Reverse Transfection of Mammalian Cells for Functional Screening: Results from Testing the BioOdyssey™ Calligrapher™ MiniArrayer

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Introduction

RNA interference (RNAi) is a recent advancement that can reduce the expression of specific target genes, with potential applications on a genome-wide scale. On the other hand, overexpression of organelle-specific markers (GFP-tagged cDNAs) using reverse transfection can be the method of choice for functional screening-based experiments. By using 8-well Lab-Tek Chamber Slides (Nunc), it is possible to have eight replicates of a subgrid, consisting of different spotted small interfering RNAs (siRNAs) or cDNAs that are ready to transfect and use for different applications. One such use in drug discovery is to increase the efficiency of transfection or treatments by influencing downregulation or overexpression by adding certain biological or chemical compounds to each well, and simultaneously monitoring the effects by using time-lapse high-throughput screening. However, to achieve this, robust methodologies that allow automated and efficient delivery of siRNAs or cDNAs into living cultured cells, and reliable quality control of siRNA or cDNA function, must be in place.

Here, we describe optimization of reverse transfection of cultured cells with siRNA or cDNA for subsequent high-content screening microscopy applications (Figure 1). All the necessary transfection components are mixed prior to robotic spotting on noncoated 8-chambered cover glass tissue culture dishes, which are ideally suited for time-lapse microscopy applications in living cells. The addition of fibronectin to the spotting solution improves cell adherence and transfection efficiency. After cell seeding, no further cell culture manipulation, such as medium changes, is needed. Calculation of the cell density improves autofocus performance for high-quality data acquisition and cell recognition.

In this report, we confirm known mitotic phenotypes after knockdown with specific siRNAs, such as INCENP (multinucleated cells, segregation problems), PLK1 (prometaphase arrest), and TPX2 (prometaphase arrest), with a transfection efficiency of up to 80%. We also studied localization of different GFP-tagged cDNAs in different organelles and constructs in mammalian cells, with a transfection efficiency of up to 43% (data not shown).

Methods

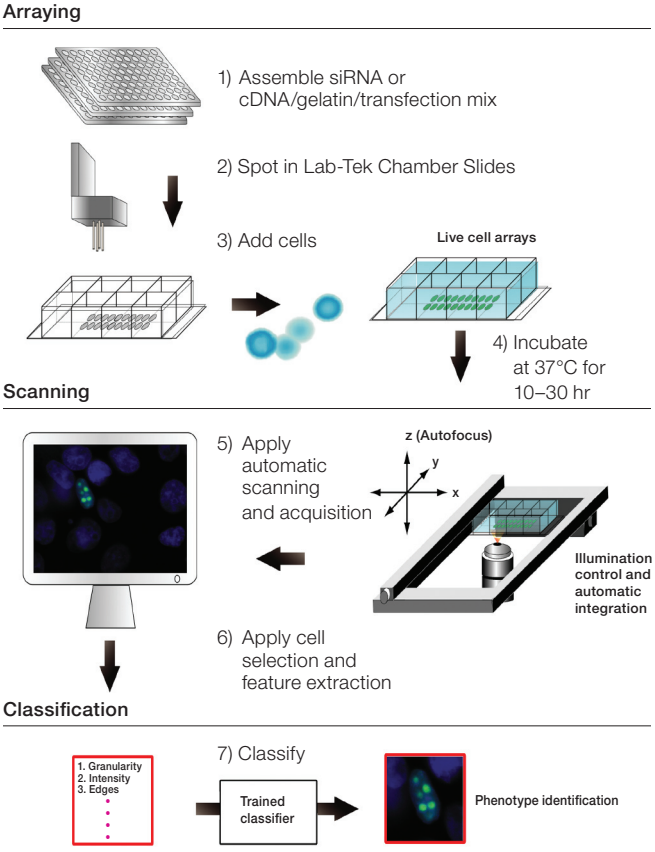

Fig. 1. Reverse transfection and automatic image acquisition workflow. **1**, different solutions of transfection mix, cDNA or siRNA, and gelatin are prepared in 384-well plates; **2**, solutions are spotted in arrays into chambered cover glass tissue culture dishes (Lab-Tek Chamber Slides, Nunc), using the BioOdyssey Calligrapher miniarrayer; **3**, cultured cells are added to the Chamber Slides and transfected with cDNA or siRNA on the arrayed spots; **4**, after 10–30 hr of incubation, the fluorescent signal of the expressed GFP-labeled protein can be visualized; **5**, on the automatic scanning platform, a motorized stage and motorized z-stepper perform scanning of live cells; **6**, known phenotypes are selected and further extracted with different software algorithms; **7**, phenotypes are identified by trained classifier (Conrad et al. 2004).

Preparation of Transfection Mix

siRNA — In a 384-well plate, 3 µl of 0.4 M sucrose in Opti-MEM I with GlutaMAX (Invitrogen Corporation) was mixed with 3.5 µl of Lipofectamine 2000 (Invitrogen) and 5 µl of siRNA (390 ng/µl). The mixture was incubated at room temperature for 20 min, so that the transfection complexes or siRNA (Integrated DNA Technologies, IDT) could form. Then 7.25 µl of gelatin/fibronectin mixture with a ratio of 100/1 (v/v), was pipetted into each well, bringing the total volume to 18.75 µl. The mixture was incubated again for 15 min followed by short centrifugation (Erfle et al. 2007).

cDNA — The same protocol that was used for preparing the siRNA mix was used to prepare the cDNA mix, with the exception that 2 µl of cDNA (500 ng/µl) was used and the total volume of the mixture was 16.75 µl.

Spotting

The BioOdyssey Calligrapher miniarrayer was used to print eight replicate subgrids with 25 (5 × 5) spots in each well of a Lab-Tek Chamber Slide, for a total of 200 spots printed on a single slide (Figure 2). One solid pin used for spotting was washed with distilled water. Humidity was set at 57% to avoid sample evaporation.

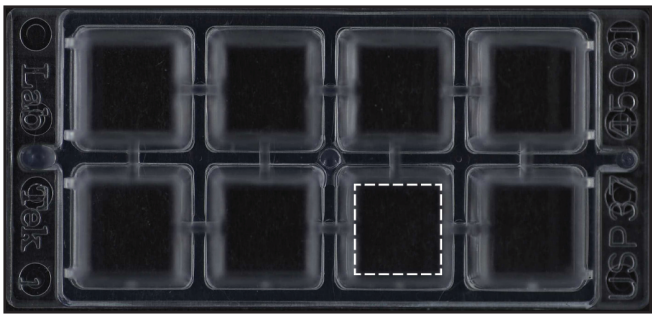

Fig. 2. 8-well Lab-Tek Chamber Slide. Each slide is 74 × 22 mm with a thickness of 0.13–0.17 mm and culture area of 0.8 cm^2. The dashed square presents a well of the Chamber Slide (see Figure 3 for a zoomed spotted version).

The middle spot/point of each well was marked with a pen before seeding of cells to provide the position coordinates for the microscope software (Figure 3). Each well contained several different siRNAs (Figure 4).

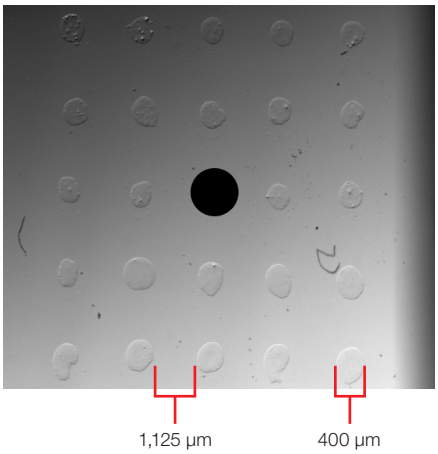

Fig. 3. Dried spots in one well of a Lab-Tek Chamber Slide. A total of 25 spots with a distance between each spot of ~1,125 µm and diameters of ~400 µm. The black dot in the center is a mark made by a pen on the external surface of the slide to provide the position for the microscope software.

INCENP-A1	INCENP-A2	INCENP-A3	TPX2-A1	TPX2-A2
TPX2-A3	TPX2-B1	TPX2-B2	TPX2-B3	PLK1-A1
PLK1-A2	PLK1-A3		PLK1-B1	PLK1-B2
PLK1-B3	INCENP-B1	INCENP-B2	INCENP-B3	TPX2-C1
TPX2-C2	TPX2-C3	INCENP-C1	INCENP-C2	INCENP-C3

Fig. 4. Different siRNAs spotted in a single well of a Lab-Tek Chamber Slide.

Seeding of HeLa "Kyoto" (H2B-GFP) Cells

Cells were passaged the day before they were seeded. For each well of a Lab-Tek Chamber Slide, approximately 3 drops (100 µl) of a cell suspension was determined to be optimal for ease of pipetting and to prevent dessication. HeLa "Kyoto" cells (Neumann et al. 2006) were seeded at a density of 0.4 x 10^{-5} cell/µl using a 10 ml pipet to plate drops in the middle of the well while shaking slightly (in a figure-eight movement) to obtain a homogenous distribution of the cells on the surface of each well. Because the seeding procedure stresses the cells, they were immediately returned to the 5% CO_2 incubator and incubated for ~24–30 hr at 37°C.

Acquisition of Images

Images were taken with a high-throughput automated microscope (scan^R screening station, Olympus Europa GmbH). By fixing the midpoint of each well, and calculating the distance between spots and number of subgrids, the microscope captures images from the position of interest using a laser autofocus system (Figure 5).

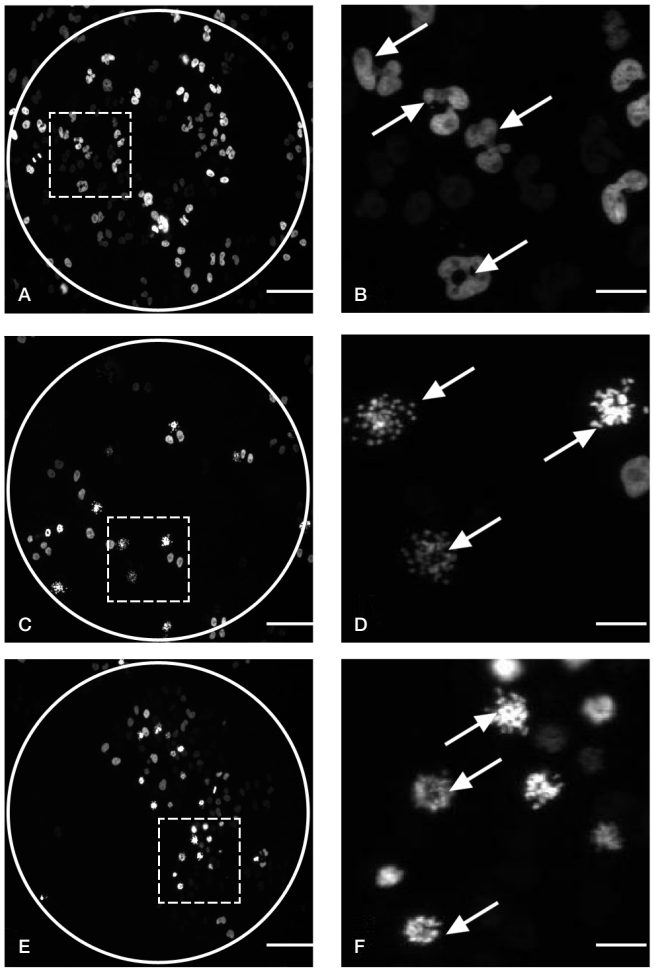

Fig. 5. Knockdown of three targets in HeLa "Kyoto" (H2B-GFP) cell line. Confirmation of known mitotic phenotypes, such as INCENP (multinucleated cells, segregation problems) (**A** and **B**); PLK1 (prometaphase arrest) (**C** and **D**), and TPX2 (prometaphase arrest) (**E** and **F**), with an efficiency of up to 80%. Panels B, D, and F are high-magnification versions of panels A, C, and E, respectively. Arrows point to phenotypes with mitotic defects. Bars, 20 µm (A, C, and E) and ~5 µm (B, D, and F).

Conclusions

We have demonstrated that effective gene silencing can be obtained using reverse transfection in plates with multiple wells. Each well of the Lab-Tek Chamber Slide possesses a number of different siRNAs, allowing us to assay multiple conditions simultaneously. In order to use these plates, an instrument that is capable of accurate spotting is required. The BioOdyssey Calligrapher miniarrayer fulfilled this requirement, allowing accurate placement of spots within the grids that were then imaged after cells were transfected. The BioOdyssey Calligrapher miniarrayer can be easily integrated into a reverse transfection protocol to successfully perform functional screening experiments.

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