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CRISPR screening in xenograft models for *in vivo* drug MOA analysis

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Introduction

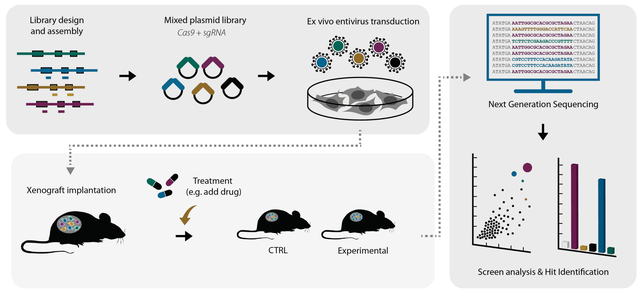
Functional genomic screening with CRISPR has provided a powerful and precise new way to interrogate the phenotypic consequences of gene manipulation in high-throughput, unbiased analyses. Rapid development of pooled lentivirus and deep sequencing-led approaches have allowed us and others to exploit this technology in target ID, target validation, drug Mechanism of Action (MOA) analysis, and patient stratification.

Until now, these exquisitely powerful screening technologies have been deployed predominantly in simple *in vitro* analyses. Whilst these *in vitro* approaches allow for high quality hit discovery and at whole-genome level scale, they are not able to adequately capture the impact of tumour microenvironment and heterogeneity on the genetic perturbations under study.

Here we present the development and validation of a platform for pooled CRISPR knock-out (CRISPRko) screening in xenograft models of tumour growth. We use both Patient Derived Xenograft (PDX) and Cell-line Derived Xenograft (CDX) approaches to evaluate the fundamental ability of these screens to identify cellular phenomena, and also explore the necessary analytical approaches to adapt these tools to explore drug mechanism of action. We find that, with careful design, the quality of hit identification from such pooled *in vivo* screening efforts can be exceptionally high.

Method

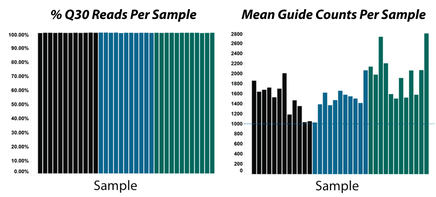
To evaluate the application of pooled screening techniques to *in vivo* analyses, we adapted our existing tools for *in vitro* screening. Thus, a library is first designed to target the genes of interest, ahead of lentivirus manufacture. Cells are then transduced *ex vivo* and selection applied to eliminate untransduced cells. At this point, a time sample is taken to establish baseline and cells can be implanted as a Matrigel plug into suitable animals. Outgrowth of the tumour is monitored and cells are harvested at a suitable time point. In the case of drug-gene interaction, animal groups are established and treated to partially ablate the tumour growth.



NGS and Quality Control

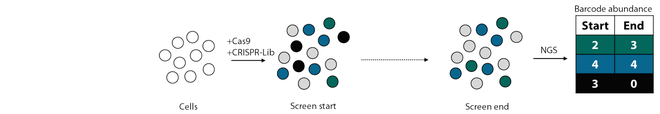
Following the completion of the *ex vivo* phase, all tumour samples are harvested, dissociated and genomic DNA is extracted. The sgRNA cassette is then amplified from each sample and deep sequenced to measure the changes in abundance of each genotype in each sample.

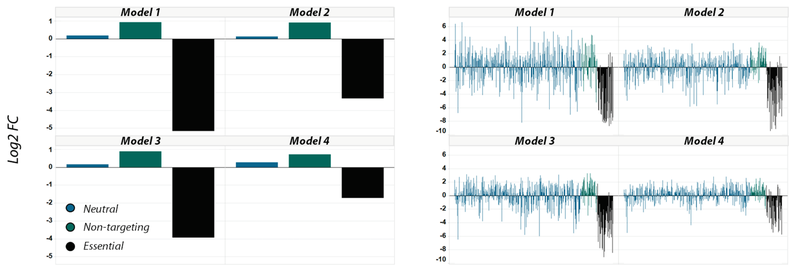
We determine the read counts of all samples in each study to establish the quality of the deep sequencing. The likelihood of base-calling accuracy and the read depth per mapped guide are valuable indicators of confidence for hit scoring and precision. In the example shown, each sample has high Q30 and >1000 reads per guide in the library.



A PDX Screen for Gene Essentiality

To examine the rate of drop out for essential gene controls in an *ex vivo* pooled screening paradigm, we took four distinct PDX models and introduced a library of 1000 guides to test the ability of our platform to robustly detect the expected loss of key essential genes from a population of engrafted cells.





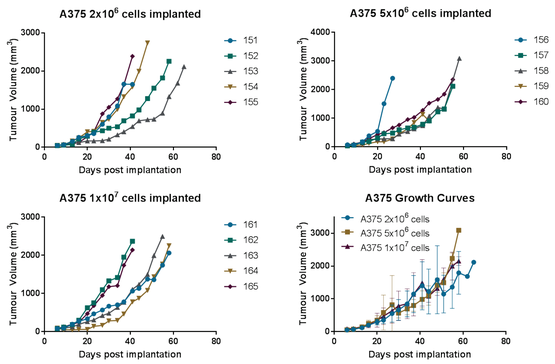
Overall QC group performance

Individual guide QC performance

As a crucial measure of screen success, we evaluate the dynamics of control gene behaviour by comparing the baseline NGS data to the end-point of the control-treated cells. Essential genes are expected to drop out of the screen over time, whilst neutral guides are expected to remain largely unaffected. In CRISPRko studies, non-targeting guides should modestly enrich in the study owing to the lack of any DNA damage effect from these reagents. In this study, each PDX model showed robust overall drop-out for essential gene controls, and expected response of other key control groups.

Implantation Rate Analysis for a Key CDX Model

The success of *in vivo* screening is highly contingent upon the successful uptake of the model in the engrafted tumour. This take-rate then in turn defines the library complexity limit for any given model. We evaluated several CDX models for implantation rate by titration of the cell number in the implantation.



We find that in the case of A375, the rate of progression of the tumour varies per implantation volume, as expected, but importantly that even at a high rate of engraftment (1x10^7 cells per tumour) the take rate is 100%.

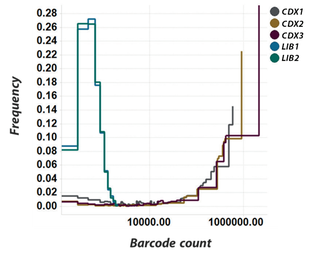
This important consideration contributes to the definition of a successful screening study, since the maximal coverage or representation of the library can be derived from this criteria.

Barcode Divergence and Distribution Analysis

Following establishment of an appropriate cell number for implantation, it is crucial to understand how the cells once implanted will individually contribute to the tumour. For instance, a dramatic divergence of barcode abundance can be observed if cells are allowed to propagate in tumours for an extended period.

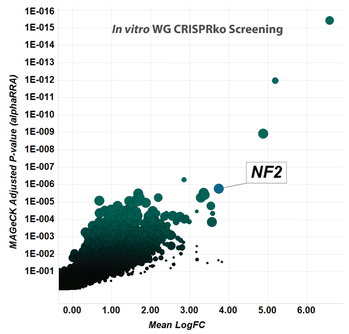
In order to define the appropriate library size compatible with any given cell type and experimental paradigm, a detailed barcode divergence experiment is recommended (see experimental layout, left).

Stochastic loss of barcodes (or guides) is a major concern in pooled screening, and necessitates inclusion of multiple technical replicates in the form of coverage of barcodes per sample. *In vitro*, this loss can be observed in samples which grow for greater than 16 population doublings. In the analogous *in vivo* experiment, tumour mass can be used as an approximation of cell division rate, but in most cases it is crucial to empirically determine the endpoint with a dedicated barcode divergence test. In this example (right), CDX samples show high skew and loss of barcodes at the endpoint.

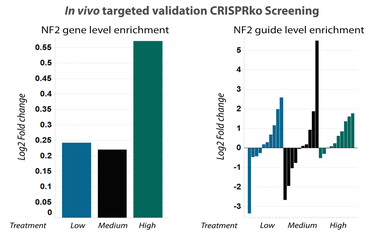


An *in vivo* MOA Validation Platform

To validate our *in vivo* screening platform for the study of drug-gene interactions, we first conducted whole-genome *in vitro* screening using our CRISPRko platform to identify genes which provide a resistance to the BRAF^{V600E} inhibitor, Vemurafenib. This screen identified and corroborated multiple genes which drive this phenomenon in A375 cells *in vitro*. In our *in vivo* validation screen, we targeted cells with a library of around 500 genes found frequently mutated in cancers. We used multiple escalating doses of the drug to elicit phenotypic response from the tumour *in vivo*. Tumours were extracted and deep sequenced to identify enriched (resistance-causing) genotypes.

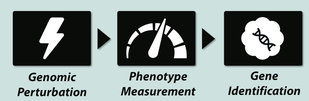


Amongst other observations, we found that the loss of Neurofibromatosis type 2 (NF2) caused a pronounced resistance to Vemurafenib in the xenograft models, validating the *in vitro* observations in a model which more closely mimics the tumour state in patients.



Conclusion

We find that an *in vivo* CRISPRko-based strategy for identifying genetic interactions, including with small molecule inhibitors, provides a robust and powerful strategy. Experimental design and selection of appropriate models remains a crucial factor for screen success and contributes directly to the possible scope of a successful campaign.



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