

OriGene GFC-Arrays™ for High-throughput Overexpression Screening of Human Gene Phenotypes

High-throughput Gene Function Validation Tool

Introduction

siRNA screening libraries enable scientists to identify cellular phenotypes of interest by downregulating genes through RNA interference. Once high-throughput siRNA screening identifies a cellular phenotype, by inference the downregulated gene behind the phenotype is also identified. Genome-wide siRNA screening libraries are particularly useful for analyzing the functions of human genes.

GFC-Arrays from OriGene allow a different approach to screen human genes for cellular phenotypes—high-throughput screening by gene overexpression. No longer must gene overexpression experiments be done one gene at a time. This white paper describes how GFC-Arrays work and a variety of ways in which overexpression arrays have been used.

Overexpression screening technology, first developed for GFC-Arrays in 2004, has been validated by peer-reviewed publications and is suitable for a wide range of biological studies and automated readout assays. The format for a GFC-Array using full-length human cDNAs (OriGene TrueClones™) is a single-use 384-well plate. These plates are expressly manufactured to minimize the work of preparing experimental materials. GFC-Arrays are economical (as low as \$2 per gene), so that systems-biology approaches to study subsets of the human genome (transcription factors, for instance) are feasible for any laboratory. At the same time, GFC-Arrays can be customized for the needs of bio-pharmaceutical companies devoted to genome-wide high-throughput screening.

Thus, GFC-Array overexpression screening complements downregulation screening based on siRNA. One might say GFC-Arrays provide the other half of the story.

GFC-Arrays Maximize Convenience

A typical GFC-Array overexpression screening experiment proceeds in four steps, as illustrated by the adjacent figure.

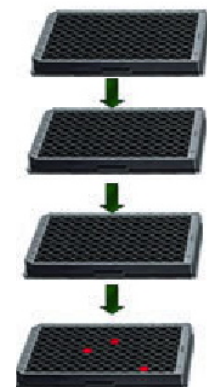
First, open the sealed GFC-Array 384-well plate, where human cDNAs for overexpression screening are already distributed for transfection. Every plate includes two vacant columns that are included for the flexible arrangement of the researchers control samples.

Second, if the readout assay will use a reporter gene such as luciferase, add the reporter plasmid along with a transfection reagent. The user's own controls are then added to the empty columns.

Third, add cells and incubate the plate for high-throughput transfection.

Fourth, add assay reagents and use a plate reader to score the plates.

- ⇒ Open plate.
- ⇒ Add reporter plasmid (if any) and transfection reagent.
- ⇒ Add cells and incubate.
- ⇒ Add assay reagents and read.





Reverse Transfection. GFC-Arrays are designed to allow use of the reverse transfection method for high-throughput overexpression as an assay format. OriGene has optimized the reverse transfection reaction with regard to DNA quantity, cell number and transfection reagent. The standard protocol is appropriate for most commonly used cell types.

Stable Platform. A notable aspect of GFC-Arrays is the use of lyophilized DNA which makes long term storage possible. OriGene carefully lyophilizes cDNAs to be transfected to the plate during plate manufacture and then seals the plate for storage. Transfection is appropriate for most cell types when used with low-toxicity transfection reagents.

Hit Validation. After screening with GFC-Arrays, the next step is usually to validate hits. It is important to verify that an observed phenotype is due to cDNA overexpression and is not an experimental artifact. We recommend validating a hit by a standard transfection with the specific cDNA plasmid. All cDNAs in GFC-Arrays are available separately in 1µg or 10 µg amounts.

Minimal User Requirements. A true high-throughput screening system minimizes the time and effort to obtain accurate, reproducible data. GFC-Arrays minimize user-supplied materials to these:

- A cell line for DNA introduction
- A readout assay to score the GFC-Array plates
- A 384-well plate reader

Standard Features. To maximize user convenience, these GFC-Array features are standard:

- **Full-length cDNAs.** Each well contains verified full-length cDNA plasmid
- **No Subcloning.** Each cDNA is subcloned into the same expression vector, pCMV6; the vector's Cytomegalovirus promoter drives high expression in virtually all mammalian tissues
- **Preloaded Plasmids.** Every GFC-Array 384-well plate comes with cDNA expression plasmids individually distributed into the wells; 60 ng of each is standard
- **Quick Transfection Optimization.** An Optimization plate enables fast determination of optimal transfection conditions and reagent concentrations
- **Standard Controls.** Every plate is designed with empty wells for addition of user supplied positive and negative controls
- **Data in Triplicate.** OriGene supplies GFC-Array plates in triplicate so there are three replicate assay data points per cDNA

GFC-Array Versatility

GFC-Arrays can be used to study a wide range of biological processes and are provided in many different human gene subsets. GFC-Arrays are also easily adapted to many different readout assays.

Biological Processes. Processes that have been studied with overexpression assays include:

- Cell growth
- Cell differentiation
- Apoptosis
- Tissue-specific gene expression
- Developmental control

Human Gene Subsets. Sometimes the cost of screening OriGene's entire TrueClone collection may be prohibitive or the effort to assay such a large number of cDNAs may not be practical. OriGene's solution to this problem is to offer GFC-Arrays tailored to the study of specific human gene subsets for which there is wide research interest. Because these subsets contain hundreds of cDNAs rather than thousands, high-throughput screening costs drop drastically and become affordable for any laboratory.

Human gene subsets currently available for GFC-Array overexpression studies include:

- Transcription Factors
- Kinases
- Transmembrane Proteins
- Druggable Genes (i.e., genes for enzymes and receptors to which drugs can be targeted)
- Screening Sets

More cDNA subsets are currently in development.

Assay Readouts. GFC-Arrays fit most plate readers for assay readouts that include:

- Transcription reporter gene assays
- Receptor activation assays (e.g., G protein-coupled receptor assays)
- Nuclear translocations of protein markers
- Enzyme assays
- Immunoassays
- Single cell morphology assays
- Protein interaction assays
- Cell proliferation assays
- Apoptosis assays
- Cell toxicity assays

Overexpression Array Experiments from the Scientific Literature

High-throughput genome-wide overexpression assays for functional genomics have been validated in published papers. Here are three examples.

Screening for modulators of a transcription factor. In this study, 20,000 cDNAs were arrayed into 384-well plates in order to screen for proteins modulating the transcriptional activity of the p53 tumor-suppressor protein [1]. The cDNAs were then introduced into human colon cancer HCT116 cells via high-throughput reverse transfection. Each transfection also introduced a reporter plasmid containing a luciferase gene controlled by a p53-responsive transcription element. A plate reader measured each well's luciferase luminescence.

This genome-wide cDNA transient overexpression screen identified two known p53 activators and nine proteins whose ability to modulate p53 activity were previously unknown. Seven of the genes upregulated p53 activity and two downregulated p53 activity. Follow-up phenotype characterization in zebrafish, chick embryos, and mouse embryonic fibroblasts revealed that some of the nine proteins regulated transcription of the p53 gene and others that regulated p53 activity at the post-translation level.

Screening for genes that ameliorate oxidative-stress toxicity. Neuronal damage caused by reactive oxygen species (ROS) is associated with several neurodegenerative disorders. In this study, 5000 human cDNAs were arrayed into 96-well plates and then transfected into a mouse hippocampus cell line [2]. The screening objective was to identify genes capable of counteracting ROS damage. To model oxidative stress-induced toxicity, after transfection the cells were challenged with hydrogen peroxide. The following day, the cells received a metabolic dye for detecting cell survival and proliferation. The automated assay



readout scored cell viability by measuring dye fluorescence, which was directly dependent upon the level of hydrogen peroxide toxicity.

The experiment identified six genes for known antioxidative enzymes, and one gene for a transcription factor that regulates genes for oxidative-stress protection. The screen also revealed (and follow-up experiments confirmed) that a neuronal gene called GFPT2 (glutamine-fructose-6-phosphate transaminase 2) also enabled cells to survive hydrogen peroxide toxicity. This GFPT2 phenotype was previously unsuspected.

Screening for tumor suppressor phenotypes. In this study, researchers transfected almost 600,000 independent cDNA library clones into HEK293 cells in order to identify novel tumor suppressor genes [3]; maximum throughput was 40,000 transfections/24h. The assay readout used colorimetric beta-galactosidase assays to detect transient expression of a *lacZ* gene controlled by a cAMP response element (CRE). As an indirect assay the phenotype of interest were not high levels of beta-galactosidase but the loss of that signal, a possible indication of apoptosis induction or loss of cell viability, traits associated with tumor suppressor proteins.

Genome-wide screening detected several genes known to be tumor suppressors, indicating that the screening protocol was valid. Apoptosis associations were confirmed by further transfections in which cDNA-transfected cells were analyzed for apoptosis-specific events such as DNA fragmentation and caspase activation. In all, the experiment detected 89 genes previously unknown to be associated with apoptosis. Of them, 70 had not been previously associated with any phenotype.

Customized GFC-Arrays

For experiments with special requirements, OriGene offers customized GFC-Arrays. Customization options include, but are not limited to:

- Increasing or decreasing the cDNA amount per well
- Full-length human cDNA subsets specified by the client
- Special well coatings
- Special plates (including 96-well plates)

Conclusion

One of the greatest frustrations in research is the time it takes to prepare experimental materials. So once a new time-saving technology becomes validated in the scientific literature, it is entirely predictable that the technology will become commercially available in a convenient, cost-effective form.

This has now happened for high-throughput gene overexpression for human functional genomics and the breadth of OriGene's TrueClone content makes this possible. GFC-Arrays make timesaving gene overexpression screening available and affordable for complementing gene downregulation screening with siRNA. The day of doing gene overexpression experiments one at a time—thanks to GFC-Arrays—is over.

References

1. Huang Q, et al., (2004) Identification of p53 regulators by genome-wide functional analysis. *Proc Natl Acad Sci USA*. 101: 3456-61.
2. Zitzler J, et al., (2004) High-throughput functional genomics identifies genes that ameliorate toxicity due to oxidative stress in neuronal HT-22 cells: GFPT2 protects cells against peroxide. *Mol Cell Proteomics*. 3: 834-40.
3. Koenig-Hoffmann K, et al., (2005) High throughput functional genomics: identification of novel genes with tumor suppressor phenotypes. *Int J Cancer* 113: 434-39.



For More Information

To learn more about available GFC-Array human cDNA subsets, visit:

<http://www.origene.com/cdna/gfc-array/default.msp>

To learn about other overexpression array experiments and assay readouts, see "Large Scale Functional Profiling Using cDNA Clone Collections" at:

http://www.origene.com/cdna/trueclone/systems_biology.msp

To learn about OriGene's collection of more than 30,000 full-length human cDNAs, visit:

<http://www.origene.com/cdna>

Learn about the full range of OriGene products by visiting:

<http://www.origene.com>

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Notice

GFC-Arrays are for research use only. Use for diagnostic or therapeutic purposes is strictly prohibited. Reverse engineering of individual cDNA clones is not allowed.