FLEX™ TECHNOLOGY
Synthesizing Full-Length cDNA Libraries

PROVIDES THE COMMERCIAL KEY TO THE HUMAN GENOME

WHAT IS IT?

FLEX™ IS A TWO-TIER PROCESS:

1. Allows rapid synthesis of Full-Length (FL) genes - Proprietary technology

2. Allows rapid synthesis of Full-Length Expressed (FLEX™) genes - Patent protected

   Patents:
   Scheele, G. and Fukuoka, S.-I., Synthesis of full-length, double-stranded DNA from a single-stranded linear DNA template. Patent no. 5,162,209, issued on 11-10-92.

   Scheele, G. and Fukuoka, S-I, Synthesis of full-length double-stranded DNA from a single-stranded linear DNA template. Patent no. 5,643,766, issued on 7-1-97.

WHAT DOES IT DO?

FLEX™ provides a unique capability for rapid, bulk analysis of groups (tens to hundreds) of genes associated–with and responsible-for human disease.

HOW IS IT DIFFERENT THAN COMPETING TECHNOLOGIES?

Increases the rate of gene processing 10-100 fold – Competing companies working on one gene at a time

Thereby decreases the time and costs of:

- Medical discoveries
- Patent protection
- Diagnostic test development
- Lead drug discovery
- Drug optimization
- Diagnosis, palliation or cure of the patient

CAN THE PROCEDURE BE SCALED UP?

FLEX™ technology can be scaled up to work on thousands of genes or more.

Competitors are scaled up to work on tens of genes

FLEX Technology
(Reversible Tailing at the 5' End of cDNAs)

FLEX encompasses a reversible tailing procedure at the 5' end of cDNA to accomplish two things:

- Ensure that the cDNA clone is full-length or very long, i.e. the degradative step involved in cDNA synthesis is circumvented.
- Preserves the functionality of the clone.

During the early development of the FLEX technology at the Rockefeller University, we observed that a poly (G)-poly (C) tail (homopolymer G-C tail) at the 5' end of the cDNA clone abolished promoter-dependent expression of the cDNA clone into protein

For example, using in-vitro expression systems, the use of the T3 or T7 promoters in a plasmid to transcribe mRNA followed by introduction of this transcript into in-vitro translation systems (either wheat germ or reticulocyte lysate systems) to synthesize corresponding proteins, we observed that the 5' homopolymer G-C tail, conferring tight secondary structure to this region of the RNA, completely abolished the expression of protein.

In contrast, we could show that a homopolymer AT tail, when placed 5' to a coding sequence, allowed the expression of proteins at the correct molecular weight and in high quantities. An A-T tail has less-tight RNA secondary structure.

Hence the need for using the tailing procedure to maximize the length of cDNA, but the further need to remove the G-C tail to preserve in-vitro protein expression.

One can, of course, circumvent the G-C tail by conducting PCR to eliminate the 5' homopolymer sequence, but this has to be done on a one-by-one basis after isolation of individual cDNA clones, a very costly and time-consuming process.

Put another way, the FLEX reversible tailing procedure allows efficient expression cloning, during industrial protein expression or during whole library screening, in insect and mammalian cells, while at the same time maximizing the full-length composition of cloned genes. Thus, the FLEX patent provides a double benefit by maximizing the full-length character of cloned genes and by preserving their functionality during protein expression studies.

THE BROAD REACH OF FLEX™ TECHNOLOGY

The patented FLEX™ (Full-Length EXpression) technology, combined with the company's biochip technology for discovery of differentially expressed genes and its protein interaction technologies for discovery of protein pathways provides a unique capability for rapid, bulk analysis of full-length expressed genes associated-with and responsible-for human disease. This proprietary platform technology, representing a sustainable competitive advantage for AlphaGene, provides the commercial key to the human genome in the third millennium.

ADVANTAGES OF FULL-LENGTH (FL) GENES IN COMMERCIALIZING THE HUMAN GENOME

1. Full-length cDNA ends are essential for the virtual sequencing of the human genome with respect to expressed genes and therefore provide the key for determining the inventory of druggable targets in the human genome.

2. Full-length cDNAs are required for protein expression and functional studies.

3. Full-length cDNA ends facilitate the discovery of isoform or isotype signatures which lead to the discovery of alternative drug targets.

4. Full-length cDNAs facilitate the discovery of sequence signatures for signal peptides in novel secretory proteins, which reside near the 5' end of the gene and therefore promote discovery of novel biological drugs.

5. Full-length cDNAs facilitate discovery of alternatively spliced genes that are expressed in the cell, which provides alternative targets as biological drugs.

6. Full-length sequences in gene families are essential for homology modeling of protein families and discovery of novel family members as new drug targets.

7. Full-length sequences are essential for computational prediction of chemicals (lead drugs) that show in silico binding to modeled proteins.

8. Full-length gene sequences provide the "bridge" necessary for protein-domain "threading" required in mass spectrometry analysis of protein mixtures and therefore promote the functional understanding of protein expression studies.

9. Rapid analysis of full-length genes is required to determine the meaning of gene expression studies that lead to inferences about coordinate gene expression and novel biochemical pathways.

10. Full-length genes provide a decided advantage in rapidly obtaining both the structure and function required for patent protection.

ADVANTAGES OF FULL-LENGTH EXPRESSED (FLEX™) GENES IN COMMERCIALIZING THE HUMAN GENOME

Protein expression of purified proteins is central to identifying function, obtaining patents identifying drug targets and making drug discoveries. High efficiency protein expression of purified proteins is required for:

1. Determination of function, necessary for patent filings.

2. Industrial expression of drug targets including splice variants that correlate with disease states.

3. Construction of expression libraries to identify antigens in cancer, immune and autoimmune diseases

4. Expression of (i) secretory proteins and (ii) cell surface receptors to identify hormone-receptor pairs and the biological drugs that stimulate or inhibit signal transduction pathways.

5. Purification of proteins to determine x-ray crystal structure, valuable in computer modeling of drug targets and drugs.

6. Use of full-length antigens in the production of monoclonal antibodies, leading to genomics-based drugs.

7. Development of successful biochemical assays for the discovery of small molecule drugs.

FULL-LENGTH IS KING IN GENOMICS AND PROTEOMICS