Long-term proliferation arrayed CRISPR screening by colour competition assay at scale

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1. Abstract

Pooled genome-wide CRISPR screening allows high-throughput identification of new genetic vulnerabilities or resistance mechanisms to drug treatment. Validation and further characterisation of the effect of the genetic perturbation using orthogonal arrayed methods is critical to build confidence in the observed phenotype. However, **long-term proliferation assays in plate format are generally limited to short-term kinetics** as introducing cell splitting usually exacerbates background noise. This prevents the screening for drugs which require multiple cell cycles before showing an effect, such as PARP inhibitors (PARPi).

Here, we report the establishment of a **miniaturised and scalable colour competition assay workflow in 96-well plates** using in-house automated platforms. **This lentiviral-based assay allowed us to hit triage 16 pooled screen hits sensitising to PARPi olaparib** in DLD1 cells, providing key data for identifying and prioritising both clinical markers and new potential drug targets.

2. Colour Competition Assay: Concept

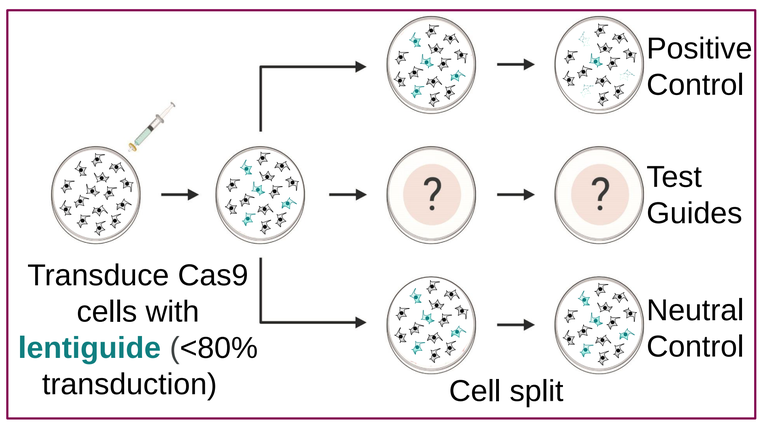


Figure 1: Analysis of cell proliferation effect by colour competition assay. Following transduction with a pseudo-virus expressing both a guide RNA of interest and the mClover3 fluorescent marker (Lentiguide), the percentage of green (= transduced) cells is measured after each cell split.

3. PARPi sensitisers: Hit triage strategy

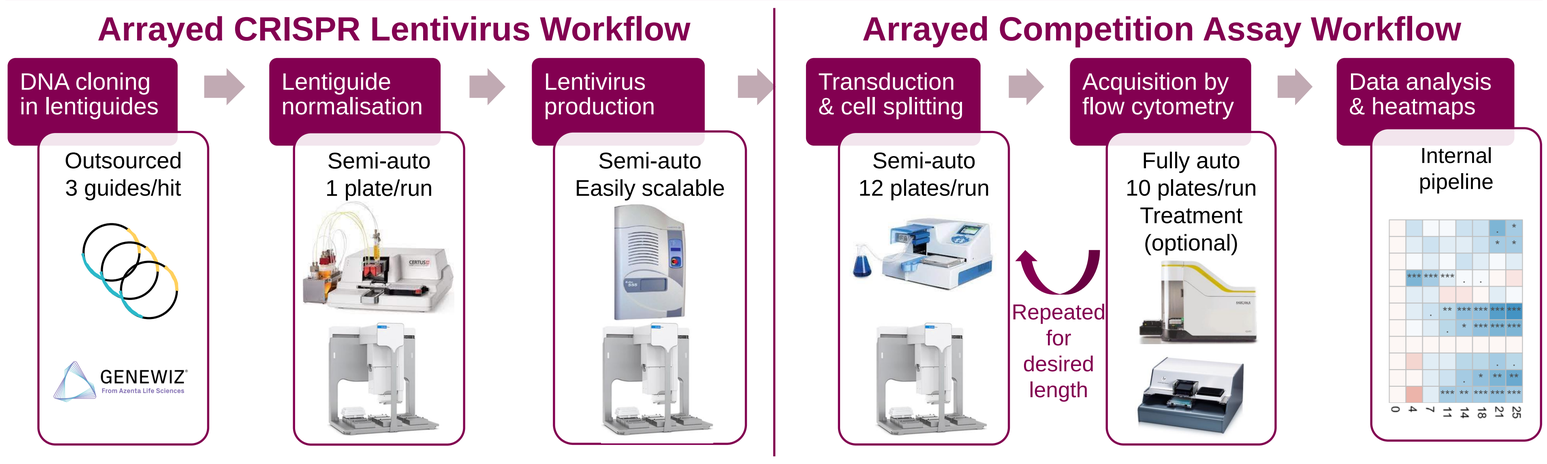
Pooled **genome-wide** sensitisation screen
(3 cell lines)

DNA damage response profiling
~200 genes
High-content imaging screen in 1 cell line

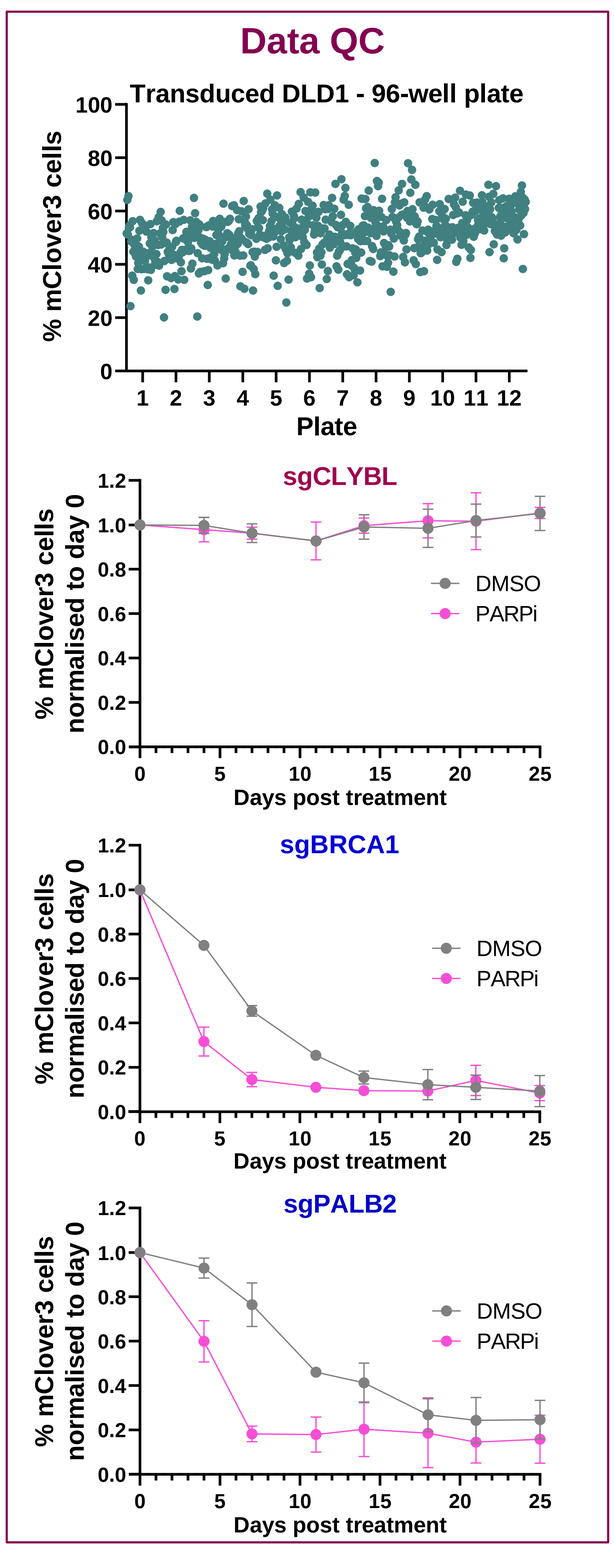
Hit triage
16 genes selected
Proliferation assay for 25 days in 1 cell line

Novel targets
& biomarkers

4. Workflow: a semi-automated modular process in 96-well plates



5. Pilot screen: Sensitisation to PARPi olaparib by colour competition assay



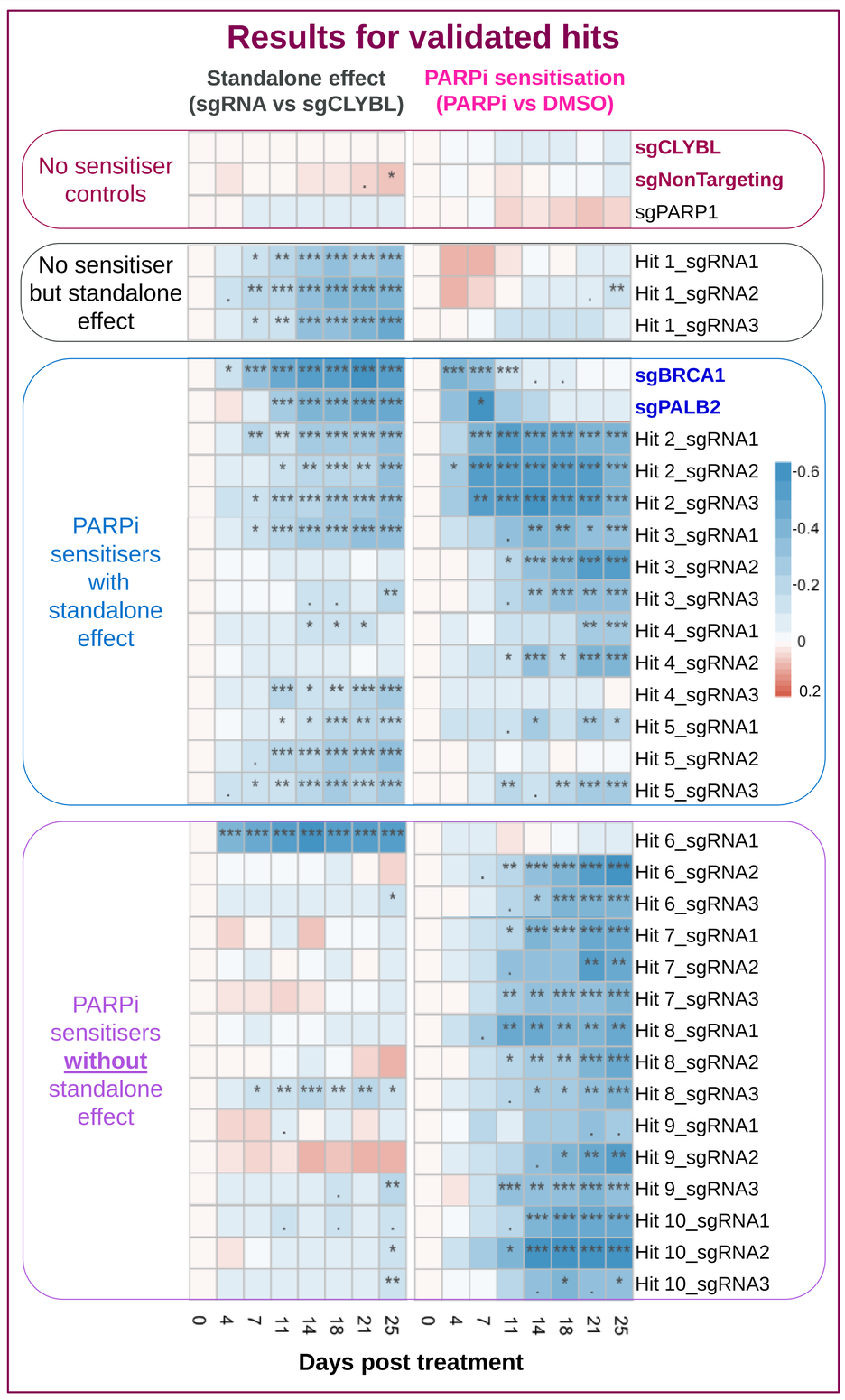


Figure 2: Analysis of cell fitness defect caused by PARPi treatment. 5µL of each lentivirus were transduced in DLD1 cells and at least triplicate wells were treated with PARPi or vehicle (DMSO) after day 0. *Left top*, effective transduction was determined by mClover3 expression (1 well = 1 dot). *Left bottom*, the evolution of the percentage of mClover3 edited cells normalised to day 0 was plotted for both neutral (sgCLYBL) & positive controls (sgBRCA1 & PALB2). *Right*, heatmaps represent proliferation effect sizes calculated at each timepoint for each guide for gene deletion alone (left) and for treatment (right) for the top 10 hits. Number of stars indicates statistical significance (p value).

Conclusions

We have successfully established **an industry leading arrayed CRISPR screening workflow** analysing long-term proliferation kinetics in 96-well plates. Our pilot screen experiment allowed us to measure olaparib sensitisation kinetics after parallel knock out of 16 genes by CRISPR in DLD1 colorectal cells (12 plates) and led to the identification of new biomarkers and potential drug targets.

Next, we would like to:
1. Scale even further the workflow by fully automating it on a HighRes laboratory automation system.
2. Add additional flow markers for deeper mechanism insights (i.e., apoptotic & cell cycle markers).

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