



December 2008

# DELphi™

## Protein Discovery

Cytos' high-throughput functional genomics platform, known as DELphi™, enables the identification and prioritization of new drug targets and biopharmaceutical candidates. The platform is based on an integrated viral expression screening and production system, which allows:

- i) functional reproduction of the approximately 100,000 human proteins encoded by the genome from a chosen tissue in a one-gene-per-cell or one-gene-per-well format;
- ii) experimental selection of new genes and proteins according to a set of desired functional criteria, which include a binding specificity or an activity that induces a whole cell response in a target cell (e.g. proliferation, differentiation or activation of a transcription factor);
- iii) immediate recovery of the gene and rapid production of the correctly folded and glycosylated protein.

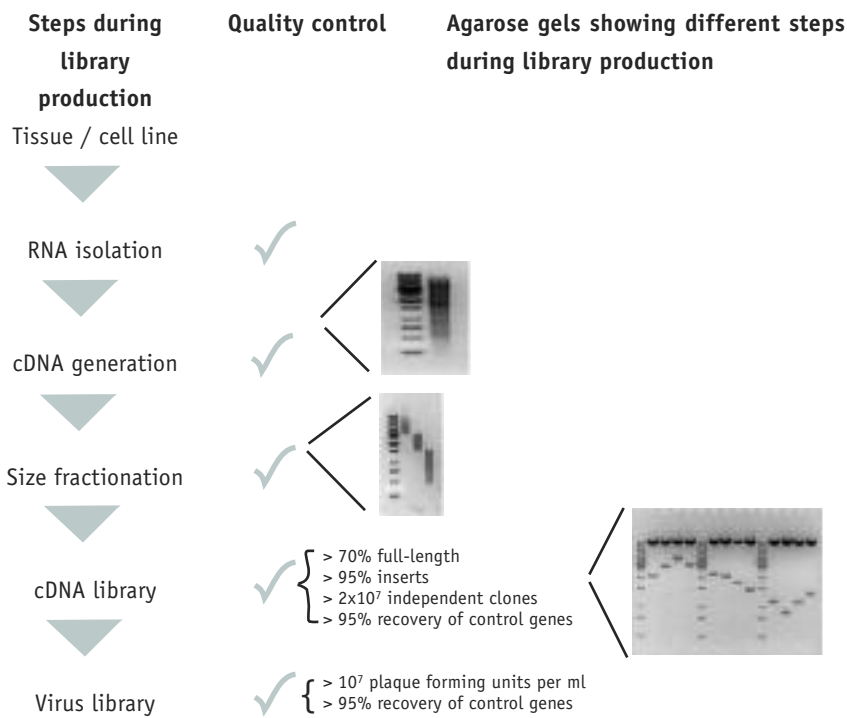
High-throughput sequencing has triggered an explosive growth in the identification of new genes. However, while gene discovery has been accelerated, the understanding of new gene and protein functions has not kept pace. Cytos' DELphi™ technology addresses this bottleneck of identifying new proteins based on their function. The DELphi™ technology has first been published in *Nature Biotechnology*, 2001, 19:851.

## Production of an alphaviral expression library

In a first step, a cDNA library derived from a tissue of relevance to a particular disease is converted into an alphaviral expression library. The library can be chosen from a particular tissue, from diseased cells, or it can be a differential library containing only genes expressed in one particular cell type.

The production of a representative cDNA library is one of the key steps for functional genomics approaches. This process has therefore been standardized at Cytos and stringent quality controls have been implemented. The different steps in generating a viral library are depicted in Figure 1.

**Figure 1**

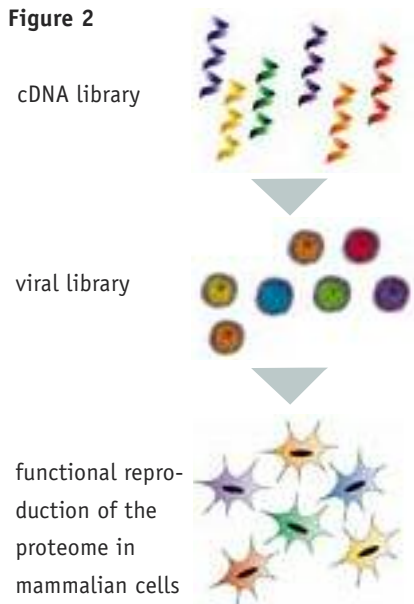


The alphavirus expression system employed by Cytos has five key properties:

- i) each virus in this library contains one single exogenous cDNA
- ii) one physical viral particle infects one cell and initiates an infectious cycle
- iii) high levels of gene expression are obtained
- iv) the progeny virus can readily be isolated from infected cells
- v) the cloning procedure does not involve homologous recombination steps, which occur rarely and are time-consuming

Infection of mammalian cells with this viral library at defined titers results in spatially segregated, high level expression of the corresponding proteins (see Figure 2). The generation of subtractive and normalized viral libraries has also been established at Cytos. The mammalian expression system ensures correct processing and glycosylation of each protein in the array, an important feature for subsequent functional screening.

**Figure 2**



## Screening for new surface receptors, targets, and molecules which induce a whole cell response

There are two different screening approaches applied at Cytos.

- 1) Expression of an alphaviral library in a one-gene-per-cell format:  
Novel surface receptors for known ligands can be identified with unprecedented speed and accuracy. It takes roughly 10 weeks from the generation of the cDNA library to the isolation and sequencing of the gene of interest using high quality bait molecules.
- 2) Expression of an alphaviral library in a one-gene-per-well format:  
This approach allows screening for secreted molecules displaying specific functions such as e.g. cytokine-like or chemotactic activities involved in complex biological responses like inflammation or tissue regeneration.

### 1) High-throughput screening for new surface receptors and targets using the one-gene-per-cell format

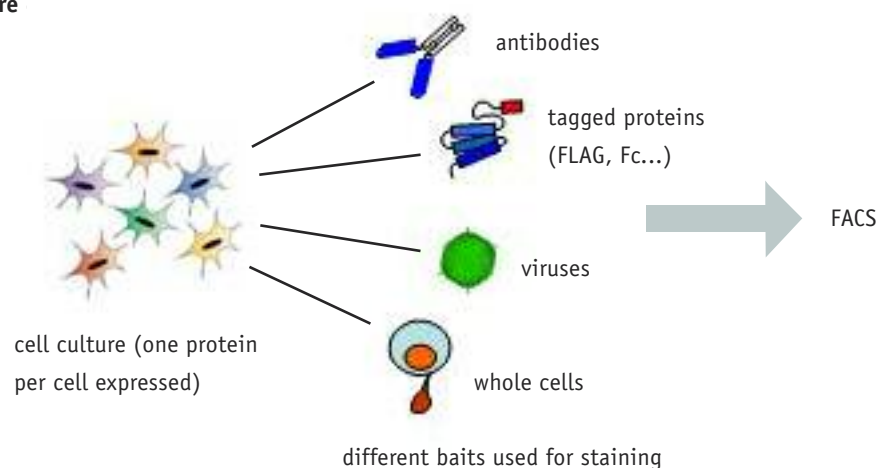
A culture of suspended single cells is infected with the alphaviral expression library at a multiplicity of infection (MOI) below one, resulting in high level expression of only one gene per cell. Single cells expressing a desired phenotype, which results from the exogenous gene are sorted in a fluorescence activated cell sorter (FACS) (see Figure 3). Using the FACS, a cell pool of up to  $10^8$  cells (corresponds to  $\sim 2 \times 10^7$  genes) can be screened within a day.

Figure 3



The infected cell culture is then stained with a ligand of interest, the bait, which typically is an antibody or a tagged protein. Fluorescently labeled secondary reagents allow for detection by FACS. The DELphi™ technology was further developed such that also whole cells or microorganisms can be used as baits for screening. Figure 4 summarizes the type of baits successfully used for FACS screening at Cytos.

Figure



After staining, every infected cell is analyzed by FACS according to specific selection criteria (e.g. fluorescence intensity, size etc.) and is single-cell sorted into 24-well plates containing uninfected mammalian cells. The virus released from the sorted single cell infects the other cells and is rapidly amplified. This system leads to the amplification of the selected cDNA clone within 1-2 days, thereafter it is subjected to RT-PCR and sequencing.

Cytos has applied DELphi™ to identify various proteins, some of which are summarized in Table 1, using different types of baits. For identification of adhesion molecules and receptors interacting with membrane proteins of another cell, fluorescently labelled cells were used as baits. As an example, the identified molecules CD22 and CD106 are shown in Table 1. Furthermore, using a fluorescently labelled adenovirus as bait, a new receptor (i.e. CD46) for this adenovirus strain was identified. And applying a recombinantly produced form of the T and NK cell receptor KLRG1 as bait for screening, E-cadherin was identified as its interaction partner.

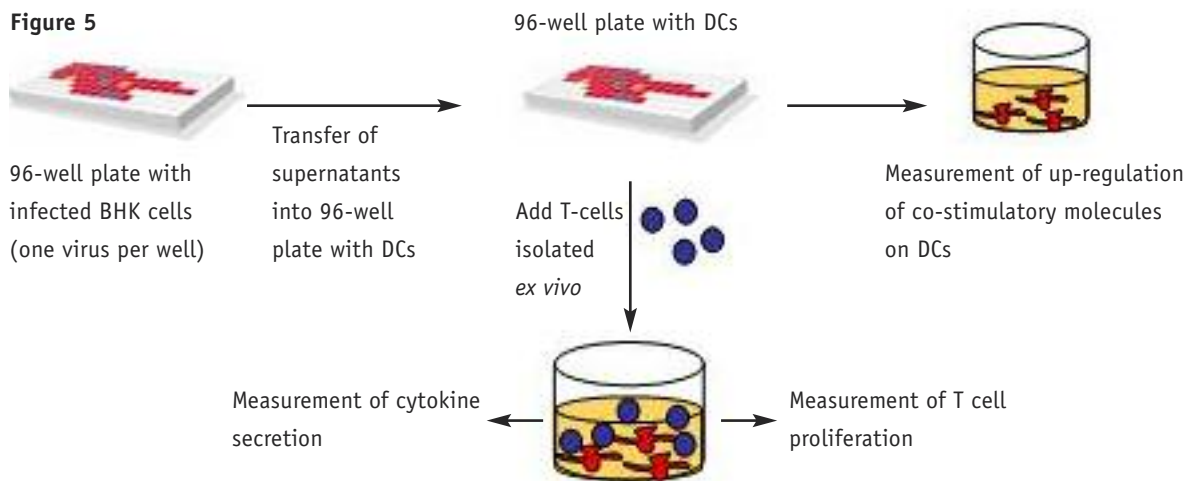
| Protein identified | Species | Bait     | Scientific publication                |
|--------------------|---------|----------|---------------------------------------|
| CD22               | m       | cell     | Biotechniques, 2004, 37:912           |
| CD106 (V-CAM)      | m       | cell     | Biotechniques, 2004, 37:912           |
| CD46               | h       | virus    | Journal of Virology, 2004, 78:4454    |
| E-cadherin         | m       | ligand   | Journal of Immunology, 2006, 176:1311 |
| CCL19              | m       | function | Immunity, 2005, 22:493                |

**Table 1:** Selection of identified proteins using different types of baits. The proteins identified are shown on the left, the corresponding scientific publication on the right. "m" stands for mouse, "h" for human proteins. The libraries were screened using the following baits: i) whole cells, ii) whole viruses, and iii) recombinant ligand. The chemokine CCL19 was identified to promote dendritic cell maturation using a one-gene-per-well approach (see below).

## 2) Screening for new molecules which induce a whole cell response using the one-gene-per-well format

Cytos used the one-gene-per-well format to screen for new regulatory molecules, which are involved in the activation and maturation of dendritic cells (DCs). DCs play a key role as antigen presenting cells during an immune response. Properly activated and mature DCs are a prerequisite for efficient activation of T-cells. A normalized alphavirus expression library was constructed from activated spleen mRNA. Viruses were used to infect BHK cells in a one-virus-per-well format. Supernatants of each well containing secreted proteins encoded by the recombinant viruses were harvested and used to measure their ability to induce maturation of *ex vivo* isolated DCs. As shown in Figure 5, maturation of DCs can be measured by different read-outs such as upregulation of co-stimulatory molecules on DCs or proper activation of T-cells as characterized by proliferation or cytokine secretion. Viruses from wells containing supernatants with proteins inducing DC maturation were subcloned and re-tested. In this way, the chemokine CCL19 was identified to promote DC maturation.

**Figure 5**



After identification and sequencing of clones that express proteins with the desired combination of functional properties, the next step is the production of working quantities of the corresponding purified protein for further functional studies in animal models or cell culture systems. Cytos' protein production capabilities give access to milligram quantities of the protein of interest within several weeks.

### **Extension of the DELphi™ technology for isolation of fully human antibodies**

Cytos Biotechnology has extended the DELphi™ technology to allow for rapid isolation of fully human antibodies. Specific B cells (i.e. the immune cells producing a certain antigen-specific antibody) are isolated from the blood of human donors and the genetic antibody information of the B cells is used for the subsequent DELphi™ screening procedure in mammalian cells. The antigen-specific antibodies resulting from the screening are of high affinity and can be readily produced in large quantities in mammalian cells. With this method, fully human monoclonal antibodies have been isolated within very competitive timelines of about 3 months. This new technology has been published in the Proceedings of the National Academy of Sciences (PNAS), 2008, 105:14336.

## DELphi™ at a glance

Cytos' DELphi™ technology encompasses a complete system for the rapid identification of genes and proteins according to their function. Using the high throughput one-gene-per-cell format it takes roughly 10 weeks from generation of the cDNA library to isolation and sequencing of the gene of interest using high quality bait molecules.

The functional screening criteria may include specified combinations of a binding specificity, a biological response in a target cell, or combinations thereof.

The technology delivers the full length sequence of the gene identified.

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