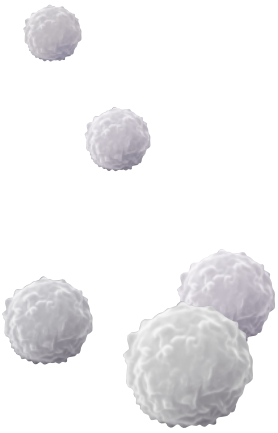
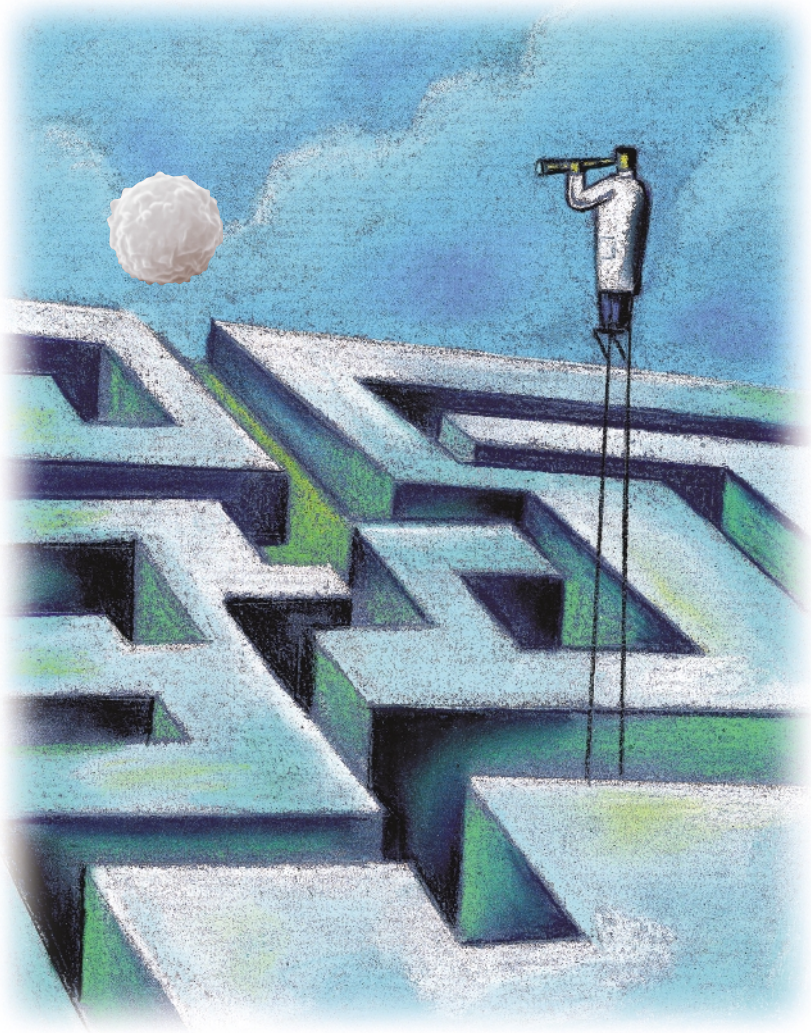


Imagine

a technology



that can test **thousands** of properties

of cells for faster discovery of better drugs

...and it's available now

There is a problem in drug discovery: The economics and efficiency of the drug development process are more important now than ever before, while at the same time that process is undergoing radical change. With the advent of gene sequencing, gene expression analysis, and combinatorial chemistry, a new problem has arisen. Now there are too many unqualified targets and leads. A critical need has developed for technologies that allow researchers to quickly, efficiently, and accurately characterize and sort through these targets and leads. **Target Validation** is a key step in the drug discovery process. Advances in genomics have created an intensely competitive environment in which thousands of genes are being mapped, proposed as important new targets, and patented. Selecting the best target genes is difficult because the function of most of these genes is unknown. **Functional Genomics** technologies will provide the means to gain understanding of the function of these important genes. With such information

one can greatly speed up the selection or elimination of a particular gene as a drug target. Therefore, rapid access to gene function information provides a competitive advantage in target validation. ◉ It is also essential in gaining a strong patent position.

Lead Validation follows the process of target validation. Companies now have ready access to large chemical libraries either from natural product sources or from combinatorial synthesis. ◉ With high-throughput robotic assays they can test these libraries against their target gene/protein of interest. But these technologies provide only part of the picture – major hurdles remain. Knowing that a chemical is active against its target is the first of many steps. The ability to rapidly gain information on the specificity of these drug leads is also of great value. ◉ Technology that provides insight at the cellular level about the interaction of a lead candidate with the thousands of other cellular proteins, would offer significant benefits. Optimal drug candidates will have specific effects on the target without causing undesired side effects.

PHENOTYPE MICROARRAYS™ A MAJOR NEW TECHNOLOGY

Phenotype MicroArrays (PMs) represent a breakthrough in technology, alongside DNA Microarrays and Proteomic Technologies (Figure 1), to complete the information needed in the genomic era of drug development.

Just as DNA Microarrays and Proteomic Technologies have made it possible to assay the expression level of thousands of genes or proteins all at once, Phenotype MicroArrays make it possible to measure, quantitatively, thousands of cellular phenotypes all at once.

DNA Microarrays and Proteomic Technologies allow scientists to detect genes or proteins that are coregulated and whose patterns of change correlate with something important such as a disease state. However, there is no assurance that these changes are really significant to the cell. Phenotype MicroArrays are a complementary technology providing the needed information at the cellular level ... and much more.

Phenotype MicroArrays are comprehensive cellular profiles that can be used to identify gene function, validate targets, and assist lead validation and optimization.

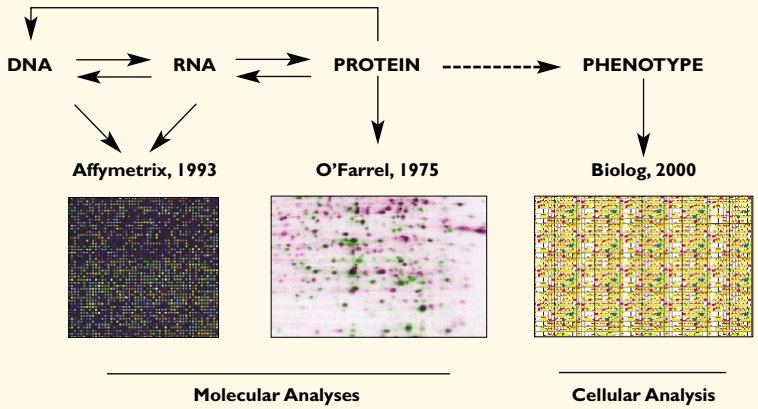


Figure 1

HOW THE TECHNOLOGY WORKS

Phenotype MicroArray technology is an integrated system of cellular assays, instrumentation, and bioinformatic software. The testing process is diagrammed in Figure 2.

Biolog preconfigures a wide range of phenotypic tests into sets of arrays. Each well of the array is designed to test a different phenotype.

Cells are tested after a genetic change or exposure to a drug lead, and the researcher can directly evaluate cellular response to that change. The scientist simply inoculates a standardized cell suspension into the MicroArray panels, thereby testing thousands of phenotypes at once. The MicroArray is then incubated, typically for 24 to 48 hours.

PMs use Biolog's patented redox chemistry, employing cell respiration as a universal reporter. If the phenotype is strongly "positive" in a well,

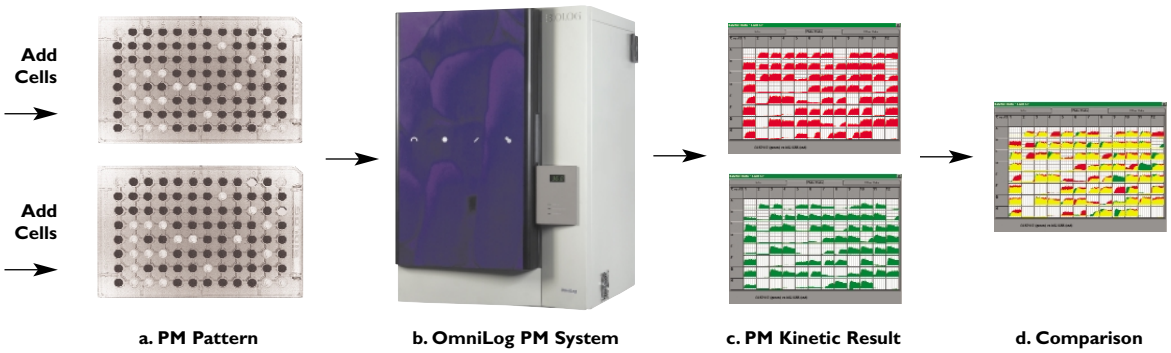


Figure 2

the cells respire actively, reducing a tetrazolium dye and forming a strong color (Figure 2a). If it is weakly positive or negative, respiration is slowed or stopped, and less color or no color is formed. The redox assay provides for both amplification and precise quantitation of phenotypes.

Incubation and recording of phenotypic data is performed by the patented OmniLog™ PM instrument (Figure 2b). The OmniLog captures a digital image of the MicroArray color change several times each hour, and stores the quantitative values into computer files. The computer files can be displayed to the scientist in the form of kinetic graphs. Thousands of phenotypes are monitored simultaneously by the OmniLog and more than 450,000 data points can be generated in one 24-hour run.

To compare the phenotypes of two cell lines, one is recorded as a red tracing and one as a green tracing (Figure 2c). These graphs can then be overlaid by the bioinformatics software to detect differences. Areas of overlap (i.e., no change) are colored yellow, whereas differences are highlighted as patches of red or green (Figure 2d).

Phenotype MicroArrays can monitor, either directly or indirectly, most aspects of cell function. The range of phenotypes includes:

- cell surface binding and transport functions
- catabolism of carbon, nitrogen, phosphorus, and sulfur
- biosynthesis of small molecules
- biosynthesis of polymeric macromolecules
- formation of cellular structures
- cellular respiratory functions
- stress and repair functions
- other cellular properties

Currently, PMs are available for use with bacterial and fungal cells. They are being developed also for mammalian cells.

TARGET VALIDATION:

Determining Gene Function

In many cases, researchers have already determined genes they

believe may be good drug targets. As diagrammed in Figure 2, a direct cell-to-cell comparison can be made between a normal cell and a cell with the gene of interest inactivated by a genetic mutation (i.e., knocked out).

Genetic changes result in phenotypic changes. Any gene of unknown function can be knocked out and the knockout cells can be assayed for phenotypic changes. An example is shown in Figure 3 comparing the nitrogen metabolism of a normal *E. coli* strain and a mutant that has lost function of a pathway for metabolizing arginine and ornithine as nitrogen sources. PMs provide a direct cellular assay of gene function.

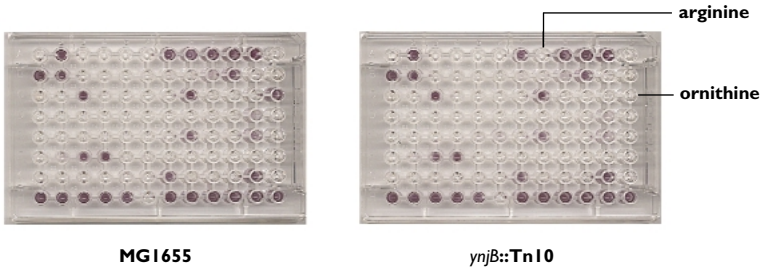


Figure 3

TARGET IDENTIFICATION:

Establishing New Drug Targets

PMs have many other uses in cellular studies for characterizing and defining phenotypic properties of cells.

For example, PM cell line-to-cell line comparisons can be performed to identify new drug targets. Researchers may want to find phenotypes that can be targeted by drugs toxic to:

- pathogenic bacteria, but not their non-pathogenic relatives
- bacteria and fungi that infect plants and animals, but not the plant and animal hosts
- cancerous cells, but not their non-cancerous precursors
- virus-infected cells, but not uninfected cells
- diseased cells but not their non-diseased neighbors

LEAD VALIDATION:

Assaying Drug Candidates

PM technology provides a revolutionary new tool for evaluating drug leads. In this application, testing is again performed in a comparative mode. A model cell line is tested without drug exposure (as a control) and then tested against a collection of drug leads of interest. The testing procedure is the same, except that the drug leads are added to the cell suspension just before the cells are distributed into the MicroArray wells (Figure 4a). Here again, the OmniLog instrument (Figure 4b) monitors, records and compares the assays automatically.

The PM pattern that forms in the absence of drug is different than patterns formed with various drugs added. From this comprehensive cellular assay, data from the MicroArrays is analyzed by pattern recognition and clustering software (Figure 4c) to sort drugs based on mode of action.

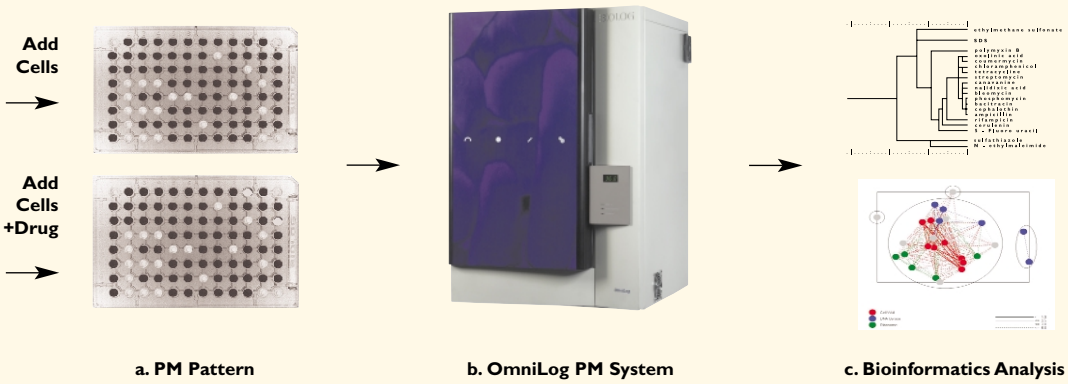


Figure 4

PM technology allows researchers to analyze chemical libraries in a rapid, high-throughput method with several unique and significant advantages.

First, it measures the effect of drugs on cells *under thousands of physiological states.* When the cell changes its physiological state it also changes its surface receptors and internal protein make-up, in a sense becoming a different cell. Current assay technologies using cells grown under one condition give a very limited, incomplete view of the range of biological possibilities.

Second, PM technology allows scientists to see the effect of a drug on *non-target sites*. These effects can be critically important if they result in unacceptable side effects.

Third, one can use PMs containing other drugs as part of the overall phenotypic analysis. This aspect of PM technology allows you to test the drug candidate's *interaction with other drugs*. Normally, this would be done inefficiently as a separate study. Discovering an unexpected synergy or antagonism with a known drug early on can change the entire course of drug development.

Soon, PM technology will also be used as a basis for detailed *toxicological studies*. With mammalian cell PM assays, it will be possible to assay drug candidates against a battery of cell lines that represent the major organs and tissues of the body. This will permit rapid and cost effective screening to help eliminate unsatisfactory drug candidates earlier in development.

The PM approach to assaying drug leads is unique and powerful. It provides a highly efficient means to assay the effect of a drug on thousands of cellular targets under thousands of physiological states of the cell.

Benefits in Drug Discovery

Phenotype MicroArrays:

- Complement DNA microarrays. DNA microarrays allow scientists to measure the expression of thousands of genes of a cell under one growth condition, whereas Phenotype MicroArrays allow scientists to measure the effect of one gene in the cell under thousands of growth conditions.

- Provide rapid answers to critical questions about cellular properties in a simple, rapid, efficient, and cost-effective process.

- Provide rapid answers to how a gene of unknown function affects a cell at a physiological level.

- Provide cell-to-cell comparisons to find novel drug targets based on *in vivo* physiological differences.

- Provide a means to assay the effect of a drug on thousands of cellular targets under thousands of physiological states of the cell.

- Provide rapid answers to questions about how a new lead affects a cell at a biochemical level.

- Provide information on how new drug leads compare to and interact with existing drugs.

- Reduce the time and cost of slow and cumbersome animal, plant, or human studies in late development.

- Eliminate unproductive leads and projects, saving substantial R&D budget dollars that would be wasted in later development.

BIOLOG 3938 Trust Way Hayward, CA 94545 Telephone: 510-785-2564 Fax: 510-782-4639 www.biolog.com

04/01