BioOdyssey™ Calligrapher™ MiniArrayer: Screening for Gene Silencing

David Primrose,* Luis Ugozzoli,** and Edan Foley*
* Dept. of Medical Microbiology and Immunology, University of Alberta, Edmonton AB Canada T6G 2S2
** Bio-Rad Laboratories, Inc., Hercules, CA 94547 USA

Introduction

As a direct consequence of the completion of sequencing the human and other genomes (Venter et al. 2001, Nierman et al. 2001), there is a growing collection of gene sequences that require high-throughput approaches for studying and characterizing gene function. The discovery of RNA interference (RNAi) has provided a new tool for functional analysis by silencing specific genes, allowing elucidation of biochemical pathways.

RNAi is a natural, evolutionarily conserved mechanism for sequence-specific posttranscriptional gene silencing. RNAi, which is triggered by double-stranded RNA (dsRNA), is thought to inhibit the replication and synthesis of genome invaders such as viruses and transposons (Zamore 2002). Biochemical characterization demonstrates that RNAi is mediated by the production of 21- to 23-base pair dsRNA molecules, named small interfering RNAs (siRNAs). Molecules of dsRNA are cleaved by Dicer, an RNase III-like enzyme, to generate siRNAs, which subsequently direct the recognition and degradation of homologous messenger RNA (mRNA) by a multiprotein complex. Although it was discovered as a natural process (Hannon 2002), RNAi has proven to be a powerful research tool for efficient gene silencing, allowing scientists to characterize the function of individual genes.

Recently, reverse transfection, a novel application of microarray-based analysis, has been applied to cDNA library screening (Silva et al. 2004, Mousses et al. 2003, Williams et al. 2003, Wheeler et al. 2004). In this method, plasmids suspended in gelatin are arrayed on a microscope slide. After drying, the slide is covered with a lipid-based transfection reagent. Following removal of excess lipid, cells are grown in a monolayer on the slide. The functional effects of cell-based transfection in the microarray can be assessed visually after 24–48 hr. The technique requires a phenotype that is readily identifiable, such as apoptosis, or the use of a reporter gene, such as GFP, for visualization.

In this paper, we demonstrate the utility of a personal benchtop arrayer, the BioOdyssey Calligrapher miniarrayer, for creating microarrays for such screens. To demonstrate proof-of-principle of this technique, we spotted dsRNAs that target the *Drosophila melanogaster* caspase inhibitor dIAP1 or a control gene and then grew S2 cells on the slides. The inactivation of the dIAP1 gene led to cell death, which was visualized by staining the arrays with a fluorescent dye that is taken up only by live cells.

Methods

A 5 x 5 grid of dsRNA that generates siRNA selectively targeting dIAP1 was printed onto DS8 slides (Erie Scientific Co.) using a BioOdyssey Calligrapher miniarrayer equipped with a single pin. Default miniarrayer conditions were used for washing. Each spot was approximately 200 µm in diameter. One grid was printed with dsRNA specific for dIAP1, while another was printed with dsRNA targeting a nonessential gene to serve as a negative control. S2 cells were allowed to grow on the array for 48 hr to allow RNAi to occur. Cells were then stained with Alexa Fluor 568-labeled phalloidin, and live cells taking up the dye were visualized using a microarray laser scanner.

Results

Removing dIAP1 results in extensive caspase activation, leading to apoptosis of S2 cells. Following a 2 day incubation, the arrays were assayed for actin by staining with labeled phalloidin to reveal localization of live cells (Figure 1). Discrete areas lacking cells corresponded to the grid created by spotting the dIAP1-specific dsRNA.

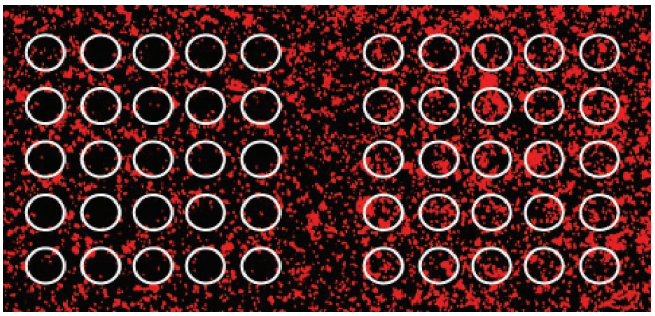

Fig. 1. S2 cells grown on a microarray of dsRNA. RNAi specific for the dIAP1 gene (left-hand grid) or a nonessential gene (right-hand grid) was generated by the cells.

Conclusions

We have demonstrated accurate assessment of gene silencing using microarray-based cellular transfection screening. The BioOdyssey Calligrapher miniarrayer allowed us to readily create grids on a microscope slide, and the grids were then overlaid with cells. We were easily able to identify which siRNA caused cell death. We will now use this technique to screen our entire cDNA library.

References

Hannon GJ, RNA interference, Nature 418, 244–251 (2002)

Mousses S et al., RNAi microarray analysis in cultured mammalian cells, Genome Res 13, 2341–2347 (2003)

Nierman WC et al., Complete genome sequence of *Caulobacter crescentus*, Proc Natl Acad Sci USA 98, 4136–4141 (2001)

Silva JM et al., RNA interference microarrays: high-throughput loss-of-function genetics in mammalian cells, Proc Natl Acad Sci USA 101, 6548–6552 (2004)

Venter JC et al., The sequence of the human genome, Science 291, 1304–1351 (2001)

Wheeler DB et al., RNAi living-cell microarrays for loss-of-function screens in *Drosophila melanogaster* cells, Nat Methods 1, 127–132 (2004)

Williams NS et al., Identification and validation of genes involved in the pathogenesis of colorectal cancer using cDNA microarrays and RNA interference, Clin Cancer Res 9, 931–946 (2003)

Zamore PD, Ancient pathways programmed by small RNAs, Science 296, 1265–1269 (2002)

Information in this tech note was current as of the date of writing (2006) and not necessarily the date this version (rev A, 2006) was published.

BIO-RAD

Bio-Rad Laboratories, Inc.

Life Science Group

Web site *www.bio-rad.com* ***USA*** *800 4BIORAD* ***Australia*** *02 9914 2800* ***Austria*** *01 877 89 01* ***Belgium*** *09 385 55 11* ***Brazil*** *55 21 3237 9400* ***Canada*** *905 712 2771* ***China*** *86 21 6426 0808* ***Czech Republic*** *420 241 430 532* ***Denmark*** *44 52 10 00* ***Finland*** *09 804 22 00* ***France*** *01 47 95 69 65* ***Germany*** *089 318 84 0* ***Greece*** *30 210 777 4396* ***Hong Kong*** *852 2789 3300* ***Hungary*** *36 1 455 8800* ***India*** *91 124 4029300/5013478* ***Israel*** *03 963 6050* ***Italy*** *39 02 216091* ***Japan*** *03 5811 6270* ***Korea*** *82 2 3473 4460* ***Mexico*** *55 5200 05 20* ***The Netherlands*** *0318 540666* ***New Zealand*** *64 9415 2280* ***Norway*** *23 38 41 30* ***Poland*** *48 22 331 99 99* ***Portugal*** *351 21 472 7700* ***Russia*** *7 095 721 14 04* ***Singapore*** *65 6415 3188* ***South Africa*** *27 0861 246 723* ***Spain*** *34 91 590 5200* ***Sweden*** *08 555 12700* ***Switzerland*** *061 717 95 55* ***Taiwan*** *886 2 2578 7189/2578 7241* ***United Kingdom*** *020 8328 2000*