Introduction to CRISPR-Cas9 KO screening

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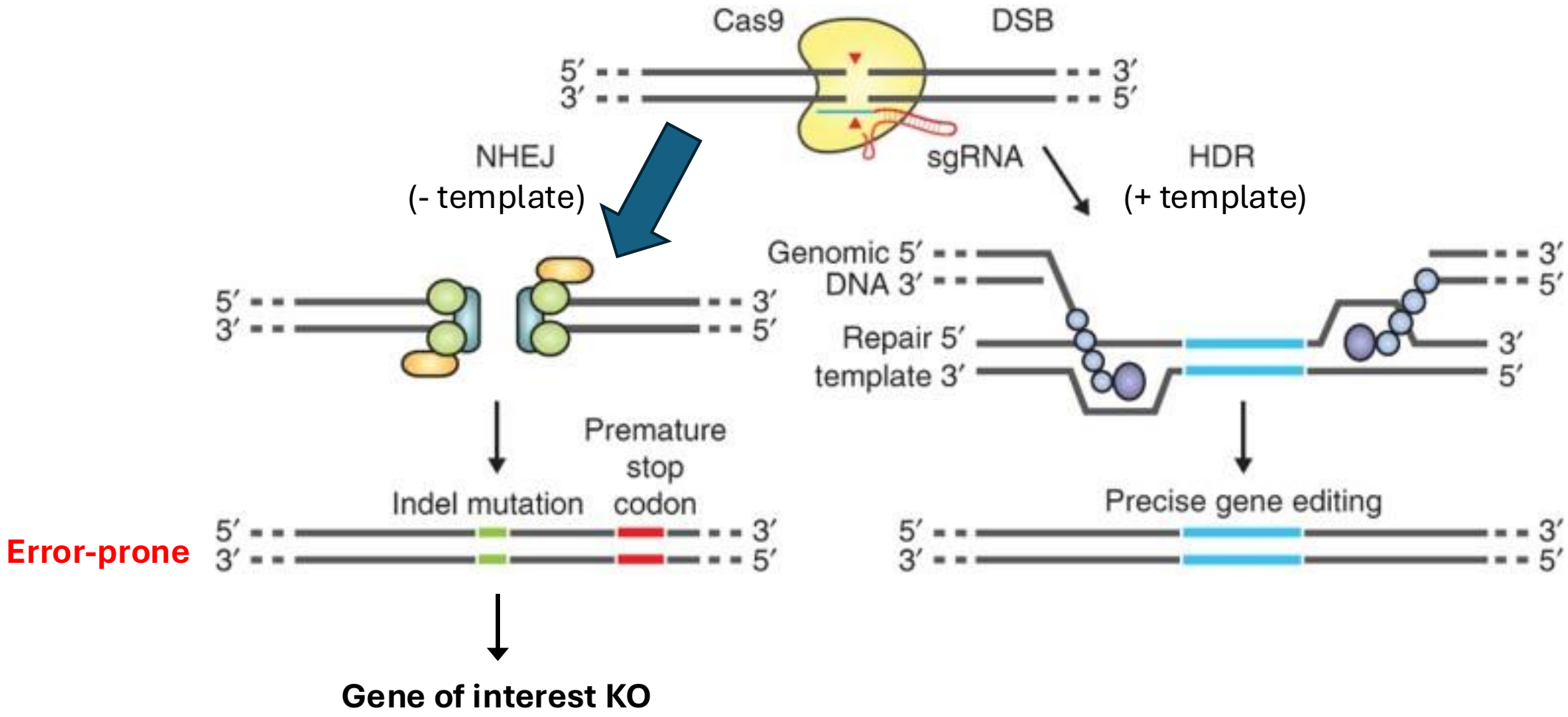
Introduction to CRISPR-Cas9 KO screening

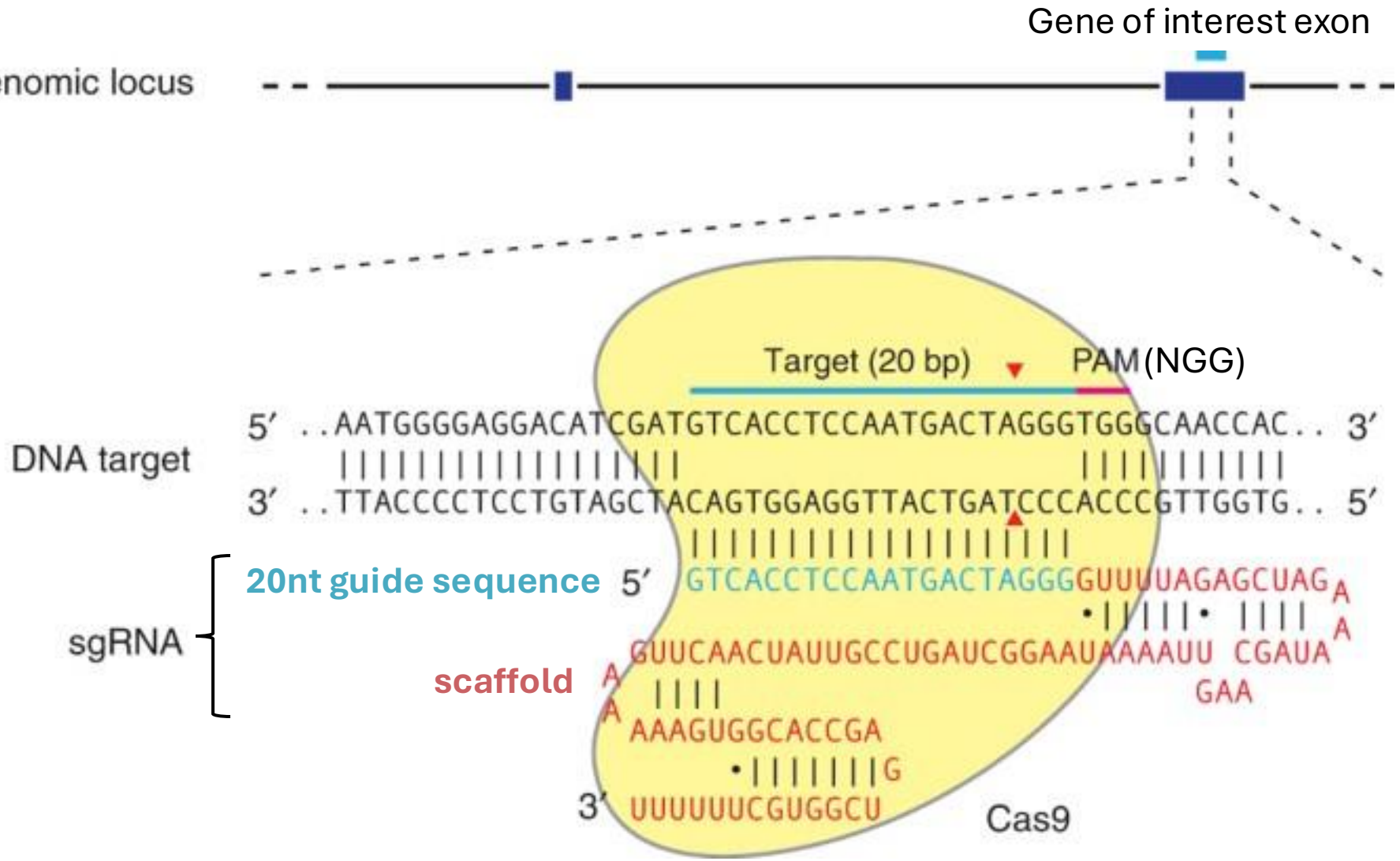
1. The CRISPR-Cas9 system
2. Overview of pooled CRISPR-Cas9 KO screens
3. CRISPR-Cas9 sgRNA libraries
4. Lentiviral Transduction
5. Functional assays
6. Overview of arrayed screening
7. Pooled vs arrayed screening
8. Applications
9. Parallel approaches

The CRISPR-Cas9 system



Adapted from Ran et al. Nature Protocols 2013

The CRISPR-Cas9 system



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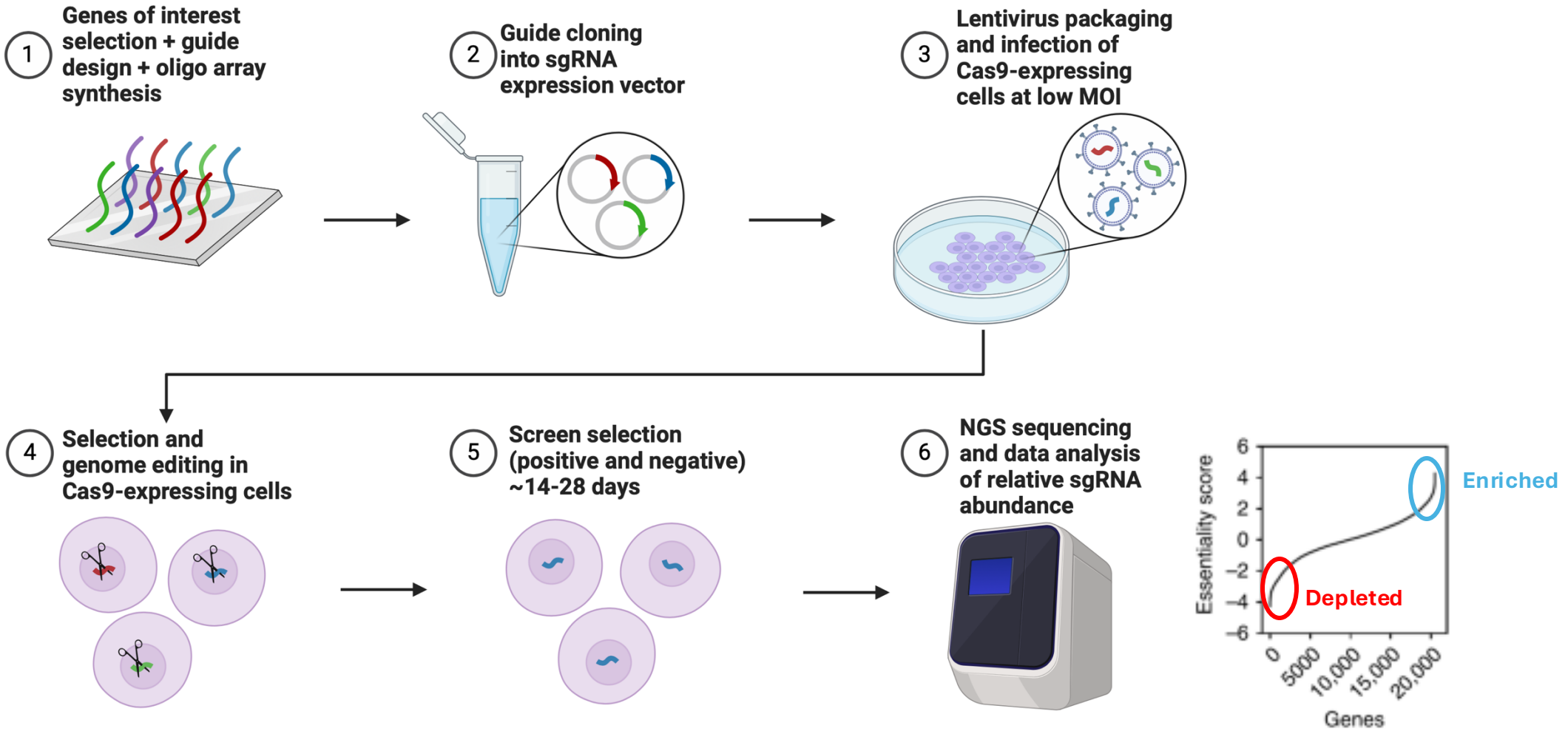
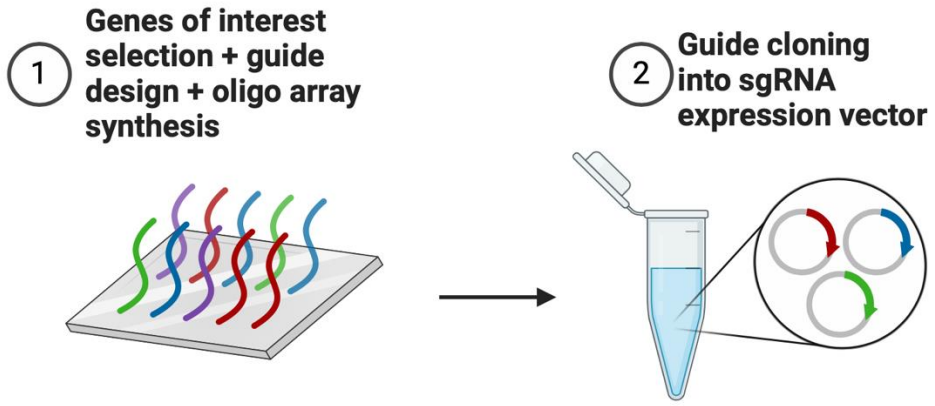
Overview of pooled CRISPR-Cas9 KO screens

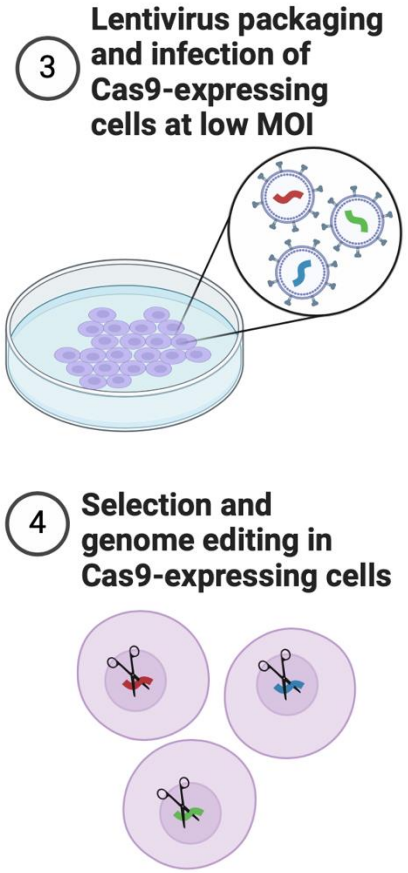


CRISPR-Cas9 KO sgRNA libraries



- Guide design: many web-based tools for picking effective sgRNAs (e.g. CRISPick from Broad Institute, Vienna Bioactivity Score)
- At least 2 guides per gene to increase confidence in data and mitigate potential off-target effects (4 is recommended)
- Positive and negative control guides need to be included within each library
- Guide cloning: guide distribution should be as homogeneous as possible to improve data quality
- For Human genome-wide libraries, various plasmid libraries available on Addgene
- Multiplexing (more than 1 sgRNA per vector, e.g. dual perturbation)

Lentiviral Transduction



- **Low MOI** (0.3-0.5) = 25-40% positive cells pre-selection= 86-77% single infected cells
- Cell model chosen for screening needs to be able to grow at scale and be transduced
- Cas9-expressing cells: high Cas9 efficiency is essential, measure Cas9 activity prior to screening (>75%)
- Cas9 expression single cell clones with high Cas9 activity can be isolated from cell line of interest to ensure more homogeneous population
- Cas9 expression or sgRNA expression can be DOX-inducible
- Selection via antibiotic resistance marker or FACS for fluorescent marker
- Coverage: ~100-1000X per vector, is dependent on sgRNA distribution within the library (libraries with even distribution require less coverage) and on positive vs negative selection

Functional Assays

1) Viability assays (conventional)

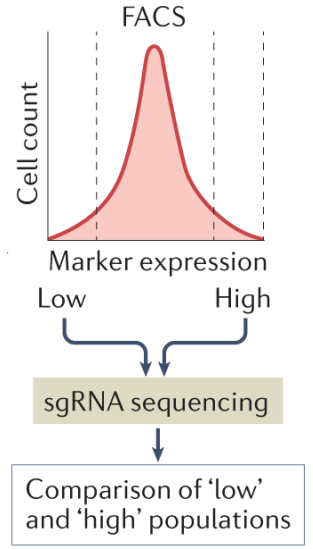
- Edited cells are left to proliferate for 14-28 days
- sgRNA abundances are compared between the start (from cells at day 0-4 or plasmid) and end of the screen
- Cells with sgRNAs that **promote cell survival and/or proliferation** will be **enriched in the population (positive selection)-** robust, requires lower coverage
- Cells with sgRNAs that **negatively** impact cell proliferation will be depleted from the population **(negative selection)- these sgRNAs target genes essential for cell survival and/or proliferation-** more challenging, requires higher coverage

e.g. Cancer Dependency Map
Behan et al. Prioritization of cancer therapeutic targets using CRISPR-Cas9 screens. Nature 2019

2) FACS based assays

- Edited cell populations can be sorted for desired phenotype (e.g. levels of endogenous proteins or engineered reporters) and sgRNA abundances compared in sorted populations

e.g. Finding regulators of endogenously tagged gene expression
Zaini et al. Endogenous HIF2A reporter systems for high-throughput functional screening. Scientific Reports 2018



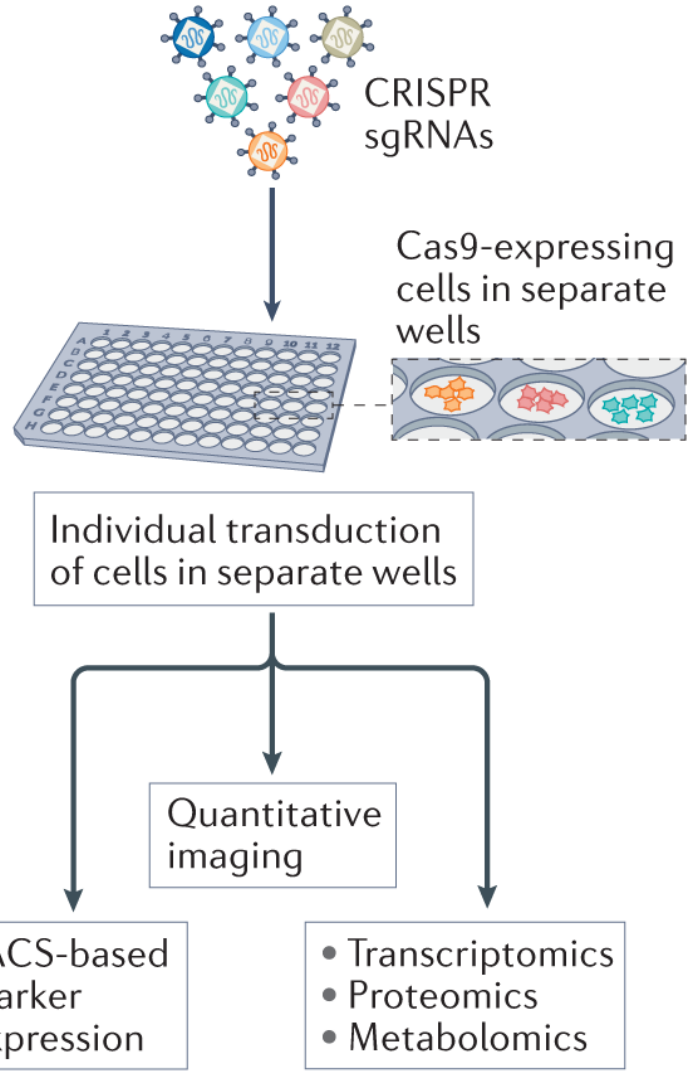
3) Perturb-seq assays

- Single-cell transcriptomic read-out for each edited cell
- Shorter assays (typically less than 7 days)

e.g. Genome-wide perturb-seq
Replogle et al. Mapping information-rich genotype-phenotype landscapes with genome-scale Perturb-seq. Cell 2022

Arrayed Screening

Multi-well plate format
(typically 96 or 384 well)

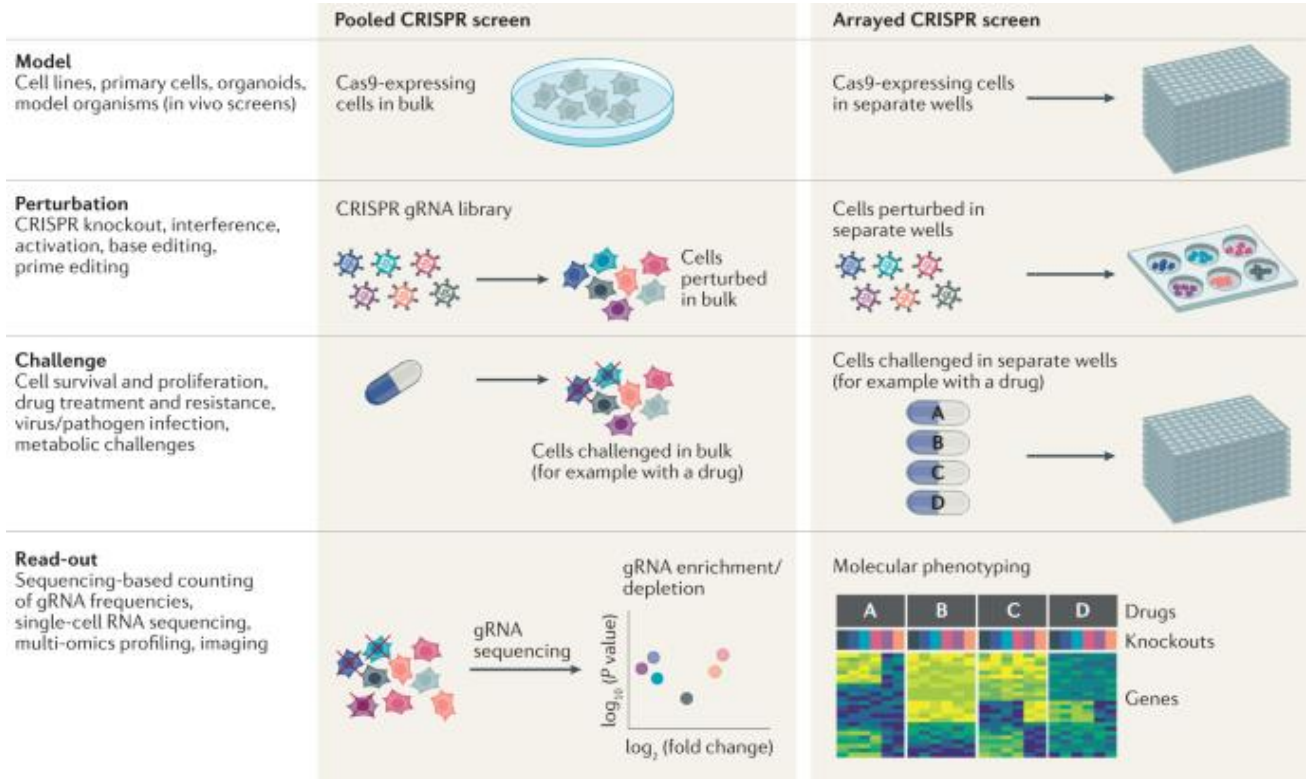


sgRNA vector or synthetic guides
transfected in individual wells

Viability read-out (e.g. Cell Titre Glo)

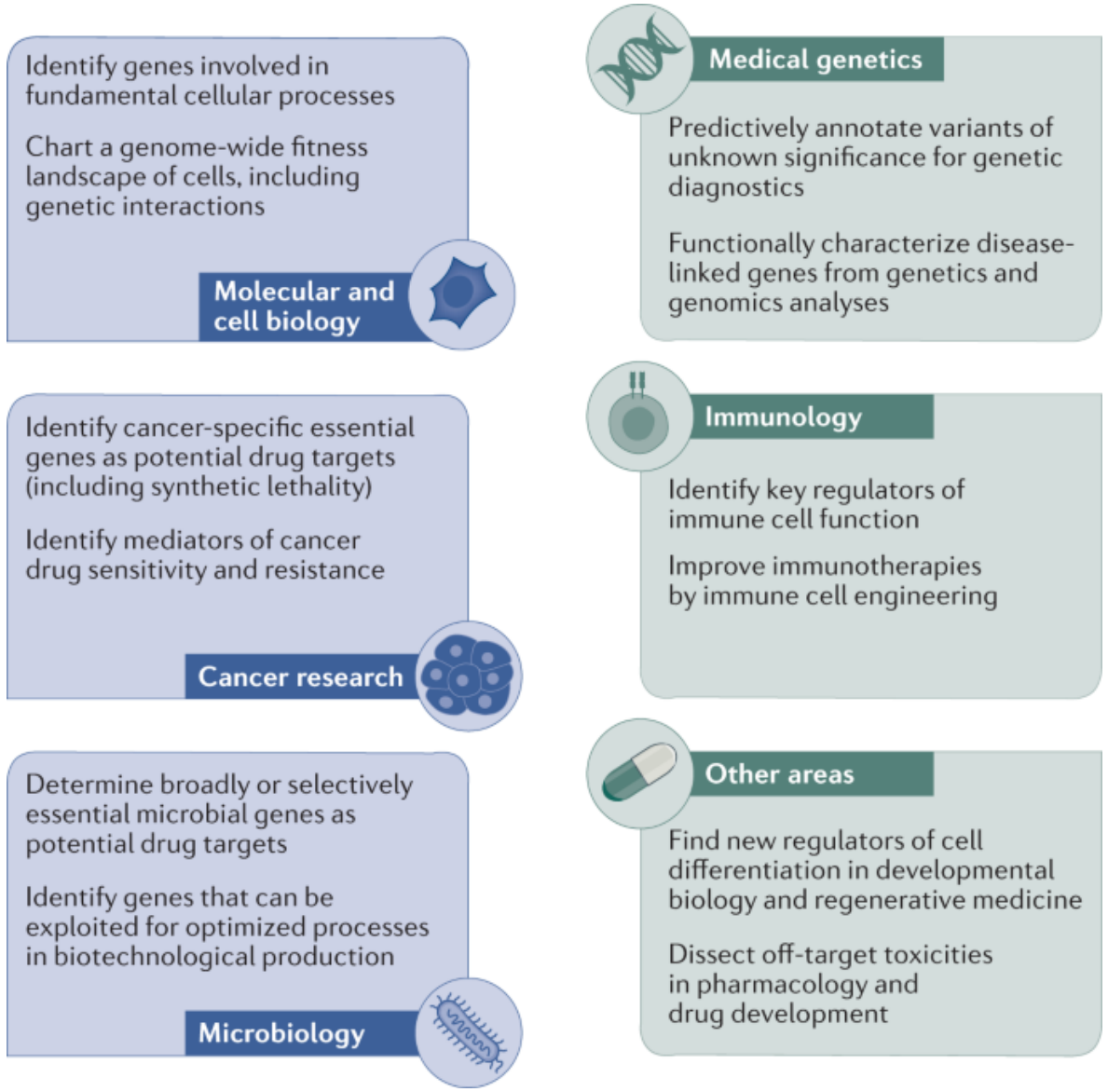
Adapted from Shi et al. Nature Reviews Immunology 2022

Pooled vs Arrayed Screening



Bock et al. Nature Reviews Methods Primers 2022

Applications of CRISPR-Cas9 KO Screening



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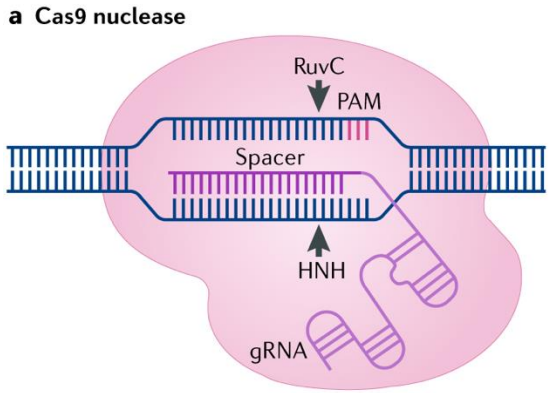
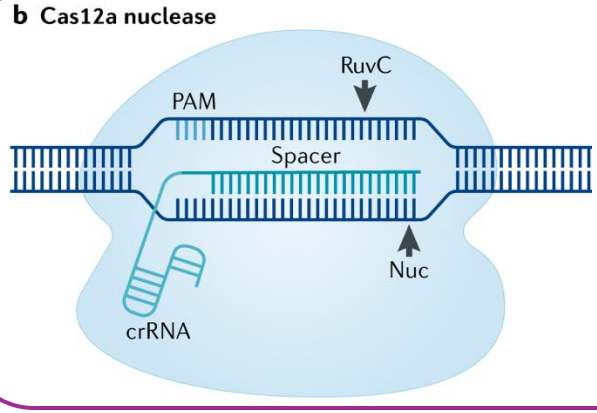
Parallel approaches to CRISPR-Cas9 KO screening



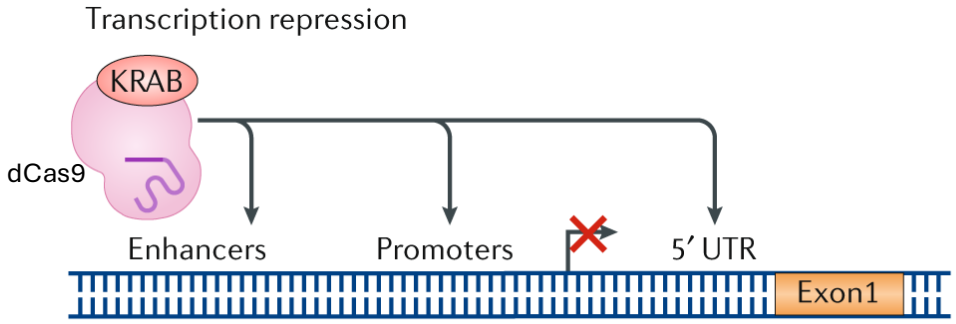
Cas9

- Blunt DNA cut
- NGG PAM
- Guide length 20nt + scaffold
- Multiplexing possible with multiple promoters or tRNA
- Gene knock-out



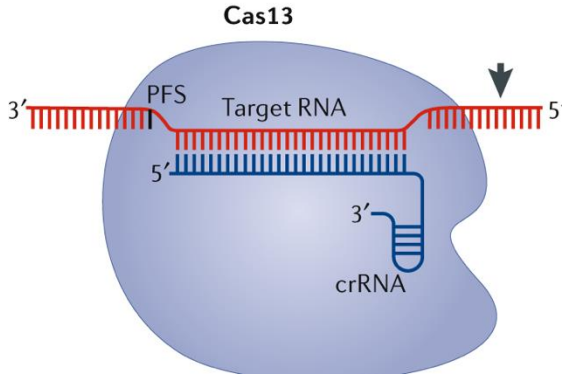
Cas12a

- Staggered DNA cut with 5' overhang
- TTTV PAM
- Guide length 20-24nt + short constant section 20nt
- Multiplexing from a single RNA
- Gene knock-out



CRISPRi

- Deactivated form of Cas9
- No DSB
- No permanent edits
- Reversible
- Represses gene expression
- Inhibition instead of knock-out

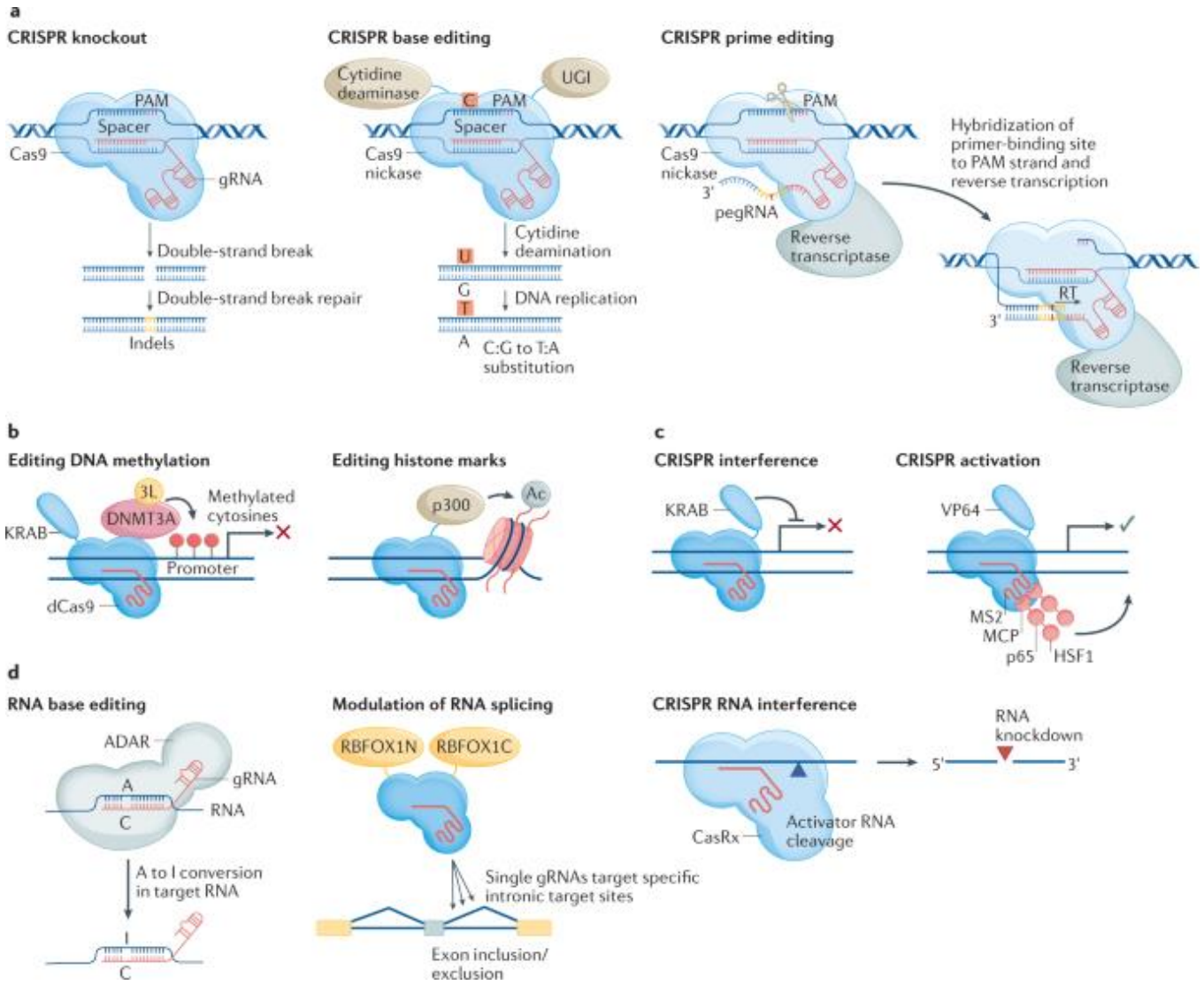


Cas13d

- Small er than Cas9
- No PAM required
- RNAse activity of ssRNA
- 22nt guide + short conserved scaffold
- No permanent edits in genome
- Represses gene expression
- Knock-down instead of knock-out

Adapted from Pickar-Oliver et al. Nature Reviews Molecular Cell Biology 2019

Alternate CRISPR Perturbation Technologies



Bock et al. Nature Reviews Methods Primers 2022

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Any questions?

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