CRISPR-Cas9: a tool for rapid target discovery and validation

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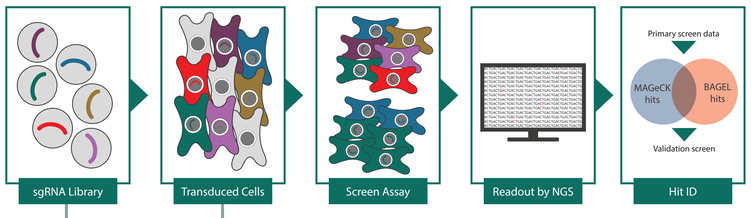
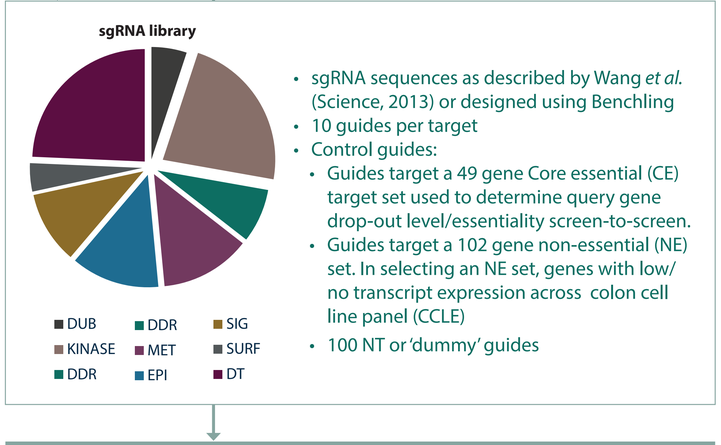
Background

The cancer mutation landscape is dominated by gain-of-function mutations in oncogenes such as *KRAS*, and loss-of-function mutations in tumour suppressors, such as *TP53*. These latter alterations have proven difficult to drug because a direct approach requires targeting truncated, under-expressed or absent proteins rather than hyper-activated enzymes. Exploiting dependencies on proteins that are only evident in the presence of a certain cancer mutations provides an alternative strategy and a host of new potential drug targets.
At Horizon, we have carried out CRISPR-Cas9 screens aimed at identifying novel synthetic lethal interactions in >30 colon and lung cancer cell lines, focussing on major cancer genotypes such as *KRAS* activation and *TP53* loss. Our secondary screens, which target functional domains with high density sgRNA tiling, have been used to verify and prioritise hits: these have now moved into arrayed target validation, involving the use of inducible Cas9 expressing cell lines and functional assays.

Method: CRISPR-Cas9 Synthetic Lethal Screens





- sgRNA sequences as described by Wang *et al.* (Science, 2013) or designed using Benchling
- 10 guides per target
- Control guides:
  - Guides target a 49 gene Core essential (CE) target set used to determine query gene drop-out level/essentiality screen-to-screen.
  - Guides target a 102 gene non-essential (NE) set. In selecting an NE set, genes with low/no transcript expression across colon cell line panel (CCLE)
  - 100 NT or 'dummy' guides

Cell Line Panel

Cell Line	Tissue	Cell Line	Tissue	Cell Line	Tissue	Cell Line	Tissue
C2BBE1	colon	GP2D	colon	H522	lung	H661	lung
HT29	colon	HCT116	colon	HCC15	lung	H2122	lung
HT55	colon	SW480	colon	TYKNU	ovary	HCC44	lung
KM12	colon	DLD1	colon	H520	lung	H838	lung
LS411N	colon	H1299	lung	HCC827	lung	A549	lung
RKO	colon	H1703	lung	Ishikawa	endometrial	SKHEP1	liver
SW1417	colon	H1793	lung	LCLC103H	lung	H460	lung
LS513	colon	TOV-21G	ovary	OVISE	ovary		

Figure 1: CRISPR-Cas9 screening workflow: library design; cell line selection and transduction; screening under low serum conditions to better reveal dependencies on genotypes, such as *PIK3CA* and *KRAS*, with 300 fold guide representation and through 12 cell doublings or 30 days in culture; and NGS data analyses using a bioinformatics pipeline developed at Horizon with the publically available algorithms of MAGeCK and BAGEL.

Screen results: rediscovery and novel target identification

Primary screen data confirmed previously reported genetic interactions, such as a dependence on MDM2 and PPMID for survival in *TP53* wildtype cancer cell lines, and SMARCA2 dependence in most SMARCA4 mutant lung cancers. Putative novel interactions were also identified, such as an increased dependence on the anti-apoptotic protein MCL1 in colon lines bearing point mutations in the F box factor responsible for MCL1 turnover, FBXW7 (Figure 2A). This dependence was confirmed in a secondary high density tiled screen, with good drop out observed in guides targeting functional BH1-3 domains (Figure 2B). FBXW7 mutant cells have problems with spindle assembly, potentially increasing their dependence on MCL1 expression (Bailey *et al* 2015).

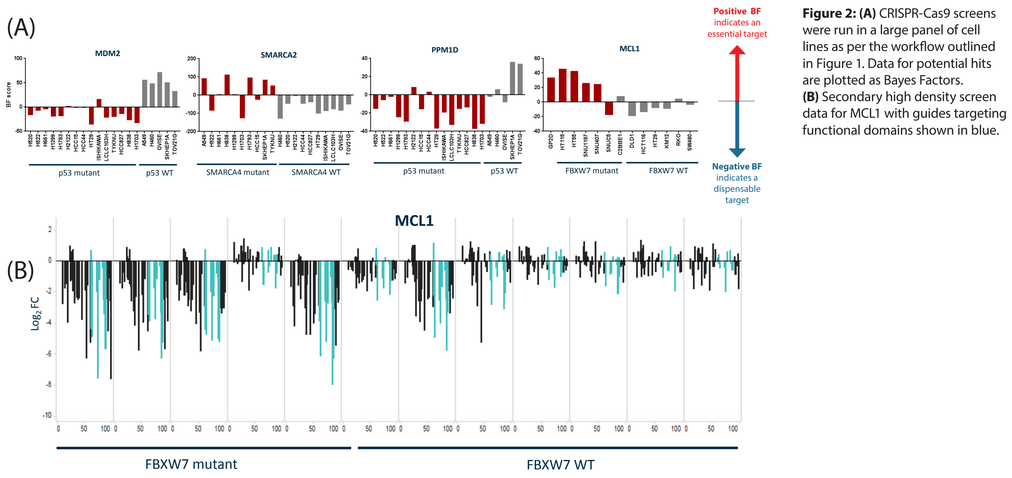


Figure 2: (A) CRISPR-Cas9 screens were run in a large panel of cell lines as per the workflow outlined in Figure 1. Data for potential hits are plotted as Bayes Factors. **(B)** Secondary high density screen data for MCL1 with guides targeting functional domains shown in blue.

Primary screens in the colon cancer cell line panel identified several novel hits that appeared more essential in cells expressing mutant KRAS. Subsequent validation screens using Horizon's isogenic cell lines and a high density tiled CRISPR screen highlighted a novel, highly druggable target, which not only exhibited a strong co-dependency with mutant KRAS but also did not score as essential in a 'normal' cell line background (Figure 3).

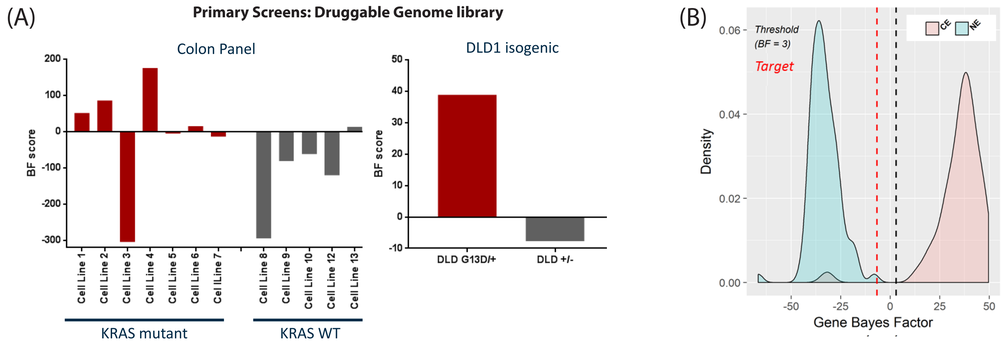


Figure 3: (A) Primary screens using the druggable genome library in a panel of colon cancer cell lines and isogenics reveal target dependency on mutant *KRAS*, but a lack of essentiality in MCF10A cells used here as a 'normal' cell background **(B)**. Data are represented as Bayes Factor, where guides are ranked relative to controls and a positive score indicates essentiality.

To further validate this target we have generated a panel of cell lines expressing a dox-inducible Cas9 construct and individual sgRNAs against the target. Lentiviral infection of target guides into iCas9 cell lines and treatment with doxycycline results in cas9 expression, target depletion, as judged by western blotting, and a striking anti-proliferative phenotype of a similar magnitude to a known essential control guide (Figure 4).

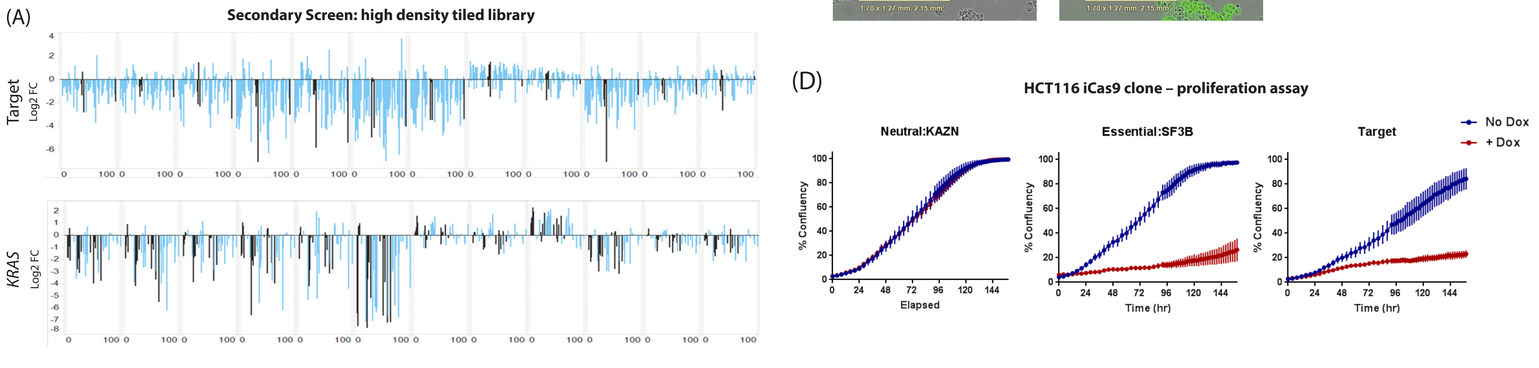


Figure 4: (A) For secondary screens, a small target library was designed with guides targeting every 5-10 amino acids. Data are plotted as log fold change or guide drop out, and show a correlation between *KRAS* and target sensitivity. Guides targeting functional domains are shown in blue. **(B)** HCT116 cells were lentivirally transduced with Dharmacon Edit-R CRISPR-Cas9 at low MOI, blasticidin selected and single cell cloned. Clones were screened for Cas9 expression following 24h doxycycline treatment by immunofluoresence using Cas9 antibody and imaged in an Incucyte ZOOM™. Cells transduced with an sgRNA to the target showed target depletion by western blot **(C)** and a failure to proliferate **(D)** only in the presence of doxycycline.

Summary and conclusions

CRISPR-Cas9 is proving to be an immensely valuable screening tool with sufficient penetrance and power to identify novel interactions, and to generate reagents to streamline target validation approaches.

References:
Bailey ML, Sing T, Mero P, Moffat J and Hieter P (2015) Dependence of human colorectal cells lacking the FBW7 tumour suppressor on the spindle assembly checkpoint. *Genetics* 201(3) 885-895