Biomarker-based pooled CRISPR-Cas9 screening: Platform development and validation

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INSPIRED CELL SOLUTIONS

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Introduction

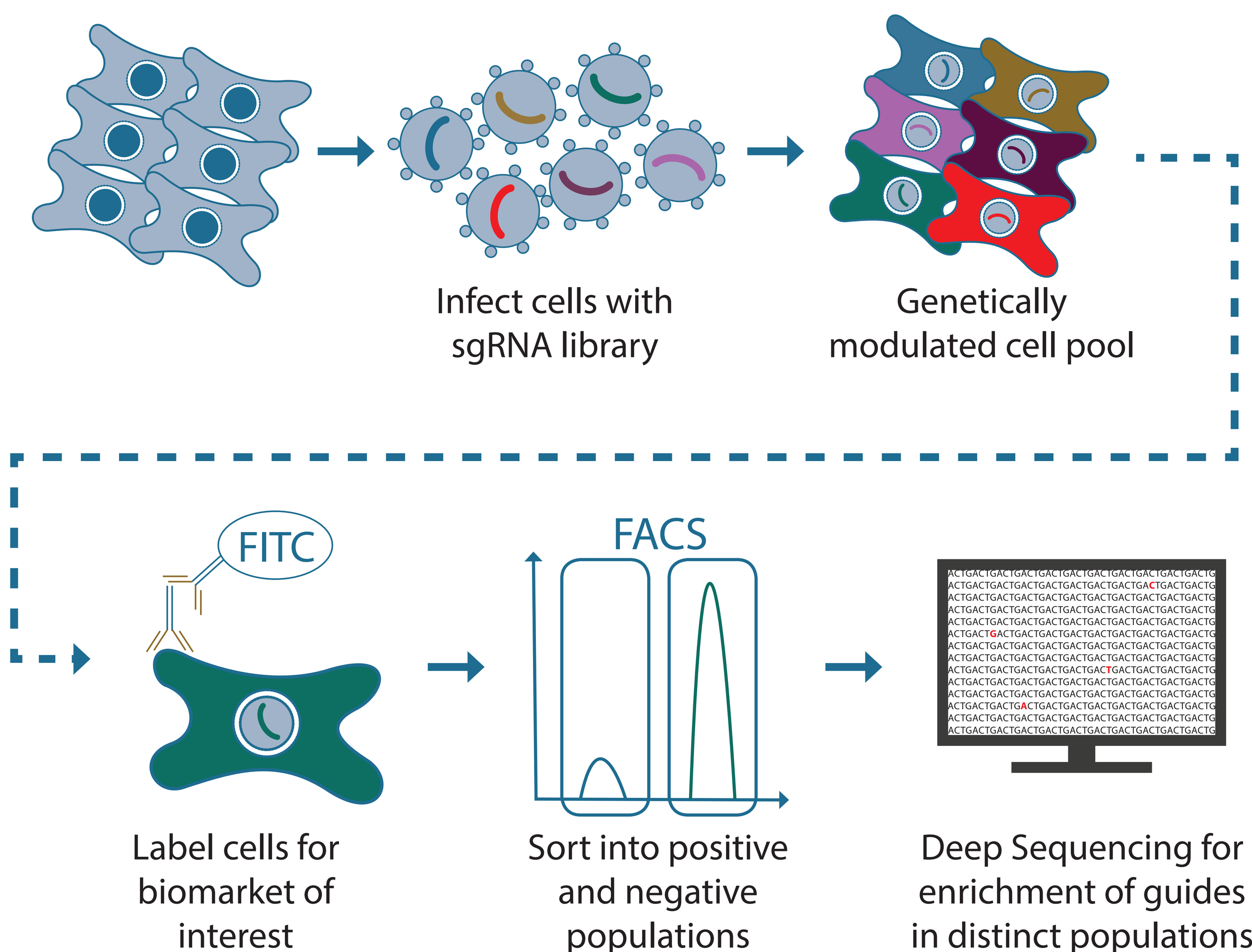
Pooled CRISPR-Cas9 screening has provided a powerful and precise method to interrogate consequences of gene loss in a high-throughput manner.

Currently the majority of screens have been based on analysis of sgRNA abundance in surviving cell populations, for example for *in vitro* resistance analysis to a cytotoxic treatment.

In situations where cellular pathophysiology is uncoupled from cell health, the enormous power of pooled CRISPR-Cas9 screening has largely been untapped.

Here we show development and validation of a FACS-based platform which can be used to monitor biomarker-based cellular response in combination with high-throughput genetic screening.

Platform overview



Development and Validation Screen Overview

The endogenous interaction between CD28 and CD80 was used as a proof-of-concept system to develop and validate the platform. Jurkat cells were depleted of CD28 using CRISPR-Cas9 (five guides used to establish five separate cell lines).

As a negative control, five cell lines (using five separate guides) in which the ROSA26 locus had been targeted were also generated.

For simulation of target identification from a complex population, cells were also infected with a control library (CTRL library). The CTRL library contained a core essential gene collection (CTRL_POS), a neutral gene collection (CTRL_NEG) and a non-targeting guide collection (CTRL_NT). The CTRL library consisted of over 2,500 guides in total.

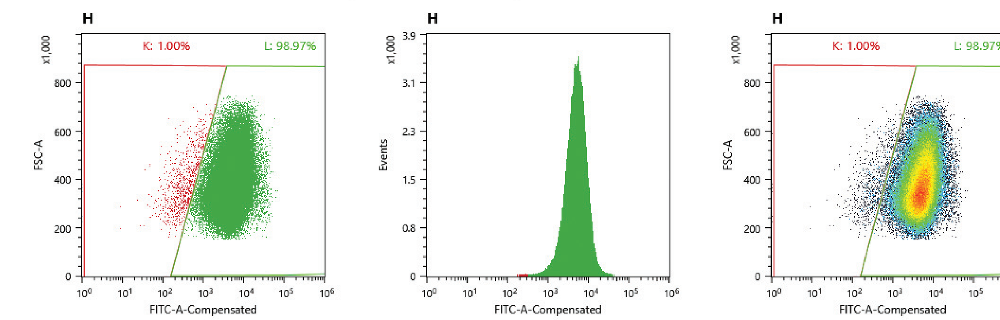
Flow cytometry was used to separate cells into "Bound" or "Unbound" populations based on their labelling with a CD80-Fc fusion in combination with an anti-human Alexa Fluro-488 secondary antibody.

Genomic DNA was extracted from sorted cell populations, and amplified and prepared by PCR for deep sequencing. Sequencing was then performed using an Illumina NextSeq next generation sequencing platform and analysed using Horizon's in-house pipeline (SG3).

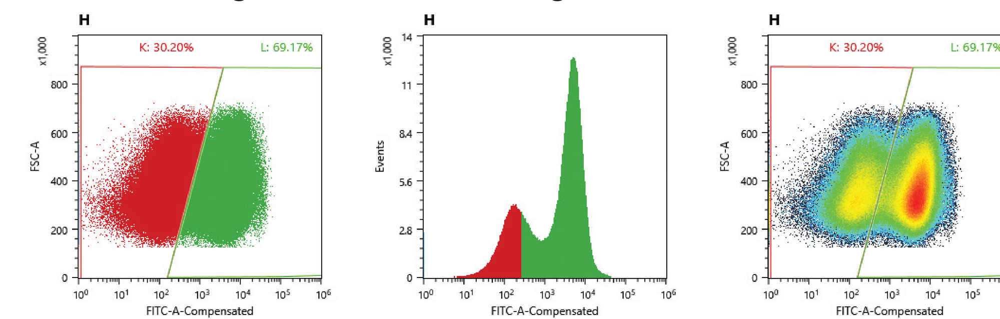
Results – Platform optimisation

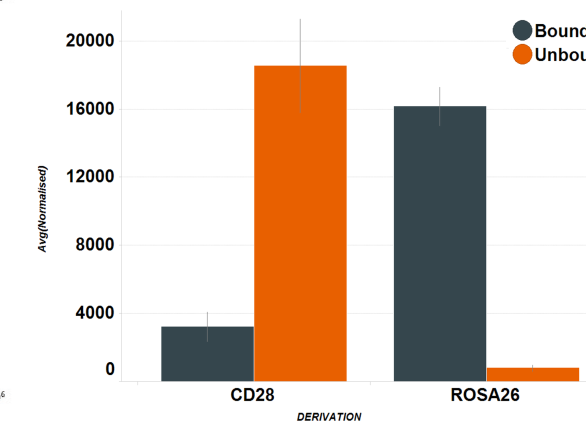
The five CD28 targeted and five ROSA26 locus targeted cell lines were mixed in equal quantities and sorted into populations that either bound ("Bound") or did not bind ("Unbound") to CD80.

FACS: Pooled ROSA26 targeted lines – gate calibration



Pooled CD28 targeted + ROSA26 locus targeted lines

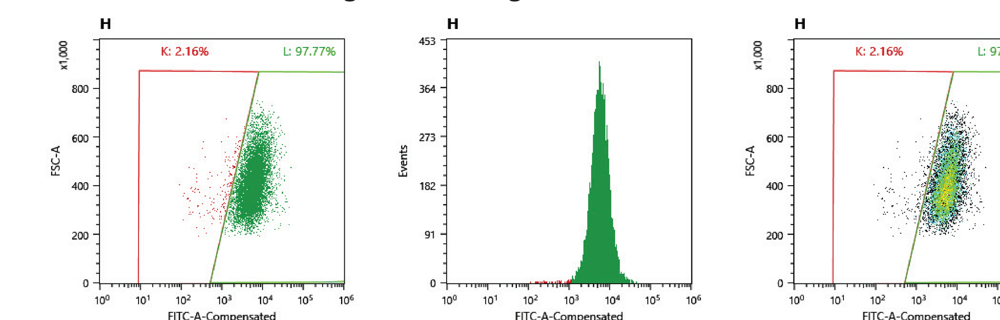




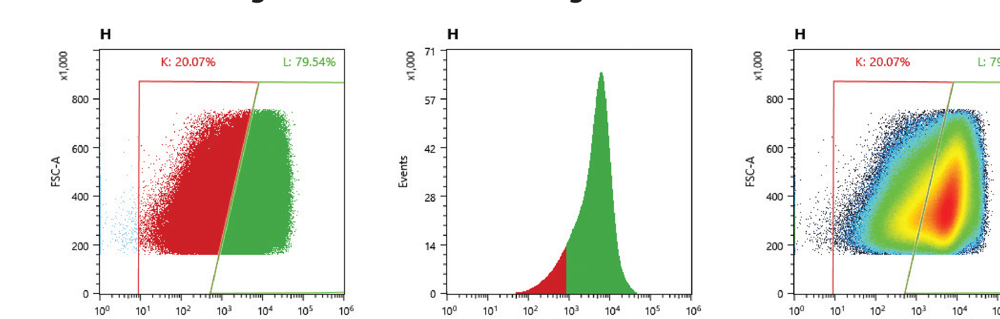
Flow cytometry data showed clear and distinct populations based on biomarker expression. Moreover, the normalised guide count analysis following deep-sequencing, comparing Bound and Unbound populations indicated clear enrichment of CD28 guides in the Unbound population and depletion in the Bound population. As expected, the reverse was apparent for the ROAS26 guides.

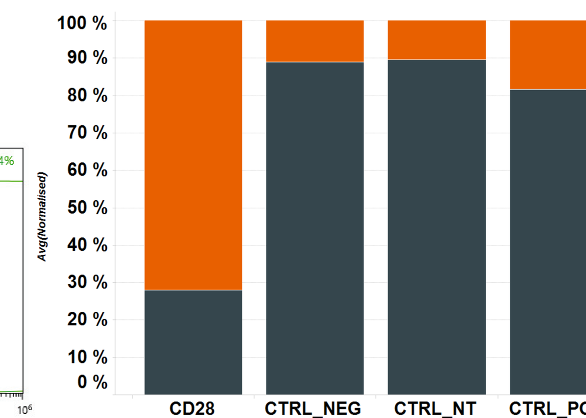
The five CD28 targeted lines were pooled and mixed with an equal number of CRTL library infected cells, before being sorted into Unbound and Bound populations.

FACS: Pooled ROSA26 targeted lines – gate calibration



Pooled CD28 targeted + ROSA26 locus targeted lines





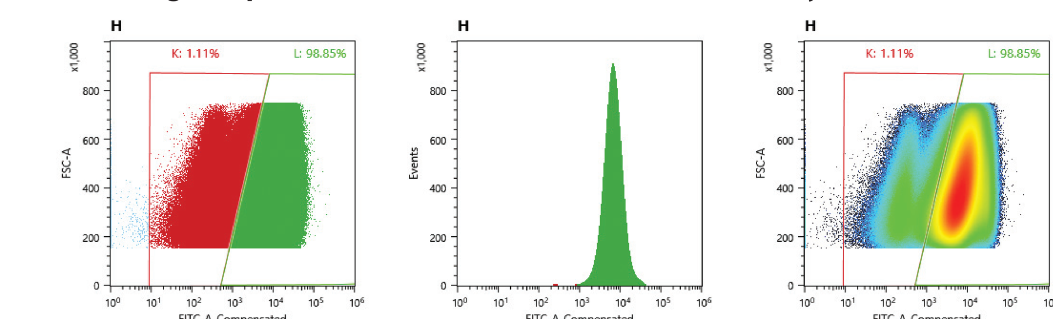
Within-group percentage distribution of guides (to account for different complexity in each group) were determined. CTRL_NEG; guides targeting neutral genes CRTL_NT; non-targeting guides CTRL_POS; guides targeting essential genes.

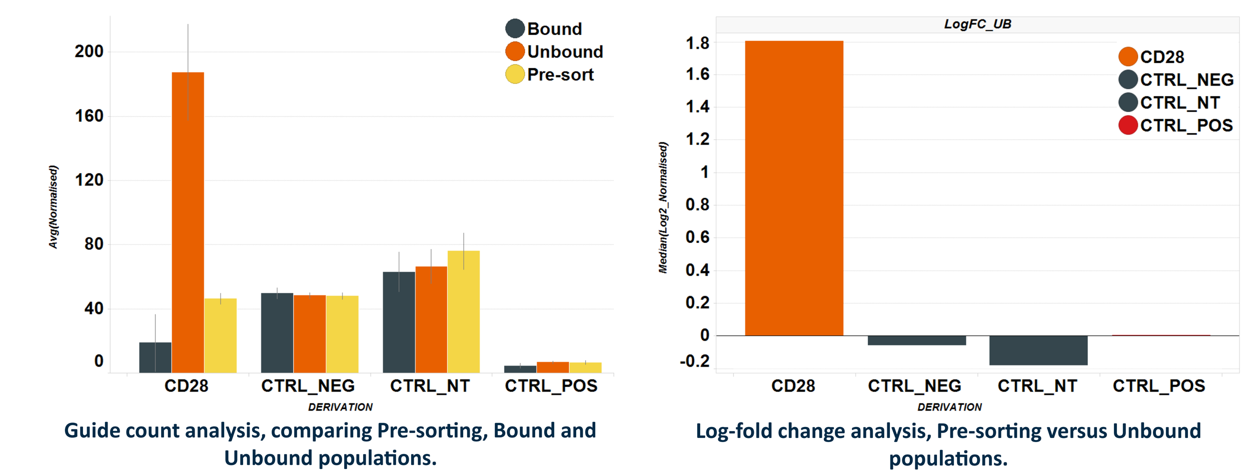
We found substantial enrichment of target (CD28) guides in the Unbound population.

Results – Screen Simulation

The five CD28 targeted cell lines were then mixed into a 12,500 excess of CTRL library infected cells, simulating single target gene identification from a 2,500 gene library. Gating calibration as per previous panel.

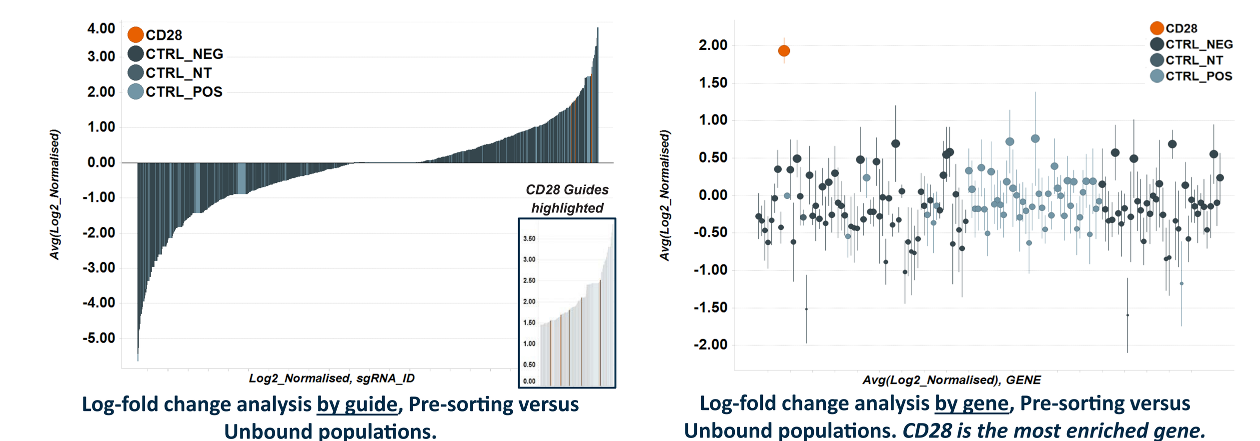
CD28 targeted pool diluted in a 12,500 excess of CTRL library infected cells, 78e6 cells sorted





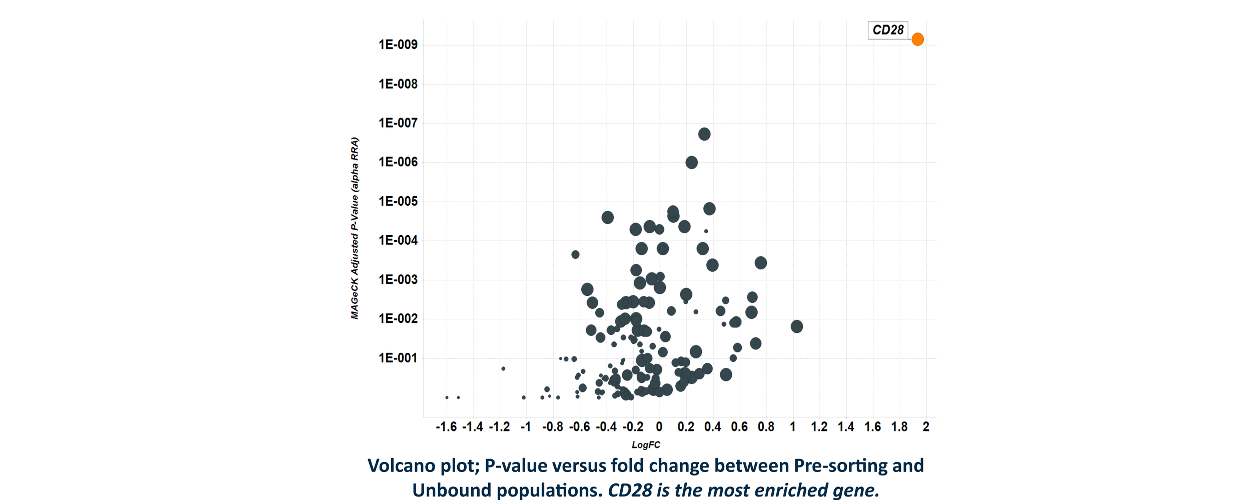
Guide count analysis, comparing Pre-sorting, Bound and Unbound populations.

Log-fold change analysis, Pre-sorting versus Unbound populations.



Log-fold change analysis by guide, Pre-sorting versus Unbound populations.

Log-fold change analysis by gene, Pre-sorting versus Unbound populations. *CD28 is the most enriched gene.*



Volcano plot; P-value versus fold change between Pre-sorting and Unbound populations. *CD28 is the most enriched gene.*

Conclusions

In this screen-simulating study, we were able to robustly identify CD28 as the target gene. Importantly, flow cytometry alone did not present a pure enriched sample, even despite highly defined control populations. Experiment, library and control design are therefore of crucial importance for successful screening. Here, five guides targeting the CD28 were sufficient to show unambiguous enrichment, but we expect a higher complexity library would provide even greater clarity in the data.

This platform opens myriad exciting discovery opportunities for identification of genes interacting with complex biomarker response across the drug discovery continuum.

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