Given that Chromos has enough cash to last to Q2 2005, access to cash may be difficult if the management is not convinced that the cell-line engineering business is low risk. By taking resources away from the R&D function, Chromos risks decreasing their productivity and achieving scientific milestones will take longer. Becoming a long term cell-line engineering business is not currently a goal of the Company; however, it does represent an opportunity to earn profits that would support the growth of gene-based cell therapy and cannot be ignored.

The cell-line engineering business is different from how Chromos normally functions on a daily basis. The Company is focused strictly on R&D and has no commercialisation capabilities. Building in-house a service function where timelines and pressures to engineer a cell-line to satisfy clients can cause a change in work culture. There may be conflicts between the cell-line engineering and R&D groups in equipment or reagent usage. The needs of the engineering and R&D groups must be balanced. There is a risk that the current work culture may become biased since managers tend to support processes that bring in profits. The Company may also need to develop in-house a sales support/marketing function to support the growth of the cell-line engineering business when clientele becomes larger. The individual in-charge of this function should be hired from in-house since they would be familiar with the technology and its capabilities. Developing commercialisation capabilities in-house builds knowledge in this area which could be useful for the Company’s future growth.

The ACE System has yet to be used for the manufacturing of a regulatory approved product, thus it is not as widely known in the industry. The reputation of the ACE System needs
to be stronger in terms of being able to produce a regulatory approved product, consistently express high yields of proteins of all types, and licensed to many biopharmaceutical partners. Chromos’ main competitor is Lonza Biologic’s G.S. Expression System, which has been used to manufacture marketed products. Protein yields over 4.0g/L were achieved using the G.S. System and it has been licensed by over 75 biotechnology and pharmaceutical companies. If Chromos does not increase the ACE System’s profile in the biopharmaceutical community, finding clients may be difficult. Chromos risks its positive reputation if the ACE System fails to deliver on its stated claims.

Finding a suitable partner to promote the ACE System may be difficult depending on the goals of the partner, terms of the agreement, how well the ACE System works with their internal processes, and the culture of the partnering company. If the goal of the partner is to focus on building downstream processes, where greater profits are extracted, they may not be concerned about promoting upstream processes and cell-line engineering. In addition, if their goal is to build cell-line engineering capabilities in-house, they may choose not to refer the ACE System to clients and engineer cell-lines using their own processes. Chromos risks their financial position and reputation of the ACE System if partners do not promote it diligently.

Negotiating terms of an agreement that would be equally favourable to both parties may be difficult as the ACE System is ‘unproven’ as a technology to produce marketed products, thus Chromos’ system risks being undervalued and Company would get the shorter end of the deal. Until the ACE System builds a stronger reputation as a protein producing cell-line, the initial deals with other companies may be of relatively lower value.

The upstream and downstream processes that companies use to develop manufacturing processes are not all the same, thus Chromos and their potential partner may encounter compatibility issues between the ACE System developed cell-lines and the partner’s processes. If such a situation is encountered, there is no value in pursuing the relationship.

The culture of the partnering company should be similar to Chromos’ in order for the partnership to be successful. Although the two Companies do not directly work with each other, the more dedicated the partner is to making the ACE System work in their processes, the greater the success of the ACE System technology. The partner’s culture must promote hard work, good documentation processes, rewards for successes, and fair treatment of their employees.
5.3 Exit Strategy

Chromos has a few exit strategies of the cell-line engineering business which will be dependent on its success and market conditions. The strategies include: 1) spinning off the business into a separate Chromos business unit; 2) selling the business to another company; and 3) dissolving the business and selling off the assets.

Spinning off the cell-line engineering service into a separate business unit will allow Chromos to focus on R&D and develop the gene-based cell therapy business without being distracted by the cell-line engineering service. As a new entity, the spun-off unit would be able to incorporate more upstream and downstream services, essentially transforming into a CMO. However, this strategy is dependent on the competitiveness of the CMO market, the needs of biopharmaceutical companies, and the global economic environment.

Selling the cell-line engineering service unit will allow Chromos to focus solely on R&D and develop the gene-based cell therapy business. Finding a suitable buyer depends on the needs of Chromos and the acquirer, competitiveness of the cell-line engineering market, and global economic conditions. The value of the service will be dependent on its future earnings potential among other factors. The cash infusion to Chromos due to the acquisition will greatly help the Company achieve their long-term goals.

If the cell-line engineering service is not as successful as projected and does not become profitable or self-sustaining, Chromos may have to dissolve the service and sell off the assets to salvage as much as possible. Chromos may or may not be able to recover all the service unit’s development costs.

5.4 Summary

Based on the external analysis of the cell-line engineering market and internal analysis of the Company, the recommendation offered is to build the cell-line engineering service in-house, and find suitable partners who will promote the system to their clients and incorporate the ACE System into their processes. The timing of implementing the service should be taken into consideration in order to maximize the acceptance of the ACE System as a manufacturing tool in the cell-line engineering and biopharmaceutical market. The challenges and risks associated with the recommendation includes garnering support from executive management, incorporating the service in Chromos’ R&D driven environment, building the reputation of the ACE System in the biopharmaceutical industry, and finding suitable partners who will support the growth of the ACE
System in the market. The exit strategy chosen by Chromos to leave the cell-line engineering service entirely depends on the how well Chromos meets their challenges and risks, how successful the service becomes, biopharmaceutical market needs, and global economic conditions. Exit strategies that are available to the Company include spinning off the service as a separate business unit and developing it by incorporating more upstream and downstream services; selling the service unit to a buyer; or dissolving the service unit and selling its assets.
6 APPENDIX

6.1 Appendix 1: The ACE System

For those readers who are technically minded here is a description of the technology. Worldwide, Chromos has 24 issued and 59 pending patents surrounding the ACE System technology and its applications. There are no known companies developing mammalian artificial chromosomes the same way that Chromos is developing them, nor are there any known mammalian gene expression systems that have similar characteristics as the ACE System.

The ACE System enables the rapid and flexible engineering of cell-lines for biopharmaceutical production. The System is composed of three components: 1) Platform ACE; 2) ACE Targeting Vector; and 3) ACE Integrase. Chromos (2003a, p. 1) describes the ACE Systems as:

"...a unique, proprietary gene expression platform technology that functions as a vehicle for transporting target genes into the nucleus of a target cell where they can be expressed to produce one or more proteins in a controlled, predictable and stable manner."

6.1.1 Platform ACE

The Platform ACE is made up of predominantly of satellite sequences found in the heterochromatic regions of a mammalian chromosome. The Platform ACE, containing a functional centromere and telomeres, replicates alongside host chromosomes and is passed onto daughter cells through cell division. Multiple acceptor sites are engineered onto Platform ACE thereby enabling single or multiple insertions of similar or different genes, or gene complexes. The Platform ACE is functional in mammalian cell-lines utilized by industry for commercial manufacturing, such as CHO, BHK, and NS0 lines, and Platform ACE containing targeted genes could be isolated allowing transfer between different cell-lines. The ACE System has been shown to express a foreign protein in mouse, rat, hamster, human, bovine, chicken, plant, primary mammalian and stem cell-lines.

6.1.2 ACE Targeting Vector

The ACE Targeting Vector ('ACE Vector') is a proprietary integrase enzyme mediated site-specific recombination targeting plasmid vector containing an ACE-specific "donor" site.
The ACE Vector containing the target gene of interest (also called ‘product gene’ as it is assumed the gene of interest encodes a product) in combination with integrase enzyme, allows unidirectional, site specific integration of the product gene onto pre-existing target sites on the Platform ACE at one or more “acceptor” sites. A foreign gene integrated on the Platform ACE creates a ‘Product ACE’. The gene of interest on the ACE Vector is flanked by expression enhancing sequences providing enhanced, reproducible gene expression. In addition, the ACE Vector contains selectable markers which only work correctly when site specific integration occurs on the “acceptor” sites of the Platform ACE, thereby allowing a highly efficient and rapid engineering process.

### 6.1.3 ACE Integrase

ACE Integrase, a proprietary enzyme expressed from a different plasmid than the ACE Vector, catalyzes the integration of the ACE Vector carrying the gene of interest and the Platform ACE. The integration event is unidirectional, i.e. integrase enzyme does not cause excision of genes on a Targeted ACE. In a single step, multiple copies of the target gene can be integrated onto the Platform ACE.

### 6.1.4 Targeted Integration

The creation of an ACE expressing a product gene can be summarized in 5 steps:

1. ACE Vector carrying the gene(s) of interest and ACE Integrase are combined with the Platform ACE chromosome which is housed in a mammalian cell-line such as Chinese Hamster Ovary (CHO).

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Diagrams were used with permission from Chromos Molecular Systems Inc.

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3 Diagrams were used with permission from Chromos Molecular Systems Inc.
2. ACE Vector and ACE Integrase migrate into the cell using standard transfection techniques.

3. Site specific integration of the ACE Vector onto the Platform ACE at multiple "acceptor" sites is catalyzed by integrase enzyme.

4. Multiple copies of the product gene are incorporated into multiple acceptor sites in a single step, thereby creating a Product ACE.

5. Product-specific candidate cell lines express the targeted gene of interest at high levels.
6.2 Appendix 2: Financial Outlook

The financial projection for building in-house is modeled using the discounted cash flow model, and are based on a conservative estimate of cell-lines produced for clients per year and assumptions listed below.

Table 6-1: Assumptions for pro-forma statements

<table>
<thead>
<tr>
<th>Line Item</th>
<th>Assumption</th>
</tr>
</thead>
<tbody>
<tr>
<td>Service revenue</td>
<td>Price per cell-line is $500,000 (based on standard industry FTE rates).</td>
</tr>
<tr>
<td>License revenue</td>
<td>Client licenses cell-line for 2 years for research purposes and annual license fee is $100,000. The annual fee is waived in the first year.</td>
</tr>
<tr>
<td>Media &amp; reagents</td>
<td>The cost of consumables used per cell-line which is $8,400. This includes culture media and flasks, enzymes, chemicals, DNA isolation kits, CO₂, and others.</td>
</tr>
<tr>
<td>Legal</td>
<td>The cost of a lawyer to administer a licensing deal which is $5,000 per deal.</td>
</tr>
<tr>
<td>External testing</td>
<td>The cost of validating cell-lines for adventitious agents (viruses, mycoplasma) which is $500 per cell-line.</td>
</tr>
<tr>
<td>General and administrative</td>
<td>This includes salary for accountant/administrative assistant and equipment such as computers, software, and office supplies. Insurance, telephone, utilities, and repairs and maintenance are based on a percentage of Chromos’ expenses.</td>
</tr>
<tr>
<td>R&amp;D Salary</td>
<td>Salaries of a lead scientist ($80,000), project manager ($60,000), and technician ($40,000). 15% is added to salaries to account for benefits. A project manager is responsible for about 5 cell-lines and starts in 2006. Technicians can produce 3 – 4 cell-lines per year.</td>
</tr>
<tr>
<td>Rent</td>
<td>This is based on a percentage of Chromos’ R&amp;D space rental costs.</td>
</tr>
<tr>
<td>Capital asset amortization</td>
<td>This is based on an estimation of amortization of Chromos’ capital assets. The cell-line engineering service is allocated a percentage based on its usage. In these statements, 10% is allocated. The amortization of purchased equipment for cell-line engineering is completely absorbed by the cell-line engineering business. The number reported is a sum of the total amortization of both old and new equipment. The original prices of equipment are estimated.</td>
</tr>
<tr>
<td>Tax</td>
<td>Corporate tax rate is 40%.</td>
</tr>
</tbody>
</table>
The pro-forma statements below look out to 2009.

**Table 6-2: Pro-forma income statements**

<table>
<thead>
<tr>
<th>Year</th>
<th>2005</th>
<th>2006</th>
<th>2007</th>
<th>2008</th>
<th>2009</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell lines</td>
<td>2</td>
<td>4</td>
<td>6</td>
<td>9</td>
<td>12</td>
</tr>
</tbody>
</table>

**Revenue**

<table>
<thead>
<tr>
<th>Service</th>
<th>2005</th>
<th>2006</th>
<th>2007</th>
<th>2008</th>
<th>2009</th>
</tr>
</thead>
<tbody>
<tr>
<td>Service</td>
<td>$1,000,000</td>
<td>$2,000,000</td>
<td>$3,000,000</td>
<td>$4,500,000</td>
<td>$6,000,000</td>
</tr>
<tr>
<td>License Maintenance</td>
<td>-</td>
<td>200,000</td>
<td>400,000</td>
<td>600,000</td>
<td>900,000</td>
</tr>
<tr>
<td><strong>Total Revenues</strong></td>
<td>1,000,000</td>
<td>2,200,000</td>
<td>3,400,000</td>
<td>5,100,000</td>
<td>6,900,000</td>
</tr>
</tbody>
</table>

**Expenses**

<table>
<thead>
<tr>
<th>Service</th>
<th>2005</th>
<th>2006</th>
<th>2007</th>
<th>2008</th>
<th>2009</th>
</tr>
</thead>
<tbody>
<tr>
<td>Media &amp; Reagents</td>
<td>16,800</td>
<td>33,600</td>
<td>50,400</td>
<td>75,600</td>
<td>100,800</td>
</tr>
<tr>
<td>Legal</td>
<td>10,000</td>
<td>20,000</td>
<td>30,000</td>
<td>45,000</td>
<td>60,000</td>
</tr>
<tr>
<td>External Testing</td>
<td>1,000</td>
<td>2,000</td>
<td>3,000</td>
<td>4,500</td>
<td>6,000</td>
</tr>
<tr>
<td><strong>General &amp; Administrative</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Admin Salary</td>
<td>57,500</td>
<td>60,375</td>
<td>63,394</td>
<td>66,563</td>
<td>69,992</td>
</tr>
<tr>
<td>Supplies and Equipment</td>
<td>1,500</td>
<td>1,725</td>
<td>1,898</td>
<td>2,087</td>
<td>2,296</td>
</tr>
<tr>
<td>Insurance</td>
<td>800</td>
<td>800</td>
<td>800</td>
<td>1,150</td>
<td>1,150</td>
</tr>
<tr>
<td>Telephone</td>
<td>550</td>
<td>550</td>
<td>550</td>
<td>800</td>
<td>800</td>
</tr>
<tr>
<td>Utilities</td>
<td>3,500</td>
<td>3,500</td>
<td>3,500</td>
<td>5,000</td>
<td>5,000</td>
</tr>
<tr>
<td>Repairs &amp; Maintenance</td>
<td>2,500</td>
<td>2,500</td>
<td>2,500</td>
<td>3,500</td>
<td>3,500</td>
</tr>
<tr>
<td><strong>R&amp;D Salary</strong></td>
<td>161,000</td>
<td>207,000</td>
<td>253,000</td>
<td>368,000</td>
<td>368,000</td>
</tr>
<tr>
<td>Rent</td>
<td>49,000</td>
<td>49,000</td>
<td>49,000</td>
<td>69,000</td>
<td>69,000</td>
</tr>
<tr>
<td>Capital Asset Amortization</td>
<td>28,877</td>
<td>34,326</td>
<td>53,020</td>
<td>67,620</td>
<td>46,620</td>
</tr>
<tr>
<td><strong>Total Operating Expenses</strong></td>
<td>333,027</td>
<td>415,376</td>
<td>511,061</td>
<td>708,821</td>
<td>733,058</td>
</tr>
<tr>
<td><strong>EBIT</strong></td>
<td>666,973</td>
<td>1,784,624</td>
<td>2,888,939</td>
<td>4,391,179</td>
<td>6,166,942</td>
</tr>
<tr>
<td><strong>Tax</strong></td>
<td>266,789</td>
<td>713,850</td>
<td>1,155,576</td>
<td>1,756,472</td>
<td>2,466,777</td>
</tr>
<tr>
<td><strong>Net Income</strong></td>
<td>400,184</td>
<td>1,070,774</td>
<td>1,733,363</td>
<td>2,634,708</td>
<td>3,700,165</td>
</tr>
</tbody>
</table>
### Table 6-3: Purchasing schedule of capital assets

<table>
<thead>
<tr>
<th>Year</th>
<th>Initial</th>
<th>2006</th>
<th>2007</th>
<th>2008</th>
<th>2009</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electrophoresis</td>
<td>$2,500</td>
<td>$</td>
<td>$2,500</td>
<td>$</td>
<td>$</td>
</tr>
<tr>
<td>Bench-top Centrifuge</td>
<td>-</td>
<td>-</td>
<td>16,000</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Micro-centrifuge</td>
<td>-</td>
<td>-</td>
<td>6,000</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Minus 86° Freezer</td>
<td>-</td>
<td>-</td>
<td>8,000</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fridge/Freezer</td>
<td>-</td>
<td>-</td>
<td>8,000</td>
<td>8,000</td>
<td>-</td>
</tr>
<tr>
<td>Bacterial Incubator</td>
<td>-</td>
<td>-</td>
<td>2,000</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>UV Spectrophotometer</td>
<td>-</td>
<td>9,600</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Water-bath</td>
<td>-</td>
<td>-</td>
<td>4,000</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Shaking Water-bath</td>
<td>-</td>
<td>-</td>
<td>2,500</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CO₂ Incubator</td>
<td>12,000</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Biocontainment Hood</td>
<td>-</td>
<td>30,000</td>
<td>-</td>
<td>30,000</td>
<td>-</td>
</tr>
<tr>
<td>ViCell Counter</td>
<td>26,000</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Multitron Incubator</td>
<td>50,000</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>90,500</td>
<td>39,600</td>
<td>49,000</td>
<td>38,000</td>
<td>-</td>
</tr>
</tbody>
</table>

The initial capital assets will be purchased before the cell-line engineering business commences operations. There are no capital asset purchases in 2005.
Table 6-4: Amortization schedule of old capital assets

<table>
<thead>
<tr>
<th>Year</th>
<th>2005</th>
<th>2006</th>
<th>2007</th>
<th>2008</th>
<th>2009</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bench-top Centrifuge</td>
<td>$6,400</td>
<td>$6,400</td>
<td>$ -</td>
<td>$ -</td>
<td>$ -</td>
</tr>
<tr>
<td>Micro-centrifuge</td>
<td>2,400</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Liquid Nitrogen Storage</td>
<td>2,400</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>HPLC</td>
<td>6,800</td>
<td>6,800</td>
<td>6,800</td>
<td>6,800</td>
<td>-</td>
</tr>
<tr>
<td>PCR Thermalcycler</td>
<td>540</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Platform Shaker</td>
<td>130</td>
<td>130</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Water-bath</td>
<td>800</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Flow Cytometer</td>
<td>60,000</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ViCell Counter</td>
<td>5,200</td>
<td>5,200</td>
<td>5,200</td>
<td>5,200</td>
<td>-</td>
</tr>
<tr>
<td>Multitron Incubator</td>
<td>20,000</td>
<td>20,000</td>
<td>20,000</td>
<td>20,000</td>
<td>-</td>
</tr>
<tr>
<td>ELISA Plate Reader</td>
<td>3,000</td>
<td>3,000</td>
<td>3,000</td>
<td>3,000</td>
<td>-</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>107,770</td>
<td>41,530</td>
<td>35,000</td>
<td>35,000</td>
<td>-</td>
</tr>
<tr>
<td><strong>10% Used for Engineering</strong></td>
<td>10,777</td>
<td>8,306</td>
<td>14,000</td>
<td>21,000</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 6-5: Amortization schedule of new capital assets

<table>
<thead>
<tr>
<th>Year</th>
<th>2005</th>
<th>2006</th>
<th>2007</th>
<th>2008</th>
<th>2009</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electrophoresis</td>
<td>$500</td>
<td>$500</td>
<td>$1,000</td>
<td>$1,000</td>
<td>$1,000</td>
</tr>
<tr>
<td>Bench-top Centrifuge</td>
<td>-</td>
<td>-</td>
<td>6,400</td>
<td>6,400</td>
<td>6,400</td>
</tr>
<tr>
<td>Micro-centrifuge</td>
<td>-</td>
<td>-</td>
<td>1,200</td>
<td>1,200</td>
<td>1,200</td>
</tr>
<tr>
<td>Minus 86* freezer</td>
<td>-</td>
<td>-</td>
<td>1,600</td>
<td>1,600</td>
<td>1,600</td>
</tr>
<tr>
<td>Fridge/Freezer</td>
<td>-</td>
<td>-</td>
<td>1,600</td>
<td>3,200</td>
<td>3,200</td>
</tr>
<tr>
<td>Bacterial Incubator</td>
<td>-</td>
<td>-</td>
<td>400</td>
<td>400</td>
<td>400</td>
</tr>
<tr>
<td>UV Spectrophotometer</td>
<td>-</td>
<td>1,920</td>
<td>1,920</td>
<td>1,920</td>
<td>1,920</td>
</tr>
<tr>
<td>Water-bath</td>
<td>-</td>
<td>-</td>
<td>800</td>
<td>800</td>
<td>800</td>
</tr>
<tr>
<td>Shaking Water-bath</td>
<td>-</td>
<td>-</td>
<td>500</td>
<td>500</td>
<td>500</td>
</tr>
<tr>
<td>CO₂ Incubator</td>
<td>2,400</td>
<td>2,400</td>
<td>2,400</td>
<td>2,400</td>
<td>2,400</td>
</tr>
<tr>
<td>Biocontainment Hood</td>
<td>-</td>
<td>6,000</td>
<td>6,000</td>
<td>12,000</td>
<td>12,000</td>
</tr>
<tr>
<td>ViCell Counter</td>
<td>5,200</td>
<td>5,200</td>
<td>5,200</td>
<td>5,200</td>
<td>5,200</td>
</tr>
<tr>
<td>Multitron Incubator</td>
<td>10,000</td>
<td>10,000</td>
<td>10,000</td>
<td>10,000</td>
<td>10,000</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>18,100</td>
<td>26,020</td>
<td>39,020</td>
<td>46,620</td>
<td>46,620</td>
</tr>
</tbody>
</table>

Table 6-6: Amortization schedule of total capital assets

<table>
<thead>
<tr>
<th>Year</th>
<th>2005</th>
<th>2006</th>
<th>2007</th>
<th>2008</th>
<th>2009</th>
</tr>
</thead>
<tbody>
<tr>
<td>10% Used for Engineering</td>
<td>$10,777</td>
<td>$8,306</td>
<td>$14,000</td>
<td>$21,000</td>
<td>$ -</td>
</tr>
<tr>
<td>New Amortization</td>
<td>18,100</td>
<td>26,020</td>
<td>39,020</td>
<td>46,620</td>
<td>46,620</td>
</tr>
<tr>
<td><strong>Total Amortization</strong></td>
<td>28,877</td>
<td>34,326</td>
<td>53,020</td>
<td>67,620</td>
<td>46,620</td>
</tr>
</tbody>
</table>

The total amortization of capital assets from the old and new assets is incorporated in the pro-forma financial statements in Table 2.
LIST OF REFERENCES


