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Transfected Cell Arrays for Assessment of Estrogen Receptor Activation in Breast Cancer Cells

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Transfected cell arrays represent a high-throughput approach to correlate gene expression with functional cell responses, based on gene delivery from a substrate that supports cell adhesion. These arrays provide the ability to express, in parallel, thousands of exogenous genes in live cells, giving real-time information on cellular physiology and gene function. While there have been advances in transfected cell arrays, improvements are needed to this technology, including increasing the cells types that can be efficiently transfected and developing better quantification and normalization methods. We have created an array using soft lithography techniques to pattern DNA-lipid complex deposition. Specifically, a mold was fabricated by curing polydimethylsiloxane (PDMS) into thin, flat disks. Rods of precise diameters were then used to punch holes into the mold, with diameters ranging from 1 mm to 3 mm. The PDMS mold was oxidized and then reversibly sealed to polystyrene slides. The holes in the mold, termed microwells, served as reservoirs for complex deposition onto the polystyrene slides. After deposition, the PDMS mold was peeled away from the polystyrene slide, which was then rinsed thoroughly. DNA complexes were immobilized on the slide in distinct regions, replicating the pattern of microwells in the PDMS mold. Transfection of cells seeded onto these slides was also confined to the patterns.

The array was then used to deliver and express DNA sequences that report on the activity of the estrogen re-

ceptor (ER) pathway in ER-positive, estrogen-responsive human breast cancer cells. Estrogen receptor α (ER α) expression is the most important biomarker for determining treatment course for clinical breast cancer. Estrogens, via ER α , act as potent mitogens of ER-positive breast cancer. To assess ER α activity, arrays were formed with DNA complexes containing an estrogen-responsive reporter plasmid, pERE(3x)TK-Luc, which contains three repeats of the estrogen response element (ERE) sequence upstream of a minimal thymidine kinase (TK) promoter. This regulated promoter directs firefly luciferase expression in response to transcriptional activation by estradiol (E₂)-bound ER α , followed by recruitment of cofactor complexes and basal transcriptional machinery. To normalize for transfection efficiency, DNA complexes also included the pTK-RenLuc plasmid, which contains the same TK promoter driving Renilla luciferase expression. ER-positive MCF-7 breast cancer cells were seeded onto the immobilized DNA-lipid complexes and treated with combinations of E₂, the complete antiestrogen fulvestrant or vehicle controls. Firefly and Renilla luciferase expression were analyzed using real-time luciferase imaging, accomplished by adding both luciferase substrates into the media above the cells. Luciferase imaging allowed rapid and quantitative imaging of two luciferase reporter genes in the same cell, which enabled normalization of ER activity.

