RNAi is a powerful tool to study gene function by silencing transcription. RNA interference (RNAi) is a biological process in which RNA molecules inhibit gene expression, typically by causing the destruction of specific mRNA molecules [1–3]. Gene silencing with siRNA is one of a set of functional genomics technologies that enable researchers to identify the precise role a gene plays in a specific biological process, which is one of the key steps in identifying therapeutic targets with well understood mechanisms of action.

While phenotypic screening is undergoing a resurgence, its use in conjunction with RNAi remains an expensive research strategy, mostly driven by costly assay reagents and the liquid handling and high content imaging instrumentation required to perform screening. Recently, these barriers have been lowered through the development of affordable, high quality, automated microscopy/imaging systems and the invention of miniaturized RNAi screening technologies that eliminate the requirement for automation infrastructure and reduce cost/data point by orders of magnitude. It is now possible to screen entire RNAi libraries at the bench, eliminating any need for liquid handling robotics.

Persomics technology is fundamentally different than the multi-well microplates that have become the norm in RNAi screening. Persomics plates replace the experimental well with a printed array of spots on an optical glass insert embedded in the base of a 96-well standard plate format. Each of these spots is a droplet of siRNA that contains all the reagents necessary to silence genes in cells grown over them. This library screening solution requires a limited number of workflow steps for phenotypic screening using a cellular strategy, mostly driven by costly assay reagents and the liquid handling and high content imaging instrumentation required. RNAi transfected cultures were imaged using a Cytation. Unlike microplate wells, there is no physical barrier between the individual RNAi experiments, making workflows highly parallel and technically straightforward.

Materials and Methods

Cell Culture

HeLa cells were cultured in Advanced DMEM supplemented with 10% fetal bovine serum and penicillin-streptomycin at 37 °C in 5% CO₂. Cultures were routinely trypsinized (0.05% Trypsin-EDTA) at 80% confluency. For RNAi experiments, 1 × 10⁵ cells in 10 ml of Optimem supplemented with 5% FBS and penicillin-streptomycin were plated into the ImageArray™ dish and incubated at 37 °C in 5% CO₂ for 48 hours.

Imaging

RNA transfected cultures were imaged using a Cytation 5 Microplate Imager (BioTek Instruments, Winooski, VT). Configured with DAPI, GFP and RFP light cubes. The imager uses a combination of LED light sources in conjunction with bandpass filters and diatomic mirrors to promote appropriate wavelength light. The DAPI light cubes use a 337/50 excitation filter and a 440/45 emission filter, GFP light cube uses a 469/33 excitation filter and a 525/39 emission filter, while the RFP light cube uses a 531/40 excitation and 593/40 emission filters.

Image Analysis

Three-color overlaid digital images were electronically stitched using Gen5® software. Object cell counting of the RFP channel was used to identify specific locations of microspots. Subpopulation analysis was used to determine the mean fluorescence intensity of the GFP channel as a means to assess RELA knockdown. Poly-nuclear determination was done by manual assessment of the number of nuclei in cells within the microspot.

Automated Assay Process

- **Printed RNAi Arrays can be used to consolidate Genomic-wide Expression Screens**
  - **Significance Testing**
  - **Time**
  - **Reagents**

- **Quantitative and Phenotypic Changes can be Observed**
  - **INCENP knockdowns interfere with mitosis and can be observed by polynuclear HeLa cells**
  - **RELA knockdowns can be observed by reduction in specific fluorescent antibody staining to the Nkappa-b p65 subunit protein**

- **MultiFlo FX Reagent Dispenser**
  - **Automates the Liquid handling tasks necessary for ImageArray™ Fixing and Staining**

- **Cytation 5 Cell Imaging Multi-Mode Reader**
  - **Evaluates RNAi knockdowns using the ImageArray™ Plate**

Conclusions

- **Printed RNAi Arrays can be used to consolidate Genomic-wide Expression Screens**
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- **Significance Testing**
- **Time**
- **Reagents**

- **Quantitative Image Analysis Using Gen5® Software**
- **Montage Stitching**
- **Mean Signal Determination**
- **Population analysis**

References