CRISPR SCREENS: A FISHING EXPEDITION

Sara & Milica HWYDI

Versteeg lab

10th February 2020

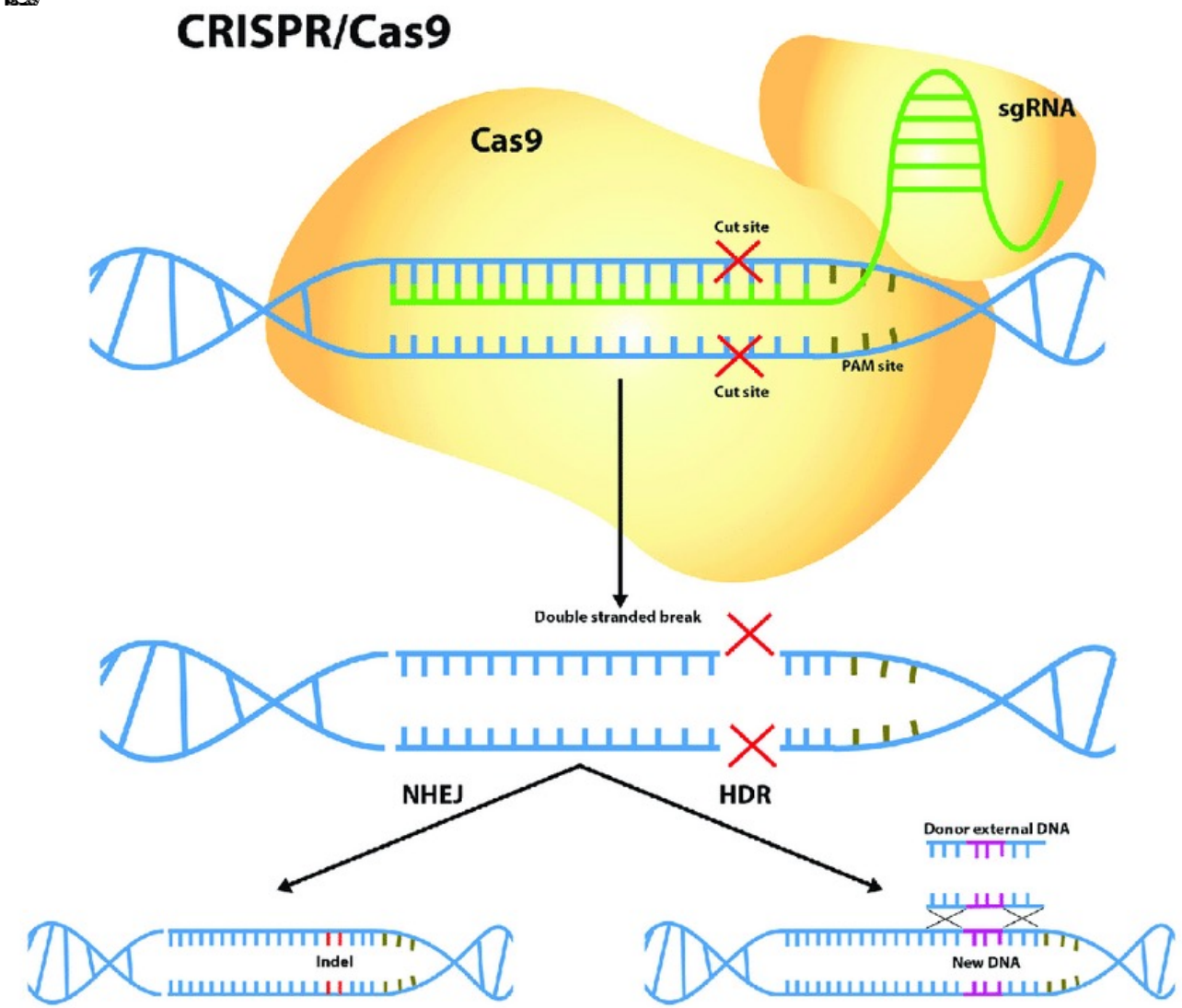
WE ARE GOING TO TALK ABOUT…

Why CRISPR?

Model system and library design

When to use it?

CAS9 BIOLOGY: A NEW TOOL FOR BIOLOGICAL QUESTIONS

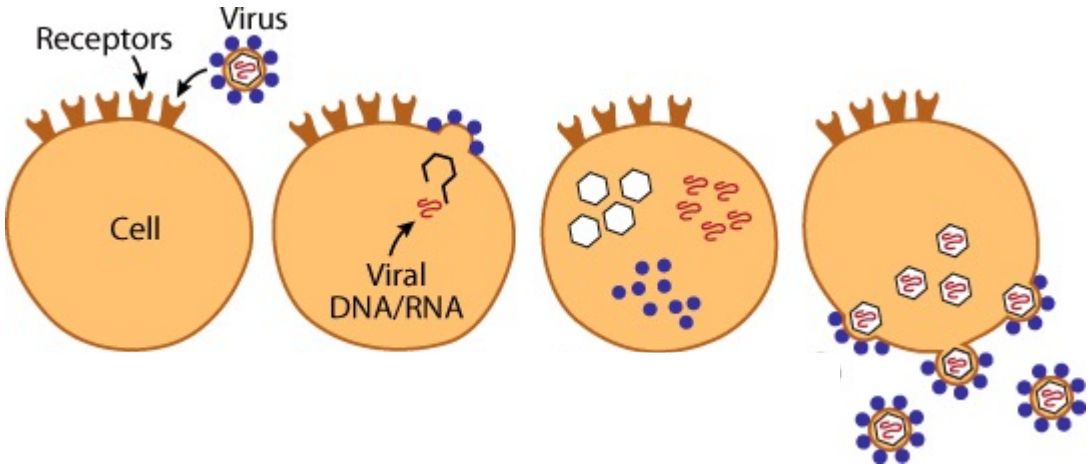


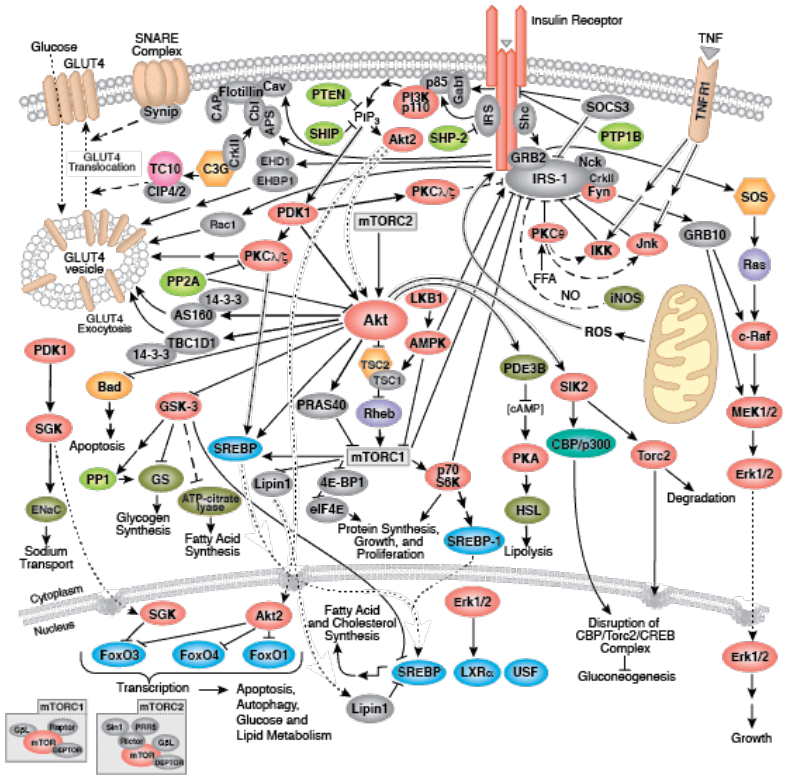
Cribbs et al. The Yale journal of biology and medicine 2017.

WHY CRISPR SCREENING?

Biological question & hypothesis

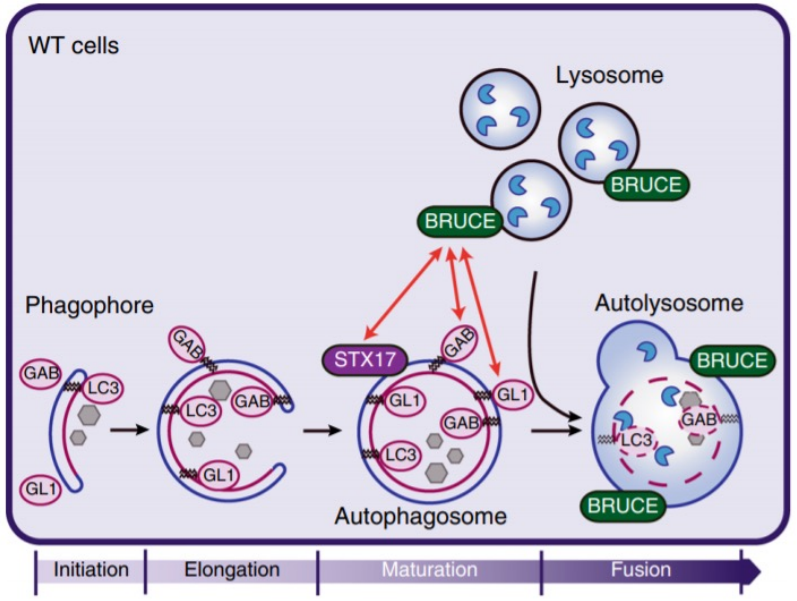
Phenotype-cell death/survival



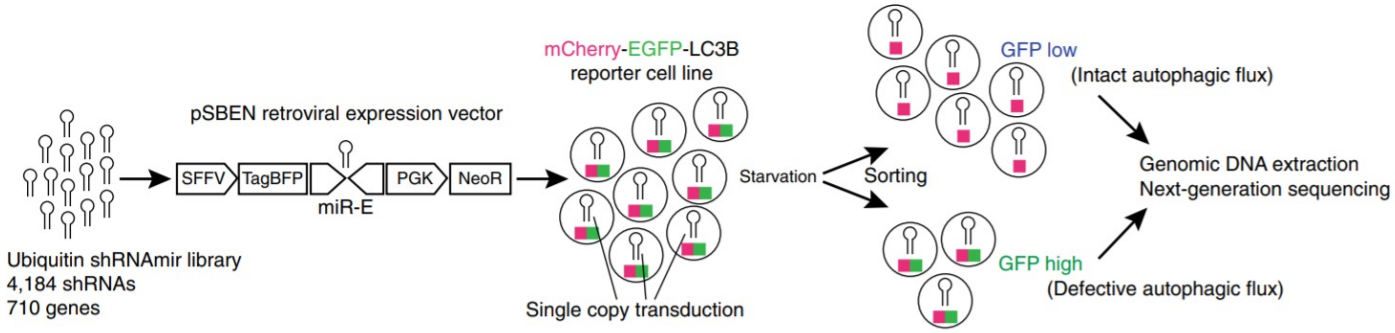


WHY CRISPR SCREENING?

Biological question & hypothesis



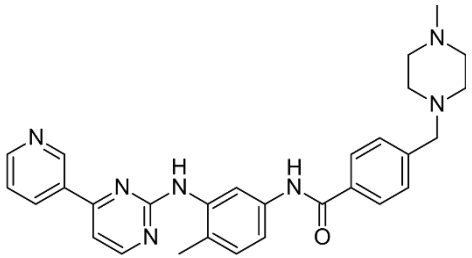
Advantage of the fluorophores (Ab)



Ebner et al. Nature Communications 2018.

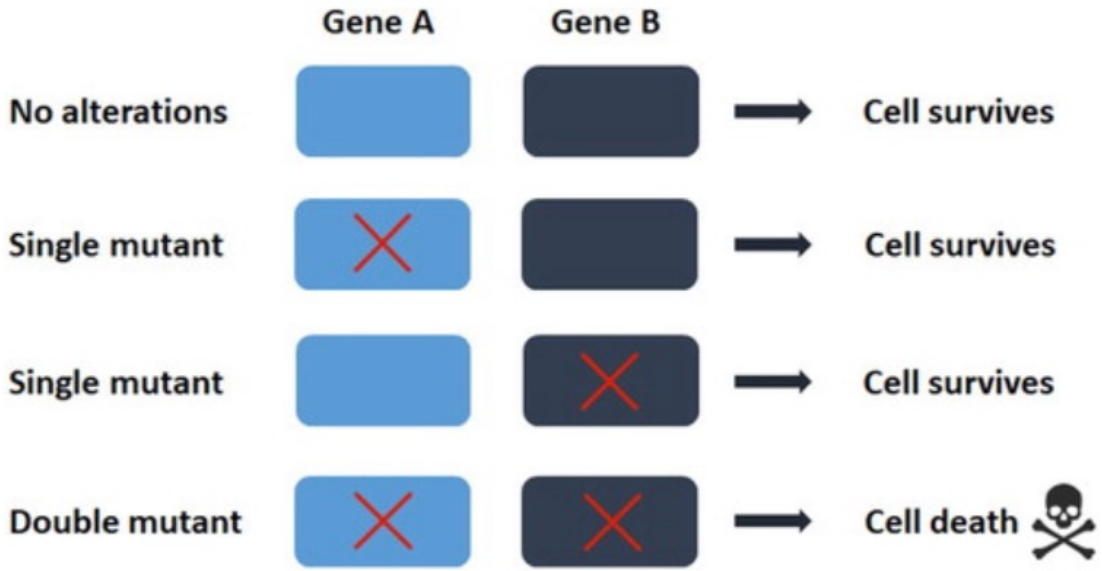
WHY CRISPR SCREENING?

Genotype vs phenotype



imatinib

"oncogenic escape"



PARP inhibitors cancer models (BRCA1/BRCA2)

Liu et al. Frontiers in Science 2019

TYPES OF CRISPR SCREENS OVERVIEW:

CRISPR using single sgRNAs (single knock-out):

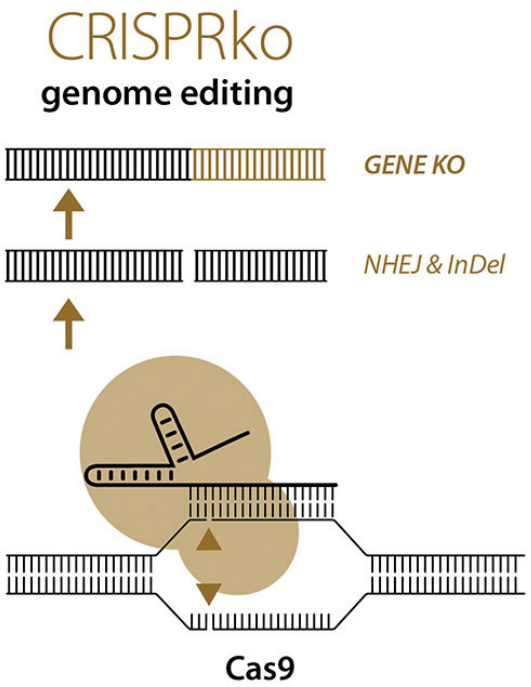
1. knock-out screens:

- positive – gain of function
- negative – drop-out screens

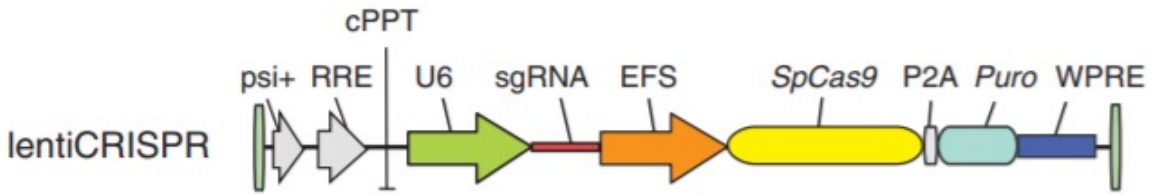
2. CRISPRi
3. CRISPRa
4. CRISPR *in vivo*

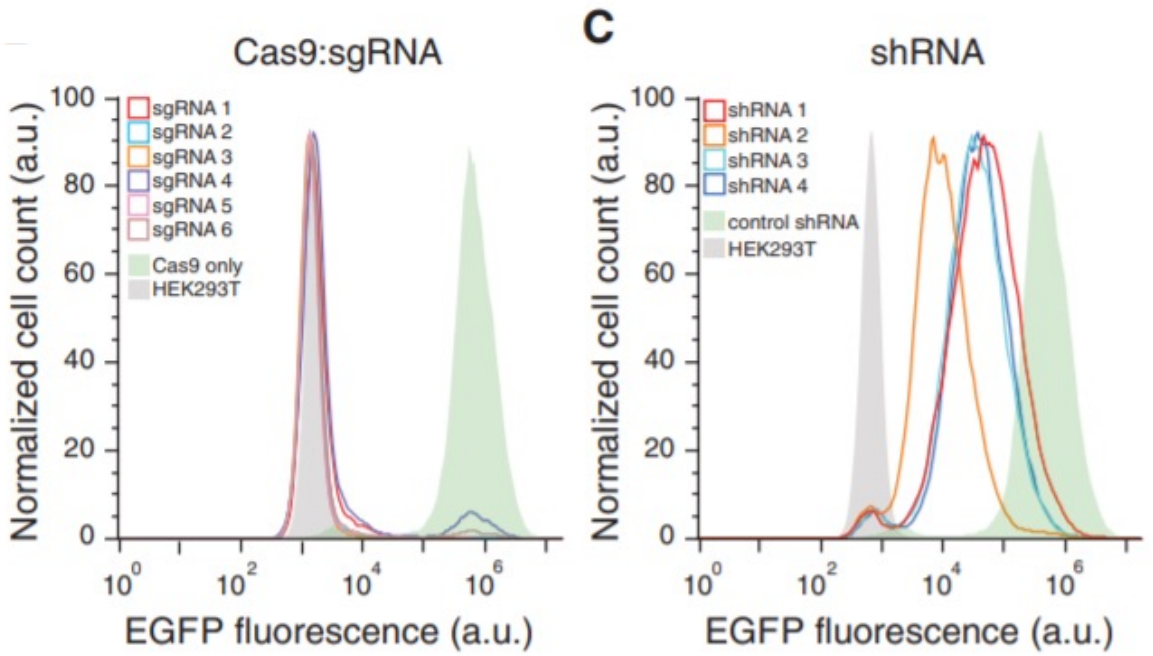
Doench Nature 2018

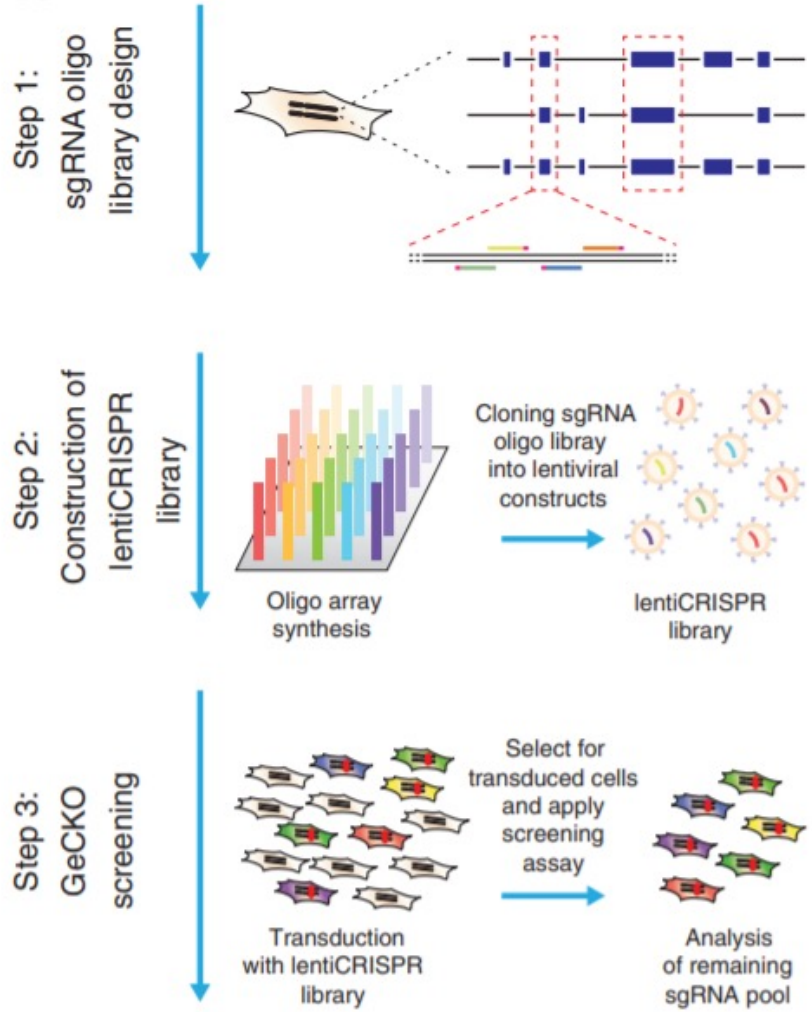
TYPES OF CRISPR SCREENS OVERVIEW:



TYPES OF CRISPR SCREENS: KNOCK-OUT → POSITIVE SELECTION



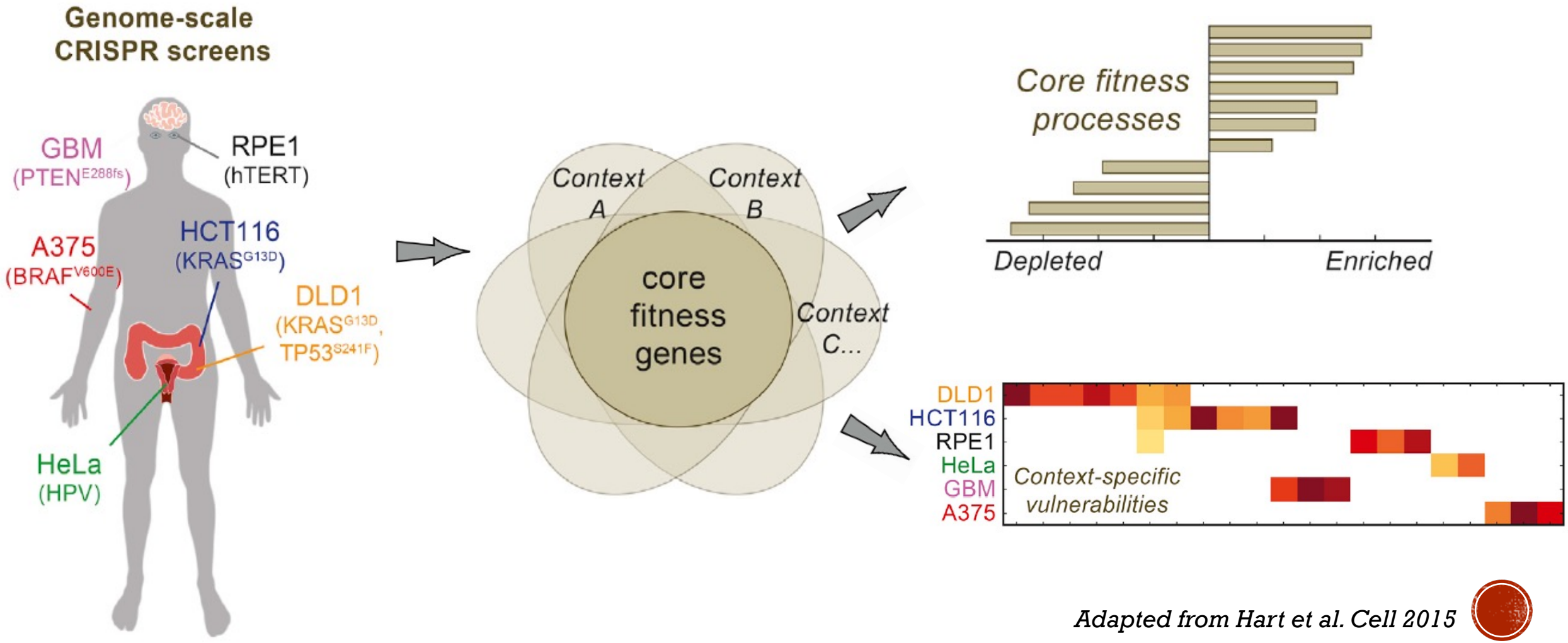




Carcinoma- RAF inhibitor vemurafenib

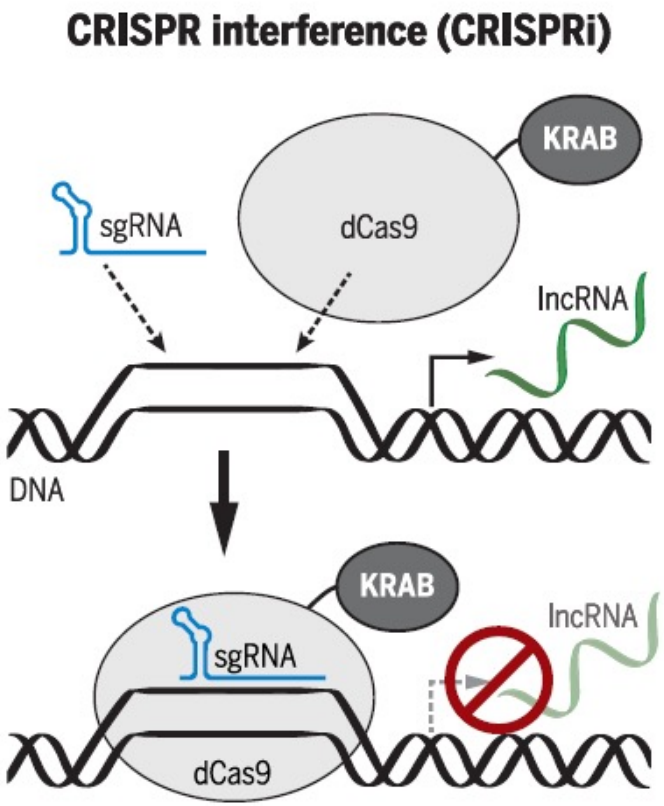
Shalem et al , Science 2014

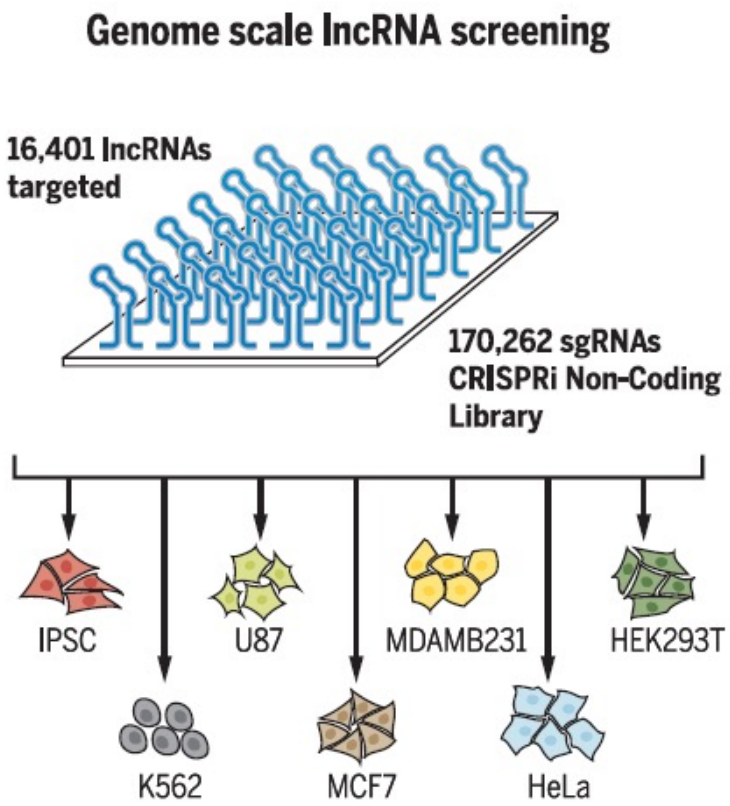
TYPES OF CRISPR SCREENS: KNOCK-OUT → NEGATIVE SELECTION

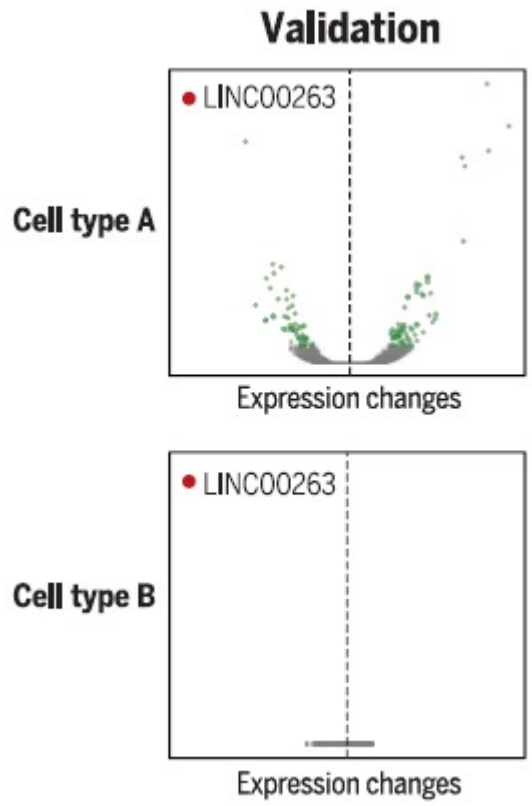


Adapted from Hart et al. Cell 2015

TYPES OF CRISPR SCREENS: *INTERFERENCE*

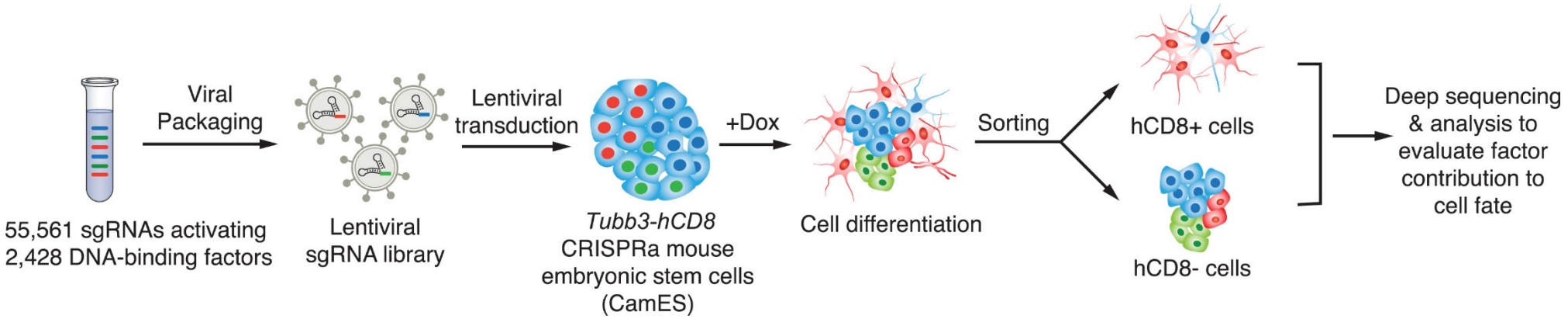


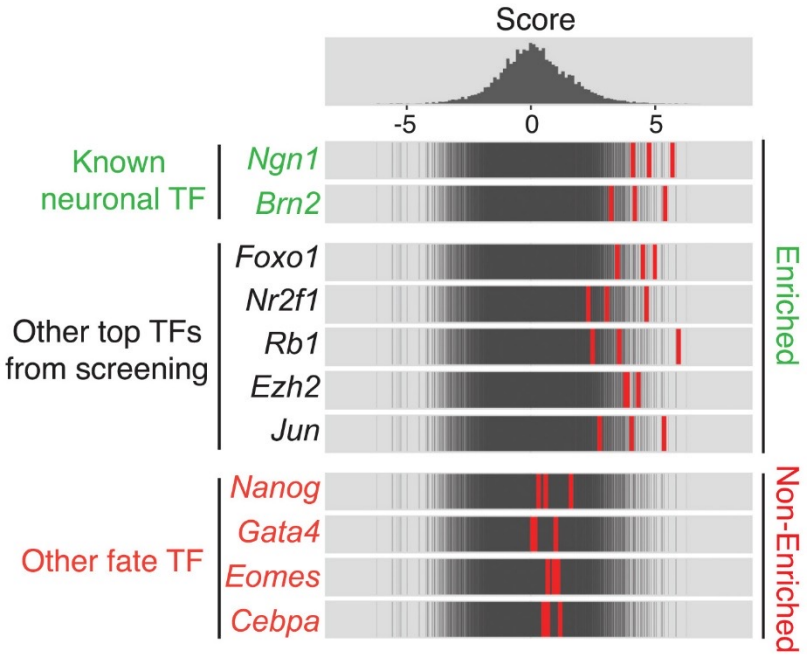


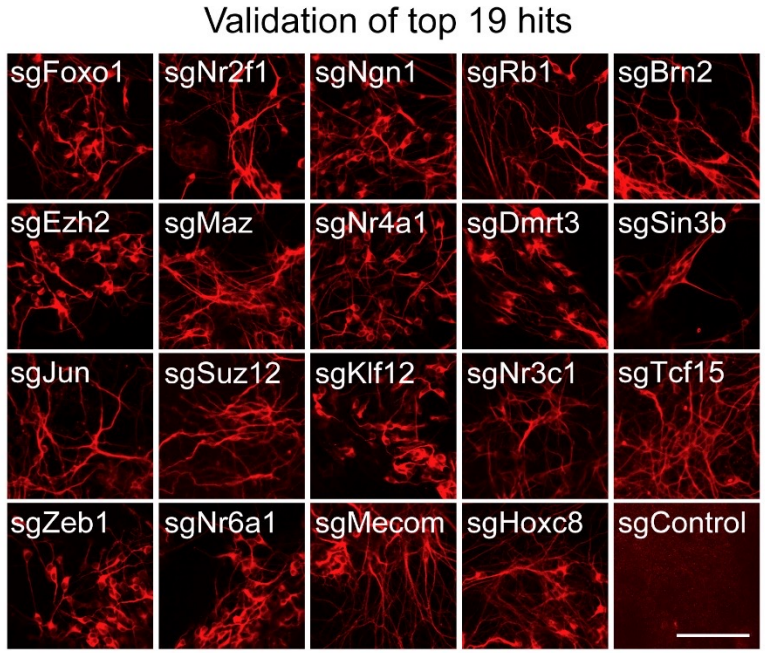


Liu et al., Science 2017

TYPES OF CRISPR SCREENS: *ACTIVATION*



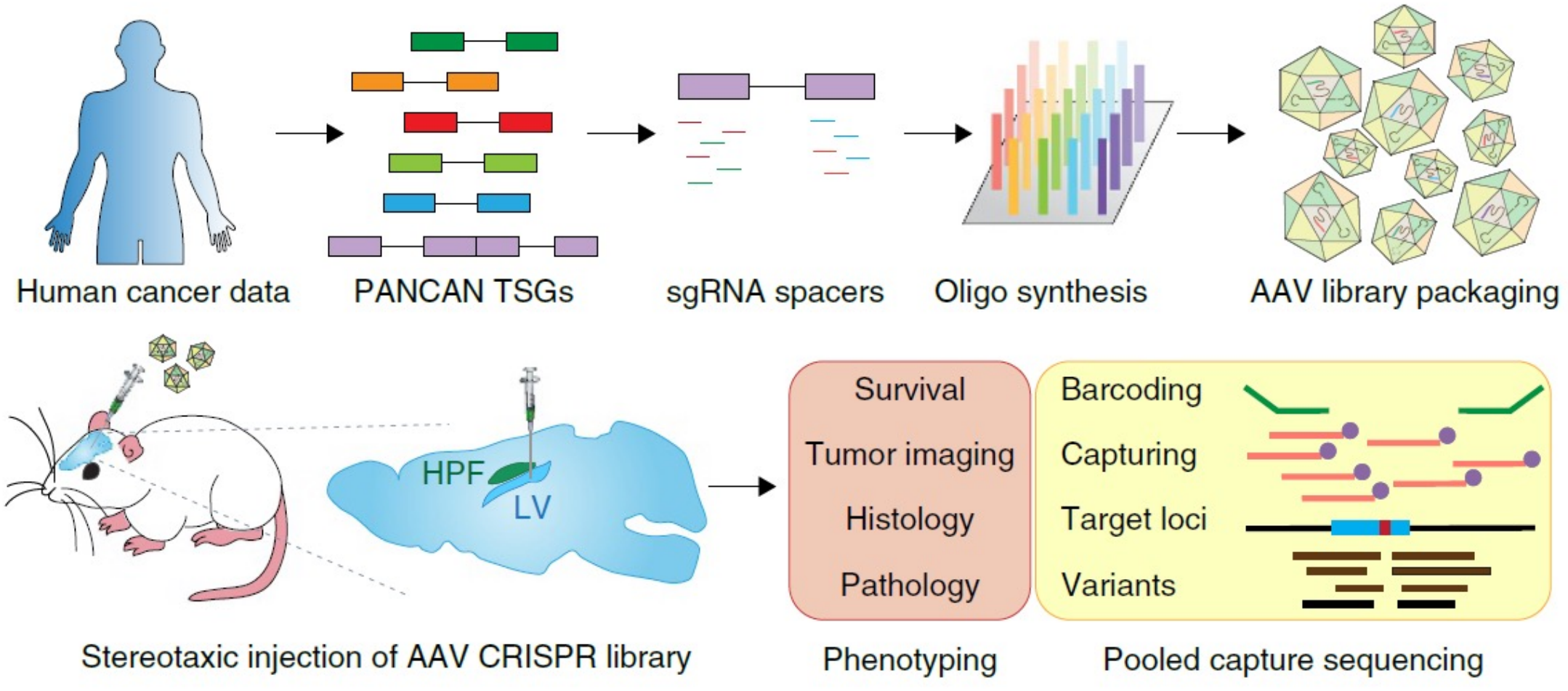




MAP2-stained neurons

Liu et al., Science 2018

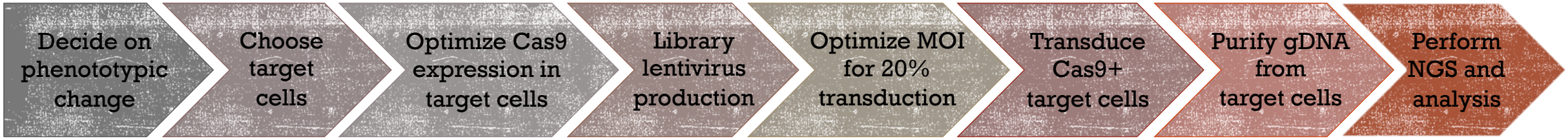
TYPES OF CRISPR SCREENS: *IN VIVO*



Chow et al. Nature Neuroscience 2017

WORKFLOW OF CRISPR SCREEN

An example of the CRISPR knock-out screen → gain of function



WHICH CELLS TO USE?

- Cell type to be used
- Primary cell vs. cell lines
- Easy to transduced and cultivate

CAS9 EXPRESSION?

- Cas9 robustness
- Expression levels and “leakiness”
- Inducible vs. constitutive

LIBRARY DESIGN FOR CRISPR SCREEN

A) Designed by you

B) Commercially available

LIBRARY DESIGN FOR CRISPR SCREEN

A) Designed by you

- How many sgRNAs per gene
- Library size (genome-wide or sublibrary)
- What characteristics an sgRNA must have

Candidate sgRNAs → **Target specificity**: Must contain ≥ 2 mismatches to off-target sited → **Target positioning**: Preference for constitutive exons dowstream of the start codon → **Sequence criteria**: Preference for <4 nt homopolymers, %GC between 20% to 80% → **sgRNAs chosen for synthesis**

Small library → small noise

Adapted from Wang et al. Science 2014

LIBRARY DESIGN FOR POOLED CRISPR SCREEN

A) Designed by you

Name	Graphical user interface	Available species	Input	Output	Ranked list
CHOPCHOP Citation »	Yes	23	DNA sequence, gene name, genomic location	Candidate guide sequences and off-target loci	Yes
CasFinder Citation »	No (Perl script)	User input	DNA sequence	Candidate guide sequences and off-target loci	Yes
Cas-OFFFinder Citation »	Yes	11	Guide sequence	Off-target loci for guide sequences	No
FlyCRISPR Citation »	Yes	18	DNA sequence	Candidate guide sequences and off-target loci	No
E-CRISP Citation »	Yes	31	DNA sequence or gene name	Candidate guide sequences and off-target loci	Yes
Guide RNA Sequence Design Platform Citation »	Yes	10	DNA sequence	Candidate guide sequences and off-target loci	No
CasOT Citation »	No (Perl script)	User input	Guide sequence	Off-target loci and additional guide sequences	No
CRISPR-ERA Citation »	Yes	9	DNA sequence, gene name, or TSS location	Candidate guide sequences and distances to TSS	Yes
Benchling	Yes	5	DNA sequence or gene name	Candidate guide sequences and off-target loci	Yes

A) DESIGNED BY YOU

Prerequisite: Have a skilled bioinformatician ☺

- Introduce control sgRNAs (Rosa Locus)
- Barcoding of the sgRNAs
- Resistance marker or fluorophore

LIBRARY DESIGN

B) Commercially available

Library	ID	Species	Lentiviral Generation	PI	Genes Targeted	gRNAs per gene	Total gRNAs
Bassik Human CRISPR Knockout Library Description	101926 — 101934	Human	3rd	Bassik	Varies (~20,500 in total)	~10	Varies
Bassik Mouse CRISPR Knockout Library Description	1000000121 — 1000000130	Mouse	3rd	Bassik	Varies (~23,000 in total)	~10	Varies
Mouse Tumor Suppressor Gene CRISPR Knockout Library Description	113584 *EFS backbone* 113585 *TBG backbone*	Mouse	N/A	Chen	56	~4	286
Mouse Metastasis CRISPR-Cpf1(Cas12a) Double Knockout Library Description	123361	Mouse	3rd	Chen	26	4	11,934 arrays
Brie mouse genome-wide library Description	73632 *(1 plasmid)* 73633 *(2 plasmid)*	Mouse	3rd	Doench and Root	19,674	4	78,637
Brie mouse kinome library Description	75317 *(1 plasmid)* 75316 *(2 plasmid)*	Mouse	3rd	Doench and Root	713	4	2,852
Brunello human genome-wide library Description	73179 *(1 plasmid)* 73178 *(2 plasmid)*	Human	3rd	Doench and Root	19,114	4	76,441
Brunello human kinome library Description	75314 (Guides 1-4); 75315 (Guides 5-8) *(1 plasmid)* 75312 (Guides 1-4); 75313 (Guides 5-8) *(2 plasmid)*	Human	3rd	Doench and Root	763	4	3,052

LIBRARY DESIGN

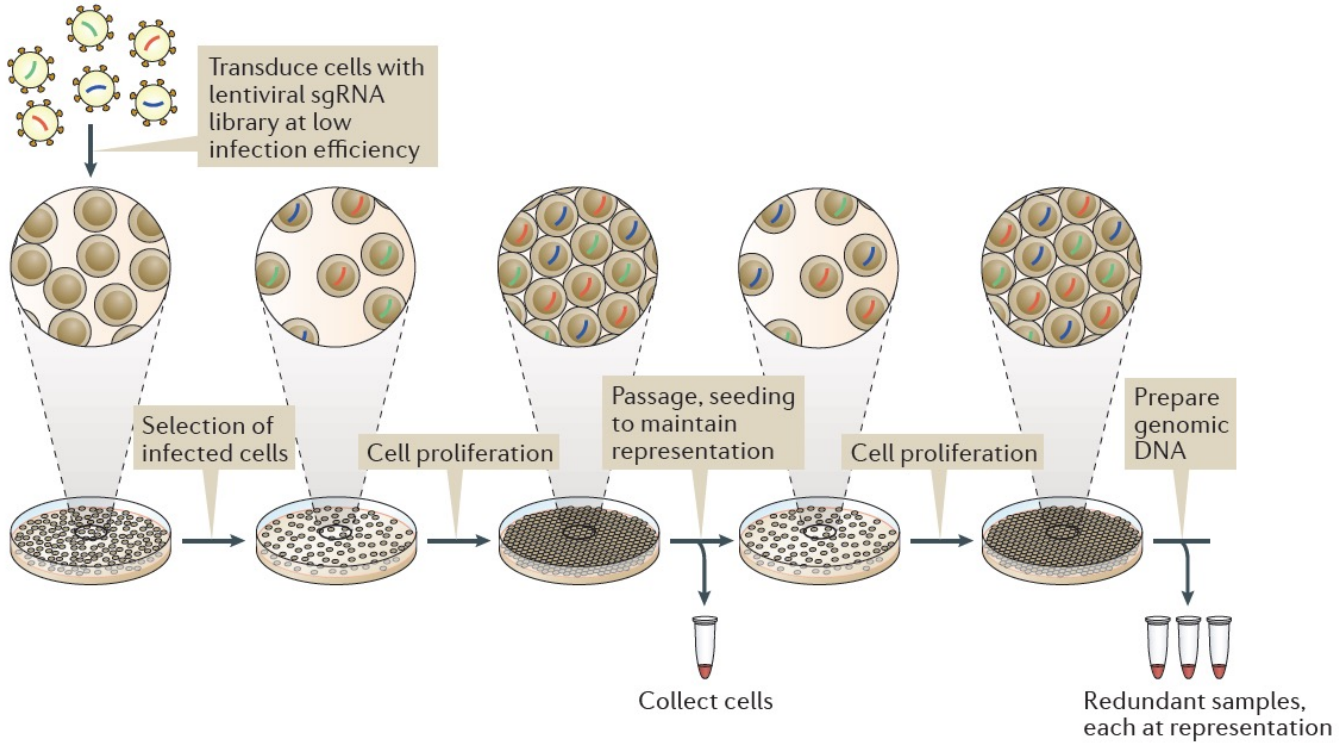
B) Commercially available

Library	ID	Species	Lentiviral Generation	PI	Genes Targeted	gRNAs per gene	Total gRNAs
Mouse CRISPR/Cas9-assisted Removal of Mitochondrial DNA (CARM) Library Description	82480	Mouse	N/A	Xie	gRNAs target every ~40bp over mitochondrial genome	N/A	395
Human improved genome-wide library v1 Description	67989	Human	3rd	Yusa	18,010	5	90,709
Mouse genome-wide library **Discontinued**	50947	Mouse	3rd	Yusa	19,150	5	87,897
Mouse improved genome-wide library v2 Description	67988	Mouse	3rd	Yusa	18,424	5	90,230
Cas13a/C2c2 Protospacer flanking site (PFS) Library Description	79153	Bacterial	N/A	Zhang	4,096 Protospacer flanking sites	N/A	N/A
Human GeCKO v2 genome-wide library Description	1000000048 *(1 plasmid)* 1000000049 *(2 plasmid)*	Human	3rd	Zhang	19,052	6	123,411
Mouse GeCKO v2 genome-wide library Description	1000000052 *(1 plasmid)* 1000000053 *(2 plasmid)*	Mouse	3rd	Zhang	20,661	6	130,209

https://www.addgene.org/crispr/libraries/

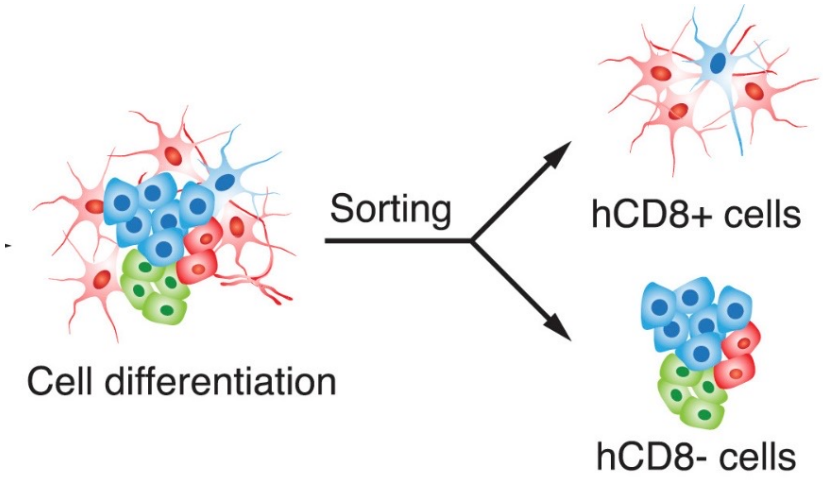
DELIVER sgRNAs VIA LENTIVIRUS

virus production → transduction efficiency → selection (markers)



Doench Nature 2018.

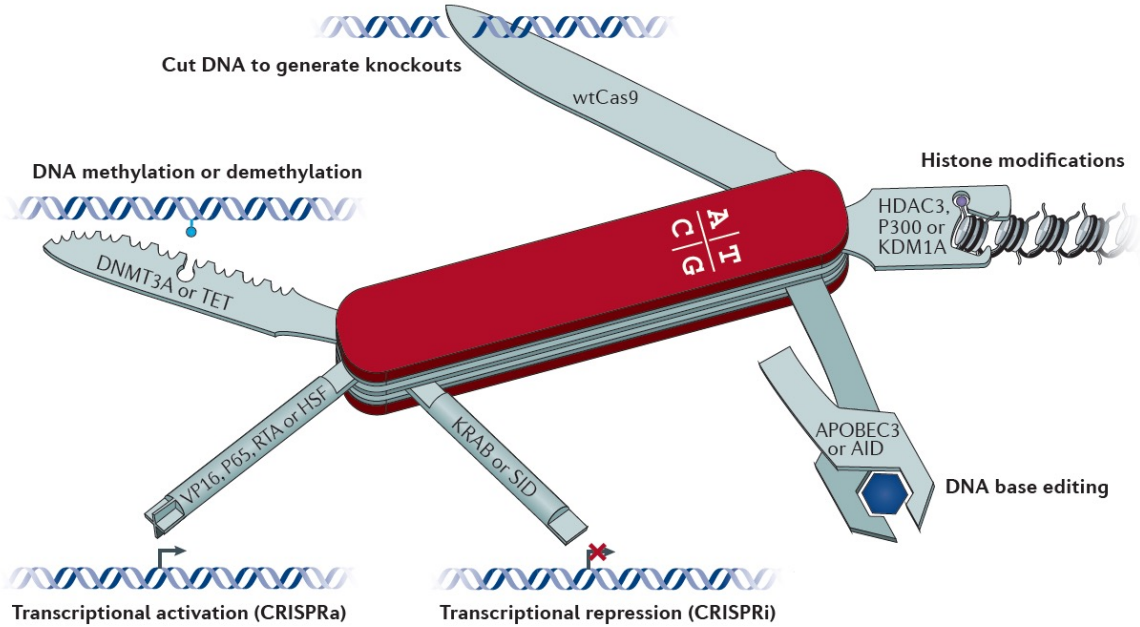
SELECT FOR DESIRED CELLS



FACS

(fluorophore, Ab)

SO WHEN I SHOULD VENTURE IN CRISPR SCREENS?



Time and cost expenses!

Redundancy of cellular pathways

Genetic interaction-mechanism?

Off target effect- false positive

Cancer: amplified genome regions: compared to RNAi

Doench Nature 2018.

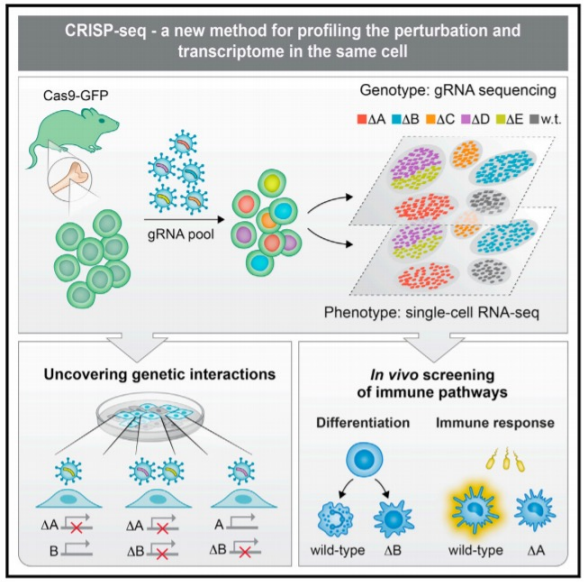
FUTURE PERSPECTIVES:

In vivo screens! ☺

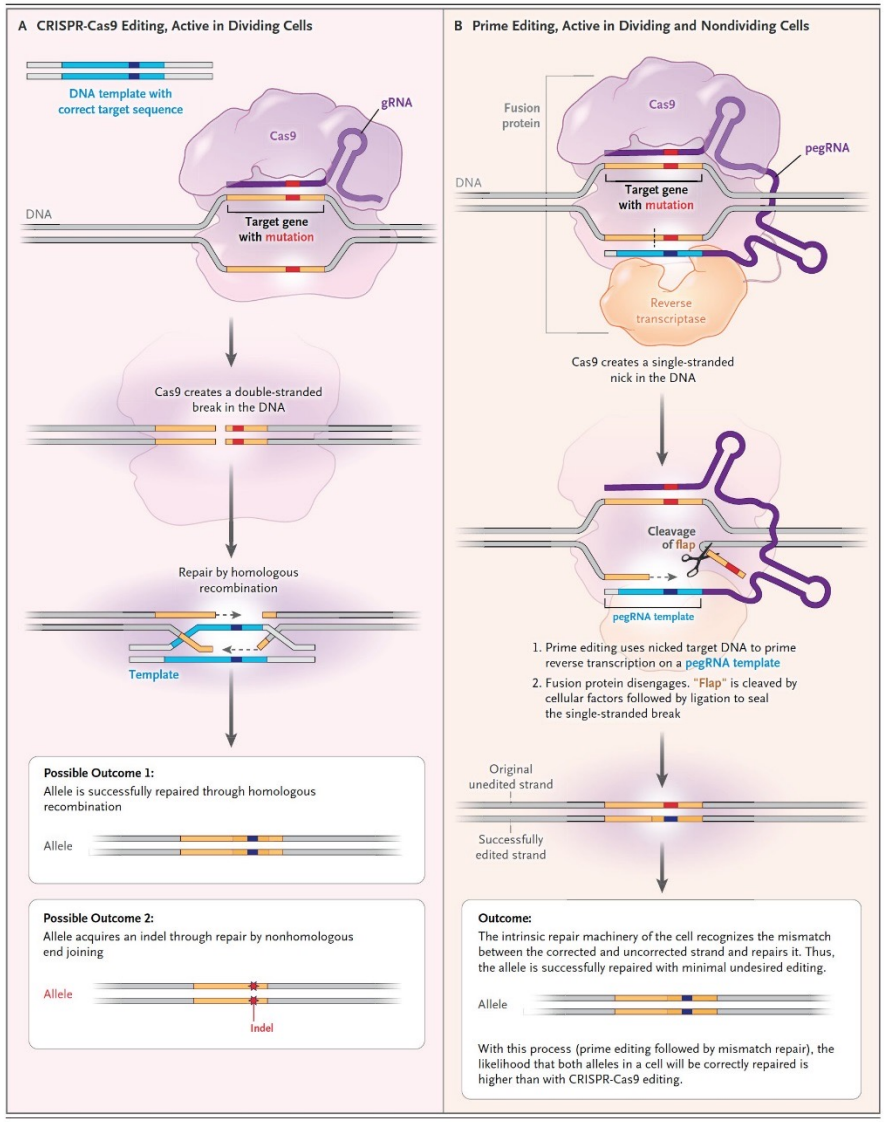
CRISPR and single RNAseq (CRISPseq)

Prime editing-> CRISPR knock-in screens?

New algorithms…
Mosaicseq
CRISPRiseq



Jatin et al. Cell 2016.



AV Anzalone et al. Nature 2019.