

GFC-Transfection Array™

Genome-Wide Full-length cDNA

384 Well Plate Transfection Ready Clone Sets

APPLICATION GUIDE

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PACKAGE CONTENTS AND STORAGE CONDITIONS

Package Contents

- Three sets of one (1) sealed, 384-well plates containing 60 ng lyophilized TrueClone plasmid DNA per well. The last two columns (columns 23 and 24) are left empty for assay controls
- Two empty 384-well plates (Greiner Bio-One black#781091 or white #781098) for transfection and assay optimization
- Five (5) sheets white bottom cover (for luminescence assay - white plates orders only)
- One (1) vial pCMV-XL5 (pCMV6-XL5 vector, 10 ug lyophilized)
- One (1) vial pCMV-Luc (pCMV6-Luciferase vector, 1.25 ug lyophilized)
- One (1) vial pIFN-Luc reporter gene (12 ug lyophilized - Transcription Factors array orders only)
- GFC-Transfection Array™ Application Guide

Storage Conditions

The plasmid plates and vials are shipped at room temperature but must be stored sealed at -20°C. Once a seal is broken, the plate needs to be used immediately.

Notice to Purchaser

This product is for research use only. Use in and/or for diagnostic or therapeutic purposes is strictly prohibited. **Reverse engineering of the assay plate including isolation or copying of any individual clone is not allowed.**

OTHER REQUIRED MATERIALS AND REAGENTS

Reagents

- Low toxicity transfection reagent suitable for transfection with serum such as TurboFectin 8.0 (OriGene - TF81001), FuGENE 6 (Roche), Lipofectamine LTX (Invitrogen) or similar. TurboFectin 8.0 (TF81001) has been thoroughly tested at OriGene with GFC-Transfection Arrays
- Opti-MEM I Reduced Serum Medium (Invitrogen)
- Routine tissue culture supplies
- Transfectable cell line (such as HEK293T or HeLa cells)
- Assay readout reagents such as Luminescence reporter assay reagent (e.g. BriteLite, Luciferase assay system Perkin-Elmer 6016976 or Promega Bright-Glo)

Equipment and Instruments

- 8 or 12-Channel electronic pipettor, Matrix Impact 2 or similar
- Tabletop centrifuge with plate adaptor rotor
- Plate reader able to read 384-wells plate with the appropriate capability for your assay readout such as Victor 3 microplate reader (Perkin Elmer).
- Inverted microscope

Optional Reagents and Equipment

- pCMV-GFP vector for transfection positive control and optimization (OriGene PS100010 & PS 100019)
- Positive controls (known activator of your reporter gene or assay) specific for your reporter gene or assay
- Fluorescent microscope for fluorescence assays and imaging assays
- Microplate mixer (micro-plate Genie SI-0400 or SI-401 or MixMate Eppendorf)
- Angled plate stand (ISC Bioexpress W-1000-2 or similar)

Related Products

TrueClone™ cDNA clones <http://www.origene.com/cdna/>

HuSH™ shRNA Plasmids <http://www.origene.com/rna/>

Transfection Reagents <http://www.origene.com/cdna/transfection/>

Validated Antibodies <http://www.origene.com/antibody/>

Functional Proteins <http://www.origene.com/protein/>

PrecisionShuttle Vectors <http://www.origene.com/trueorf/>

INTRODUCTION

Overview

OriGene's GFC-Transfection Arrays (**G**enome-wide **F**ull-length **c**DNA Transfection Arrays) are a revolutionary tool for high throughput functional screening by protein overexpression. GFC-Transfection Arrays provide a set of expression-ready cDNA clones in a convenient 384-well format optimized for high-throughput transfection and rapid screening. Each well contains a standardized amount of 60 ng of plasmid DNA from OriGene's large selection of human full-length cDNA clones (TrueClones) in the robust pCMV6 mammalian expression vector. The procedure is simple; just add your reporter, transfection agent and cells then score the array after 48 hours. Gene over-expression provides additional information over other methods like gene silencing by providing gain of function screening and the ability to target lethal genes that cannot be silenced.

An overview of the procedure is presented in the diagram below.

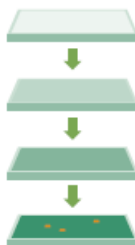
Figure 1. Procedure Overview and Preliminary Experiments

Open screening plates with 60 ng plasmid per well

Add reporter plasmid (if applicable) and transfection reagent

Add cells and incubate

Add assay readout reagents and read plates

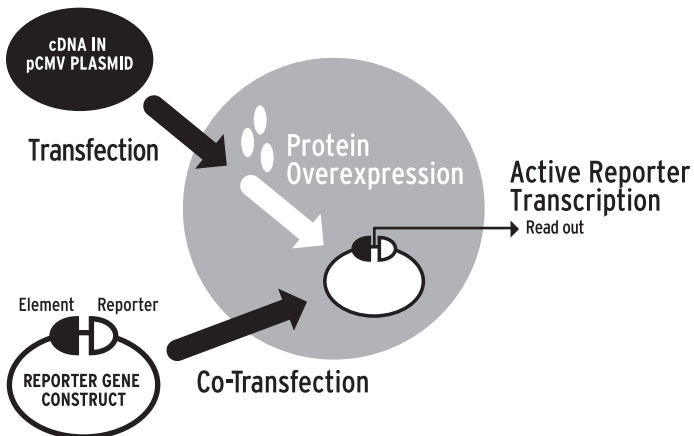


Background

Leading pharmaceutical companies and academic researchers established the proof of principal for GFC-Transfection Arrays. High throughput overexpression screening technology used in GFC-Transfection Arrays was first developed in 2003 (11). Since then, additional leading publications describing this technology have further demonstrated its validity, simplicity and versatility. Among these publications are articles describing the discovery of modulators of transcription factors and pathways (regulating elements of the Wnt and AP-1 pathways, the p53 tumor suppressor pathway and novel functions of TORC proteins) by scientists from the Genomics Institute of the Novartis Research Foundation, The Scripps Institute and The Salk Institute (8-13). Other studies published the identification of the role of BMP-9 in glucose homeostasis by scientists from Human Genome Sciences (14) and the identification of genes that ameliorate oxidative-stress toxicity and genes associated with tumor suppression (6). All are testimonial evidence for the versatility and usefulness of GFC-Transfection Arrays

An illustration of the GFC-Transfection Array assay using co-transfection with a luciferase reporter plasmid is presented below.

Figure 2. Transfection Overview



GFC-Transfection Arrays are suitable for a wide range of biological studies and are easily adapted to many different assay readouts. Biological processes that have been studied using overexpression assays include but are not limited to: cell growth and differentiation, apoptosis, tissue-specific gene expression, toxicity and sensitivity to small molecules. Many assays, including immunoassays, enzymatic assays and promoter/ reporter assays can be adapted to the GFC-Transfection Array format using an appropriate bioassay readout and transfectable cell line. OriGene scientists can work with you to convert or adapt your assay to the required format.

For convenience and affordability, OriGene offers several GFC-Transfection Arrays of genome subsets for which there is wide research interest. Larger sets covering up to the entire OriGene TrueClone collection or custom arrays of clone sets at your own configuration can be purchased as custom-made arrays. If necessary, a limited number of genes can also be added to the array by purchasing individual TrueClones from OriGene and arraying them yourself into some of the provided empty wells.

Working with 384-well plates in High Throughput

Working with 384 wells plates in high throughput screening is very different than regular screens using 96 wells plate. If this is your first high throughput screening assay with 384 well plates you should take some time to become comfortable working with this type of format. Using an electronic multi-channel pipettor that can pick up a large fluid volume and repeatedly dispense smaller volumes is vital. It will save you time, hand strain and more importantly, prevent the formation of bubbles. We recommend placing the plate on an angled plate stand and pipetting the volume to the side of the well followed by a brief centrifugation to bring the entire volume down to the bottom of the well. The use of a microplate mixer is highly recommended to achieve adequate mixing and to recover the maximal amount of material from the lyophilized plasmid DNA.

OPTIMIZATION OF TRANSFECTION & ASSAY CONDITIONS

Before embarking on a broad use of the GFC-Transfection Arrays, it is important to optimize your transfection conditions, validate your reporter (if applicable) and assay readout and identify the best conditions to maximize the assay signal to noise ratio.

Optimization should include:

- Identifying the most appropriate transfection reagent
- Choosing the most effective DNA to transfection reagent ratio
- Choosing the optimal number of cells to be used
- Validating your reporter gene (when applicable) and assay controls
- Choosing the most effective activator / reporter ratio (e.g. arrayed clones to reporter gene / modifier ratio)

OriGene provides basic guidelines and recommendations for optimization based on its scientific experience with GFC-Transfection Arrays. OriGene scientists and Customer Support staff will be happy to provide advice and assistance with assay optimization for your specific needs. Additional information about transfection conditions can be found on the website of your transfection reagent provider.

Optimization of Transfection

GFC-Transfection Arrays are designed for high throughput reverse transfection protocol. In reverse transfection, DNA and the transfection reagent are added to the plate first and allowed to form a complex before cells (in suspension) are added. With this method, cells are transfected as they settle down and adhere to the plate. Reverse transfection is easier and faster to perform in high throughput than traditional transfection. Reverse transfection is compatible with most cell types and many transfection reagents. However, transfection conditions should be optimized for your

cells and transfection reagent.

OriGene supplies two control 384-well assay plates with positive and negative control pCMV based vectors for use in luminescence-based assays. In addition, the pCMV-GFP vector (OriGene PS100010 and PS100019) and any other tagged pCMV based vectors can be used in fluorescence-based visual and other quantitative optimization assays.

Selecting your transfection reagent.

A low toxicity serum compatible agent must be used. We routinely use TurboFectin 8.0 but other transfection reagents such as FuGENE 6 (Roche) or the Lipofectamine family of transfection reagents are also suitable. A database with transfection results and recommendations for many established and primary cell lines is available on the OriGene website (<http://www.origene.com/cdna/turbofectin.msp>) as well as on Roche's web site (<https://www.roche-applied-science.com/sis/transfection/index.jsp>). A large selection of transfection reagents and cell lines transfected with these agents is also available on Invitrogen's website (<http://www.invitrogen.com/content.cfm?pageid=4005> and <http://www.invitrogen.com/celllines>).

You will need to optimize the transfection conditions for each transfection reagent. This can be performed simultaneously using the reagents provided in this kit

Optimizing the amount of transfection reagent.

Plasmid DNA amounts in GFC-Transfection Arrays are pre-set at 60 ng. We recommend optimizing your conditions for this amount and making adjustments based on this ratio for the actual total plasmid DNA amount used (activator + reporter). We recommend performing optimization assays in triplicates.

Use the transfection reagent manufacturer's application guide for designing your specific optimization protocol. Optimize your transfection reagent conditions before making the final selection of your transfection reagent.

The following sample protocol describes transfection optimization for TurboFectin 8.0 and HEK293T cells using pCMV-luciferase (supplied) and pCMV-GFP (not supplied) plasmid vectors.

The TurboFectin 8.0 application guide suggests testing TurboFectin 8.0 at three ratios: 3:2; 3:1 and 6:1 of transfection reagent (ul) to DNA (ug). However, at 100 ng DNA, a 6:1 ratio can start to show cell toxicity. We recommend adding a 4:1 or 5:1 ratio to the optimization.

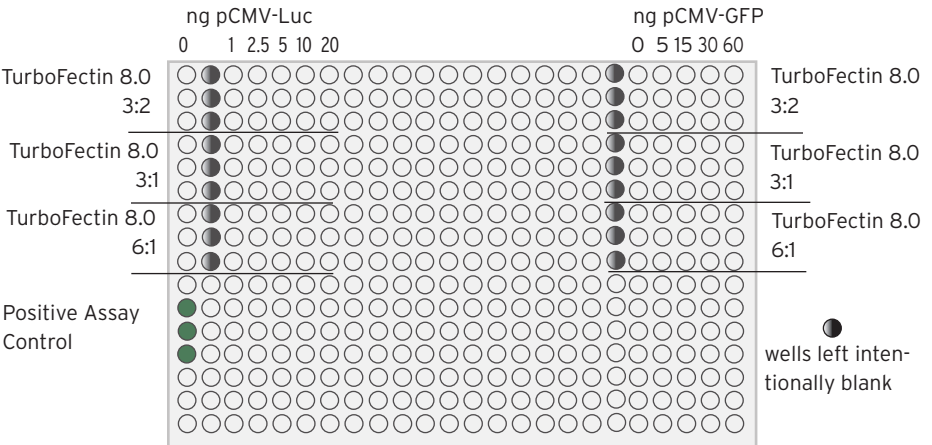
- Prepare stock pCMV-XL5 plasmid at 50 ng/ul by adding 200 ul of water to the provided pCMV-XL5 (Vector control) tube (10 ug).
- Prepare stock pCMV-luciferase plasmid at 25 ng/ul by adding 50 ul of water to the provided pCMV-luciferase tube (1.25 ug).
- After initial use, keep your stock solutions at -20° C.
- Make sure your Opti-MEM I is at room temperature (or 37° C)
- Prepare working dilution of 12.5 ng / ul of pCMV-XL5 by diluting the stock solution 1:4 in Opti-MEM I. You will need about 5 ul of solution for each well (data point)

used. We recommend preparing 330 ul of pCMV-XL5.

- Prepare 200 ul of 2.5 ng/ul working dilution of pCMV-luciferase.
- Test pCMV-luciferase at 0, 1, 2.5, 5, 10 and 20 ng. Add pCMV-XL5 to total of 60 ng and Opti-MEM I to complete the volume to 10 ul.
- Optimization with pCMV-GFP can be performed by using 0, 5, 15, 30, and 60 ng of pCMV-GFP and adding control pCMV-XL5 vector to total of 60 ng.
- The recommended amount for Luciferase optimization assay (12 data points for each concentration) are provided below.

pCMV-Luc (ng)	pCMV-Luc (ul)	pCMVXL5 (ul)	Opti-MEM I (ul)
0	0 (0)	4.8 (57.6)	5.2 (62.4)
1	0.4 (4.8)	4.8 (57.6)	4.8 (57.6)
2.5	1 (12)	4.6 (55.2)	4.4 (52.8)
5	2 (24)	4.4 (52.8)	3.6 (43.2)
10	4 (48)	4 (48)	2 (24)
20	8 (96)	3.2 (38.4)	0 (0)
Additional			
Total	15.4 (184.8)	25.8 (309.6)	20 (240)

Figure 3. Layout of Optimization Plate for Transfection Reagents



- Centrifuge the plate briefly to bring all the added content down.
- Prepare TurboFectin 8.0 at 3:2, 3:1 and 6:1 of transfection reagent (ul) to DNA (ug) ratio. First dilute the TurboFectin 8.0 1:30 in Opti-MEM I. **DO NOT let the transfection reagent touch the walls of tube; make sure to pipette directly into the medium.** To make TurboFectin 8.0 solution at 3:1 ratio, dilute 5.4ul of TurboFectin 8.0 1:30 in total of 10 ul of Opti-MEM I (add 4.6 ul of Opti-MEM I). To make a 3:2 ratio dilute 2.7 ul of TurboFectin 8.0 1:30 in total amount of 10 ul Opti-MEM I (add 7.3 ul of Opti-MEM I). You will need 10.8 ul of TurboFectin 8.0 (1:30 dilution) for each

well at 6:1 ratio.

- For our optimization scheme you have 22 wells (66 with added volume) for each ratio.

TurboFectin 8.0 calculation

Ratio	Turbo-Fectin 8.0 (1:30) (ul)	Opti-MEM (ul)	Number of Wells	Total Vol TurboFectin 8.0 (ul)	Total Vol OptiMEM (ul)	Total Vol (ul)
TurboFectin 8.0 6:1	10.8	0	22	237.6	0	237.6
TurboFectin 8.0 3:1	5.4	4.6	22	118.8	101.2	220
TurboFectin 8.0 3:2	2.7	7.3	22	59.4	160.6	220
Total (ul)	18.9	11.9		415.8	261.8	677.6

You will need a minimum of 450 ul of TurboFectin 8.0 diluted 1:30 (15 ul TurboFectin 8.0 and 450 ul of Opti-MEM I).

- Add 10 ul (10.8 ul for TurboFectin 8.0 6:1) of the appropriate TurboFectin 8.0 solution to each of the wells and centrifuge down briefly.
- Mix for 30 seconds using microplate mixer and incubate at room temperature for 25-30 minutes. (Incubation of 45 minutes or even more for some cell lines will not affect transfection efficiency). Some cell lines may require additional incubation time. In these unusual cases, the specific transfection time will need to be optimized as well.
- Prepare the HEK293T cells during the incubation time. The cells can be up to 80% confluent. (We recommend using between 5,000 and 7,500 cells per well)
- Remove the spent media. Rinse once with sterile 5 ml PBS.
- Add 1-5 ml (depend on the flask used) of pre-warmed Trypsin/EDTA solution. Incubate at 37°C for 5 min.
- Make sure during the entire procedure that the cells are well separated from each other (single cells).
- Remove the trypsinized cells to a 15 ml conical sterile centrifuge tube and add pre-warmed complete DMEM media. Mix the cells well and centrifuge at 1200 RPM for 5 minutes.
- Aspirate the liquid, leaving a small amount of media for resuspension of the cells. Re-suspend the cells by flicking the tube and add 10 ml of pre-warmed complete DMEM media. Mix the cells well and centrifuge at 1200 RPM for 5 minutes.
- Resuspend cells in 5-10 ml of pre-warmed complete DMEM media.
- For counting the number of cells, add 10 ul to a hemocytometer and count cells in 2 of the 1mm X 1mm squares. The cell concentration is $(X1+X2)/2 \times 10^4$ cells / ml.
- Adjust the cell density to about 250,000 cells / ml with complete DMEM medium.

When the total amount of serum is important, you should adjust the amount of serum in the DMEM to account for the volume of the non-serum containing component (20 ul of Opti-MEM). We routinely do not adjust the amount of serum for HEK293T cells to account for the Opti-MEM media.

- Aliquot 30ul of cell suspension into each well. Let cells settle by gravity. Do not vortex, mix or spin down the cells (unless required by the assay). This will prevent the cells from settling down evenly.
- Cover the plate and incubate 37°C in a CO₂ incubator for 48 hours.

Note: All procedures related to cells must be carried out in a sterile manner. We recommend using antibiotic in the complete medium. However, antibiotic (pen-strep) can reduce the efficiency of the transfection in some cases.

- Add white adhesive bottom cover to the bottom of the plate.
- Prepare the BriteLite reagent (Perkin-Elmer 6016976) and equilibrate to room temperature.
- Add 15-25 ul of the BriteLite reagent directly to the plate (NO WASH STEP) and incubate for two minutes (to ensure all cells are lysed). We found little difference between the recommended 25 ul amount of BriteLite reagent and addition of 15 ul reagent. Reading must be performed quickly. The activity of the BriteLite reagent is diminished significantly within 10 - 30 minutes thus it is important to have positive and negative controls in each plate
- Read luminescence with Victor3 reader (Wallace 1420 PerkinElmer) or similar using Luminescent 384 wells protocol.

Validation of reporter gene and assay conditions

Before you embark on your high-throughput screening you should validate your reporter gene and optimize and validate your assay.

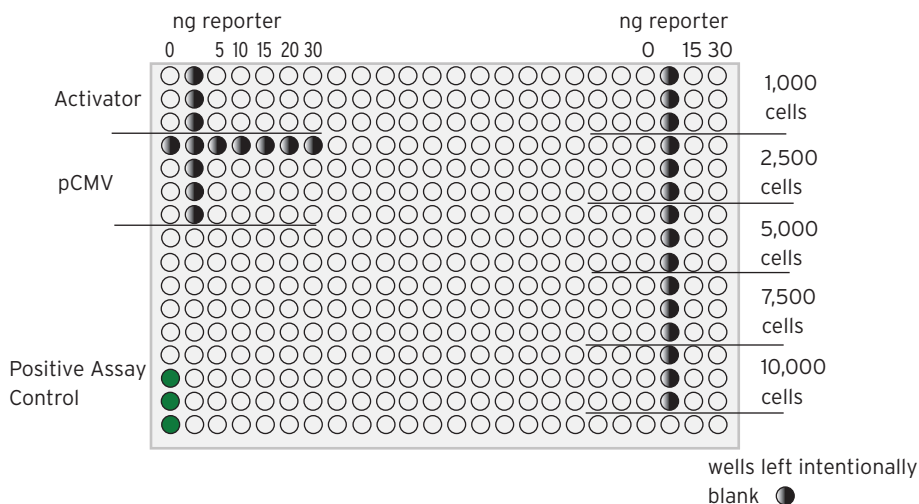
Reporter genes can be very different from each other. They can have high or low specific activity depending on the activating gene or even have high intrinsic activity. It is very important to optimize the reporter to activator (arrayed genes) ratio and select the optimal conditions for your specific assay needs and readout.

Your positive activator gene (or genes) should ideally be cloned into a pCMV-XL5 vector (provided). Spot 60 ng of the positive activator gene and test your reporter at 0, 5, 10, 15, 20 and 30 ng (see suggested plate layout). For measuring the intrinsic activity of your reporter gene, spot 60 ng of pCMV with the same layout as you did for the positive activator gene (see suggested plate layout). Remember to take into account the added amount of DNA when applying your transfection reagent. Not accounting for the added DNA amount will affect your transfection efficiency and overall results. Using the same elevated amount (calculated for 30 ng additional plasmid) is acceptable. When choosing the optimal amount of reporter gene, remember that the level of activity of positive (and negative) genes can vary significantly. Some genes will only be detected with the higher quantities of reporter plasmid. Include a positive assay control such as pCMV-luciferase or pCMV-GFP. When using pCMV-Luc as positive assay control use only a small amount (0.5-5 ng at most depending on the specific expression in your cell type) sufficient for significant readout and add

pCMV-XL5 to total of 60 ng. Adding large amount of Luciferase can cause significantly elevated readouts in the adjacent wells.

The number of cells used is also an important factor. We recommend using between 5,000 and 7,500 cells per well for HEK293T cells. We recommend optimizing the number of cells by using 60 ng of the activator plasmid and 0, 15 and 30 ng of the reporter gene. Alternatively, a range of pCMV-GFP or pCMV-luc similar to the one used for transfection optimization can be used for optimizing the number of cells. Vary the number of cells from 1,000 to 10,000 cells (see suggested plate layout). Higher amounts of adherent cells are not recommended for 384 wells.

Figure 4. Layout of Optimization Plate for Reporter Genes and Number of Cells



HIGH THROUGHPUT SCREENING

Once the assay and transfection conditions have been optimized and validated, you are ready to proceed to the high throughput screening. The GFC-Transfection Arrays contain three (3) sets of assay plates, sufficient to generate enough repetition for statistical analysis. The last two columns in the array (columns 23-24) are intentionally left empty to accommodate the negative and positive controls. Moreover, these wells can accommodate additional individual clones that were not included in the array but are important for your assay. These clones can be purchased from OriGene's large collection of TrueClones and spotted in the same way as the array controls.

Unlike the DNA used for the optimization and validation experiments, the plasmid DNA in the GFC-Transfection Array plates comes lyophilized and needs to be hydrated before use. It is important to follow the recommended procedure to minimize

the loss of plasmid DNA.

- Equilibrate the sealed plates and the Opti-MEM I medium to room temperature.
- Centrifuge the plate at 1200 RMP for 5 minutes and remove the seal.
- Prepare the assay negative and positive controls and any additional clones you like to include at 12.5 ng/ul (60 ng/5 ul) in Opti-MEM I (see above for specific instructions).
- Spot in the array empty wells:
 1. Three to four wells of pCMV-XL5 negative control
 2. Two (or more) wells of your positive activator controls
 3. Two (or more) wells of your positive assay controls (pCMV-Luc or pCMV-GFP)
 4. Any additional individual TrueClones you wish to add

Note: Do not spot the positive control in the wells next to your negative controls or the printed array wells. Use column 24 for these controls. High readouts may create false positive reading in the adjacent wells due to crosstalk.

- Prepare your reporter gene (if applicable) at the desired amount / 10 ul for each well in Opti-MEM I. For example, for the pIFN-Luc reporter at 15 ng/10 ul prepare 1.5 ng/ul solution.
- Add your reporter gene to each of the wells including your negative and positive controls. Use an electronic multi-channel pipettor to repeatedly add 10 ul to each well. Exclude wells that will not use your reporter gene, such as pCMV-GFP, pCMV-luc (if used) and pCMV background control.
- If not using a reporter gene add 10 ul of Opti-MEM I to each well to hydrate the plasmid DNA. **Remember not to add anything that will interfere with the transfection such as serum or proteins.**
- Centrifuge briefly to bring all the volume down and shake on a microplate mixer for 10-20 minutes. This step will ensure recovering the maximal amount of the spotted plasmid DNA.
- Prepare your dilution of transfection reagent at 10 ul / well (up to 15 ul can be used) based on your optimization assay. Remember to account for the added amount of reporter plasmid (60 ng spotted plasmid + reporter plasmid).
- Add 10 ul of the transfection reagent to each of the wells.
- Centrifuge briefly and shake on a microplate mixer for 30 seconds.
- Incubate at room temperature for 25-30 minutes or according to the suggested manufacturer conditions or your specific cell requirements.
- During the incubation time, prepare your cells, which should be less than 75% confluent (for HEK293T cells).
- Remove the spent media. Rinse once with sterile 5 ml PBS.
- Add 1-5 ml (depend on the flask used) of pre-warmed Trypsin/EDTA solution. Incubate at 37°C for 5 min.
- Make sure during the entire procedure that the cells are well separated from each other (single cells).
- Remove the trypsinized cells to a 15 ml conical sterile centrifuge tube and add pre-warmed complete DMEM media. Mix the cells well and centrifuge at 1200 RPM for 5 minutes.
- Aspirate the liquid leaving a small amount of media for re-suspension of the cells.

- Re-suspend the cells by flicking the tube and add 10 ml of pre-warmed complete DMEM media. Mix the cells well and centrifuge at 1200 RPM for 5 minutes.
- Re-suspend cells in 5-10 ml of pre-warmed complete DMEM media.
 - For counting the number of cells, add 10ul to a hemocytometer and count cells in 2 of the 1mm X 1mm squares. The cell concentration is $(X1+X2)/2 \times 10^4$ cells / ml.
 - Adjust the cell density to about 250,000 cells /ml or to the optimized amount of cells, with complete DMEM medium. When the total amount of serum is important you should adjust the amount of serum in the DMEM to account for the volume of non-serum containing component (20 ul of Opti-MEM). We routinely do not adjust the amount of serum for HEK293T cells to account for the Opti-MEM media.
 - Aliquot 30 ul of cell suspension into each well using an electronic multi-channel pipettor. Let cells settle by gravity. Do not vortex, mix or spin down the cells (unless required by the assay). This will prevent the cells from settling down evenly.
 - If at any time you need to add additional materials such as specific drugs, antibodies or other reagents, you may subtract it from the total volume of cells (30 ul) added. Make sure to adjust the plated cell density accordingly to give a final number of 7,500 cells / well. Cells can be plated in as little as 15 ul of complete media but the amount of serum can or should be adjusted accordingly (20% serum if using 15 ul of media).
 - Cover the plate and incubate 37°C in a CO₂ incubator for 48 hours.
 - Read the plates and analyze the results.

Note: All procedures related to cells must be carried out in a sterile manner.

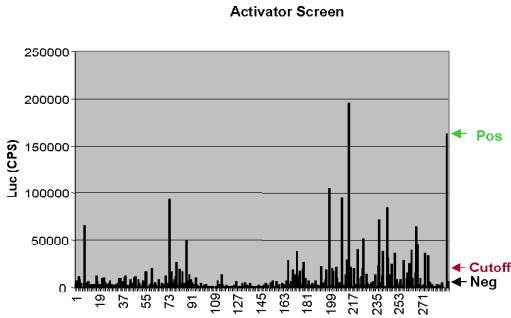
We recommend using antibiotics in the complete medium but this may reduce the efficiency of the transfection.

Data Analysis

Analysis of high throughput screening is different than assays using a single gene. Variations between experiments are expected and false positive and negative results are much more common with this screen. Confidence in the results comes from repetition of the assay to gain sufficient statistical analysis. Absolute values are less important and can be replaced by the activation factor (X times the basal level) or other values. Although three (3) repetitions are sufficient for initial statistical analysis, repeating the assay 4 to 6 times can significantly reduce the number of false positive and false negative results. You can analyze each assay separately or average the results of your three or more repetitions (both ways give similar results). Determine the baseline and maximal signal with the negative and positive controls in each of the assay plates. Determine the standard deviation (SD) of the controls. Based on the baseline and maximal signal, determine the cutoff values. For example, the cutoff can be set at 20% of the maximal signal, set at a value equal to the sum of the baseline plus minus 3 X SD or set at specific fold of activation value. Generate a list of potential hits that have a signal beyond the cutoff values. Examine the surrounding wells of each of the low-level hits. If any of these wells contain a high value hit, then your low hit may come from cross-talk between wells and may not be real. On your next experiment, you can move the content of this well (after hydration) to one of the empty wells in the array, away from any positive controls or high scoring

clones.

Figure 5. Typical Results from Interferon Reporter Activation Assay



Validating your assay results

Identify the list of TrueClones that correspond to the potential hits.

Obtain a DNA stock of all such TrueClones from OriGene.

Retest each potential hit in triplicate along with the controls.

Repeat the assay in dosage-dependent studies.

Additional validation can be made by using RNA inhibition (RNAi) assays available from OriGene <http://www.origene.com/rna/>.

QUALITY CONTROL

Plasmid DNA Validation:

All plasmids are TrueClones, human full-length cDNA clones. Both the concentration and the purity of the DNA were determined based on OD₂₆₀ and OD₂₈₀ readings. A minimum purity of 1.8 (OD₂₆₀/OD₂₈₀) is required. 5' sequencing was used to validate each clone and the orientation of the arrayed cDNA clones is validated by its unique 2D barcode. The array is printed directly from the barcoded tubes by a robotic instrument in a validated procedure developed at OriGene. The OriGene GFC-Transfection Array has been validated using a broad number of assay types and cell lines by OriGene's scientists and customers, some of which have appeared in print (see Citations). OriGene validates subsets of its GFC-Transfection Array stock in a luciferase-based assay to minimize any lot-to-lot variation.

FREQUENTLY ASKED QUESTIONS (FAQs)

Q. What format should I use, 96 wells or 384 wells?

A. This largely depends on your array readout and personal preference. Both formats provide reproducible results, although the 96 wells format appears to give tighter triplicates than the 384 wells format. Using the 384 wells format is eventually more convenient (one vs. 4 plates) and providing significant savings. It is less expensive to purchase and uses much less reagents and cells. If possible we recommend using the 384 wells format for screening.

Q. Can I use different assays with the GFC-Transfection Array?

A. Certainly. The arsenal of assays and readouts specifically designed for high-throughput rapidly growing and covers most cell based assay readouts. Reporter gene assays (Luc, GFP, beta-gal etc.) are only one of many applications. Some of the most common applications are: cell growth and viability, cytotoxicity, apoptosis, kinase activity, proteases, protein expression and even high content microscopic examination are all available. At OriGene we have tested several of these applications (see our website) and found they are easily applied to our GFC-Transfection array formats. Chances are that you will easily be able to find and adapt readout for your specific application.

Q. Which transfection reagents do you recommend?

A. We routinely use the TurboFectin 8.0 transfection reagent (OriGene Cat # TF81001) that is ideal for reverse transfection. This reagent is non-toxic and gives similar or better results than comparable transfection reagents (see data on our website) at a lower cost. However, other non-toxic transfection reagents may work as well as or even be superior to TurboFectin 8.0 depending on your cell type and assay.

Q. Why can't I use the clones provided in the array for any purpose besides screening?

A. The TrueClones provided in the array are proprietary products of OriGene available as individual clones. Charging our regular or even a substantially reduced price will make the array outside the reach of most researchers. To overcome this problem, the clones in the array are provided for screening only. Individual clones to validate your results can be purchased from OriGene and used for any in vitro purpose.

Q. What is the difference between white and black plates?

A. White plates are more suitable for luminescence assays. Black plates are appropriate for fluorescent type assays.

Q. Why do you supply all plates with clear bottoms?

A. Clear bottom plates give more assay flexibility and can be used with non-luminescence readouts like GFP and others. We provide an adhesive white bottom cover for luminescence assays. Moreover, the clear bottom plate allows you to visualize and monitor the growth and behavior of your cells.

Q. Why do you provide the white bottom covers and how do I apply them to the plate?

A. The white bottom covers are design to enhance the luminescent signal. Applying them to the plate is easy. Peel one side of the cover, place the cover with

the adhesive side up on the cover of a 250 ul Matrix tip box, place the plate on top (the wells section is perfectly aligned with the cover), press to make the adhesive adhere, lift the plate peel the second half of the adhesive cover and smooth over the plate bottom.

Q. Why are the GFC-Transfection Arrays constructed with 60-100 ng of plasmid DNA per well?

A. Based upon our experience and internal data (see below) and published data from our collaborators, 60 ng for 384 wells plate and 100 ng for 96 wells format provides sufficient material for efficient transfection with a number of transfection reagents yet is small enough to minimize cell toxicity effects.

Q. Why are the GFC-Transfection Arrays constructed by first coating the plates with pUC-19?

A. OriGene has found that pre-coating the plates with a plasmid like pUC-19 prevents the loss of the target plasmid by adherence to the plate. More importantly, it is well known that transfection efficiencies can be higher when a small percentage (10%, w/w) of a second, non-reactive plasmid is included as part of the experiment.

Q. How much DNA can be recovered from each well?

A. We are able to recover close to 90% of the added plasmid DNA. Following our procedure and using an appropriate microplate mixer are important for maximal recovery of DNA.

Q. Why aren't all the known genes in this family / pathway included in the set?

A. GFC-Transfection Arrays are designed by relevance and availability. OriGene is committed to creating additional panels over time (see our website for upcoming panels). By releasing each panel separately, OriGene keeps its GFC-Transfection Arrays affordable. Researchers can use the first array to test and verify their screening, obtain novel data and results and then proceed with other GFC-Transfection Arrays as they become available.

Q. Can I add additional clones to the array?

A. Yes, a limited number of additional clones can be added in some of the empty wells at columns 23-24 (384 wells) or column 12 (96 wells). If you require a large number of additional clones or a different set of clones, OriGene can make a custom GFC-Transfection Array that addresses your needs.

Q. Why you do not supply the pCMV-GFP as part of the optimization kit?

A. GFP plasmids and proteins are propriety reagents with legal claims by several entities. Therefore we are unable to include it as part of our commercial kit. These can be purchased separately from OriGene.

Q. How important it is to optimize the transfection conditions?

A. We found that changing the ratio of the transfection agent from 3:1 to 3:2 (ul/ug DNA) results in considerable reduction of transfection. Unless you use HEK293T cells or any other cell lines that were optimized by OriGene for reverse transfection it is critical that you optimize the conditions of your transfection reaction. Failing to do so can result in negative outcomes.

Q. How can I be sure that my transfection and screening procedures really work?

A. We always recommend including positive controls in your experimental design. You can use pCMV-Luc, pCMV-GFP or any plasmid with a known readout

to verify the conditions of the transfection. When using Luciferase or similar reagents we recommend using a very small amount (0.5-2 ng based on your cell type) on top of the 60 ng pCMV-XL5 control plasmid, placing it on the last row, well separated from array wells and other controls (for 384 wells). Using a large amount of pCMV-Luc will result in considerably higher reading in the adjacent wells. Always use an assay specific positive (and negative) control to verify the validity of your procedures and assay. If you do not have a known positive activator, OriGene's Transcription Factor GFC-Transfection Array (FTCW1003) can be used as a control under your conditions with HEK293T cells to validate your procedures. Use the pIFN-Luc reporter gene (IFN-promotor-Luciferase) provided by OriGene with the Transcription Factors GFC-Transfection Array. It should show activity with at least three clones (5 known response elements of which 2 have low activity). If no activity is apparent, verify your procedures and storage conditions and repeat the assay. If problems persist, please contact OriGene Technical Support (techsupport@origene.com).

Q. I used the pIFN-Luc reporter gene with the transcription Factors GFC-Transfection Array but did not receive any results. What am I'm doing wrong?

A. First, make sure to use HEK293T cells at least in one of the plates. This screen works well with some cell lines like HEK293T cells but may not work with other cell lines such as HeLa and more. Second, follow closely the recommended procedure in our application guide. If problems persist, please contact OriGene Technical Support (techsupport@origene.com).

Q. Should I use all three triplicates plates in one assay or in three independent assays?

A. There are specific benefits for each way and it is entirely up to each researcher to choose the most appropriate way. We prefer using one set in three independent assays. Each assay may have its own set of technical difficulties. Performing three independent assays appear to even out deviant readouts specific for each assay and provide more accurate outcome.

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