Discussion

Recent Advances in CRISPR-based Screening and Potential Applications

Yongcheng Jiang

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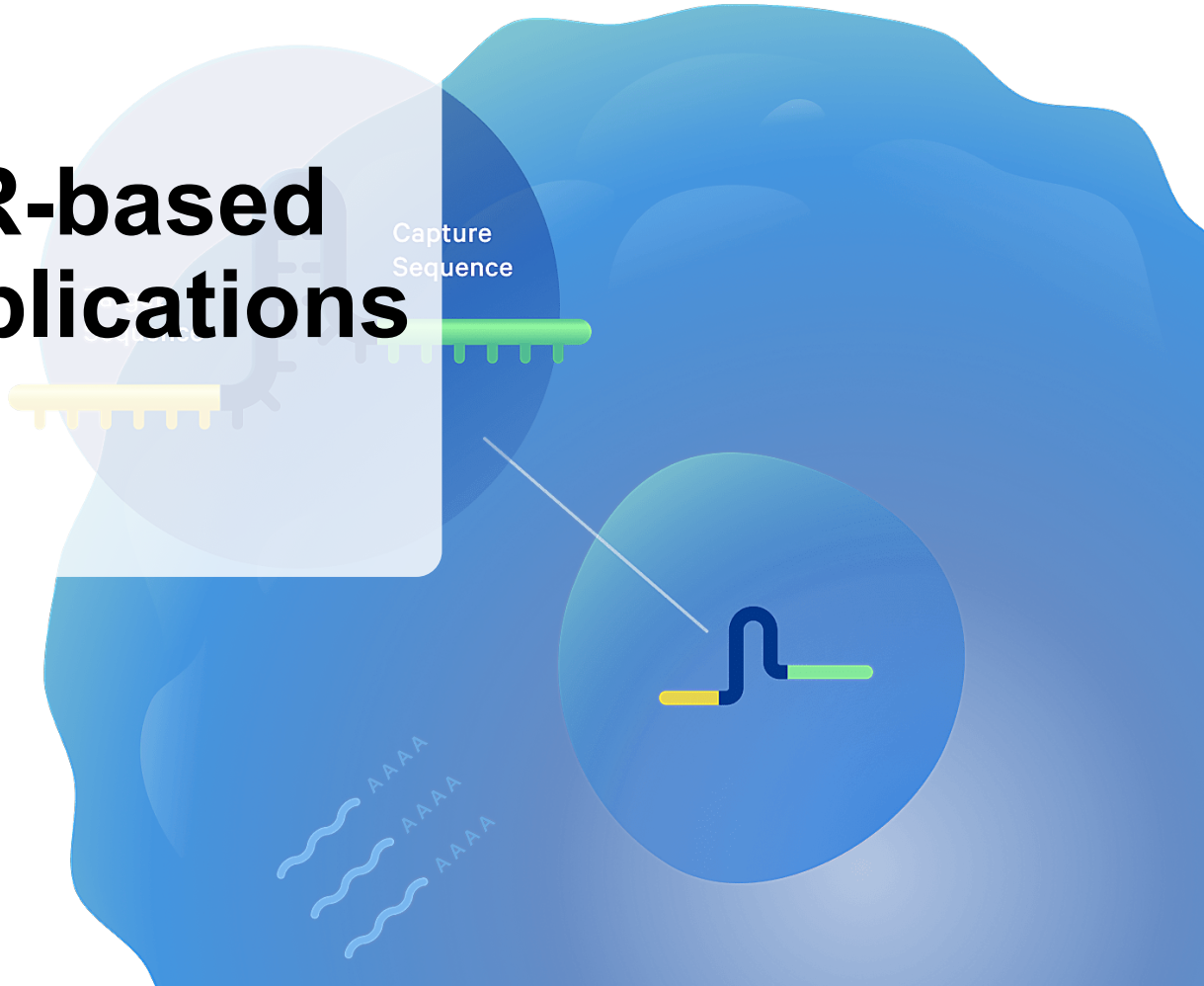


Outline of the discussion

- Recent advances and applications
- Limitations and future perspectives

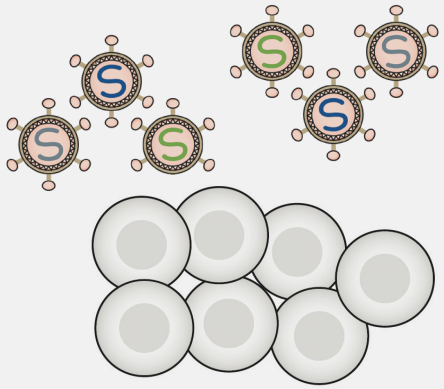
What is the big picture of the field?
What technological/biological breakthroughs could it bring?

Recent advances and applications

How could traditional CRISPR screens be improved?

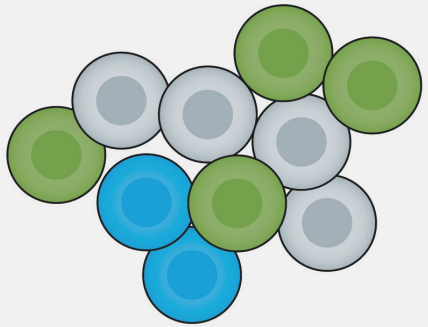
Traditional CRISPR screens pipeline

Library construction and transfection



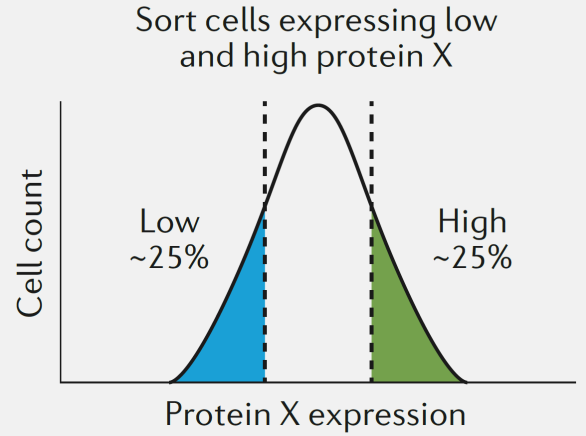
Cas9 CRISPR KO
dCas9 CRISPRi/a

Perturbation



Pooled screens

Readout phenotypes and genotypes



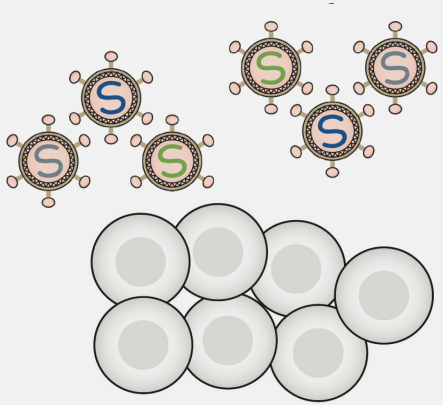
Survival/dropout
FACS markers [1]
sgRNA abundance [1, 2]

Targets: coding DNA only

Przybyla, L., & Gilbert, L. A. *Nature Reviews Genetics* (2021)

Three main research frontiers

Library construction and transfection



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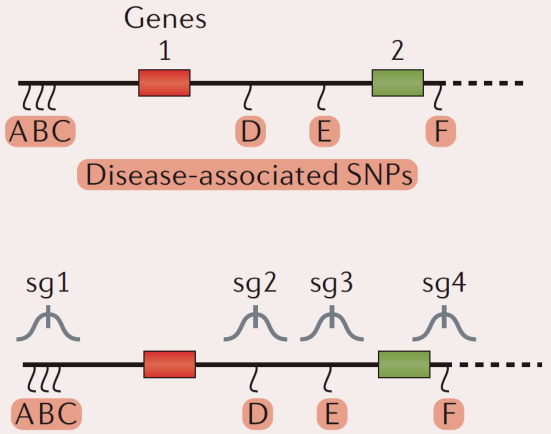

Cas9 CRISPR KO
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Cas13 CRISPRi

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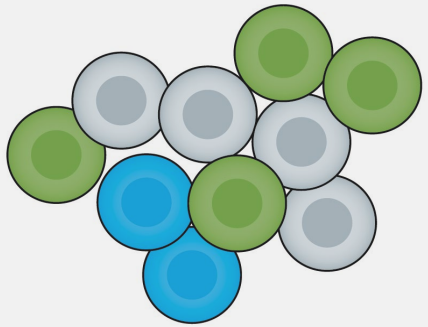
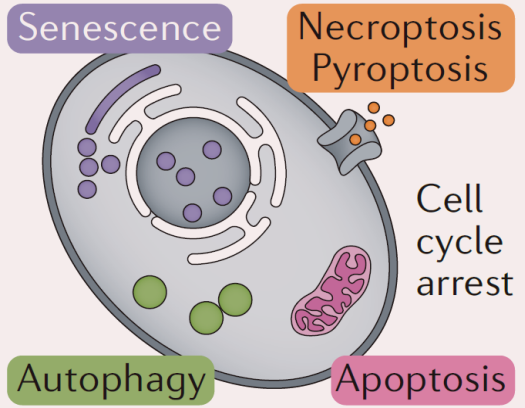


Pooled screens

Altering screening modalities



Arrayed screens
OP. screens

Przybyla, L., & Gilbert, L. A. *Nature Reviews Genetics* (2021)

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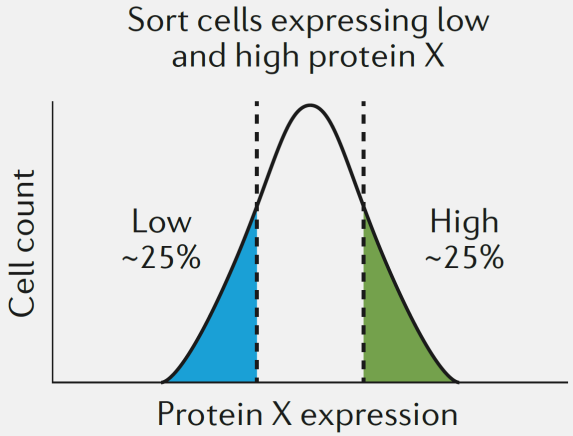
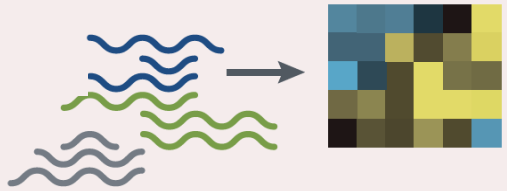


Survival/dropout

FACS markers [1]

sgRNA abundance [2]

Single-cell functional genomics



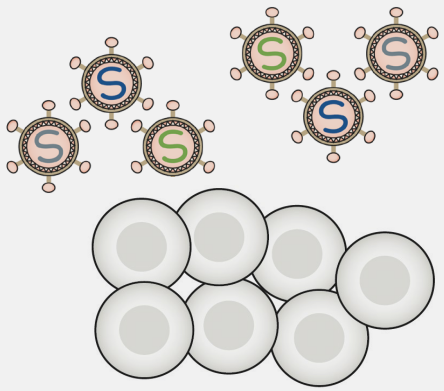
Single-cell sequencing

- ❑ Transcriptomics
- ❑ Genetic interactions
- ❑ Epigenomics

Przybyla, L., & Gilbert, L. A. *Nature Reviews Genetics* (2021)

Traditional CRISPR screens pipeline

Library construction and transfection



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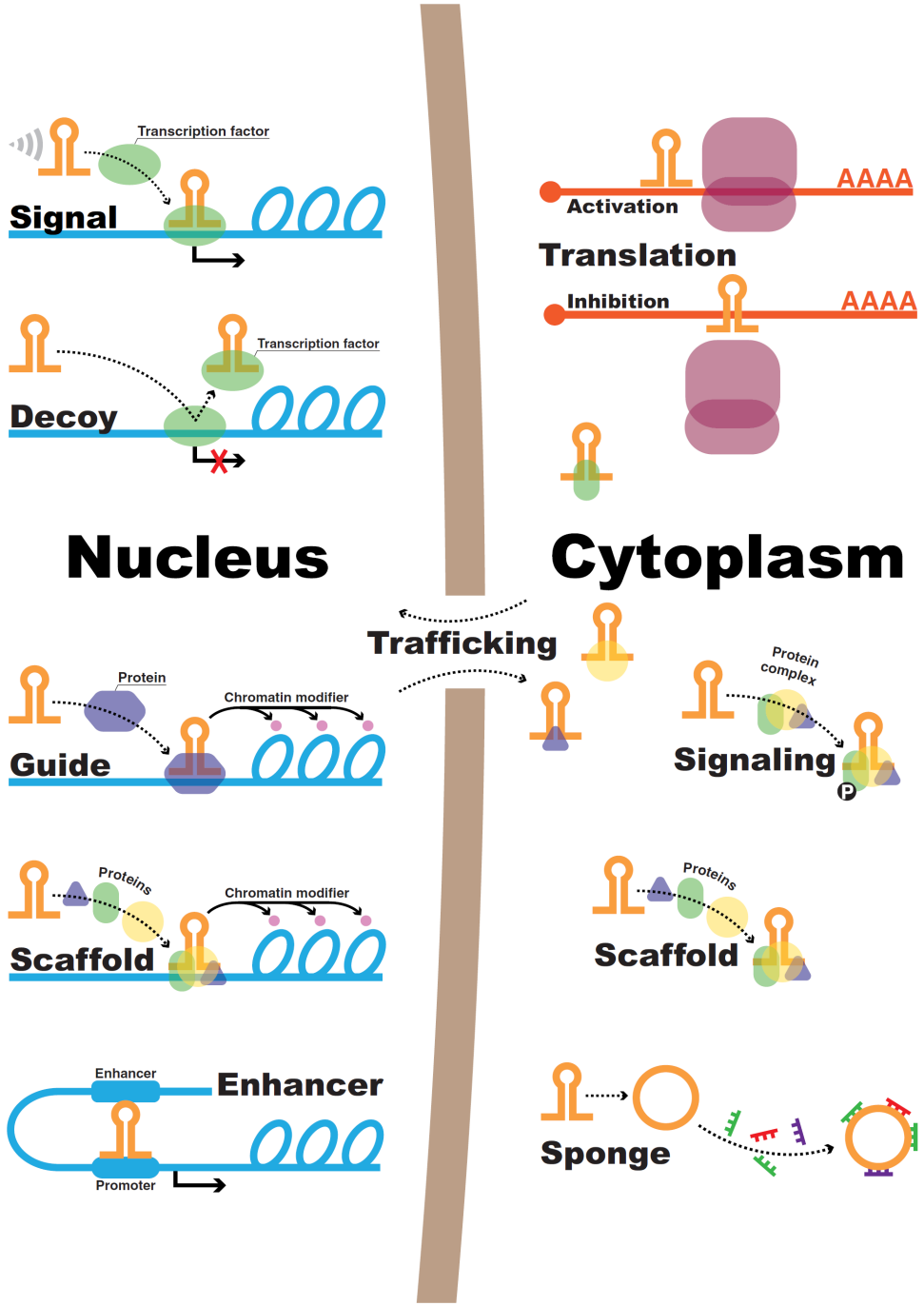
Targeting non-coding genome

Non-coding genome regulates phenotypic profiles of living systems

A large proportion (>98%) of genome is consist of non-coding sequence.

Enhancer, silencer, lncRNA…

CRISPR KO (Cas9) on struc. non-coding loci
CRISPRi (dCas9) on lncRNA loci
CRISPRi (Cas13) on RNA transcript



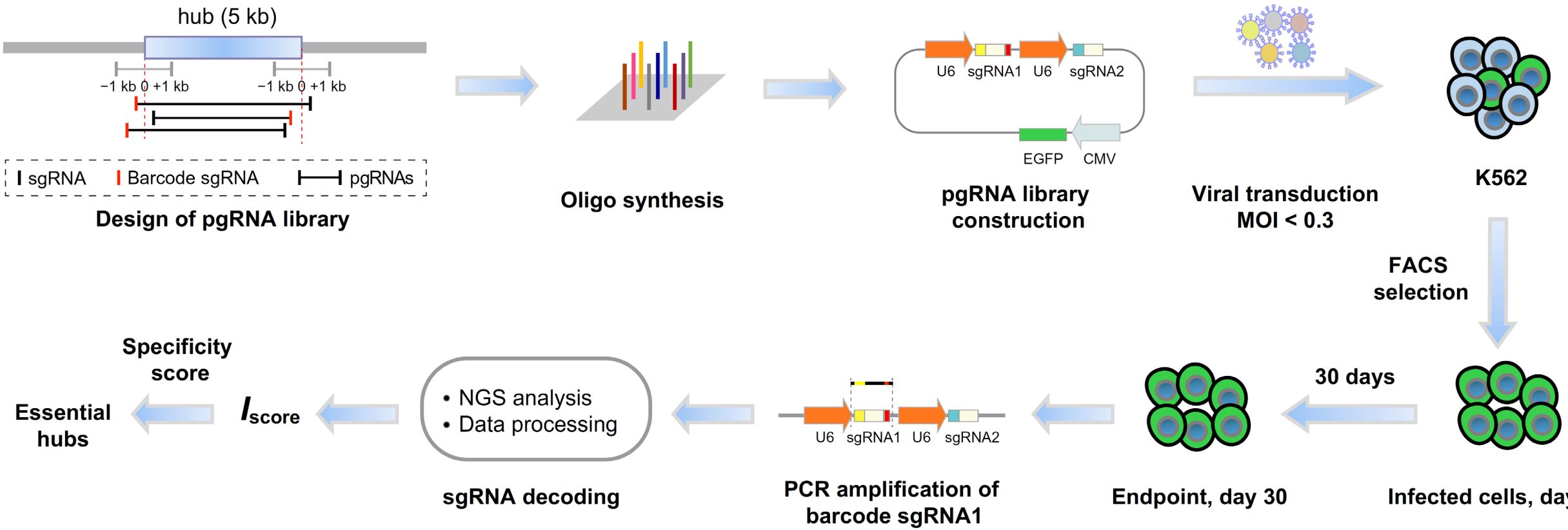
Struc. = chromatin structure related

Gomes, C. P. C, *et al. Molecular Therapy: Nucleic Acids* (2017)

Targeting non-coding genome

Cas9-mediated knockout on noncoding loci in Hi-C hubs

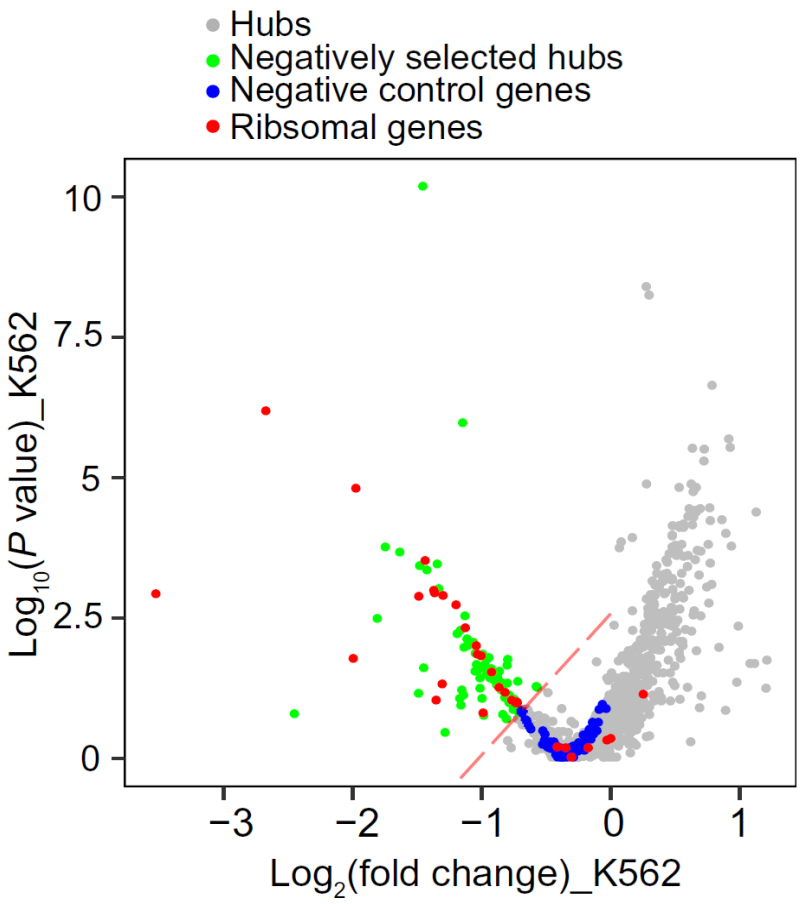
Hi-C network analysis → KO live/dead screening → Hi-C/single cell transcriptomics

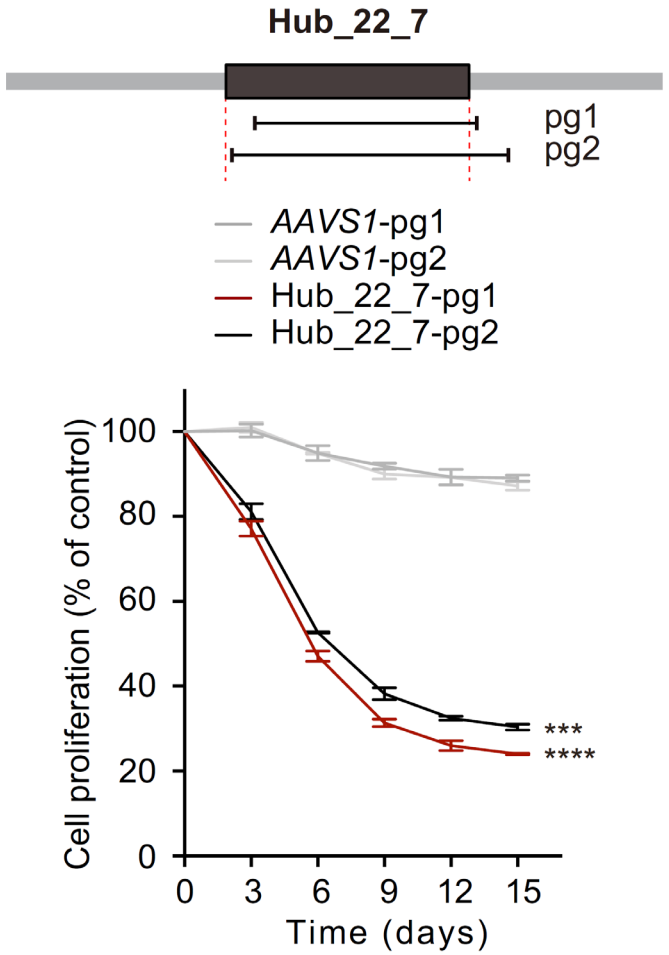


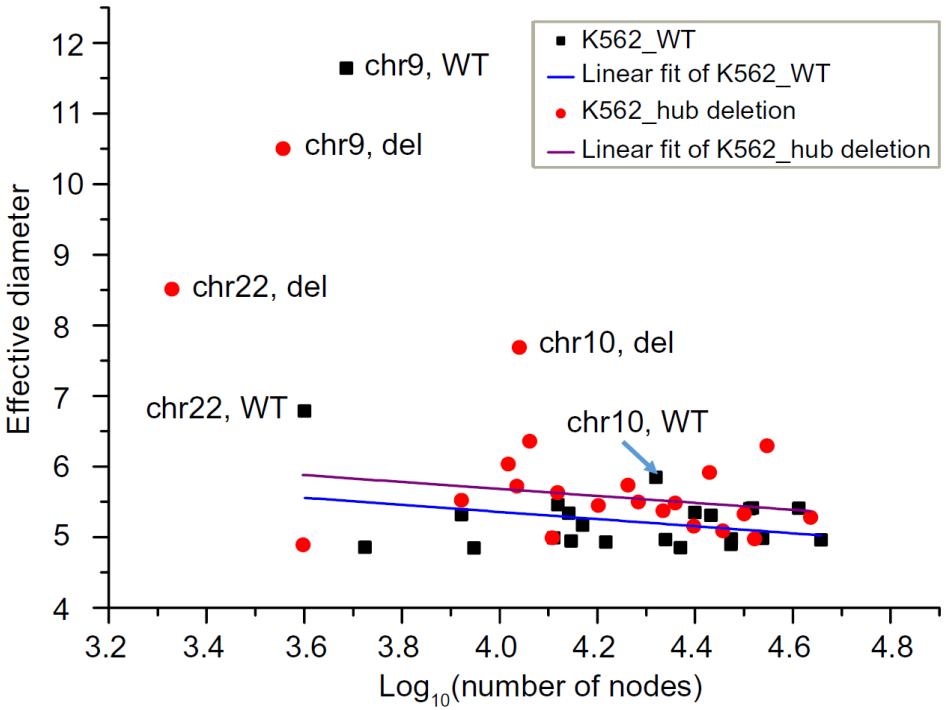
Ding, B. *et al. Science Advances* (2021)

Targeting non-coding genome

Deletion of essential hubs can alter the global chromatin structure







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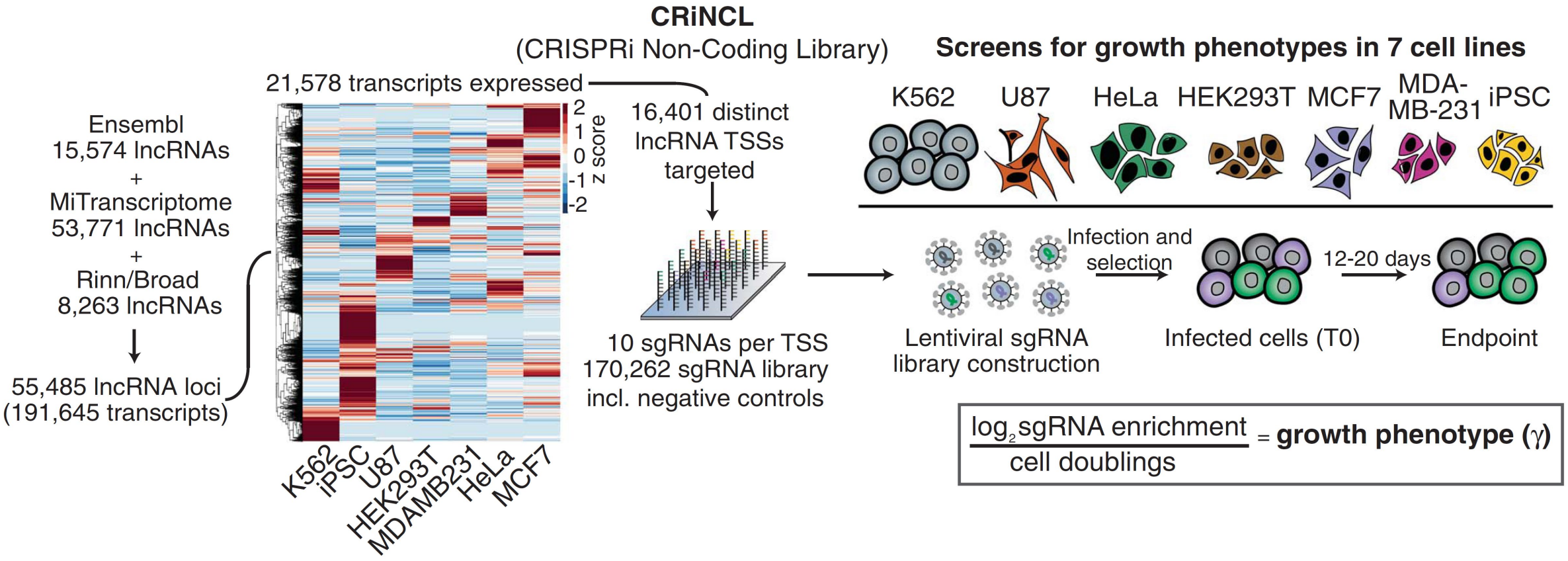
Targeting non-coding genome

dCas9-mediated interference on lncRNA loci in human cells

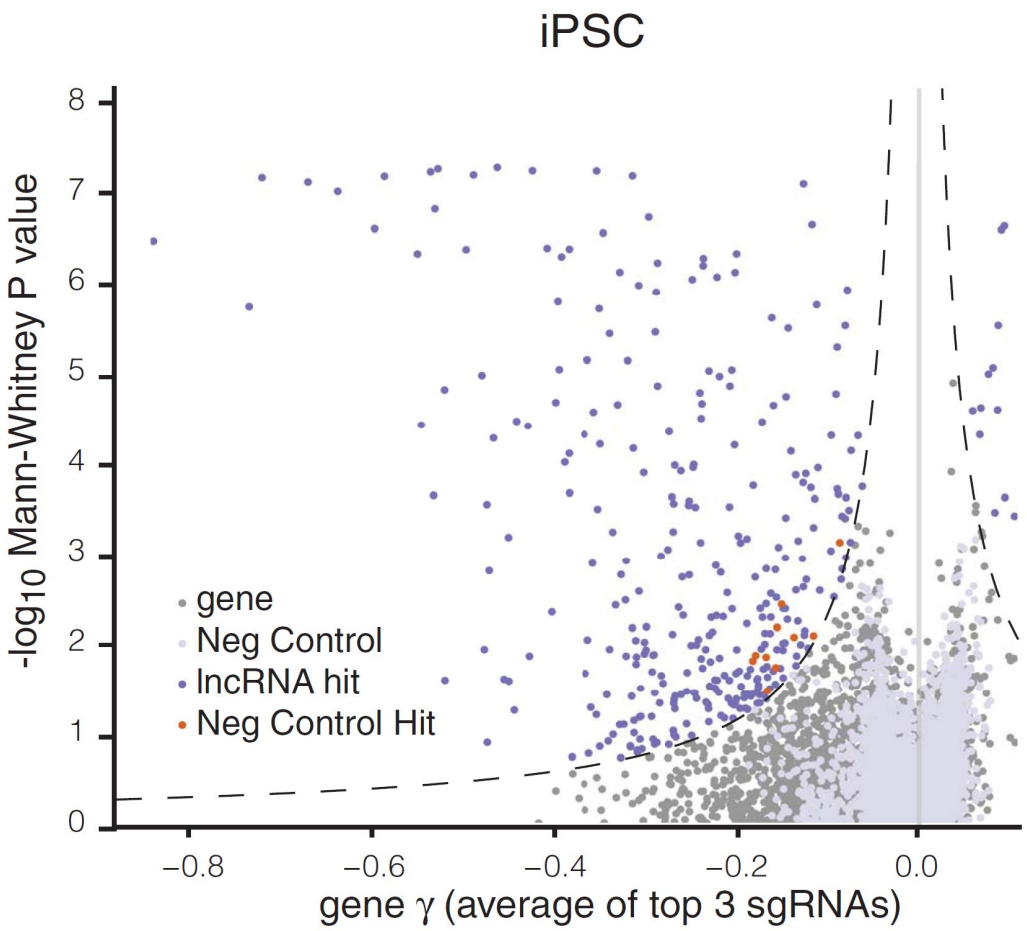


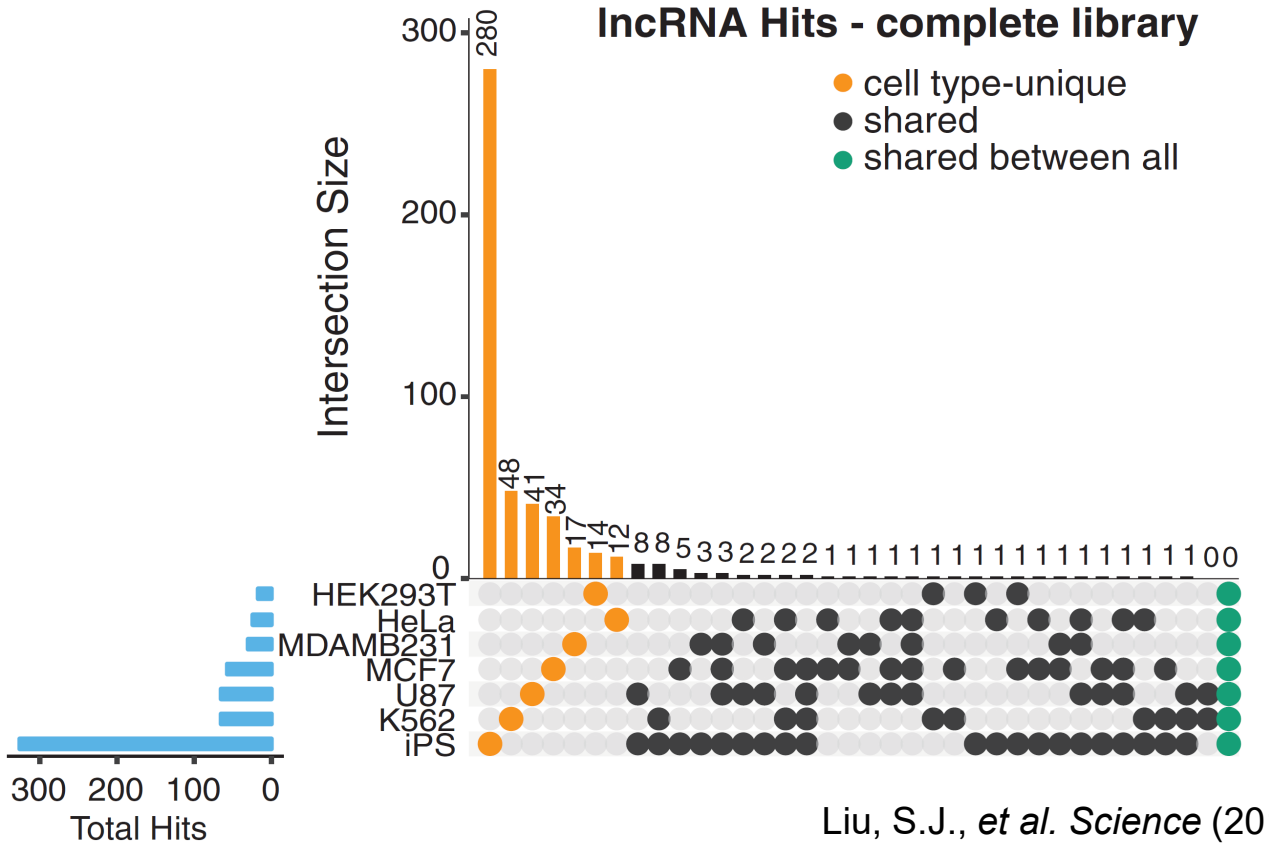
Liu, S.J., *et al. Science* (2017)

Targeting non-coding genome

lncRNA knockdown perturbs in a cell type-specific manner

	Genes targeted in screen	Total hits	lncRNA genes neighboring essential coding genes	lncRNA hits
iPSC	5,534	438	112	**326**
MCF7	5,725	117	60	**57**
U87	5,689	88	23	**65**
K562	16,401	144	79	**65**
MDA-MB-231	5,725	44	14	**30**
HeLa	6,158	52	28	**24**
HEK293T	5,785	28	11	**17**

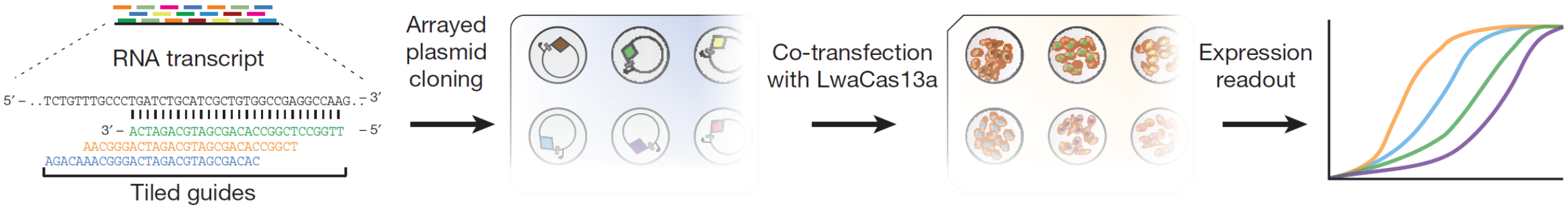


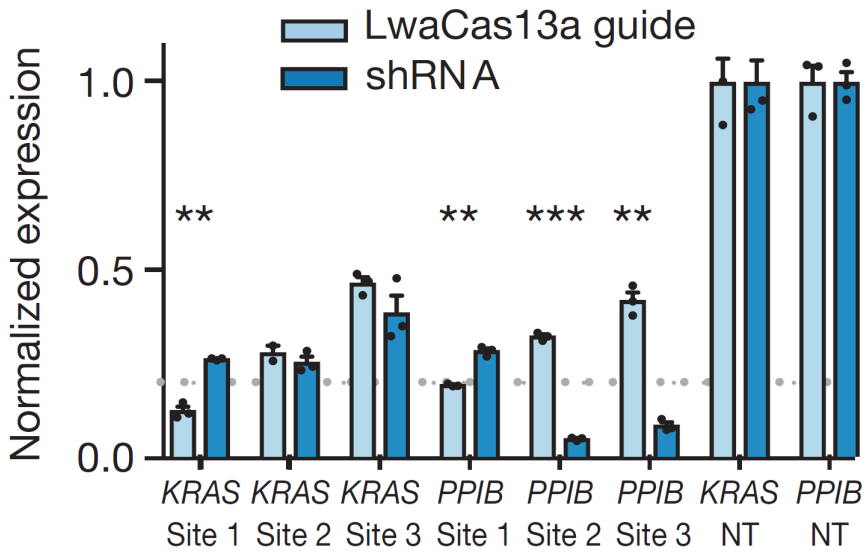


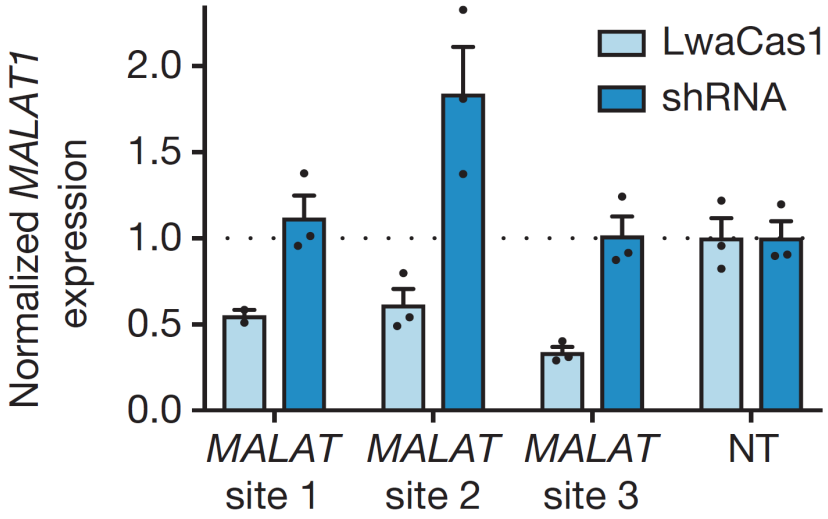
Liu, S.J., *et al. Science* (2017)

Targeting non-coding genome

Cas13-mediated interference on coding and non-coding RNAs







KRAS: endogenous gene
PPIB: endogenous gene
MALAT: nuclear transcript

Abudayyeh, O. O., *et al. Nature* (2017)

Traditional CRISPR screens pipeline

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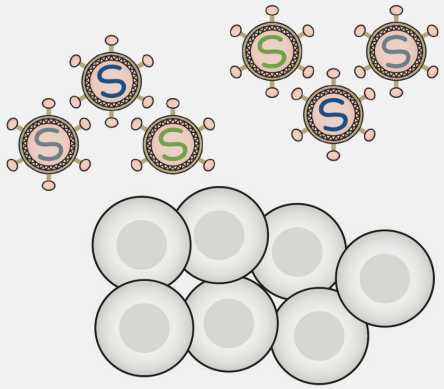

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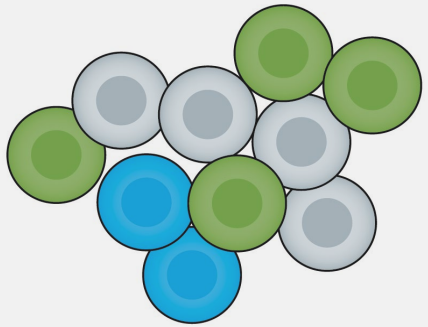

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Readout phenotypes and genotypes

Survival/dropout
FACS markers [1]
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Targets: coding DNA only

Przybyla, L., & Gilbert, L. A. *Nature Reviews Genetics* (2021)

Altering screening modalities

Pooled screens are limited to low-content readouts

Traditional pooled screens

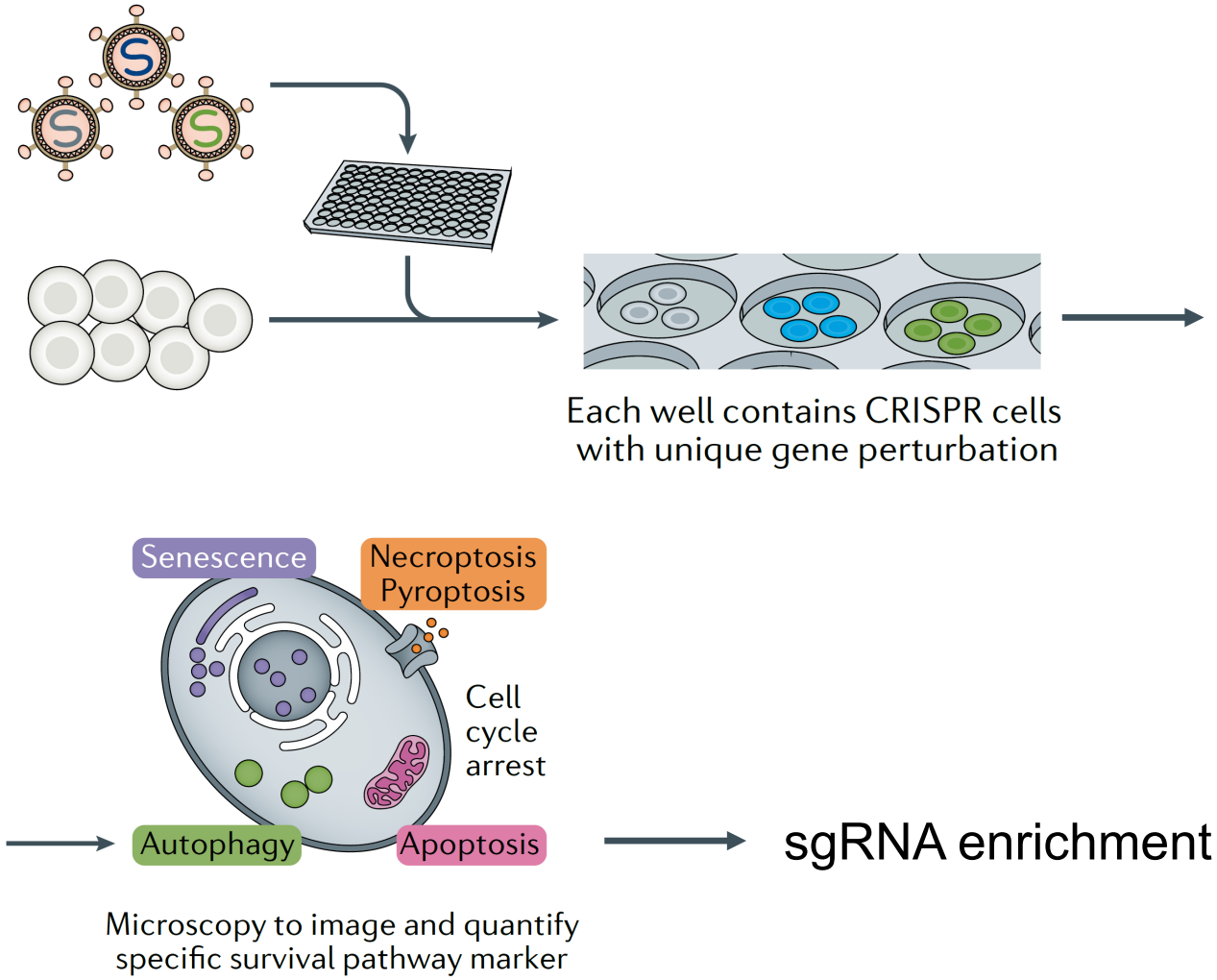
No individual information (i.e., morphology, protein dynamics)

Arrayed screens

Microscopy plus markers or dyes

Optical pooled screens

FACS or *in situ* sequencing



Yan, X., *et al. Journal of Cell Biology* (2021)

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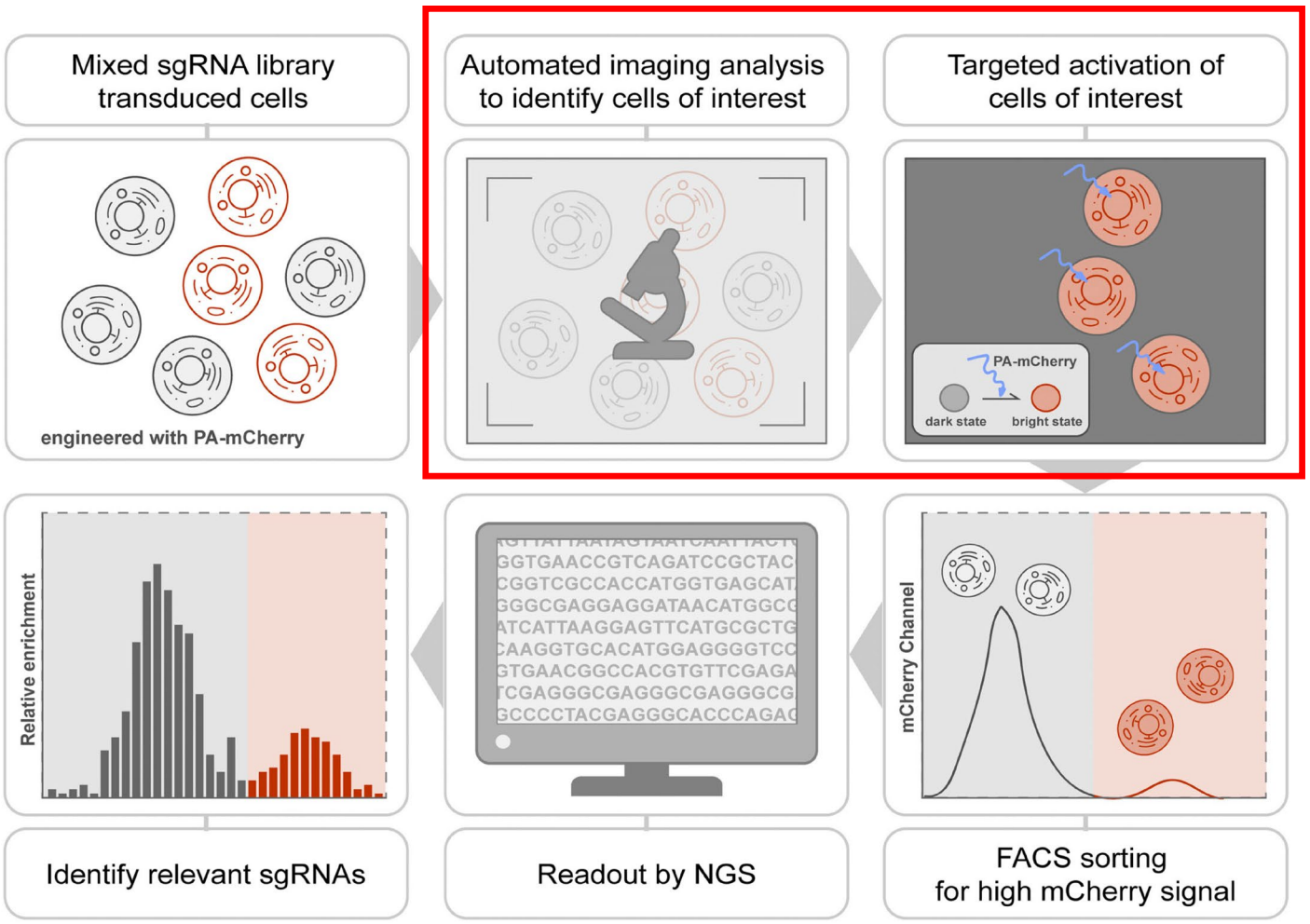
Optical pooled screens

FACS or *in situ* sequencing

Optical enrichment with **PA-mCherry**



Yan, X., *et al. Journal of Cell Biology* (2021)

Altering screening modalities

Pooled screens are limited to low-content readouts

Traditional pooled screens

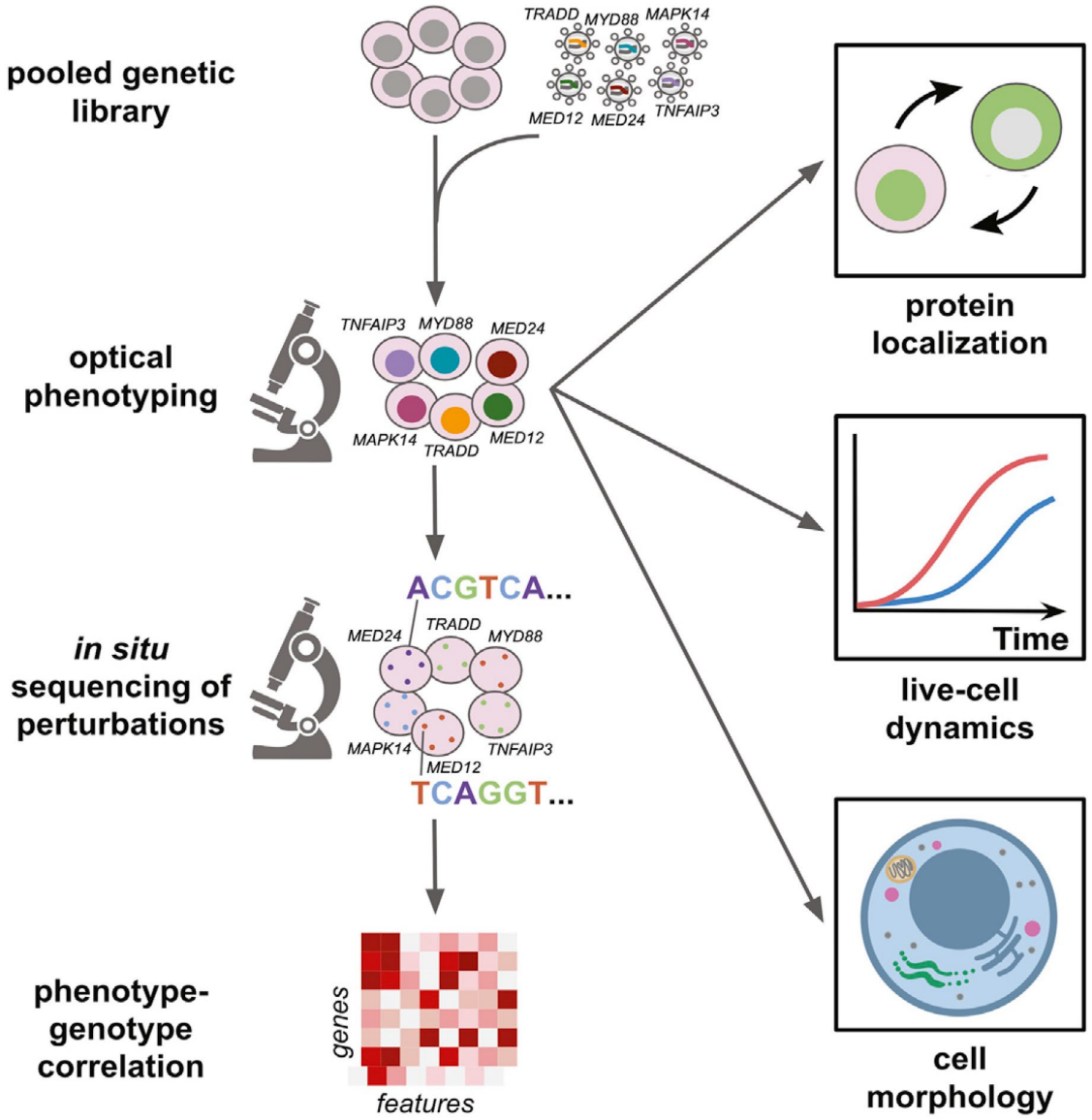
1. Only population-level data
2. Spatial data is lost
3. Relied on sgRNA enrichment

Arrayed screens

Microscopy plus markers or dyes

Optical pooled screens

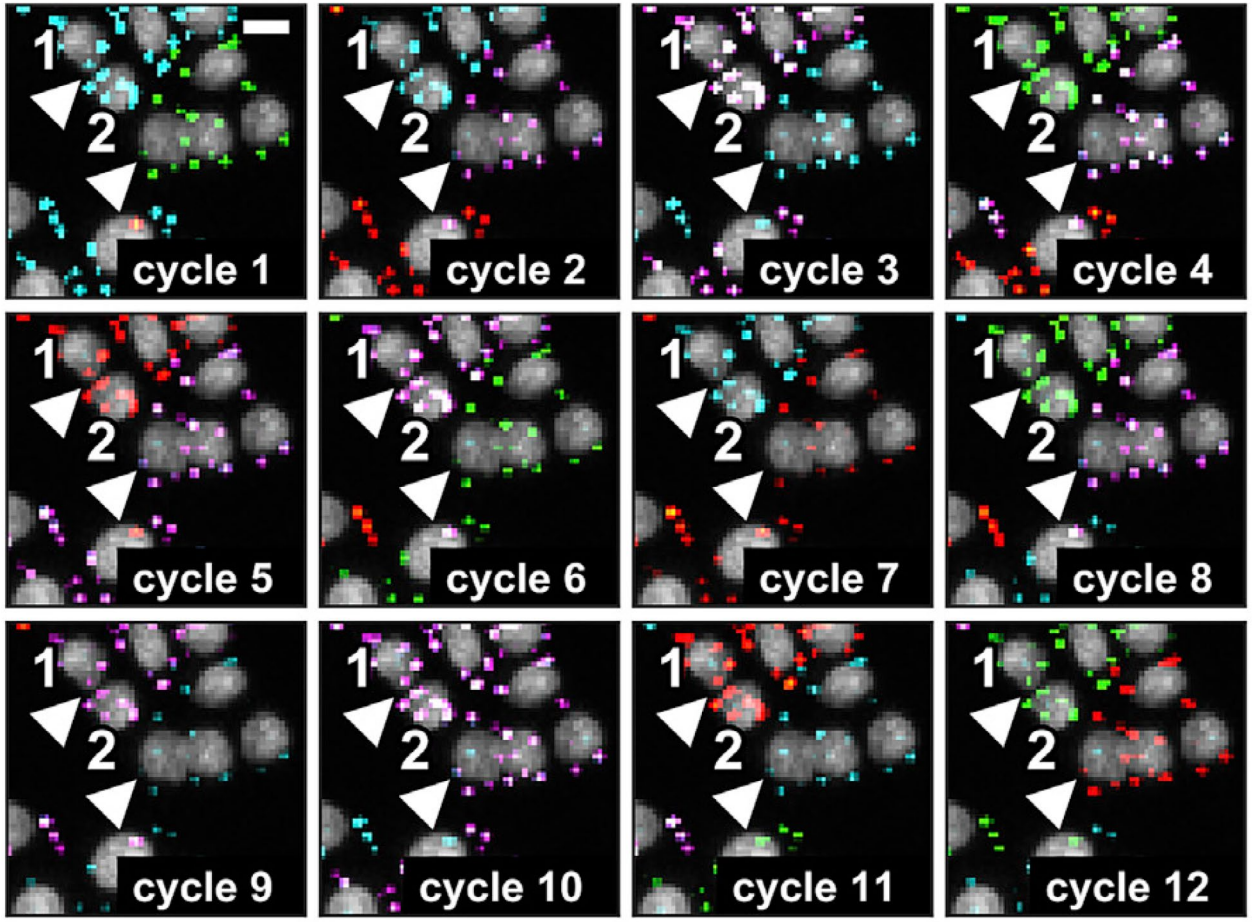
FACS or *in situ* sequencing

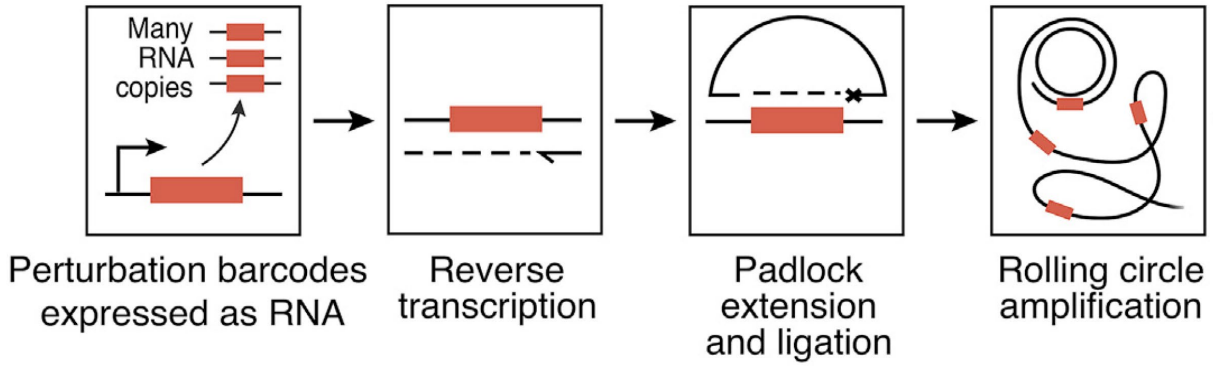


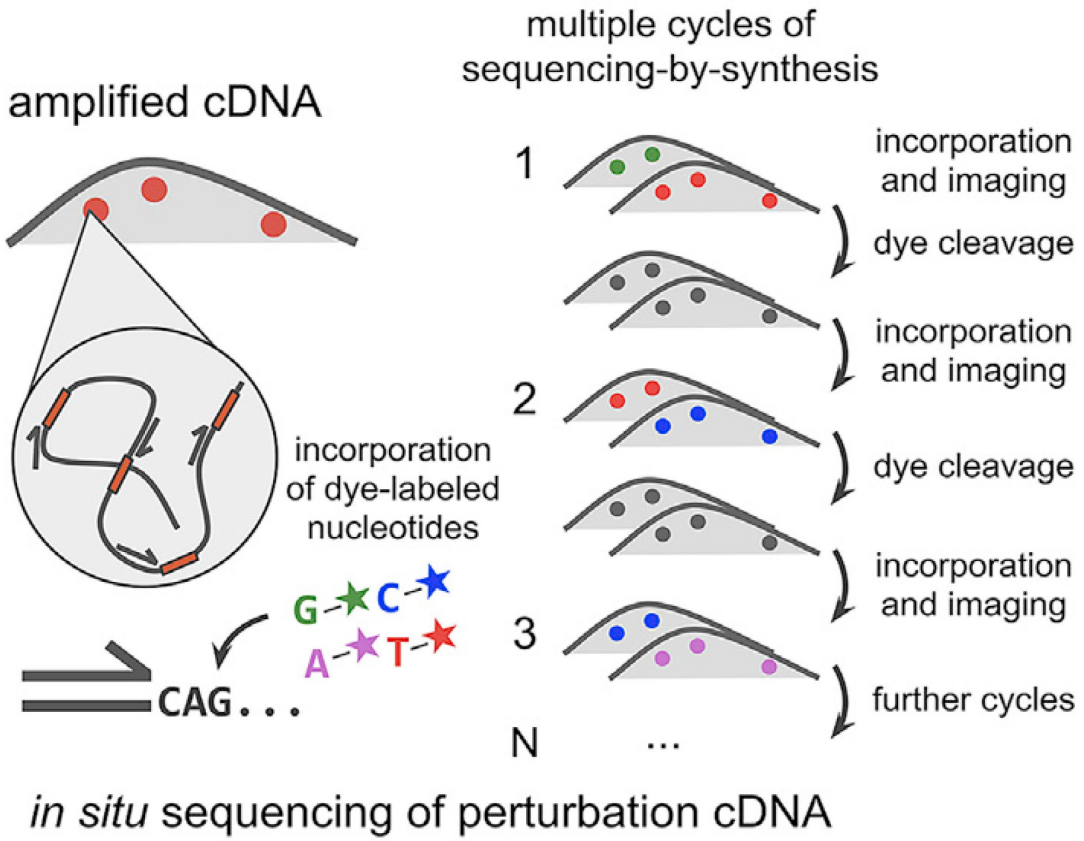
Feldman, D., *et al. Cell* (2020)

Altering screening modalities

Optical pooled screens apply *in situ* sequencing to readout spatio-temporal phenotypes



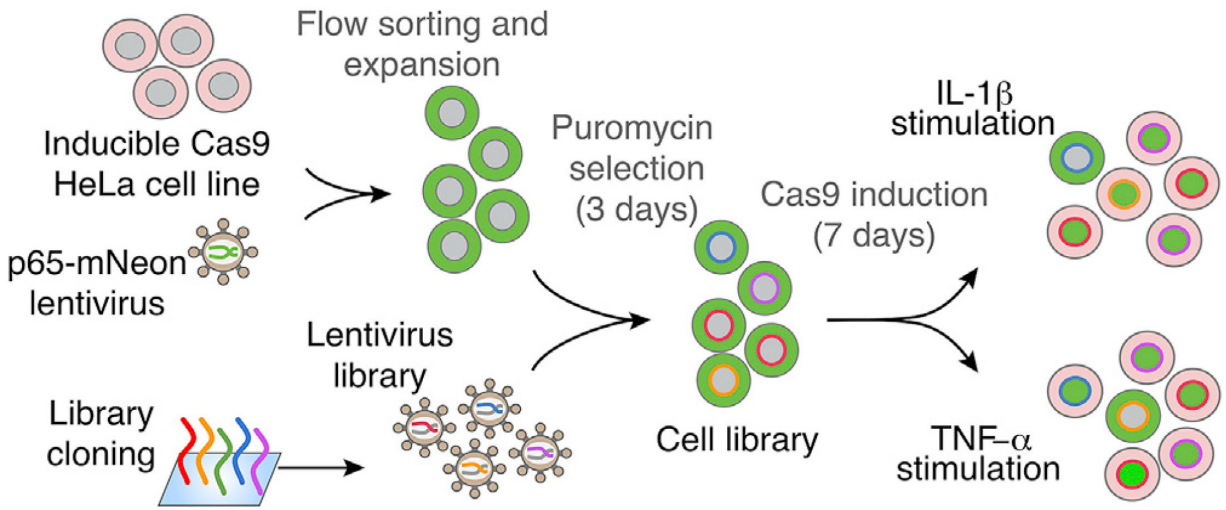




Feldman, D., *et al. Cell* (2019)

Altering screening modalities

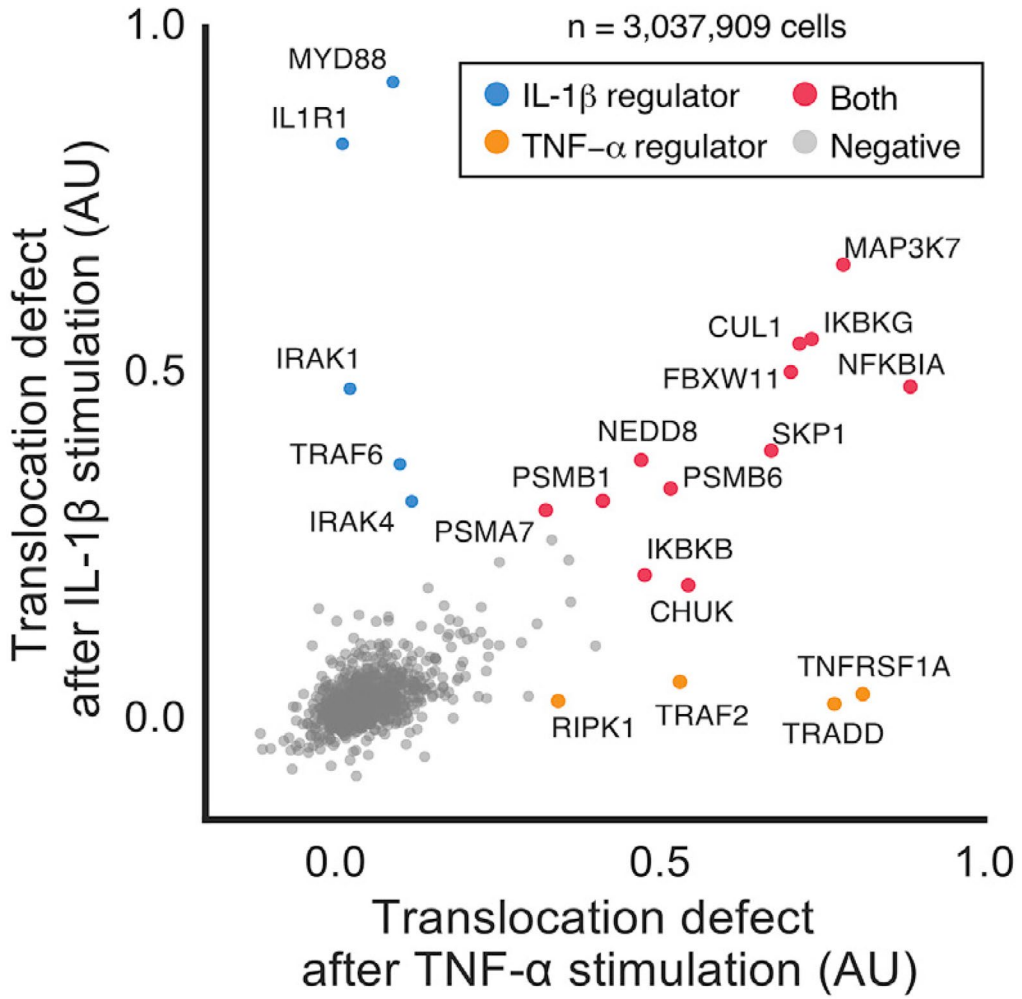
Optical pooled screens in identifying genes for activation of NF-κB



Phenotype imaging and *in situ* barcode amplification (2 days) → *In situ* sequencing (3 days) →

Gene perturbation and phenotype data for each cell

Cell	Gene	Stimulant	Translocation score
1	*Gene A*	IL-1β	x_1
⋮	⋮	⋮	⋮
M	*Gene Z*	IL-1β	x_M
1	*Gene A*	TNF–α	x_1
⋮	⋮	⋮	⋮
N	*Gene Z*	TNF–α	x_N



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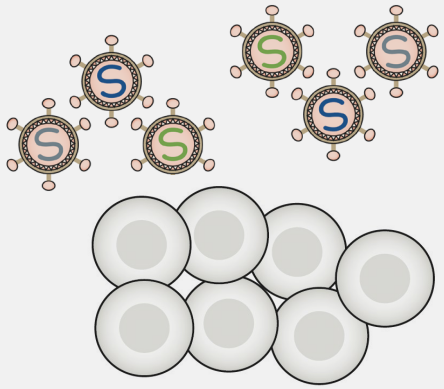

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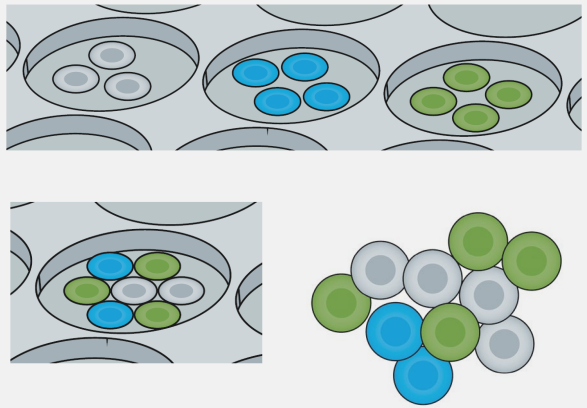
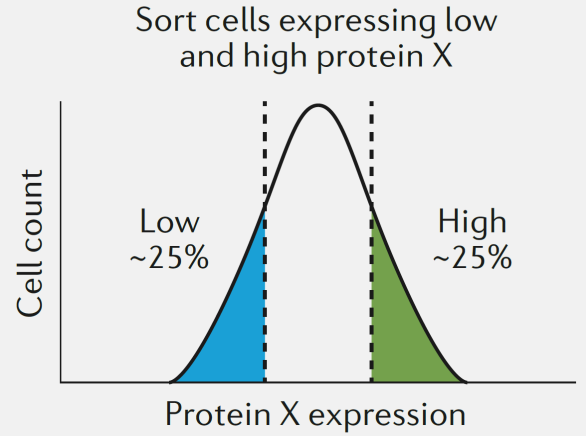


Pooled screens
Arrayed screens
OP. screens

Readout phenotypes and genotypes



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FACS markers [1]
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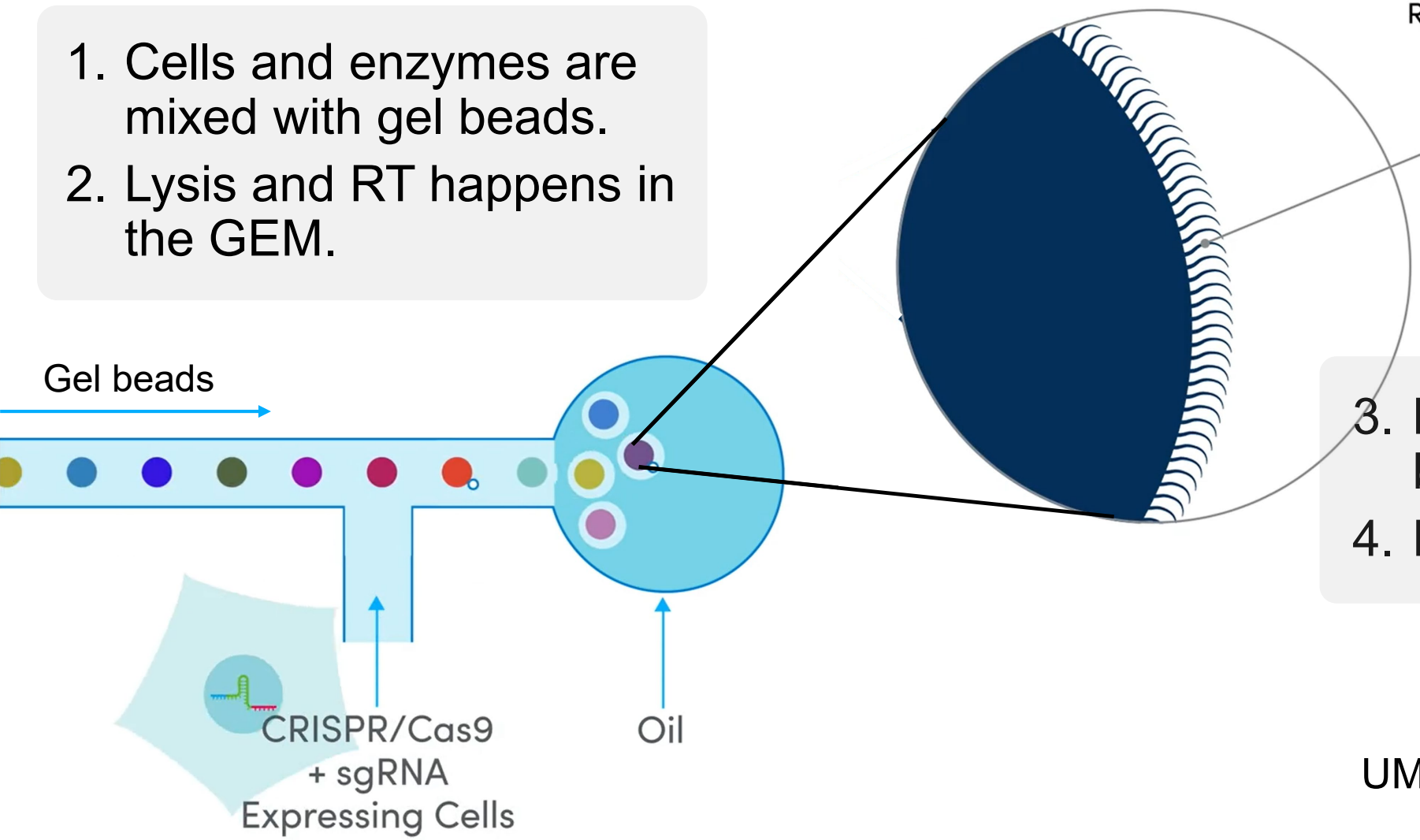
Przybyla, L., & Gilbert, L. A. *Nature Reviews Genetics* (2021)

Single-cell omics capture individual genomic profiles

1. Cells and enzymes are mixed with gel beads.
2. Lysis and RT happens in the GEM.



\+

sgRNA (GBC) or mRNA

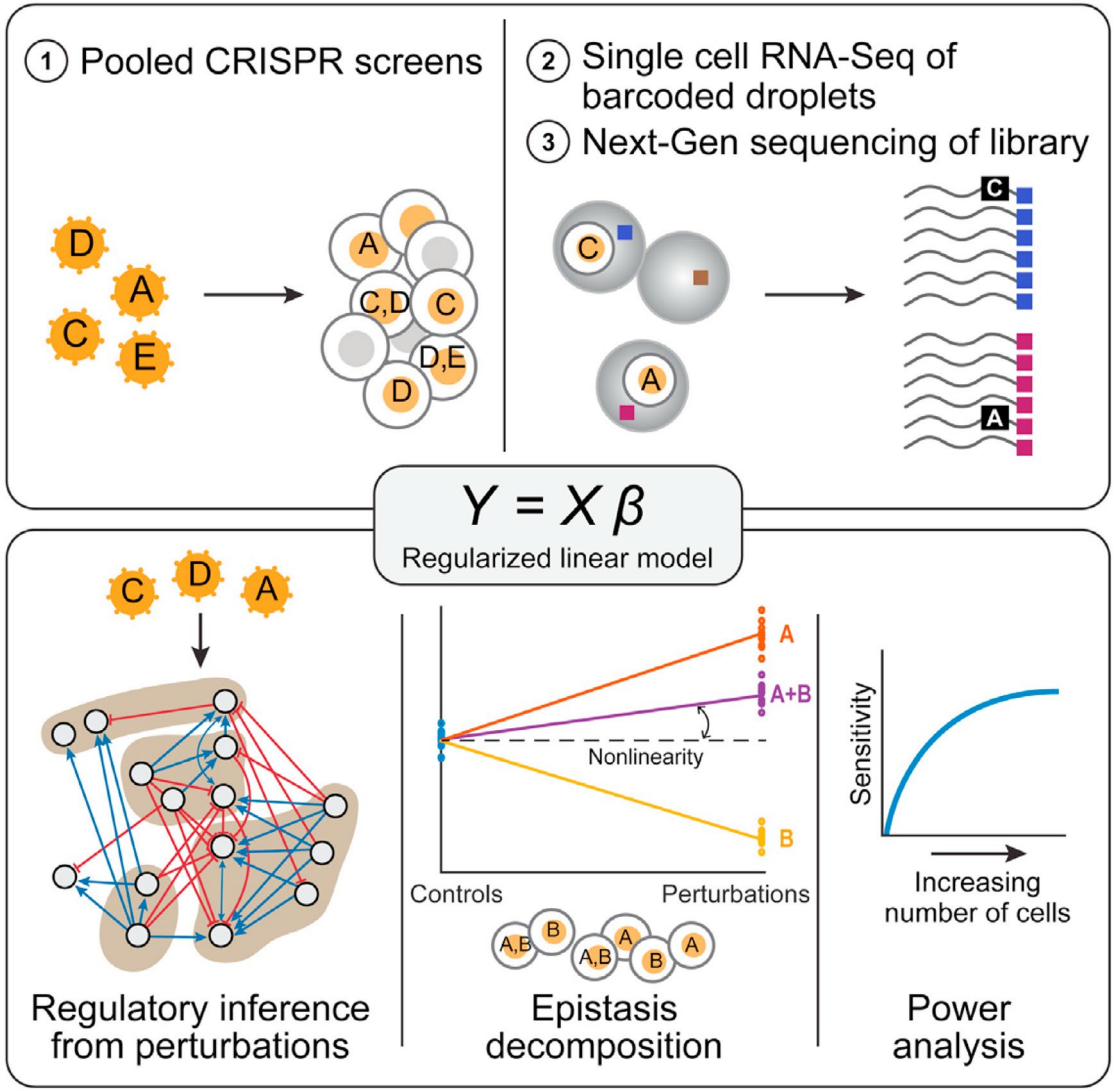
3. Library construction after breaking the emulsion.
4. NGS sequencing.

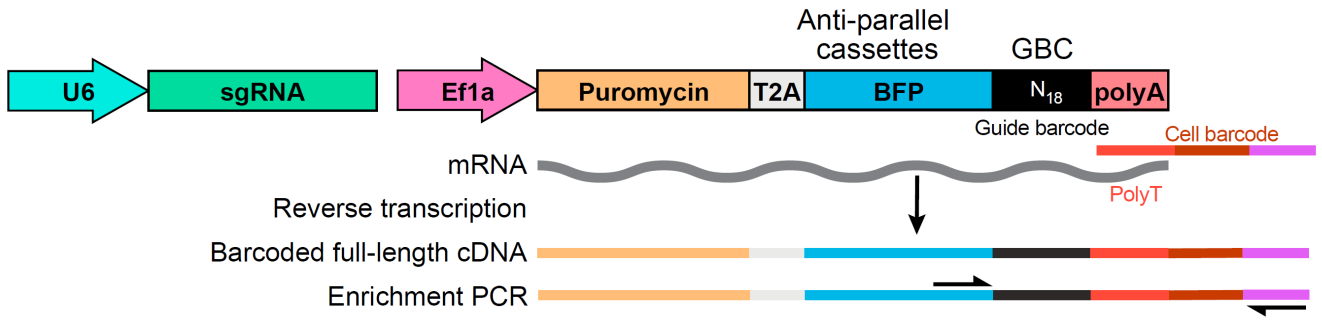
GEM = gel bead-in-emulsion
CBC = cell barcode
UMI = unique molecular identifier
GBC = guide barcode

https://www.10xgenomics.com/products/single-cell-crispr-screening

Single-cell functional genomics

Perturb-seq combines pooled CRISPR screens with scRNA-seq





$$\log(\text{Cells}\begin{bmatrix} 0\,8\,0\,1\,0\,2\,\ldots\,0 \\ 1\,2\,0\,0\,1\,1\,\ldots\,0 \\ 0\,7\,0\,1\,0\,0\,\ldots\,1 \\ \ldots \\ 0\,5\,0\,1\,0\,0\,\ldots\,0 \end{bmatrix} + 1) = \text{Cells}\begin{bmatrix} 1\,0\,0\,\ldots\,-0.1\ldots \\ 0\,1\,0\,\ldots\,0.3\ldots \\ 0\,0\,1\,\ldots\,0.2\ldots \\ \ldots \\ 0\,0\,0\,\ldots\,-0.2\ldots \end{bmatrix} \begin{bmatrix} \beta_{1,1}\,\beta_{1,2}\,\beta_{1,3}\,\ldots\,\beta_{1,G} \\ \beta_{2,1}\,\beta_{2,2}\,\beta_{2,3}\,\ldots\,\beta_{2,G} \\ \beta_{3,1}\,\beta_{3,2}\,\beta_{3,3}\,\ldots\,\beta_{3,G} \\ \ldots \\ \beta_{C,1}\,\beta_{C,2}\,\beta_{C,3}\,\ldots\,\beta_{C,G} \\ \ldots \end{bmatrix}$$

Y: Expression matrix — Genes — Signature decomposition

X: Design matrix — Covariates — Cell features; Design of experiments

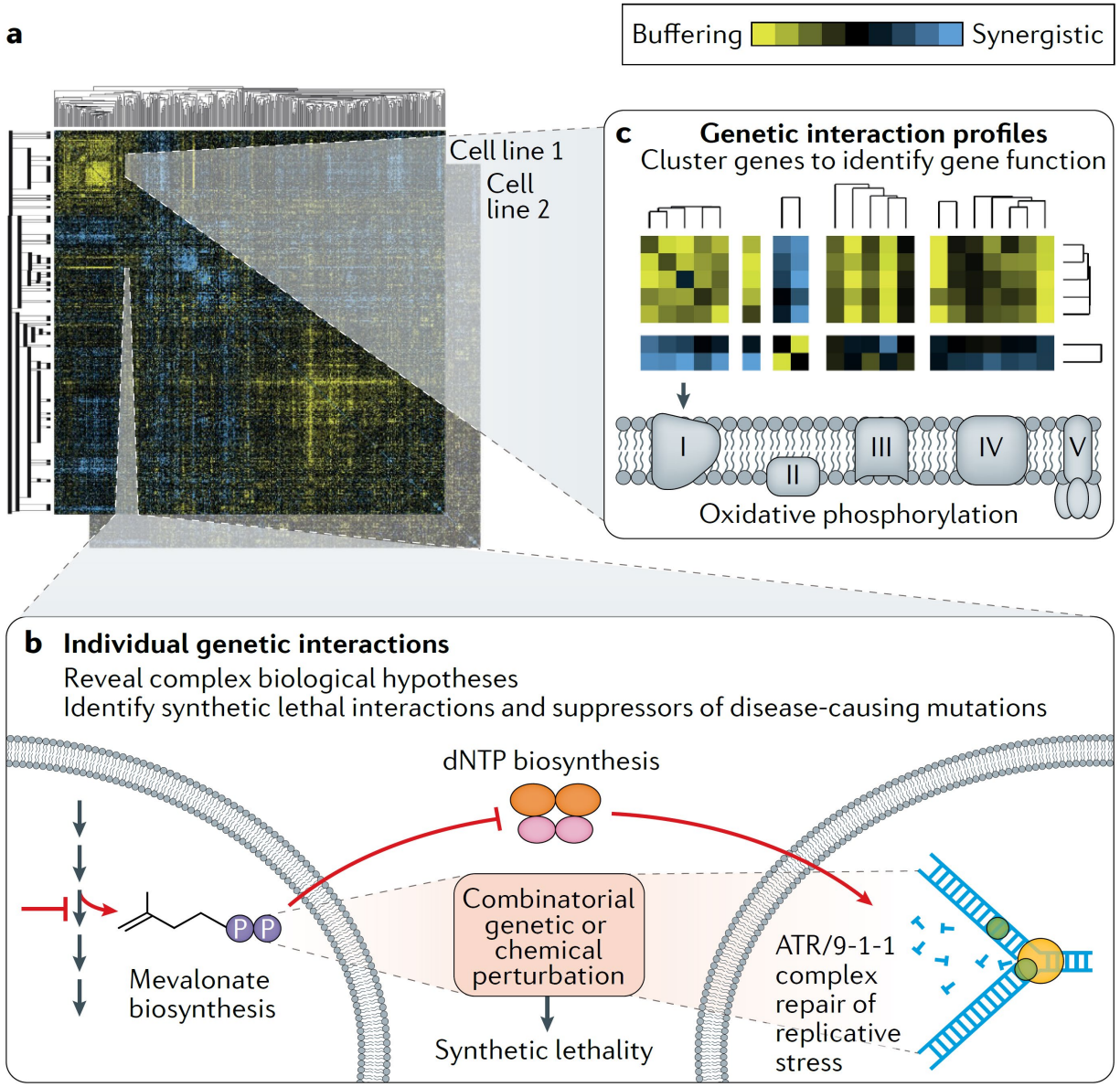
β: Coefficient (regulatory) matrix — Genes — Inference; Interpretation

sgRNAs; Other covariates; Covariates

Dixit, A., *et al. Cell* (2016)

Single-cell functional genomics

Mapping genetic interactions with dual-gene perturbations



Workflows:

1. Library construction (2sgRNA/vec.)
2. Pooled screen
3. Readout cell proliferation
4. Calculate GI scores

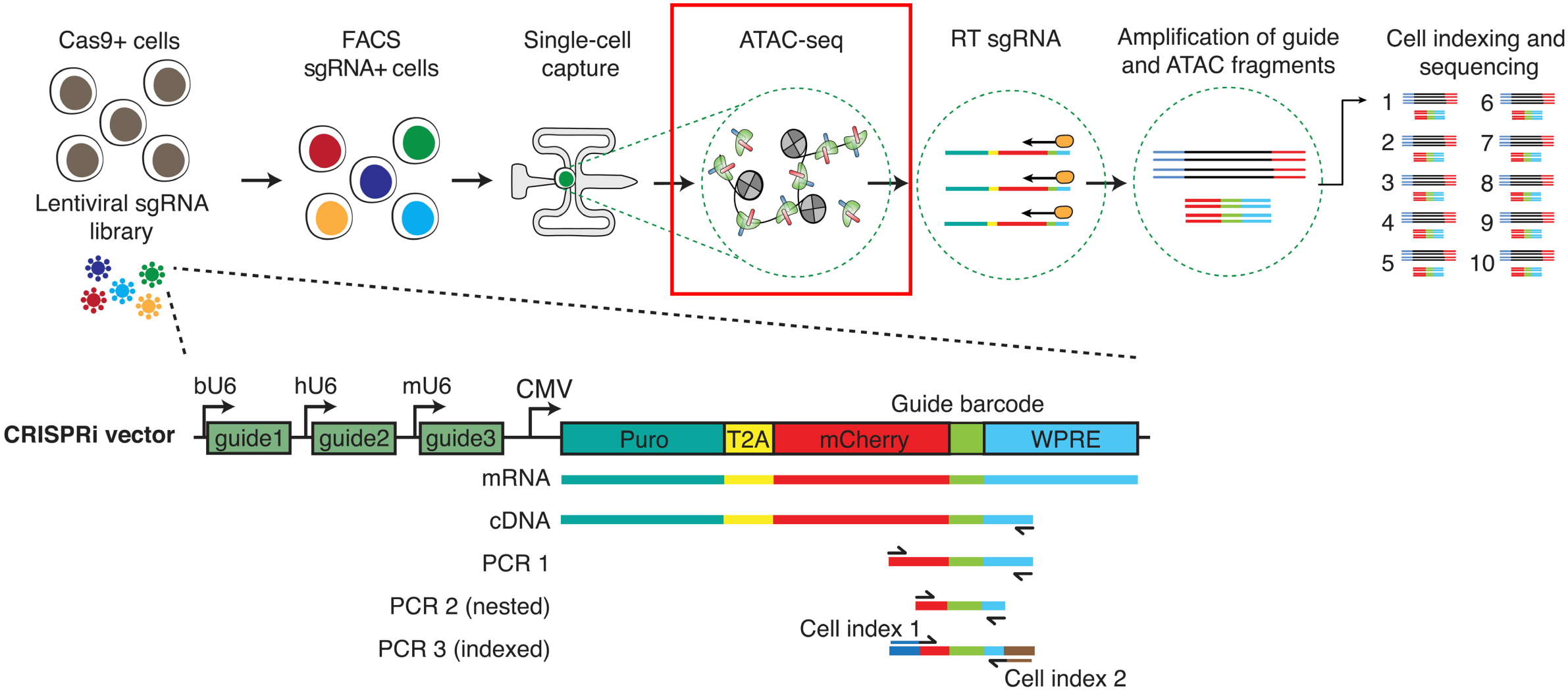
222784 gene pairs in two cancer cell lines were perturbed.

1. Identify synthetic lethal interactions.
2. Assign gene function in clusters.

Horlbeck, M. A., *et al. Cell* (2018)

Single-cell functional genomics

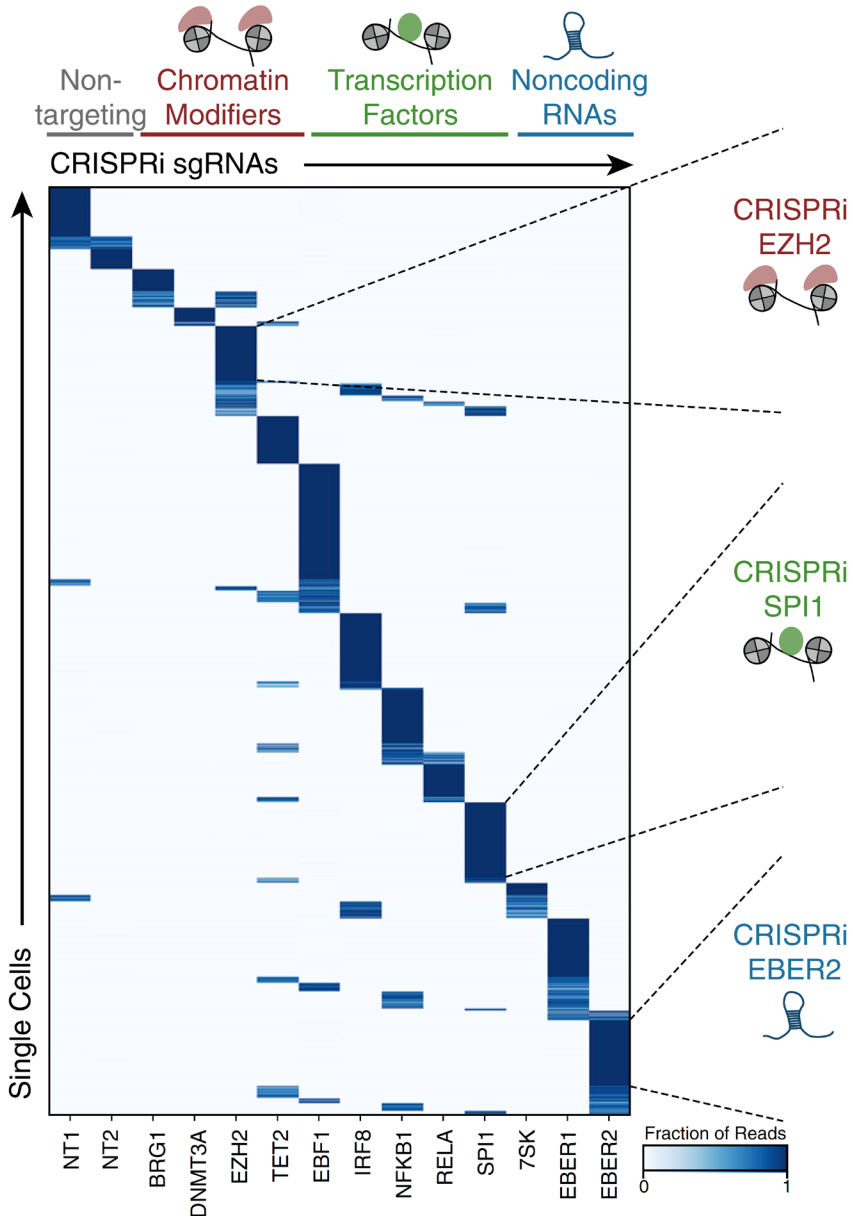
Perturb-ATAC combines pooled CRISPR screens with chromatin accessibility profiling of single cells



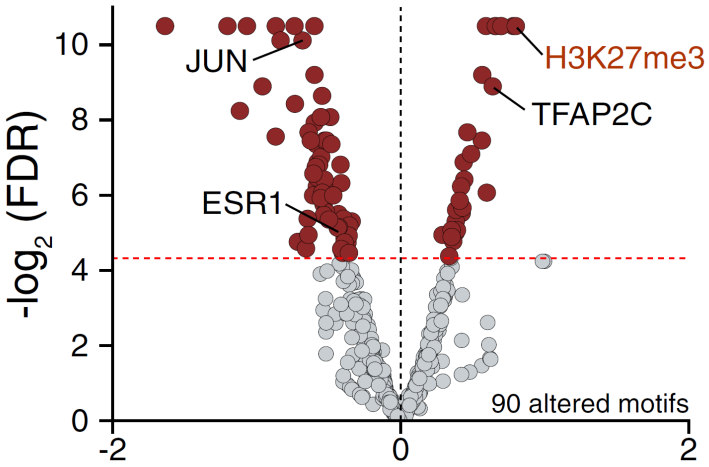
Rubin, A. J., *et al. Cell* (2019)

Single-cell functional genomics

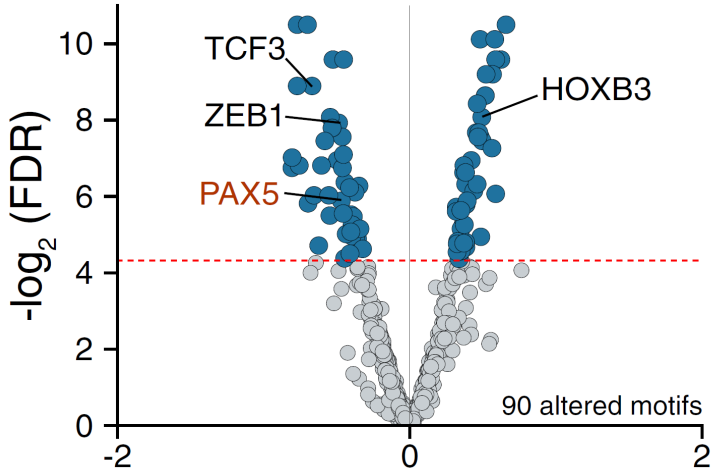
Perturb-ATAC CRISPRi screens in B lymphoblasts



ENH2 knockdown



EBER2 knockdown



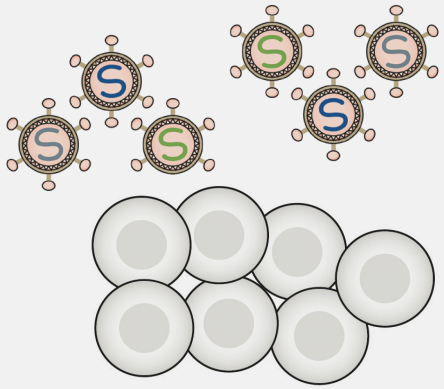
X-axis: diff. in accessibility; Y-axis: false discovery rate

Perturbations of diverse categories of *trans*-factors alter corresponding epigenomic phenotypes

Rubin, A. J., *et al. Cell* (2019)

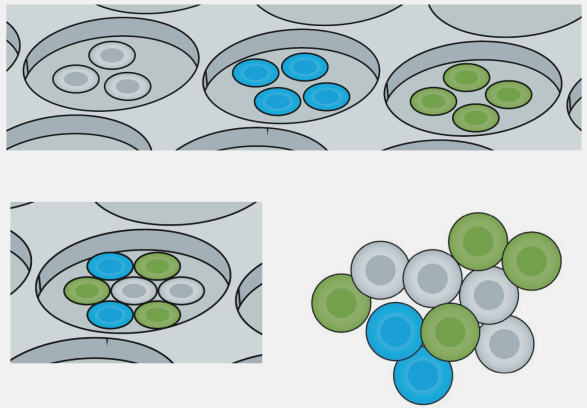
Advanced CRISPR screens pipeline

Library construction and transfection



CRISPR KO
CRISPRi
CRISPRa
Cas9/dCas9/**Cas13**

Perturbation



Pooled screens
Arrayed screens
OP. screens

Genotype-phenotype mapping

Survival/dropout
FACS markers
Imaging features
Single-cell sequencing
- ❑ Transcriptomics
- ❑ Gene interactions
- ❑ Epigenomics

Targets: every parts of the genome!
(theoretically)

Przybyla, L., & Gilbert, L. A. *Nature Reviews Genetics* (2021)

Limitations and future perspectives

What are the constraints of CRISPR screens?

Major caveats and challenges

Mutual problems

False data interpretation (i.e., ineffective guides, exon skipping, post-translational modification, off-target effect)

CRISPR KO	CRISPRi/a	GIs	Single-cell FG
Impractical indel for non-coding sequence	Low efficiency; 1kb-window around the TSS	Low throughput due to the exponential rule	Low throughput due to high cost in preparation

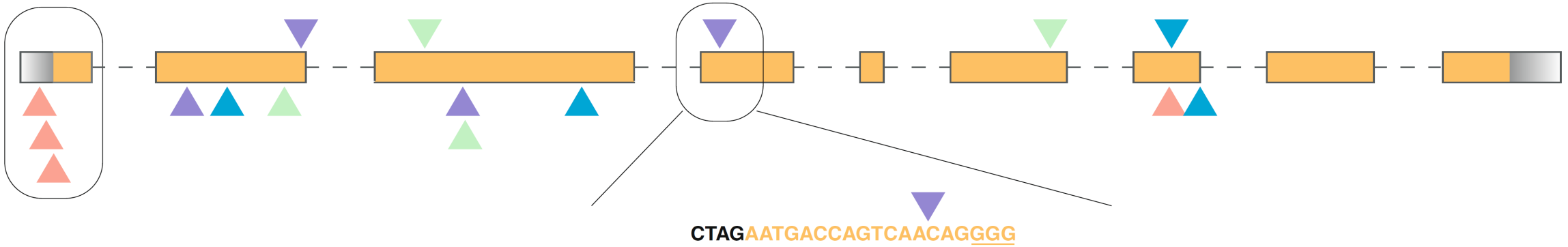
How to design better guide RNA library?

TSS = transcription start site;

sgRNA design tools make different tradeoffs

Features to consider

Species, Cas enzymes (PAM), on/off-target predictions…



CHOPCHOP
prioritizes multiple-hits transcripts

CRISPick
consider spacing requirements

GUIDES
prioritize annotated domains

Hanna, R. E. & Doench, J. G. *Nature Biotechnology* (2020)

The future of CRISPR functional genomic screening

- Better pilot studies and library design to narrow the experimental space.
- Integration with genomic database (i.e., GWAS) for complex cell models.
- Combination with single-cell multiomics (i.e., CITE-seq) more complex expression profiles.

Has there been a major breakthrough in our understanding of genomics that could not have been possible without CRISPR screens?

PNAS

Genome-wide CRISPR screen f reveals transcriptional repress of mitophagy

Christoph Potting[a], Christophe Cro Walter Carbone[a], Judith Knehr[a], R John S. Reece-Hoyes[b], Gregory R. and Stephen B. Helliwell[a,1]

nature genetics

In vivo Perturb-Seq reveals neuronal and glial abnormalities associated with autism risk genes

Xin Jin[1,2,3,4,*], Sean K. Simmons[3,5,6], Amy Guo[3], Ashwin S. Shetty[2], Michelle Ko[2], Lan Nguyen[3,6], Vahbiz Jokhi[2], Elise Robinson[3,5,8], Paul Oyler[2], Nathan Curry[2], Giulio Deangeli[2], Simona Lodato[7], Joshua Z. Levin[3,5,6], Aviv Regev[3,6,9,10,*,†], Feng Zhang[3,4,10,*,†], Paola Arlotta[2,3,5,*,†]

ARTICLE

CRISPR–Cas9 screens in human cells and primary neurons identify modifiers of *C9ORF72* dipeptide-repeat-protein toxi

Science

Cite as: S. Parvez *et al.*, *Science* 10.1126/science.abi8870 (2021).

Identifica immunot

Shashank J. Patel[1,2]*, N Jared J. Gartner[1], Li Jia Ophir Shalem[6], Eric Ti Steve Feldman[1], Glenn

LETTER

A CRISPR screen defines a pathway required by flaviv

Rong Zhang[1], Jonathan J. Miner[1], Matthew J. Gorman[1], Keiko Rausch[2], Holly Ramage[2], James P. White[1], Adam Zuiani[1], Ping Zhang[1,3], Estefania Fernandez[1], Qiang Zhang[1], Kimberly A. Dowd[4], Theodore C. Pierson[4], Sara Cherry[2] & Michael S. Diamond[1,5,6,7]

MIC-Drop: A platform for large-scale in vivo CRISPR screens

Saba Parvez[1], Chelsea Herdman[2], Manu Beerens[3], Korak Chakraborti[1], Zachary P. Harmer[1]†, Jing-Ruey J. Yeh[4], Calum A. MacRae[3], H. Joseph Yost[2], Randall T. Peterson[1]*

[1]Department of Pharmacology and Toxicology, University of Utah, Salt Lake City, UT, USA. [2]Department of Neurobiology and Molecular Medicine Program, University of Utah School of Medicine, Salt Lake City, UT, USA. [3]Department of Cardiovascular Medicine, Genetics and Network Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA. [4]Cardiovascular Research Center, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA.

†Present address: Cellular and Molecular Biology, University of Wisconsin-Madison, Madison, WI, USA.

*Corresponding author. Email: randall.peterson@pharm.utah.edu

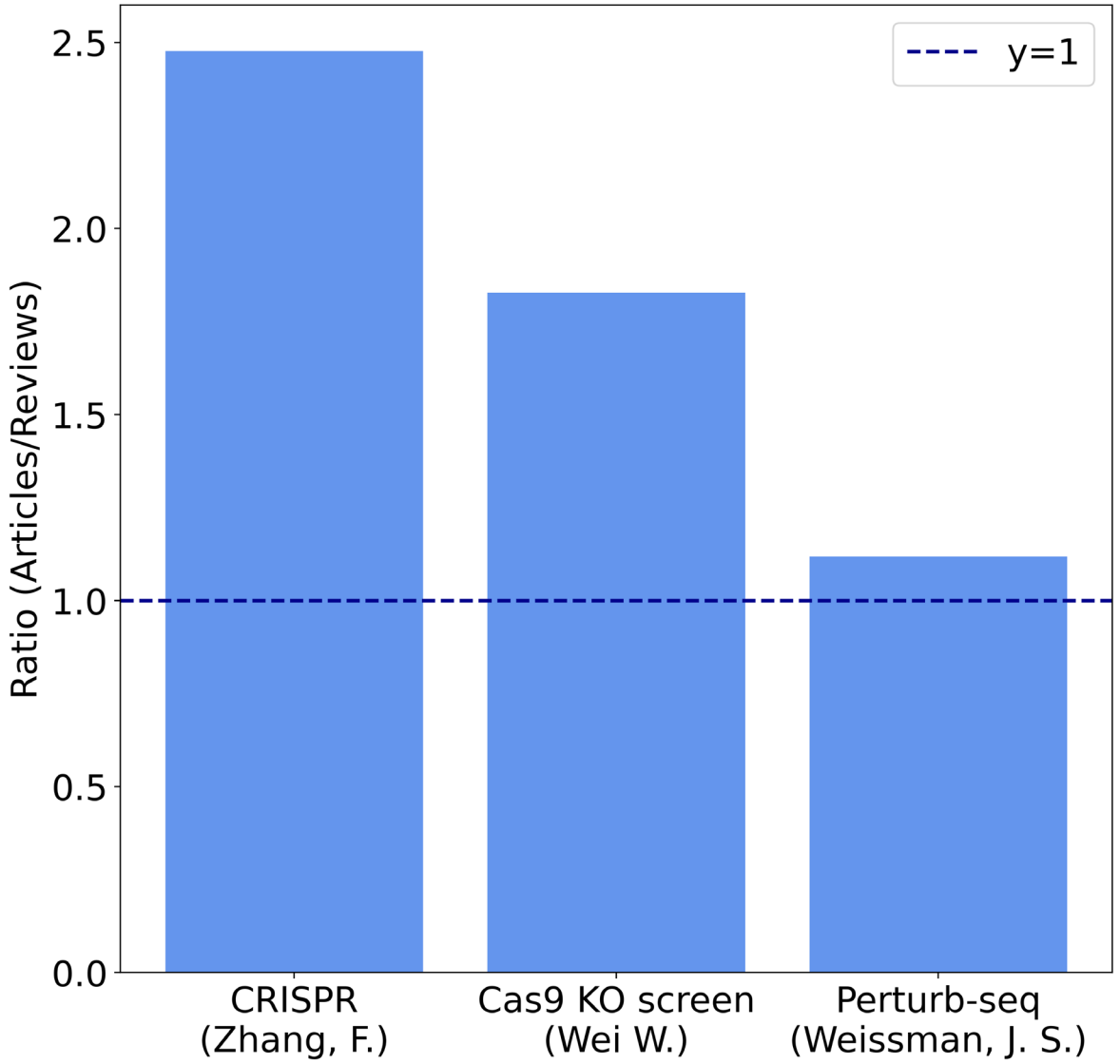
Citation analyses might give us some clues…

Presume that real-promising techniques should be adopted by other labs. We define citation ratio CR:

$$\mathrm{CR} = \frac{\#\ \text{of articles}}{\#\ \text{of reviews}}$$

The bigger the CR, the more prevailing the novel technique is.

Simple pooled screens are the major screening method, while single-cell methods are emerging.



Data was extracted on Dec. 17 from WoS Core Collection

The fates of any new technologies

Novel techniques always undergo an evolution…

The initial, hyper-enthusiastic phase is often mixed with outrageous claims about the novel method's power and specificity.

In the maturational stage, the claimed super specificity and super sensitivity issues are reduced and replaced by more sober understanding of the objective and reliable values of the method.

In the third phase, the innovation is adopted by a large community and combined with other methods. This is typically the stage when major breakthroughs are expected.

CRISPR screening is currently in the _____ phase?

Adapted from Gyuri Buzsaki's comments on optogenetics from Prof. Liangyi Chen's slides

Take-home message

1. CRISPR screening is a programmable genome-wide high throughput method for genotype-phenotype mapping.
2. Its workflow could be tailored to different Cas enzymes, guide RNA libraries, screening formats, and readout methods.
3. Limitations in guide RNA library construction still exist, and traditional pooled screens are still the most common methods.