Genome-wide pooled CRISPR screening in primary T cells at the Functional Genomics Centre (FGC), a partnership between AstraZeneca and Cancer Research Horizons

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POOLED CRISPR SCREENING AT THE FGC

The emergence of CRISPR technologies has enabled rapid and precise gene editing opportunities. The Joint AstraZeneca - Cancer Research Horizons Functional Genomics Centre (FGC) has been launched to better utilise and improve CRISPR technology within cancer research. With the goal of accelerating the discovery of new cancer medicines, the FGC aims to be a centre of excellence in genetic screening, cancer modelling and big data processing. One of our current aims is to develop workflows to screen in more complex cell models.

Pooled CRISPR screening has become a significant tool in cancer research, able to interrogate the whole genome to find potential new drug targets, combination therapies, and resistance mechanisms to aid patient stratification. In most pooled CRISPR screens guide RNAs are delivered to Cas9 expressing cells en masse via lentiviral delivery. Following selection, cells can be treated with anti-cancer agents and genes that have either conferred resistance or sensitised the cells to the drug treatment are deconvoluted by NGS.

PRIMARY CELL SCREENING CHALLENGES

Pooled CRISPR screening in primary T cells faces several technical challenges including cell number, Cas9 expression and library transduction. For this reason, screens are often performed in cell line models which are known to poorly represent primary T cell biology. We have adapted the SLICE protocol for genome-wide CRISPR screening in primary T cells, in order to utilise this platform to support the target/drug discovery and development process across the AZ and CRUK communities.

ADAPTATION OF THE SLICE PROTOCOL FOR GENOME-WIDE T CELL SCREENING AT THE FGC

The SLICE protocol (PMID: 30449619) (sgRNA lentiviral infection with Cas9 electroporation) describes a protocol for genome-wide CRISPR screening in primary T cells. This method transduces activated primary CD8+ T cells with sgRNA library virus, with Cas9 protein then introduced by electroporation. Cells are then set up in screen conditions or treated with puromycin, sorted for live cells and then setup in screen conditions. This method has been validated using arrayed screening by the same group. These screens have been bespoke and do not include timepoints beyond 2 weeks post-Cas9 electroporation, or even 1 week when selection was included. We set out to modify this protocol, in order to offer a robust broadly applicable model for T cell screening.

We started by optimising with CD3+ cells by transducing with the YusaV3 human CRISPR library. Testing across multiple donors, we optimised this to transduce using spinfection and 1µg/ml polybrene 1 day post-isolation. Most donors remained in the linear range of transduction up to 30% transduced (BFP+), then levelling off with most donors not reaching above 40% transduced (Fig.1A). We confirmed selection with puromycin with 2µg/ml, with a second round of selection after 3/4 days reproducibly generating >95% %BFP+ cells (Fig.1B).

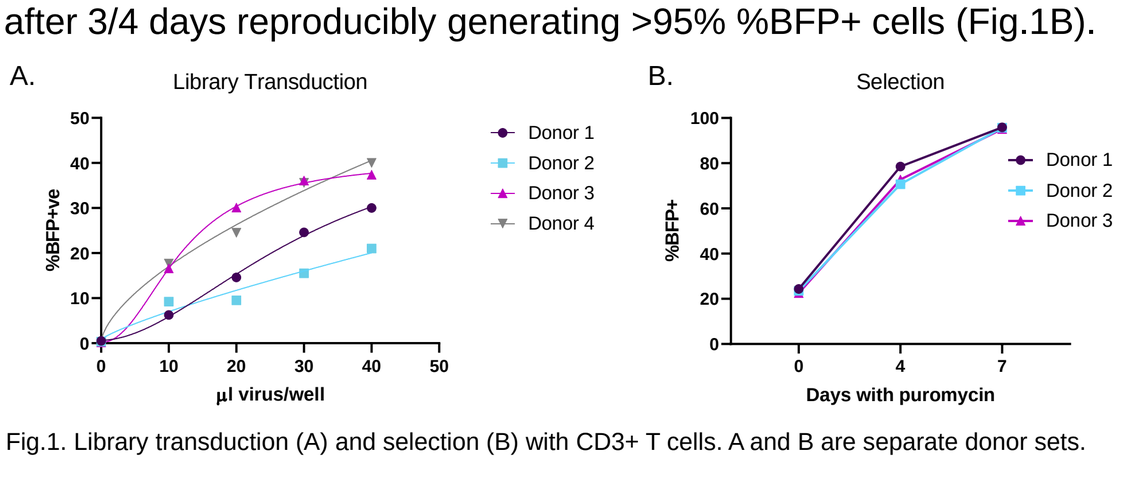


Fig.1. Library transduction (A) and selection (B) with CD3+ T cells. A and B are separate donor sets.

To optimise Cas9 electroporation we used the gGFP_BFP/GFP reporter construct for Cas9 editing efficiency. Using the MaxCyte ATx electroporation platform we optimised program, cell concentration, Cas9 protein concentration: cell number ratio and post-electroporation seeding density. We also investigated the use of non-targeting RNP, but didn't find a significant enough benefit in editing efficiency to add this to the standard workflow (Fig.2A).

The FGC successfully performed it's first full genome-wide primary T cell screen in 2021 using this workflow, combined with our standard sample preparation and NGS workflow, followed by bespoke bioinformatics analysis. QC data showed that essential gene drop out was strong, at >0.95 AUROC for the pan_cancer_Sanger gene set.

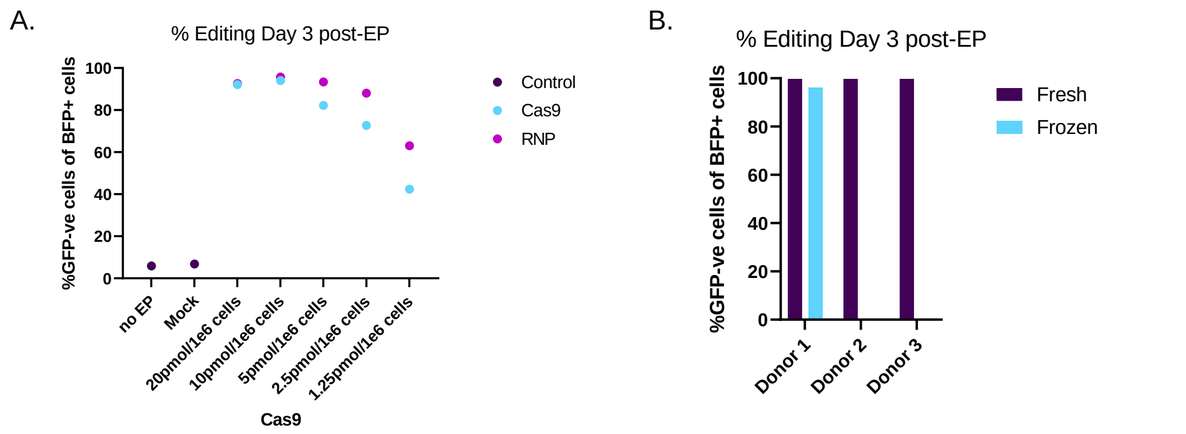


Fig.2. Cas9 editing of reporter construct after Cas9 protein electroporation in CD3+ cells (A) in modified CD8+ T cells in screen.

Based on these results, we initiated a follow-on screen with primary CD8+ cells. We knocked out 3 biologically-relevant genes 1 week pre-library transduction and screened with our VBCTop3 library using the same SLICE process, with 4 days selection and very high Cas9 efficiency achieved (>96% in 4 donors) (Fig.2B). We also determined that cell age is a key indicator of transducibility (Fig.3), which enabled us to forecast virus requirement based on how long the cells had been in culture.

We show that the adapted SLICE protocol is applicable and adaptable across major T cell subpopulations and have evolved this method further to now be able to incorporate biologically relevant genetic modifications into the process.

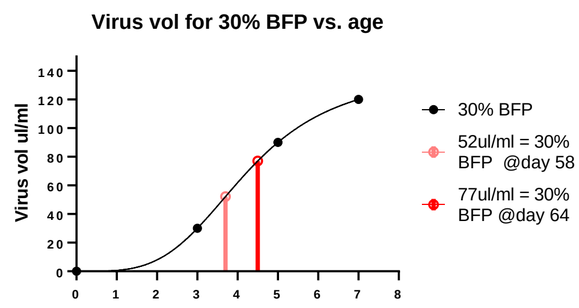
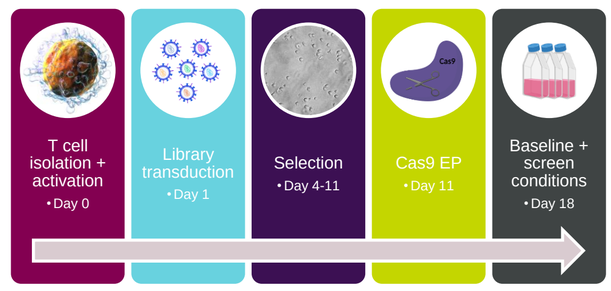
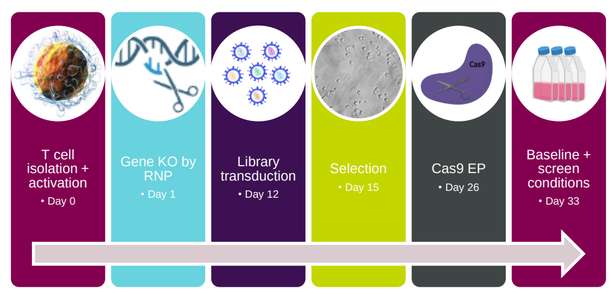


Fig.3. Transduction rate decreases with age of the cells.

CD3+ screen workflow



CD8+ modified cell screen workflow



"Working together to accelerate the development of new treatments for people with cancer"