

CRISPRi: Transcriptional repression screening with genome-wide CRISPR interference.

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Introduction

Building on the transformative impact of pooled screening using CRISPR-Cas9 researchers have developed catalytically inactive Cas9 (dCas9) and have demonstrated that it can be used to tune genetic output. These systems are known as CRISPRi (interference) and CRISPRa (activation), respectively.

Revvity have previously developed a powerful CRISPR-Cas9 knockout platform and have demonstrated improved screening quality by small but crucial modifications to the tracrRNA sequence (Cross et al., 2016). We now present a novel all-in-one human whole-genome CRISPRi platform and demonstrate its effectiveness in identifying resistance and sensitivity genes to the BRAF inhibitor vemurafenib. The ability to negatively control transcription by several orders of magnitude opens new avenues and offers researchers the opportunity to identify novel hits in both positive and negative selection screens where dCas9 is non-mutagenic.

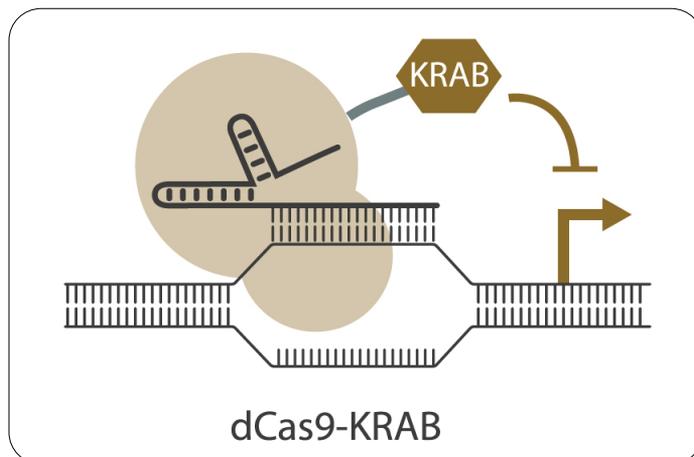
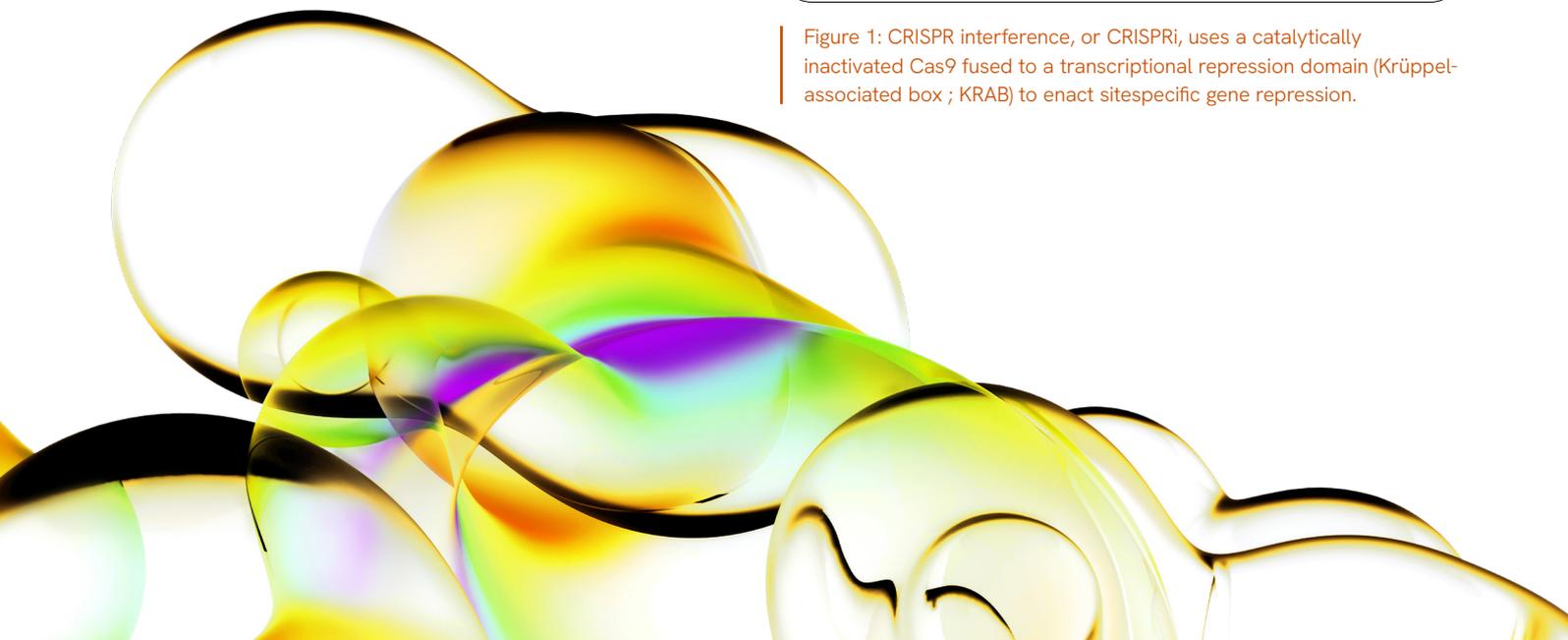


Figure 1: CRISPR interference, or CRISPRi, uses a catalytically inactivated Cas9 fused to a transcriptional repression domain (Krüppel-associated box ; KRAB) to enact sitespecific gene repression.



Platform design

For CRISPRi screening we produced a custom all-in-one lentiviral vector system containing a dCas9-KRAB fusion as well as a U6-promoter driven guideRNA cassette. The simultaneous expression of both dCas9-KRAB and CRISPR from a single lentiviral insert provides a highly efficient targeting strategy required for pooled functional screening. To further enhance performance, we implemented our modified tracrRNA which had previously been demonstrated to improve the robustness of pooled CRISPR-Cas9 drop-out screening (Cross et al., 2016). Finally, we constructed a human whole-genome guide RNA library based on the latest guide RNA design by Horlbeck et al (2016) who have demonstrated strongly improved performance of guide RNAs that bind to genomic DNA locations away from local nucleosomes. This second generation CRISPRi library contains 5 guide RNAs per gene targeting a total of 19,050 genes.

Proof-of concept screening

As proof of concept we performed a genome-wide positive selection screen to identify genes associated with vemurafenib resistance (PLX-4032). Vemurafenib is a BRAF kinase inhibitor which has been shown to act cytostatically in A375 melanoma cells, which carry a BRAF V600E gain-of-function mutation (Davies et al., 2002; Flaherty et al., 2010; Shalem et al., 2014).

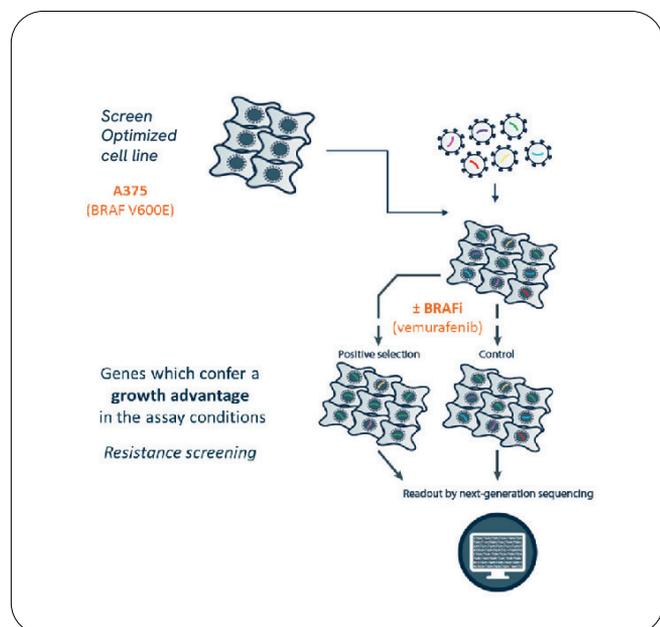


Figure 2: Screen schematic. Resistance to vemurafenib was monitored in response to CRISPRi screening to evaluate the platform performance.

We hypothesized that a CRISPRi system would allow a subset of cells in the population to become resistant to vemurafenib treatment as a result of decreased gene transcription.

CRISPRi Screening platform validation

After 16 days of vemurafenib treatment, screen end pellets were taken and the relative abundance of each of the guide RNAs was measured by next-generation sequencing. Subsequently, guide RNA and gene hit level ranking were evaluated by running the NGS data on a CRISPR-Cas9 screen analysis platform, equipped with an adapted version of the MAGeCK workflow (Li et al., 2014). We observed excellent overall QC performance, as evaluated by the drop-out of key essential genes from the cell population when targeted for repression.

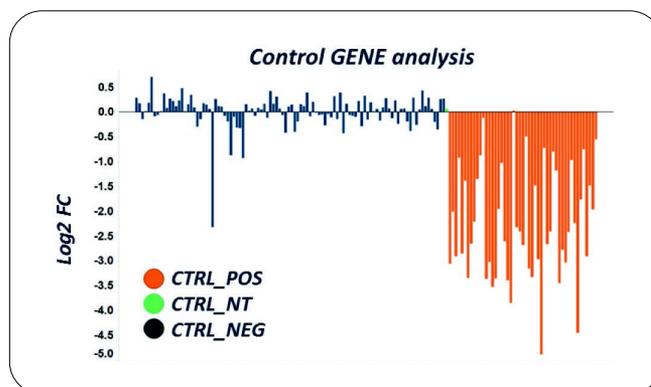


Figure 3: Comparison of guide RNA abundance in DMSO treated cells relative to the plasmid library input. We observed a strong drop-out of core essential genes, such as ribosomal genes and genes involved in replication indicating a strong transcriptional repression effect had been enacted in the screen.

CRISPRi screening hit identification

Following vemurafenib treatment we found >100 fold enrichment of 375 guide RNAs, many of which targeted the same gene.

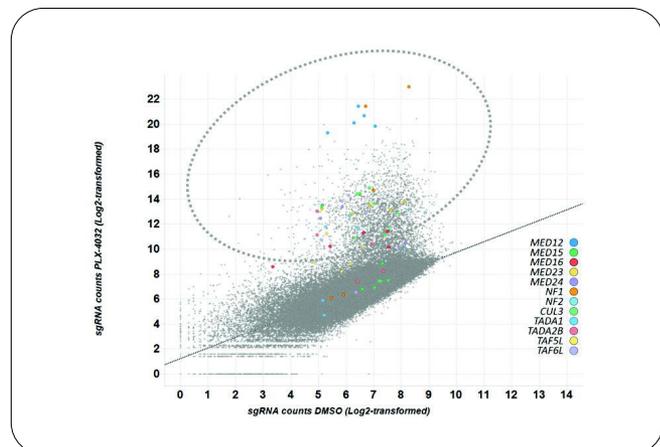


Figure 4: Enrichment of sgRNAs after vemurafenib treatment. Scatterplot of log₂-transformed guide RNA counts in screen conditions after 16 days of DMSO (x-axis) versus vemurafenib treatment (y-axis). Among the guides that were enriched in the vemurafenib treated condition (circled) was a set of guide RNAs targeting the same genes multiple times (highlighted in colour).

Gene-level hit identification was then conducted using assimilated scores from each of the guides targeting each gene. Among the highest ranking genes were *MED12*, *NF1*, *CUL3*, *TADA1*, *NF2* and *TADA2B*, genes whose loss is known to confer resistance to vemurafenib (Huang et al., 2012; Whittaker et al., 2013; Shalem et al., 2014). In addition, we find more members belonging to either Mediator (*MED15*, *MED16*, *MED23* and *MED24*) or SAGA (*TAF5L* and *TAF6L*) complexes.

Many additionally identified hits were found to be members of the of the MED complex, and these genes which are not specifically detected by the equivalent screens conducted using CRISPR knock out technology.

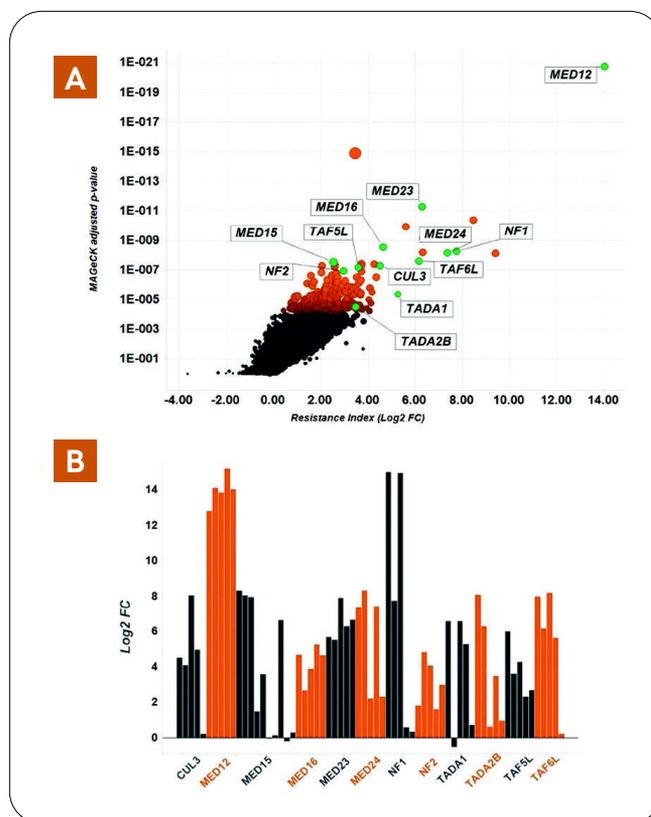


Figure 5: Data from the vemurafenib screening analysis. A. Full screen data showing log₂ fold enrichment of each gene and associated p-values. Highlighted hits have been previously identified and validated by CRISPR knock-out screening. B. Individual guide performance for each of the highlighted hits showing enrichment level in each case.

Importantly, when evaluating ranking of the individual guide RNAs of the resistance top hits, only few guide RNAs did not perform optimally, indicating the robustness of CRISPRi and reflecting its ability to effectively downregulate gene output to enable resistance to vemurafenib.

Interestingly, although the screen was specifically designed to identify vemurafenib resistance factors. Genes whose loss increase sensitivity to the compound included *EGFR* and *ITGB5*, two genes previously reported to aid in vemurafenib resistance when expression is enhanced by CRISPRa technology (Koneremann et al., 2015).

This finding underlines the high level of performance of the guide RNAs enabling hit identification even when the window of detection is small due to the high selective pressure treatment conditions for a given phenotype.

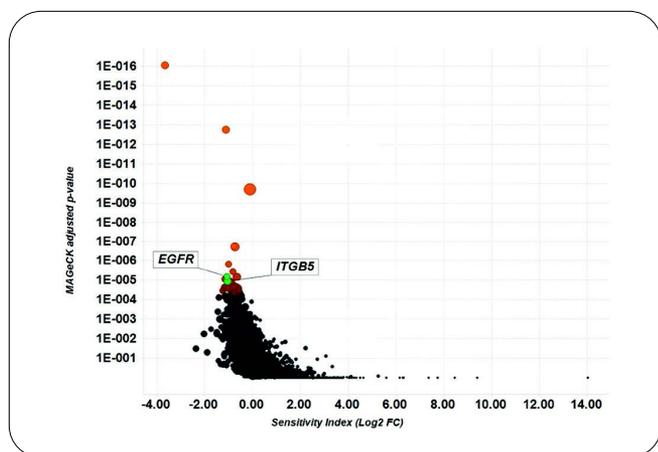


Figure 6: Genes which were found depleted in the vemurafenib-treated population included *ITGB5* and *EGFR*, which are known sensitising factors to treatment with this drug.

Summary

Taken together, we have used a proof-of-concept approach to examine the ability of our CRISPRi platform to produce resistance to the BRAF inhibitor vemurafenib. We were able to identify all validated genes previously shown to confer resistance to vemurafenib treatment by the CRISPR-Cas9 knockout technology. Overall, the platform performance was very high, with excellent precision and hit identification power.

As a new member of the genetic manipulation toolbox, CRISPRi will be invaluable to study hypomorphic phenotypes, such as those involving essential genes.

Methods

A375 melanoma cells were infected with Revvity's CRISPRi-dCas9 lentivirus at low MOI, to ensure integration of a single construct per cell. Cells carrying integrated lentivirus were selected with an antibiotic to eliminate non-transduced cells. The screen dose phase was then initiated and cell populations were treated with media containing vemurafenib (2 μ M) or control (DMSO), each in two replicates. When control treated cells reached population doubling 16, end pellets were collected, genomic DNA was extracted and guideRNA abundance in each condition measured by NGS.

References

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