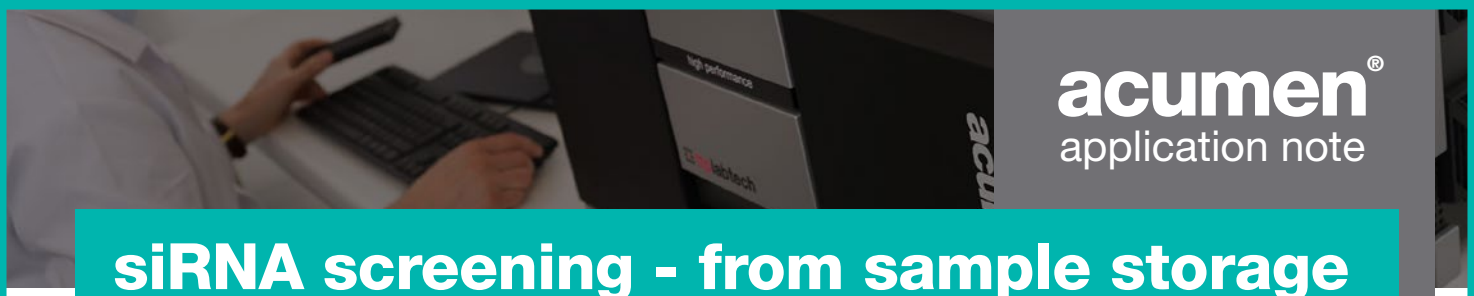


acumen®
application note

siRNA screening - from sample storage to phenotypic results

introduction

RNA interference (RNAi) is a biological process in which RNA molecules inhibit gene expression. Two types of small ribonucleic acid (RNA) molecules, microRNA (miRNA) and small interfering RNA (siRNA) are central to RNA interference. RNAi is a valuable research tool, both in cell culture and in living organisms, because synthetic dsRNA introduced into cells can selectively induce suppression of specific genes of interest.

Using these tools, genome-wide screens are commonly used to elucidate the role of individual genes in complex biological processes. By identifying gene products whose knockdown is associated with a particular phenotypic endpoint, large-scale RNA-mediated interference screens have demonstrated previously unknown components of biological pathways.

Typically siRNA screening programs are run using pooled sets of at least three siRNAs that are supplied in either a 96- or 384-well plate format and screened in a variety of ways. Hits are identified as positive results, then the individual siRNAs from that pool are re-transfected as single siRNAs and tested again to determine which siRNA elicited the effect.

This approach faces several technical challenges. The first challenge is the cost of the libraries, and how to reformat the libraries into 384-well plates (if required) for transfection. The second challenge lies in the fast generation of the phenotypic data itself. Although high content readers are now better placed to generate images for large numbers of plates and wells, the storage, analysis and retrieval of these large datasets remains challenging. Finally, the process of identifying and selecting the individual hit siRNAs from a bank of -80°C freezers imposes limitations, the process can be very labour intensive, but perhaps more importantly, it runs the risk of damaging the integrity of the sample due to warming. As the freezer doors are opened and the tubes located, there is some warming effect, especially in all the tubes stored at the front of the freezer. As RNAs are prone to temperature-induced degradation this is a big concern.

Here we present an integrated siRNA screening solution from TTP Labtech which overcomes these challenges and enable robust and fast genome-wide phenotypic screens.

miniaturising transfection reagents and plate reformatting

TTP Labtech's mosquito® HTS positive displacement liquid handler can reduce sample preparation costs associated with high-throughput applications, such as genome wide siRNA screens through miniaturisation. mosquito HV can dispense volumes between 0.5 - 5 µL, respectively, providing accurate and precise low volume pipetting, every time, irrespective of liquid viscosity or environmental conditions. It enables 8- or 16-channel pipetting compatible with any SBS microplate format (48-, 96-, 384- or 1536-well plates). Each disposable micropipette has its own individual piston, offering true positive-displacement pipetting with no risk of cross contamination. It can also be used to reformat the pooled siRNA source plates from 96-to 384-well plates or 384-to 1536-well plates, or better compatibility in HTS environments. These feature enable miniaturised sample usage both of the library itself and of the transfection reagents and therefore decrease the overall cost of running the siRNA screen.

key benefits

- educe transfection volumes and reformat plates to high density formats using mosquito
- genome-wide phenotypic screens in hours/days - not weeks with acumen
- systematic storage of entire arrayed siRNA libraries at -80°C with flexible options to re-arrary and cherry pick constructs using arktic

fast, easy phenotypic screening

Following transfection of the RNAi reagents into cells on the plates, the acumen® cellista microplate cytometer is ideally placed for rapid phenotypic screening. The acumen combines rapid whole well scanning with the ability to measure cell number as part of the same assay, which gives researchers the capacity to normalise responses to total cell number. This capability eliminates the requirement to run a separate proliferation assay alongside the assay of interest. The acumen's high-throughput capability and rapid analysis means it can conduct genome-wide screens in a matter of days rather than weeks, as illustrated in our case study using cell cycle as a phenotypic readout.

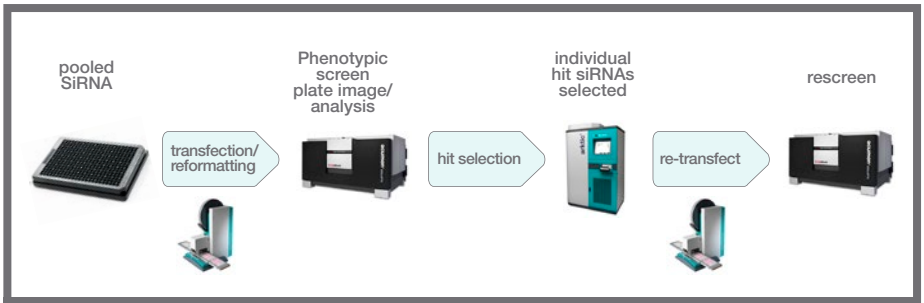


Fig 1. Typical siRNA screening format and TTP Labtech solutions.

ttplabtech

cherry picking from -80°C sample stores

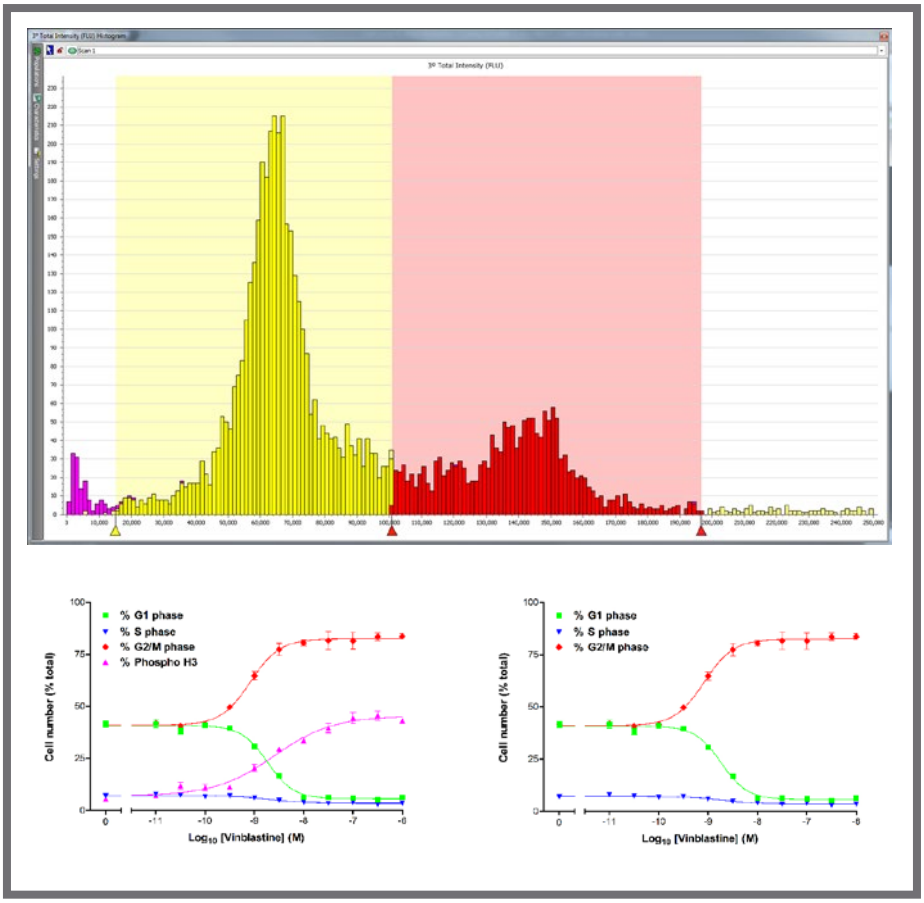
arktic is a compact, automated -80°C storage solution for effective biobanking. It enables the rackless storage of up to 139,000 individually barcoded vials – sufficient to hold all constructs required to target a whole genome of 20,000 genes with 7-fold degeneracy (reference Shalem *et al*. Nature Genetics revies 2015 for the numbers). A pre-defined ordering system arrays selected tubes to 96-well blocks for further processing, ensuring that only tubes of interest are thawed, thus reducing the risk of sample degradation by freeze-thaw cycles when samples are picked. In addition, the 2D barcoding system keeps track of the number of retrievals for sample management. The proper collection, processing, storage, and tracking of siRNAs are vital components in siRNA screening, allowing researchers to achieve accurate and valuable research results. To meet these challenges, arktic is a low cost, compact storage unit which provides unrivalled storage density, full sample tracking and rapid retrieval for individual siRNAs.

case study: genome wide screen of genes required for cell division

One of the most common uses of RNAi screening among acumen users is cell cycle analysis. Stains such as DAPI, propidium iodide or DRAQ5 are used to label total nuclear DNA in the cell. As cells progress through the cell cycle, from G1 phase through to G2/M phase, the amount of DNA doubles. The acumen Cellista software can be used to identify cells in the various cell cycle phases using the total fluorescence intensity characteristic.

method

HeLa cells were plated out in 1536-well plates and incubated with vinblastine overnight to arrest cell division. Cells were stained with Hoechst to determine total DNA content and to derive cell cycle classification gates.



results

This method of cell cycle analysis was used by Kittler *et al* (Nat Cell Biol. 2007, 9 :1401-12) where a primary screen with a genome-wide human siRNA library targeting 17,900 genes yielded 2149 primary hits (showing cell cycle arrest at G2/M phase). This primary screen was carried out on an acumen laser scanning microplate cytometer. Of these 2149 primary hits, the gene expression was verified and a secondary siRNA screen resulted in 1841 expression-verified hits. These hits then progressed through a secondary screen using the combined power of acumen high speed scanning, flow cytometry and mitotic index analysis on an image analysis platform. The work resulted in a nine-parameter fingerprint of 1389 genes essential for cell cycle progression.

summary

TTP Labtech innovative products facilities and streamline genome-wide phenotypic screens. mosquito can be used to reformat siRNA library source plates into high density plates and miniaturise the transfection reagents, thereby reducing reagent costs. Whole genome phenotypic screens can be run using the on the acumen imaging cytometer. It can image and analyse whole wells of a 384-well plate in under 5 minutes, with only small file sizes being created, therefore eliminating the requirement for large data storage solutions. Finally, arktic enables individual tube storage for cherry picking of siRNAs of interest at the hit confirmation stage. With a holding capacity of up to 139,000 0.5ml tubes, the store size is ideal to hold entire siRNA libraries targeting the whole genome. The full sample tracking capability of the system allows for faster, simpler picking and also reduces possible sample degradation associated with freeze-thaw cycles.

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