Monoclonal antibodies (MAbs) represent the fastest growing segment of the biopharmaceutical market, with sales that totaled greater than $3B in 2002 and have been projected to grow to over $5B by 2005. A survey of 144 companies by the Pharmaceutical Research and Manufacturers of America has shown that there are 75 MAbs in development among the 371 biotechnology based medicines currently in clinical development (1). The dramatic success of the use of antibodies as therapeutics has created substantial value for companies developing these products (2, 3). Moreover, the recent approvals of Erbitux, Avastin, and Xolair support the view that antibodies offer a safe, effective therapeutic treatment for human disease. This widely held view of the safety and therapeutic efficacy of antibodies is driven by the wealth of research that has accumulated over the last fifteen years demonstrating the utility of targeting the highly specific nature of disease-associated antigens.

Therapeutic Antibodies: Development Challenges

While MAbs represent safe, effective molecules for therapy, a number of bottlenecks exist in their development as drugs. The extended timelines for optimizing MAbs that can specifically bind to critical epitopes of a target antigen with sufficient affinity as well as the development of high-titer cell hosts that can generate sufficient annual product yield requirements at a reasonable cost of goods are important factors that are commonly encountered during the development of these molecules. Several technologies are currently being employed in the industry to address these critical issues for antibody and cell line development. For titer optimization of cell hosts, technologies revolve around the engineering of selectable expression vectors, the use of gene amplification, gene shuffling and codon optimization, sib selection of robust production hosts, and process optimization (media, affinity matrices, bioreactors, etc.) (4). For affinity optimization, technologies revolve around amino acid substitutions in complementarity determining regions (CDR) or framework regions in combination with protein modeling (5). While these technologies have resulted in the successful development of high-titer cell hosts or high-affinity antibodies, extended development timelines have been observed in cases where first and second generation approaches have failed. Alternative strategies are needed to optimize therapeutic MAbs or manufacturing cell hosts in a timely fashion. One approach to improve the performance of a production cell line or enhance the properties of a therapeutic antibody that it produces is through a whole genome evolution platform that can make subtle genome-wide alterations in a parental antibody-producing cell host, leading to sibs that can be screened via automated high-throughput analysis to identify subclones producing higher titers for scaleable manufacturing or higher affinity antibodies.
MORPHODOMA® Technology

We have developed a proprietary process, termed MORPHODOMA® technology that harnesses the power of evolution for the development of antibodies with improved biological properties or cell lines with enhanced titer yields that are suitable for scaleable manufacturing. Owing to the random mutagenic nature of the MORPHODOMA® process, combined with automated high-throughput screening procedures, this technology circumvents the need to understand which amino acid residue(s) are critical within the antibody light and heavy chain to enhance binding affinity to a target antigen or which genes/pathways are required within a host cell line for generating higher titers. A key distinction that separates MORPHODOMA® technology from other antibody technologies is the random in vivo nature of the process. The ability to utilize the many genes and pathways that all cells innately possess, most of which are unexploited due to our lack of complete understanding of cellular processes, permits the generation of unexpected mutants leading to cells and gene products with desirable phenotypes. As described here, MORPHODOMA® technology is time and cost efficient because it is applied in vivo to pre-existing production strains and does not entail any in vitro manipulation.

Molecular Basis of MORPHODOMA® Technology

DNA replication is a complex process that all cells undergo during proliferation in order for parental cells to pass on genetic information to sibling cells. As cells replicate their DNA, mutations occur within the newly synthesized template through a variety of mechanisms, including polymerase infidelity. A series of post-replicative DNA repair processes, such as the mismatch repair (MMR) system (6), have evolved in nature and are ubiquitously present in prokaryotic and eukaryotic cells in order for organisms to retain their genotypic identity. MMR prevents the accumulation of “naturally occurring mutations” (Figure 1, marked in red) via a secondary proof-reading process that corrects discordant genetic information that may exist between parental and sibling DNA templates. MORPHODOMA® technology is based on the reversible inhibition of MMR and is mediated by the activity of an allelic variant of the human PMS2 gene product. This gene, referred to as the morphogene, was identified in hereditary non-polyposis colorectal cancer patients (7) and when expressed in a MMR proficient cell exerts a dominant negative activity by antagonizing the host’s endogenous MMR machinery (8). Consequently, the morphogene allows naturally occurring mutations to be inherited by sib cells at higher frequencies (up to 1000-fold enhancement) than typically observed in MMR proficient cells. The genetically diverse population of sibs that are derived from MORPHODOMA® technology results in a library of cells that can be screened via automated functional high-throughput screening (HTS) to identify subclones secreting antibody with enhanced properties (i.e. affinity), exhibiting enhanced growth characteristics, or producing antibodies at higher titer levels.

Figure 1: The genetic rationale of the MORPHODOMA® technology

Process Outline
Figure 2 outlines the MORPHODOMA® process. An expression vector containing the morphogene is first introduced into an antibody producing cell via standard gene transfection methods. The morphogene is co-expressed along with a negative selection marker which allows for removal of the morphogene vector to restore the genetic integrity of an optimized subclone. Stable morphogene-expressing cells (referred to as MORPHODOMA® cells) are passaged for 30 generations during which time genome-wide mutations accumulate. The genetically diverse pool of cells are then single-cell subcloned by limiting dilution into 96-well microplates and clones are expanded for up to two weeks. At this stage, a variety of functional, automated HTS assays are performed to identify subclones yielding antibodies with higher binding affinities or cells with enhanced titer yields or growth properties that are suitable for scaleable manufacturing. Subclone(s) that exhibit the desired phenotypes are “cured” of the morphogene via negative selection to generate genetically stable sibs that no longer express the morphogene product due to loss of the morphogene expression vector via intragenic homologous recombination. This is achieved by treating optimized sib cells with a prodrug that is enzymatically converted into a cytotoxic molecule in the presence of the morphogene-linked negative selection marker. Consequently, all cells expressing the morphogene perish, while a fraction of cells that have naturally lost the morphogene-negative selection marker survive. These viable, putative non-morphogene expressing subclones are then analyzed to confirm i) the preservation and stability of the cell host’s or antibody’s enhanced properties; ii) the restoration of wild type MMR activity and stabilization of the host’s genome; and iii) the integrity of the antibody’s structure and function. An attractive feature of MORPHODOMA® technology is that it can be directly applied to a manufacturing cell line for which there is a need to further optimize growth characteristics, titer yields for scaleable manufacturing, or both.

The examples provided in this article are representative of a number of projects that have been successfully conducted and validated by collaborative studies.

**Increasing Antibody Titers**
MORPHODOMA® technology can be applied to optimize antibody products or titers of antibody producing cell lines of any species by virtue of the high degree of conservation among MMR proteins.
and the ability of the morphogene to block MMR activity in microbial, plant and mammalian cell-based systems. As an example for creating high-titer production cells, we demonstrate the utility of the process in Chinese Hamster Ovary (CHO) cells producing a recombinant human antibody. MORPHODOMA® process was applied as outlined in Figure 2. Cells were transfected with the morphogene expression vector containing a negative selection marker. A morphogene expressing cell line was propagated and subsequently subcloned to yield approximately 10,000 sibs that were screened for antibody production. Because of the automated nature of the MORPHODOMA® screening process, it is noteworthy to mention that 10,000 clones can be screened in as little as 3 days. In the absence of the morphogene-enhanced genetic diversity, a spontaneous mutation frequency of ~10⁻⁷ would necessitate the impractical screening of approximately 10 million individual sibs in order to potentially find clones exhibiting a phenotype of interest. Representative ELISA data from the initial screening for a high-production MORPHODOMA® CHO cell line are shown in Figure 3. Putative high-titer clones (indicated by red arrows in the chart) were isolated by primary screening and expanded to conduct secondary assays to confirm titer levels.

Figure 3: Representative ELISA data of HTS to identify high titer sublines

![Image](image-url)

After confirming the productivity of high-titer MORPHODOMA® cells, clones exhibiting an increase of antibody titers were selected, expanded and subsequently genetically stabilized by negative selection (see Process Outline and Figure 2). Cured clones (morphogene-null) that arose after negative selection were expanded and analyzed to confirm the absence of the morphogene by PCR analysis of genomic DNA. Figure 4 shows that the presence of the morphogene expression vector was observed in untreated parental MORPHODOMA® cells but not in negatively-selected MORPHODOMA® subclones, confirming the loss of the morphogene expression vector in the genome of these clones.

Figure 4: Curing of the morphogene expression vector from parental MORPHODOMA® cells via negative selection

![Image](image-url)
Next, antibody integrity was analyzed to confirm that subclones exhibiting an enhanced titer retained similar genetic and biochemical properties to that of the parental antibody. DNA sequence analyses of both the light and heavy chain antibody cDNAs were conducted on independent transcripts derived from the subclone to confirm the integrity of the DNA sequence and that no spurious nucleotide changes, and potentially amino acid changes, were present. Subsequently, affinity analyses were performed to demonstrate that the antibody did not change its binding characteristics, while tryptic digestion-mass spec analyses of the secreted antibody confirmed that the peptide fingerprint of the antibody derived from the high-titer subclone was identical to the parental molecule. Finally, the growth rate and viability of all high-titer subclones was determined and those which performed equally to or better than the parental cell line were chosen. The cured, genome-stable MORPHODOMA® subclone was found to maintain a 3-fold increase of titer versus the parental line over the course of a 3 month period (the duration of the project after curing, Figure 5).

Figure 5: Stable expression of antibody high titer levels over time

Increasing Antibody Affinity

MORPHODOMA® technology can also be applied to antibody-producing cells to generate higher affinity antibodies through randomized genetic changes within the variable regions of the light and heavy immunoglobulin chains. In the following example, the affinity of a therapeutic antibody was increased 34-fold by a single random point mutation in the antibody’s light chain variable region (Figure 7) resulting from the activity of morphogene. The fact that the presence of the specific mutation was revealed only at the end of the process, whereas the clone bearing this mutation was identified first by virtue of its antigen binding properties, reemphasizes that the MORPHODOMA® process obviates the need for understanding of sequence-related functions prior to generating and screening for the phenotype of choice. After transfection of the morphogene vector into the antibody producing parental line (following similar procedures as described above), MORPHODOMA® clones were expanded and single-cell subcloned. Approximately 5,000 MORPHODOMA® sibs were screened along with an equal number of parental-derived sibs. The levels of steady-state binding of the antibody to its antigen were measured using an ELISA method whereby the absorbance values can be analyzed as a function of the antibody binding affinity. Representative ELISAs comparing antigen specific binding of the antibody produced by parental or MORPHODOMA® subclones are shown in Figure 6.
Lead clones were identified after normalizing the ELISA antigen binding data against the total immunoglobulin secreted by each clone. Several clones were identified after the initial screening that consistently showed increased binding affinity as compared to the average binding value of the parental sib cells. Some of these putative high-affinity clones were further analyzed by surface plasmon resonance using a Biacore instrument. The affinity (KD) of the parental antibody was $4.8 \times 10^{-8}$ M. One MORPHODOMA® clone exhibited an affinity of $1.4 \times 10^{-9}$ M, representing a 34-fold increase. Sequence analysis of the light and heavy chain immunoglobulin cDNAs revealed a point mutation (A→G, Figure 7) in the framework of the light chain variable region that resulted in an amino acid change. During the same screening, another MORPHODOMA® clone was identified that showed an affinity 15 times higher than the parental line and several point mutations were found in the framework of its heavy chain variable region (not shown).

MORPHODOMA® clones producing higher affinity antibodies were then cured and verified using the analytical processes as described above.

**Figure 6:** Representative ELISA data to identify clones producing high-affinity antibodies

**Figure 7:** Identification of point mutation in the variable region of high affinity antibody

<table>
<thead>
<tr>
<th>nucleotide change</th>
<th>Affinity KD (M)</th>
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<tbody>
<tr>
<td>MORPHODOMA</td>
<td>$1.4 \times 10^{-9}$</td>
</tr>
<tr>
<td>Parental</td>
<td>$4.8 \times 10^{-8}$</td>
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**Extensions of Technology**

The generation of an antibody with an increased affinity is one of several applications of the MORPHODOMA® process in the development of therapeutic antibodies. On the one hand, due to the
gene cloning techniques available, it is straightforward to clone out and verify the nature of the mutation that results in the higher affinity of antibodies as derived from the MORPHODOMA® process. On the other hand, given the number of genes within the mammalian genome (conservatively 30,000), of which only a few, if mutated, could lead to a cell that exhibits increased titers, it is less straightforward elucidating which gene or combination of genes is responsible for a phenotype involved in enhanced antibody production. The MORPHODOMA® process allows for the creation of isogenic cells that can be analyzed by a variety of genomic and proteomic tools to uncover genes and pathways involved in optimized cell growth or titer production. In contrast to chemical mutagens, which induce aneuploidy as a result of chromosomal instability (9), the MORPHODOMA® process allows comparative genetic approaches to be undertaken because it results in subtle point mutations while leaving the chromosomal stability of the host cell and long-term viability intact. Morphotek scientists have used sets of isogenic parental and high-titer MORPHODOMA®-derived sibs to identify pathways involved in enhanced antibody production. RNA microarray analyses of parental and high-titer MORPHODOMA® cells have uncovered two genes whose expression was found to be suppressed in high titers clones. Follow-up studies have demonstrated that induced suppression of these genes in low-titer parental cells can recapitulate the increase in antibody titers.

The ability to isolate the genes that drive phenotypes (i.e. over-production of recombinant proteins) from a complex mammalian genome will allow for the generation of more efficient universal production cell lines that can continue to be further refined via MORPHODOMA® technology.

Although we have focused the present article on mammalian systems, by virtue of the strong homology of MMR pathways across species, an approach similar to the MORPHODOMA® process has been successfully applied to microbial production systems (such as yeast and Escherichia coli) and plants to evolve phenotypes. This permits the genetic evolution of virtually any host organism using the same morphogene reagent and results in a rapid in vivo process to generate hosts exhibiting commercially important phenotypes.

Summary

MORPHODOMA® technology can be considered a new tool for therapeutic antibody development. Its random nature and in vivo mode of action separate this process from other complementary technologies, thus providing alternative solutions to improve a cell host’s and antibody’s performance. The speed with which a pre-existing production strain can be optimized makes this process suitable for satisfying the current need for rapid cell line optimization to produce higher titers of antibody at the preclinical, clinical or commercialization stage.

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Glossary and abbreviations:
CDR = complementarity determining region
CHO = Chinese hamster ovary
ELISA = enzyme-linked immunosorbent assay
HTS = high throughput screening
MAb = monoclonal antibody
MMR = mismatch repair
PCR = polymerase chain reaction
RNA microarray = chip coated with DNA oligonucleotides that bind mRNAs allowing the measurement of the levels of expression of individual genes
Sib = sibling
Transfection = introduction of genes into mammalian cells

References:
2. George Miller, Preclinica 2003, July/August

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