

# Chapter 2

## Cell-Based Co-transfection Microarrays for Use with HEK293T Cells on a Poly D-Lysine-Coated Polystyrene Microplate

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### Abstract

Analysis of the human genome sequence has identified thousands of putative genes with unknown function; therefore, a new tool allowing for rapid identification of gene functions is needed. Reverse transfection microarray technology, which turns a DNA microarray into a cell-based microarray, has emerged for simultaneously studying the function of many genes. Since the initial demonstration in 2001, many variations have surfaced, making the technology more versatile for a broad range of applications. We have developed a protocol to make ready-to-transfect DNA microarrays in a 96-well microplate for co-transfection of two plasmids into HEK293T cells. This cell-based microarray in a microplate may be used for screening hundreds of analytes against multiple protein targets in parallel, providing a powerful tool for functional genomics and drug discovery.

**Key words:** Reverse transfection, surface-mediated transfection, co-transfection, cell microarray, microplate, GFP, LacZ.

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### 1. Introduction

Reverse transfection microarray technology first developed by Ziauddin and Sabatini is a powerful tool for bridging genomics with proteomics (1). The technology involves three basic steps to turn a DNA microarray into a cell-based microarray. First, a DNA microarray is fabricated, in which each microspot contains a plasmid DNA capable of expressing a gene of interest. Second, the DNA microarray is treated with transfection reagents. Third, adherent cells are grown on the treated DNA microarray.

Due to surface-mediated transfection enabled by the presence of transfection reagents, the cells on top of a microspot take up the plasmid DNA and express the protein encoded by it, producing a localized patch of transfected cells, a cell microspot. This cell-based microarray with the number of cell microspots corresponding to the number of DNA microspots can be used to simultaneously study the function of dozens or hundreds of genes. Recently, the technology has been extended for turning a microarray of siRNA, virus, or even chemical compounds into a cell-based microarray for many cellular and biological applications (2–4).

Over the years, many modifications have been made. To circumvent the need for extensive post-transfection processing of the cell-based microarray to detect protein activity, we have developed a reporter system using green fluorescent protein (GFP) for direct readout (5). In this system, two plasmids, one for a target protein and the other for GFP which can be turned on only when the target protein is active, are printed within a single microspot and can be co-transfected. Since the presence of GFP, which is readily visualized by an imaging system, is the indicator of the activity of the target protein, the cell-based microarray assay is substantially simplified through elimination of fixing and permeabilizing cells, as well as immunostaining with multiple antibodies.

Although  $\gamma$ -aminopropylsilane (GAPS)-coated glass (Corning Incorporated, Lowell, MA) and poly-D-lysine (PDL)-coated glass or polystyrene (PS) surfaces are suitable for reverse transfection, efforts have been made to create a ready-to-transfect surface so that the second step, treatment with transfection reagents, may be eliminated. In 2000, before the publication of reverse transfection, Zheng et al. reported that immobilizing a plasmid DNA on polyethyleimine (PEI) attached to a polymer film made of poly( $\epsilon$ -CBZ-L-lysine) (PCBZL) mixed with poly(D,L-lactic-co-glycolic) or poly(L-lactic acid) could enable surface-mediated transfection (6). In 2008, two reports described the use of PEI-plasmid complexes immobilized on self-assembled monolayers (SAMs) of ethylene glycol (EG) and carboxylic acid-terminated alkanethiols or on small intestinal sub-mucosa (SIS) for improved transfection efficiency (7, 8). Similarly, calcium-phosphate (Ca-P)-DNA co-precipitates on or encapsulated in fast-degrading polymer was also found to be adequate for transfection of HEK293, HeLa, and NIH 3T3 cells (9). Most recently, Oyane et al. reported that including a cell adhesion molecule such as laminin or fibronectin in a DNA-apatite composite layer enhanced transfection efficiency (10). Moreover, fibrin-based hydrogel embedded with lipofectamine-plasmid lipoplexes was shown to be useful for transfection of cells on top of the gel (2D) and within the gel (3D) (11).

While potentially useful, the feasibility of applying aforementioned methods to making cell-based microarrays has not yet been demonstrated.

Using a PDL-coated PS microplate, we have developed a method to print plasmid mixed with transfection reagents (effectene) and gelatin into a DNA microarray for reverse transfection (12). This ready-to-transfect DNA microarray could be stored at 4°C for up to 1 year without significant loss of transfection efficiency. While our paper was still in press, another group reported a similar method but on poly (vinyl alcohol) (PVA) surface pre-patterned with sodium hypochlorite (NaOCl) (13).

A growing list of adherent cells with different tissue or species origin such as A549, cos7, and *Drosophila* cells has been successfully used for reverse transfection. Human embryonic kidney 293T (HEK293T), a cell line derived from transforming HEK293 cells with SV40 large T gene, is one of the most widely used cell lines. The fast-growing HEK293T cells, with a doubling time of 16–20 h, are relatively easy to transfect with 40–80% of transfection efficiency.

The transfection efficiency is affected by multiple factors, including cell type, the size, purity, and amount of plasmid DNA, transfection reagents, as well as transfection formats (surface mediated vs. solution based). The last two factors have been systematically examined (14, 15). On a particular surface with a given cell type, optimizing transfection conditions are often necessary to achieve high transfection efficiency. In this chapter, using a two-plasmid model (one for GFP and one for LacZ), we have described a detailed protocol for making a ready-to-transfect DNA microarray in a 96-well microplate and optimal co-transfection of HEK293T cells for creating a cell-based microarray for two-color assays.

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## 2. Materials

### 2.1. Microarray Fabrication

1. pHMGFP, plasmid containing the gene for green fluorescent protein (GFP) (Promega, Madison, WI). Store at –20°C.
2. pcDNA3.1/V5-His/lacZ, plasmid containing the gene for LacZ, and its vector phRL-SV40 (Invitrogen Co., Carlsbad, CA). Store at –20°C.
3. Gelatin, 12% (w/v) in deionized (DI) water. Store at 4°C. Prepare working solution by mixing 10 µl of 12% gelatin with 90 µl of DI water to make final concentration of 1.2%, and store at 4°C up to 1 month.

4. Poly-D-lysine (PDL)-coated microplates (Corning Incorporated, Lowell, MA). Store at 4°C.
5. 384-well plate reservoir (Corning Incorporated, Lowell, MA).
6. Effectene transfection reagent kit, including EC buffer, enhancer, and effectene (Qiagen, Valencia, CA). Store at 4°C.
7. 1.5 M Sucrose in DI water (Invitrogen Co., Carlsbad, CA). Store at 4°C.
8. Chipmaker Micro Spotting Pin CMP10B (Arrayit Corporation, former Telechem International, Inc., Sunnyvale, CA).

## **2.2. HEK293T Cell Culture**

1. Human embryonic kidney cell line HEK293T (GenHunter, Nashville, TN).
2. Fetal calf serum (FCS). Store at -20°C.
3. Pen Strep: 10,000 units/ml penicillin, streptomycin 10,000 µg/ml. Store at -20°C.
4. Dulbecco's modified eagle medium (DMEM). Store at 4°C.
5. Complete medium: DMEM 500 ml, FCS 10% (v/v), Pen Strep 1% (v/v) (final concentration of penicillin 100 units/ml and streptomycin 100 µg/ml). Store at 4°C for up to 6 months.
6. Trypsin-EDTA: 0.025% (w/v) trypsin and 0.01% (w/v) EDTA (ethylenediaminetetraacetic acid in a phosphate buffer salt solution with 5 mM glucose) (Invitrogen Co., Carlsbad, CA). Store at 4°C.
7. Phosphate buffered saline (PBS). Store at 4°C.
8. Dimethylsulfate (DMSO).
9. Tissue-culture-treated (TCT) flasks, T-75, and T-150.
10. 15- and 50-ml centrifuge tubes.

## **2.3. Cell Microarray Assay**

1. 0.2% Triton X-100: prepare a working solution, 0.2 ml triton X-100, 99.8 ml PBS.
2. Ten percent goat serum (Invitrogen Co., Carlsbad, CA): prepare a working (blocking) solution, 1 ml goat serum, 9 ml PBS.
3. Primary antibody, anti-Lac Z mouse IgG (Santa Cruz Biotechnology, Santa Cruz, CA).
4. Secondary antibody, Cy3-labeled goat anti-mouse IgG (Jackson ImmunoResearch Laboratories, West Grove, PA).
5. Ninety-six-well microplate aluminum sealing tape (Corning Incorporated, Lowell, MA).

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### 3. Methods

#### 3.1. Transformation and Bacterial Culture

If starting with a plasmid preparation, it is necessary to make a bacterial clone for replicating the plasmid via transformation. Many companies sell *Escherichia coli* competent cells accompanied with a detailed protocol for transformation. We have used the *E. coli* HB101 competent cells from Invitrogen (Carlsbad, CA) for cloning purpose.

#### 3.2. Plasmid Amplification and Purification

A plasmid is amplified in a 200-ml bacterial culture. It is isolated and purified with an alkaline method using a QIAGEN Plasmid Plus Maxi kit according to the manufacturer's protocol (QIAGEN Inc., Valencia, CA).

#### 3.3. Microarray Sample Preparation

1. Add 1  $\mu\text{l}$  each of the phMGFP (250 ng/ $\mu\text{l}$ ) and the pcDNA3.1/V5-His/lacZ (250 ng/ $\mu\text{l}$ ) plasmid DNAs (250 ng each) to 9.5  $\mu\text{l}$  EC buffer to a final volume of 11.5  $\mu\text{l}$  (*see Note 1*).
2. Add 2  $\mu\text{l}$  of enhancer, 1.2  $\mu\text{l}$  of 1.5 M sucrose, and 2  $\mu\text{l}$  of effectene to a total volume of 16.7  $\mu\text{l}$ . The DNA concentration is  $\sim 30$  ng/ $\mu\text{l}$  (*see Note 2*).
3. Incubate the above mix at room temperature for 15 min to allow the formation of DNA and transfection reagent complexes.
4. Add 12  $\mu\text{l}$  of 1.2% gelatin to make a final DNA concentration of  $\sim 18$  ng/ $\mu\text{l}$  with each plasmid at 9 ng/ $\mu\text{l}$ .
5. Load all 28.7  $\mu\text{l}$  of the sample (mixture of plasmid DNA, transfection reagents, and gelatin) in a well of a 384-well microplate until use. Although it is desirable to use the sample for printing right away, it may be kept at room temperature for up to 2 h without any noticeable effect.

#### 3.4. Microarray Fabrication

1. Prior to sample preparation, turn on the PixSys 5,500 printer (Cartesian Technologies, Irvine, CA), set relative humidity (RH) to 70%, and let it warm up for 1 h.
2. Prior to printing, turn on warm DI water (45–50°C) circulator and vacuum pump. Warm water helps to clean the quill pin thoroughly, but is optional.
3. Place the 384-well microplate carrying the sample onto the source plate holder.
4. Pre-warm a 96-well PDL-coated microplate for 10 min to room temperature, and place it onto a sample plate holder.
5. Place a clean 3×4-inch glass slide onto a slide holder.

6. Place a CMP10B pin onto the pinhead. With the CMP10B pin, the diameter of a microspot is  $\sim 365\ \mu\text{m}$ . With a  $600\ \mu\text{m}$  center-to-center spot distance, a microarray containing up to 36 microspots ( $6\times 6$ ) may be printed in each well. If printing three replicate microspots per sample, then up to 12 samples can fit into one well.
7. Print microarrays at the bottom of individual wells of the 96-well PDL-coated microplate using the following program:
  - a. Move the pin to water bath, rinse it, and vacuum dry it for 1 s each; repeat the pin wash cycle four times.
  - b. Move the pin to source plate, dip it into the sample for 3 s to ensure that the quill (slit) is fully filled ( $0.6\ \mu\text{l}$ ).
  - c. Move the pin to the glass slide and blot 20 dots to remove excess sample outside of the pin.
  - d. Move the pin to a well of the 96-well PDL-coated microplate and print three replicate microspots at the bottom; move it to next well to print three microspots; and so forth until the first four columns of 32 wells are printed (with a total of 96 microspots).
  - e. Repeat Steps “a” through “d” twice until the entire plate is printed with a total of 288 microspots per sample.
  - f. Repeat Steps “a” through “e” to print second sample until all the samples are printed. Up to 12 samples may be printed in one 96-well PDL-coated microplate.
8. Dry the printed microarray plate in a desiccator for 1 h at room temperature. The humidity in the desiccator should be  $<20\%$ .
9. Cover the dried microplate with a lid, wrap with a piece of parafilm, and store in a desiccator at  $4^\circ\text{C}$  till use. The printed microarrays are stable for up to 1 year under this storage condition.

### **3.5. HEK293T Cell Preparation**

1. Pre-warm complete medium (DMEM with 10% FCS and 1% Pen Strep) in a  $37^\circ\text{C}$  water bath for 15–30 min. The pre-warmed complete medium is used in all subsequent steps.
2. Take out a frozen vial of HEK293T cells from a liquid nitrogen tank (typically contains  $2\text{--}4\times 10^6$  cells), while holding it, immediately place the vial in  $37^\circ\text{C}$  water, and gently swirl it until the liquid inside of the vial is completely thawed.
3. Take the vial out from water and spray it thoroughly with 70% ethanol to sterilize the surface, and place it in a laminar flow hood. All of the following steps are done in the hood.
4. Carefully open the vial and transfer all the cells inside to a T-75 flask filled with 20 ml of complete medium.

5. Place the T-75 flask into a CO<sub>2</sub> (5%) incubator set at 37°C with 95% humidity to let cells attach and grow overnight.
6. Take the T-75 flask out from the CO<sub>2</sub> incubator, gently aspirate off the used medium to completely remove DMSO contained in the frozen vial, and add 20 ml of fresh complete medium.
7. Put the T-75 flask into the CO<sub>2</sub> (5%) incubator set at 37°C with 95% humidity, and continue to grow cells until the cells reach 80–90% confluency ( $\sim 5\text{--}11 \times 10^4$  cells/cm<sup>2</sup>) by visual inspection under a microscope. It typically takes 1–2 days to yield  $\sim 4\text{--}8 \times 10^6$  cells per flask depending on the number of viable cells at beginning. It is important to avoid letting cells reach 100% confluency as many cells start to die or become unhealthy.
8. Aspirate off the medium and gently wash the cells with 5 ml of PBS to remove trypsin inhibitors that may come from serum in the medium.
9. Aspirate off the PBS and trypsinize the cells with 1 ml of trypsin–EDTA for 2–3 min at room temperature. The cells can be readily detached from the surface by gently tapping the flask. Do not shake the flask vigorously and make sure that cells do not sit in trypsin–EDTA for >10 min.
10. Add 5 ml of complete medium to the flask to stop trypsinization, break cell clumps by gently pipetting up and down several times without making bubbles, and transfer all the cells to a 15-ml centrifuge tube.
11. Centrifuge at  $\sim 1,000 \times g$  for 3 min to pellet cells (3,000 rpm in Baxter Scientific Centrifuge Model 2742 Biofuge 17, Heraeus Sepatech, Germany). Gently pour off supernatant to remove trypsin–EDTA, and resuspend the cells in 6 ml of complete medium.
12. The resulting cells may be (i) used for reverse transfection if  $< 4\text{--}8 \times 10^6$  cells are needed, (ii) further propagated for a large scale reverse transfection, (iii) split (1:10 or 1:20) for maintenance up to 10 passages, and (iv) propagated for making more frozen vials.
13. To directly use the cells for reverse transfection, proceed to Step 18 for cell count.
14. To propagate cells for a large scale reverse transfection, add 1 ml of the cells ( $\sim 7\text{--}13 \times 10^5$  cells) into each of five T-75 flasks filled with 20 ml of complete medium (*see Note 3*).
15. Culture cells in a CO<sub>2</sub> (5%) incubator set at 37°C with 95% humidity overnight to let cells attach and grow.
16. Change the medium the next day, and then every other day until the cells reach 80–90% confluency by visual inspection under a microscope.

17. Harvest cells by repeating Steps 8–11 and transfer the resulting 6 ml of cells from each T-75 flask to a 50-ml centrifuge tube (pool all the cells from multiple flasks into one centrifuge tube).
18. Take 50  $\mu$ l of the cell suspension for cell count using a Beckman-Coulter Cell Counter (Fullerton, CA) following manufacturer's instructions (*see* **Note 4**). The cells are now ready for reverse transfection (proceed to **Section 3.6**).
19. For cell passage, add 1 ml of the cells from Step 11 into a T-75 flask filled with 20 ml complete medium and repeat Step 15–16 until cells reach 80–90% confluency. To keep HEK293T cells healthy, which is critical for efficient reverse transfection, it is recommended to use the cells within 10 passages.
20. For making a large quantity of frozen vials of cell stock at passage #1, add 5 ml of the cells from Step 11 into a T-150 flask filled with 40 ml of complete medium, and repeat Step 15–16 until cells reach 80–90%. Trypsinize the cells with 6 ml Trypsin–EDTA for 3–5 min, add 30 ml of complete medium, and count cells. Pellet cells and re-suspend the cells in freezing medium (85% DMEM, 10% CFS, 5% DMSO) at  $2\text{--}4 \times 10^6$  cells/ml (as described in Step 8–11). Aliquot the cells at 1 ml per vial, store them at  $-80^\circ\text{C}$  overnight, and then transfer them to the vapor phase of a liquid nitrogen tank for long-term storage.

### **3.6. Reverse Transfection**

1. Centrifuge the cells from Step 17 (in **Section 3.5**) at  $\sim 1,000 \times g$  for 5 min, and aspirate off the medium.
2. Re-suspend the cells gently in an appropriate volume of complete medium to make final concentration of  $7 \times 10^5$  cells/ml based on the total cell number obtained from Step 18 (in **Section 3.5**).
3. Use an automatic pipettor to add 100  $\mu$ l of the cells into each well of a 96-well printed microarray plate ( $7 \times 10^4$  cells/well). Make sure that there are no air bubbles trapped in the bottom of individual wells, specifically between the cells and the surface. If there is a bubble, remove it immediately by pipetting out the cells and gently adding them back into the well. Having no barriers for cells to attach to the surface is crucial for the success of reverse transfection.
4. Incubate the plate in a  $\text{CO}_2$  (5%) incubator set at  $37^\circ\text{C}$  with 95% humidity overnight. With exogenous GFP expression, patches of transfected cells are first detected after 16–24 h. To ensure maximum transgene expression, cell microarrays are usually assayed after 48 h.



### **3.7. Cell Microarray Assay**

1. Remove media with a pipette carefully and gently to avoid dislodging cells as transfected HEK293T cells usually become less adherent and easily detached. To prevent cells from drying, it is recommended to work with no more than eight wells at a time when doing the assay manually.
2. Wash each well with 100  $\mu$ l of PBS twice.
3. Add 100  $\mu$ l of 4% (v/v) formaldehyde in PBS carefully and slowly down the wall.
4. Incubate for 10 min at room temperature; wash once with 100  $\mu$ l PBS.
5. Add 100  $\mu$ l of 0.2% Triton X-100 very carefully down the wall.
6. Incubate for 5 min at room temperature. This is a permeabilizing agent and therefore a difficult step since there is a high chance that cells may be washed off.
7. Add 100  $\mu$ l of blocking solution (10% goat serum in PBS) to each well and incubate for 15 min at room temperature.
8. Dilute primary antibody, anti-LacZ mouse IgG, in PBS (usually between 1:20 and 1:500), and add 100  $\mu$ l of the diluted primary antibody in each well.
9. Incubate for 1 h at room temperature for detecting an exogenous protein. The time needed for detecting endogenous proteins may be longer (up to 2 h).
10. Remove the primary antibody and carefully wash three times with 100  $\mu$ l of PBS.
11. Dilute fluorescently labeled secondary antibody, Cy3-labeled goat anti-mouse IgG, in PBS (usually 1:500), and add 100  $\mu$ l of the diluted secondary antibody in each well.
12. Incubate for 1 h in the dark.
13. Remove the secondary antibody and carefully wash three times with 100  $\mu$ l of PBS.
14. Cells can be stored with foil covering over the microplate at 4°C until ready to image.

### **3.8. Cell Microarray Imaging and Data Analysis**

1. Carefully flip the microplate on a piece of paper towel to drain all the liquid in the wells, and seal the wells with a piece of microplate sealing tape.
2. Scan the microplate for GFP signals with a 488-nm laser and a 532-nm filter (488 nm<sub>ex</sub>/535 nm<sub>em</sub>) at a PMT gain of 190, and LacZ signals (labeled with Cy3) with a 532-nm laser and a 590-nm filter (532 nm<sub>ex</sub>/590 nm<sub>em</sub>) at PMT gain of 210 in a Tecan LS400 fluorescent scanner (Research Triangle Park, NC).

3. The images may be analyzed with Array Pro Analyzer (provided by Tecan). Individual cell microspots are circled, and relative fluorescent units (RFU) within a circle are measured (*see Note 5*). The data output is exported to Excel, further calculated for average signal intensity of three replicate spots and standard deviation, and graphed (*see Note 6*).

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## 4. Notes

1. Affected by purity and size, the optimal DNA amount for high transfection efficiency varies from plasmid to plasmid, and sometimes even from prep to prep. For a given plasmid DNA prep, it is highly recommended to test a range of quantities first to determine the optimal amount. We typically tested the range of 100–1,000 ng, translating to 3.5–35 ng/ $\mu$ l after mixing with transfection reagents to a total volume of 28.7  $\mu$ l. With a given phMEGF plasmid DNA prep, the effect of DNA amount is shown as an example in **Fig. 2.1**. The highest transfection efficiency is achieved with 250 ng (8.7 ng/ $\mu$ l). For co-transfection, similar tests are done by varying amounts of the two plasmids in a two-way titration (e.g., 100, 250, 500, and 750 ng) to determine the optimal amount and ratio of the two plasmids. For the combination of phMGFP plasmid and pcDNA3.1/V5-His/lacZ plasmid, we have found that a 1:1 ratio (250 ng each) works the best (12).
2. Effectene is a nonliposomal lipid reagent and has been routinely used for solution-based transfection. In an effort to optimize surface-mediated transfection in a ready-to-transfect format, i.e., printing DNA together with transfection reagents, varying amounts of enhancer (4–8  $\mu$ l) and effectene (2–4  $\mu$ l) in the final DNA-transfection reagent mix have been tested. The difference seems negligible at least for the phMGFP plasmid and HEK293T cells as shown in **Fig. 2.2**. Lipofectamine 2000 from Invitrogen has also been shown to work (data not shown).
3. The way to propagate cells for reverse transfection can be very flexible in terms of the number and the size of flasks or Petri dishes and the ratio of cells to medium (1:20–5:20) used; all depend on the timing and the scale for the next reverse transfection experiment. One may use the following information as guidelines. When HEK293T cells are cultured in complete medium, approximately  $4\text{--}8 \times 10^6$  cells ( $7\text{--}13 \times 10^5$  cells/ml in a total volume of 6 ml) may be harvested from a 80–90% confluent T-75

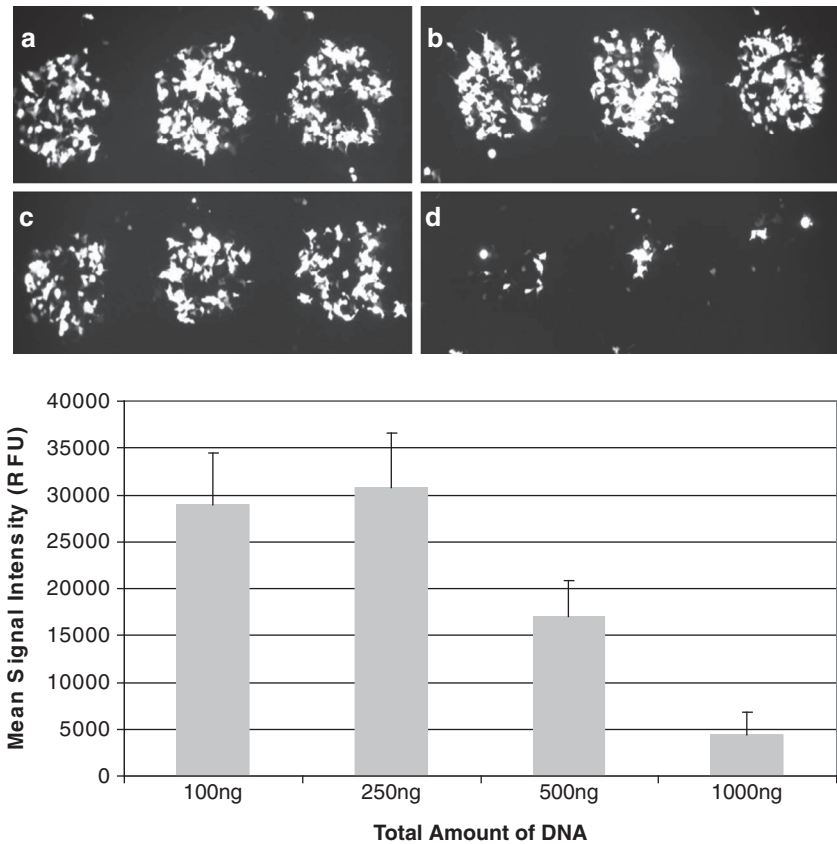


Fig. 2.1. Effect of DNA amounts on reverse transfection efficiency. *Top* is the image of a HEK293T cell microarray expressing GFP excited at 488 nm and captured at 535 nm with a Tecan scanner. HEK293T cells were transfected with different amounts of phMGFP plasmid DNA, 100 ng (a), 250 ng (b), 500 ng (c) or 1,000 ng (d) each mixed with transfection reagents and printed in triplicate spots. At the *bottom* is the histogram showing the average relative fluorescent units (RFU) of three cell microspots resulting from the reverse transfection with the amount of DNA indicated at the *bottom*.

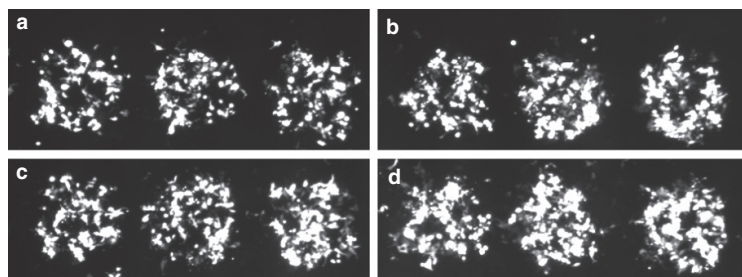


Fig. 2.2. Optimization of transfection reagents. Shown is the image, excited at 488 nm and captured at 535 nm with a Tecan scanner, of a HEK293T cell microarray expressing GFP generated by mixing 250 ng of phMGFP plasmid DNA with various amounts of enhancer and effectene. The combinations tested were (a) 4  $\mu$ l enhancer + 2  $\mu$ l effectene, (b) 4  $\mu$ l enhancer + 4  $\mu$ l effectene, (c) 8  $\mu$ l enhancer + 2  $\mu$ l effectene, and (d) 8  $\mu$ l enhancer + 4  $\mu$ l effectene.

flask ( $5\text{--}11 \times 10^4$  cells/cm<sup>2</sup>). If splitting at 1:20 (1 ml cells in 20 ml medium) ratio in a T-75 flask ( $7\text{--}13 \times 10^5$  cells,  $\sim 9\text{--}17 \times 10^3$  cells/cm<sup>2</sup>), it takes 4–5 days to reach 80–90% confluency.

4. We have used a cell counter to count cells for convenience and minimizing human error. The downside of this method is that there is no information regarding the percent of viable cells, an important indicator of the cell quality for reverse transfection. The problem may be circumvented by not using overly confluent cells (>90%). Conventional hemacytometer can also be used for cell count. Using trypan blue staining for viable cell count is desirable but not necessary.
5. Any microarray imaging system equipped with proper lasers and filters can be used for imaging cell-based microarray. Since the resolution of a microarray imaging system is typically at 5–10 microns per pixel, one can collect only average signal from multiple transfected cells within a microspot, demanding high transfection efficiency for sufficient signals. Moreover, the shape of individual cell microspots is often irregular, and signals within a microspot are blotchy; therefore, defining a microspot area can be tricky and often leads to big spot to spot variations.
6. We have also used the Discovery-1 automatic fluorescence microscope (Molecular Devices Co., Sunnyvale, CA) at 2× or 10× images and Zeiss Axiovert 135 microscope (Carl Zeiss, Thornwood, NY) at 2.5× or 20× objectives for imaging cell-based microarrays. Both imaging systems offer single cell resolution. Coupled with MetaMorph software, fluorescent signals from individual transfected cells can be measured and quantified, which is especially helpful for calculating co-transfection efficiency.

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Cell-Based Microarrays

Methods and Protocols

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