Direct comparison of CRISPR knockout and interference with Perturb-seq

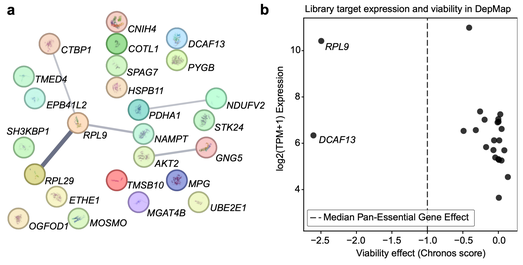
KATHLEEN B YATES[1], LAURA M DREPANOS[1], BERTA ESCUDE VELASCO[1], SMRITI SRIKANTH[1], ERIKA LI[1], ABIGAIL CHASE[1], TSUKASA SHIBUE[1], PETER S WINTER[1], ANDREW W NAVIA[1], MICHAEL GATZEN[1], HANNAH D RICKNER[1], DAN DUBINSKY[1], TONI M DELOREY[1], ELEANOR G KAPLAN[1], JOHN G DOENCH[1]

[1]Broad Institute of MIT and Harvard, Cambridge, MA • Correspondence: jdoench@broadinstitute.org

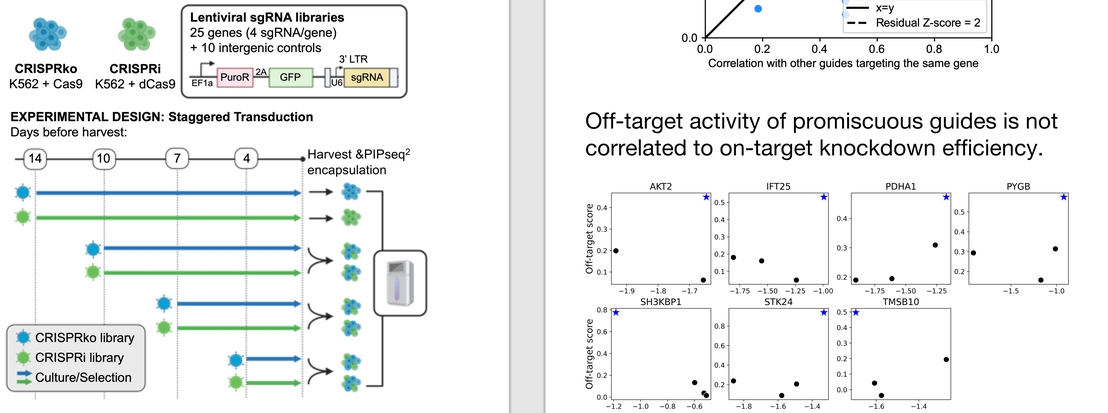
Abstract

CRISPR knockout (CRISPRko) and CRISPR interference (CRISPRi) are workhorse technologies for loss-of-function studies, yet direct comparisons remain scant. Here, we establish benchmarking libraries for Cas9-based CRISPRko and CRISPRi Perturb-seq screens. For both modalities, cells with the same gene perturbed share consistent transcriptional signatures, strong evidence of on-target signal. We also examine tradeoffs: CRISPRi guides elicit heightened rates of off-target activity, while CRISPRko produces artifacts stemming from the cellular response to double-stranded breaks. As atlas-scale Perturb-seq data increasingly train foundation models of cell state, these artifacts are learned alongside genuine biology, limiting what such models reveal about gene function. The libraries and analyses presented here provide a benchmarking resource for any group preparing a large-scale Perturb-seq screen, and practical filters for separating real perturbation responses from modality-specific artifacts.

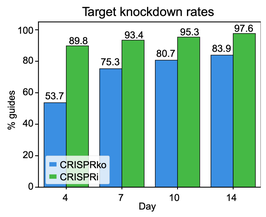
Head-to-head screen design



Matched CRISPRko and CRISPRi libraries target the same 25 functionally diverse genes[1].

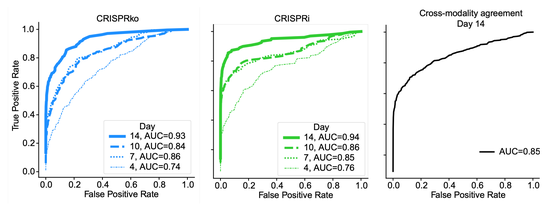


Consistent on-target activity



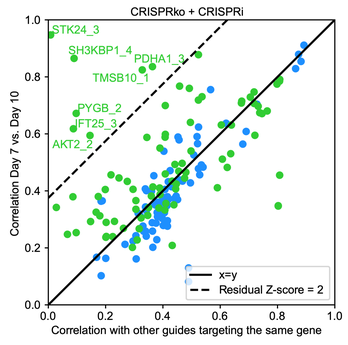
Most perturbations show significant target knockdown (SCEPTRE[3] FDR<0.1) at all time points.

Guide-to-guide signature consistency



CRISPRi shows greater sequence-dependent off-target activity

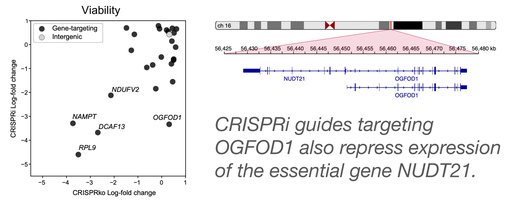
Effects reproduced by the same guide across independent transductions and timepoints, but not shared with other guides targeting the same gene, indicate off-target activity. Seed-driven dCas9 binding is a likely mechanism[4,5].



Off-target activity of promiscuous guides is not correlated to on-target knockdown efficiency.

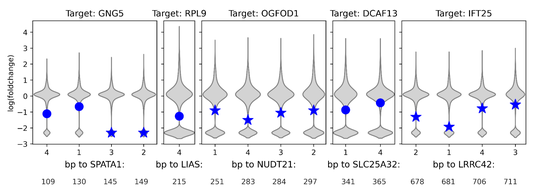
Target-dependent off-target activity

CRISPRi guides repress expression of neighbor genes.

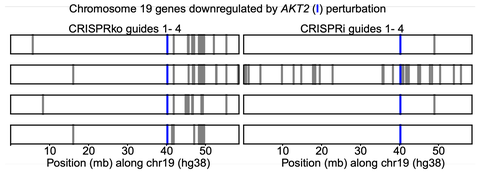


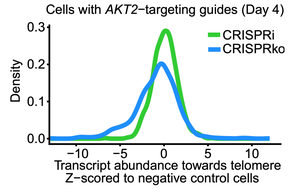
CRISPRi guides targeting OGFOD1 also repress expression of the essential gene NUDT21.

Up to 17% of protein-coding genes have a TSS within 1 kb of another gene's TSS and cannot be perturbed independently by CRISPRi[6,7].

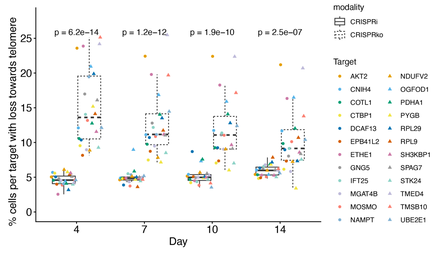


CRISPRko: reduced expression of genes towards the telomere





CRISPRko shows increased frequency of inferred arm loss[8] across all targets and time points.



Practical recommendations

Liability	CRISPRko	CRISPRi
Sequence-dependent off-target	• Use aggregate CFD at library design[9] • Remove promiscuous guides before analysis	• Avoid PAM-rich seed sequences[4] • Remove promiscuous guides before analysis[6]
Target-dependent off-target	Chromosome arm truncation • Remove cells showing arm truncation[10] • Apply geometric arm correction[8]	Dual promoters • Validate with an orthogonal technology
Mechanism-dependent off-target	DSB-induced DNA damage response • Check p53 status of the cell line[11] • Include cutting and non-cutting controls • Validate with an orthogonal technology	Effector overexpression toxicity • Use a repressor domain with low known toxicity[12]

Recommended approach:

- Use at least 4 independent constructs per gene, and require phenotypes to reproduce across them.
- Avoid vectors expressing multiple guides against the same target, as it increases off-target liability.
- Inspect guide-level data before running weighted differential expression.
- Screen (or validate) with both modalities where feasible.

Conclusions

- Both modalities give reproducible on-target signatures.
- CRISPRi elicits more guide-specific off-target activity, while CRISPRko induces chromosome-arm artifacts.
- Multiple independent constructs per gene are essential; multi-guide vectors may obscure off-target activity.

Preprint describing this comparison of CRISPRko and CRISPRi in Perturb-seq data.

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