High Throughput Profiling of siRNA and miRNA Libraries using Microplate Cytometry

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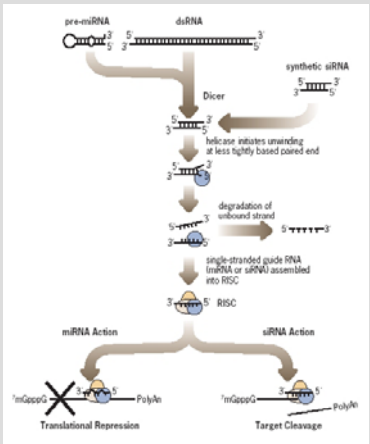
1 Abstract

The recent discovery that small interfering RNA (siRNA) technology has potent and rapid gene silencing properties has led to its rapid adoption by the scientific community for the identification of candidate drug target genes. MicroRNAs (miRNAs) are a class of small RNAs that can modulate mRNA expression in mammalian cells by blocking mRNA translation. In this study, we have used microplate cytometry for the phenotypic profiling of both siRNA and miRNA libraries in HeLa cells. Data are presented for cell proliferation in which the total cell number was determined using propidium iodide staining and an Acumen Explorer™ laser-scanning fluorescence microplate cytometer. Analysis of samples was rapid, taking about 10 minutes to process a 96 well microplate. This study supports the application of microplate cytometry for high throughput profiling of the siRNA and miRNA libraries.

2 siRNA and miRNA Processing Pathway



3 Microplate Cytometry

Microplate cytometers such as the Acumen Explorer™ are particularly suitable for running primary and secondary screens. Key capabilities include:

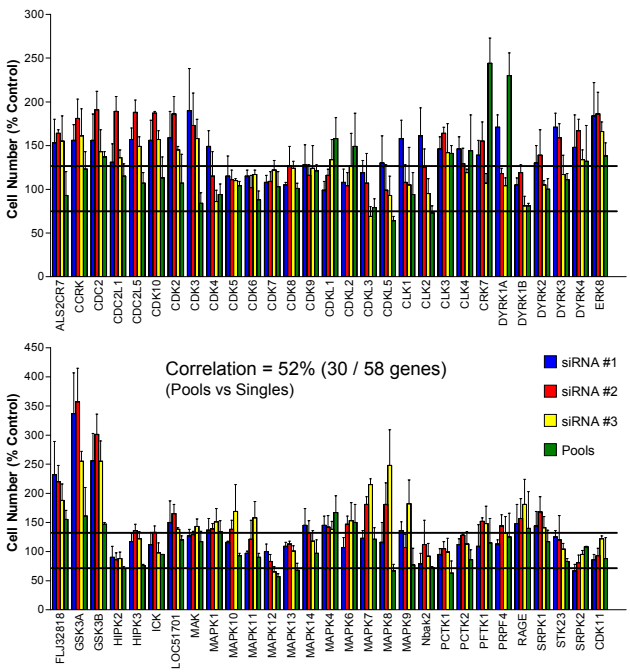
- Rapid high content screening of fluorescently labelled cells
- Simultaneous 4-colour analysis of entire wells
- Compatible with all SBS plate formats
- Screens adherent, non-adherent, live and fixed cells
- Simultaneous scanning and data processing (down to 5 minutes/plate)
- Data file sizes range from 350 MB (assay development) down to 50 KB (screening)

These features are highly compatible for the phenotypic profiling of RNAi libraries. The includes determination of the effects of RNA inhibitors on cell proliferation, essential for data normalisation.

4 Analysis of siRNA Activity in HeLa Cells

HeLa cells were screened using Silencer CellReady-96 siRNA Library Plates and examined for changes in cellular proliferation. All three design sequences and pools of Silencer CellReady-96 siRNA Library Plate sets for the CMGC subset kinases were transfected, using NeoFX, into HeLa cells in triplicate according to the recommended protocol. The cells were harvested at 72 hours post transfection and assayed for changes in cell proliferation by fixing the cells and staining with propidium iodide. After staining, cells were counted on an Acumen Explorer laser scanning fluorescence microplate cytometer (TTP LabTech).

5 Silencer Cell Ready-96 siRNA Library Screen - siRNA pools versus Singles

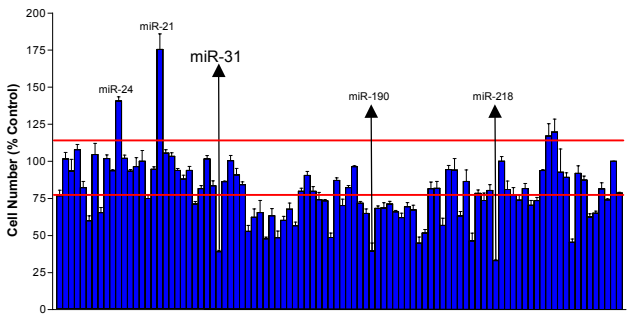


Any gene in which two of the three siRNA have an effect is considered a hit. Half (48%) of the pools of siRNA did not match with the results obtained with two single siRNA. In addition, pools of siRNA yielded more false positive hits as compared to using three single siRNA molecules per gene.

6 Analysis of miRNA Activity in HeLa Cells

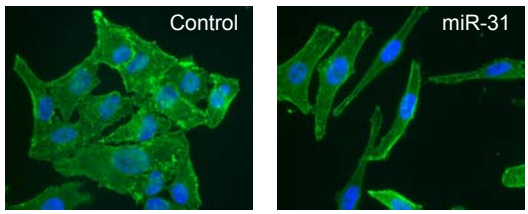
In 96-well plates, 8,000 HeLa cells were reverse transfected with miRNA inhibitors (5 pmoles) in triplicates using Ambion siPORT Neo-FX. 72 hours post-transfection, cells were fixed with 4% paraformaldehyde, permeabilised with 0.1% Triton X-100 and stained with propidium iodide. The plates were scanned using an Acumen Explorer microplate cytometer (10 minutes per plate for whole well scan) to determine total cell number. To visualize cell morphology changes in response to inhibition of miRNA function, HeLa cells were fixed post-transfection and stained with anti β-actin antibody and DAPI.

7 Identification of miRNAs That Alter Cell Proliferation



Cell counting using the Acumen Explorer revealed that inhibiting with miR-31, miR-190 and miR-216 resulted in reduced cell proliferation, while inhibiting with miR-21 and miR-24 increased cell proliferation.

8 Effect of miR-31 Transfection on Cell Morphology



Cell transfected with miR-31 inhibitor became elongated and displayed thin membrane protrusions similar in appearance to neurite outgrowths.

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