



# The genome editing revolution

Andrew Bassett

NEWS · 30 NOVEMBER 2018

# First CRISPR babies: six questions that remain



Jan 29<sup>th</sup> 2013



## RNA-programmed genome editing in human cells

Martin Jinek<sup>1,2</sup>, Alexandra East<sup>2</sup>, Aaron Cheng<sup>2</sup>, Steven Lin<sup>1,2</sup>, Enbo Ma<sup>2</sup>, Jennifer Doudna<sup>1,2,3,4\*</sup>

<sup>1</sup>Howard Hughes Medical Institute, University of California, Berkeley, Berkeley, United States; <sup>2</sup>Department of Molecular and Cell Biology, University of California, Berkeley, Berkeley, United States; <sup>3</sup>Department of Chemistry, University of California, Berkeley, Berkeley, United States; <sup>4</sup>Physical Biosciences Division, Lawrence Berkeley National Laboratory, Berkeley, United States

Scienceexpress

## RNA-Guided Human Genome Engineering via Cas9

Prashant Mali,<sup>1,5</sup> Luhan Yang,<sup>1,3,5</sup> Kevin M. Esvelt,<sup>2</sup> John Aach,<sup>1</sup> Marc Guell,<sup>1</sup> James E. DiCarlo,<sup>4</sup> Julie E. Norville,<sup>1</sup> George M. Church<sup>1,2\*</sup>

Scienceexpress

## Multiplex Genome Engineering Using CRISPR/Cas Systems

Le Cong,<sup>1,2\*</sup> F. Ann Ran,<sup>1,4\*</sup> David Cox,<sup>1,3</sup> Shuailiang Lin,<sup>1,5</sup> Robert Barretto,<sup>6</sup> Naomi Habib,<sup>1</sup> Patrick D. Hsu,<sup>1,4</sup> Xuebing Wu,<sup>7</sup> Wenyan Jiang,<sup>6</sup> Luciano A. Marraffini,<sup>8</sup> Feng Zhang<sup>1†</sup>

nature  
biotechnology

## Efficient genome editing in zebrafish using a CRISPR-Cas system

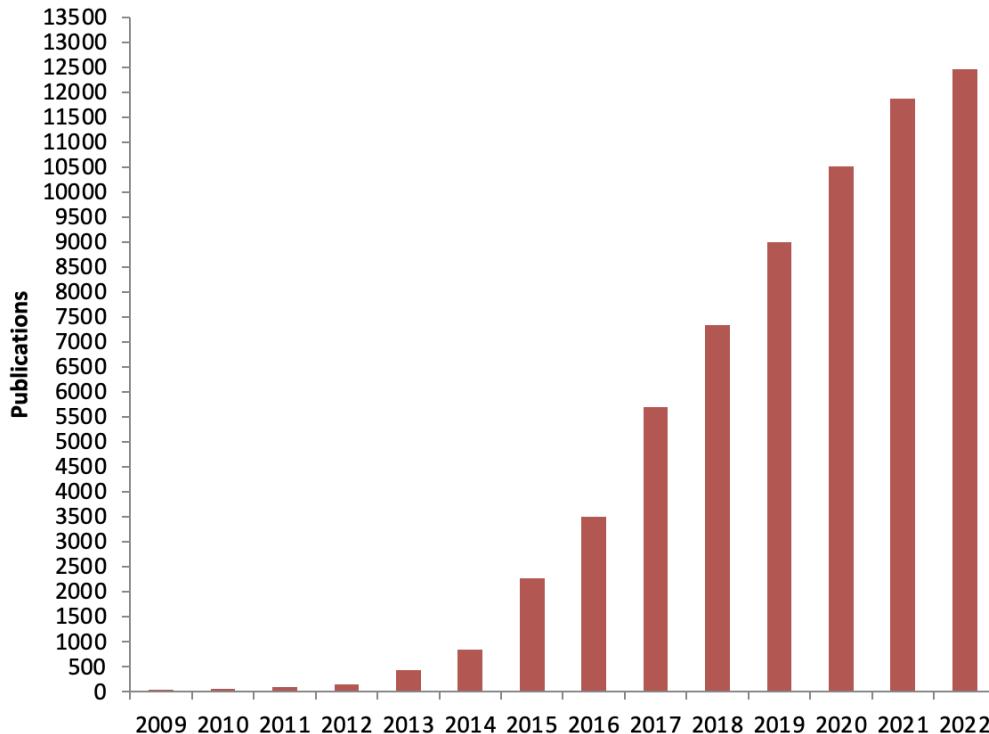
Woong Y Hwang<sup>1,7</sup>, Yanfang Fu<sup>2,3,7</sup>, Deepak Reyon<sup>2,3</sup>, Morgan L Maeder<sup>2,4</sup>, Shengdar Q Tsai<sup>2,3</sup>, Jeffry D Sander<sup>2,3</sup>, Randall T Peterson<sup>1,5,6</sup>, J-R Joanna Yeh<sup>1,5</sup> & J Keith Joung<sup>2-4</sup>

Jan 29<sup>th</sup> 2013



## ScienceX RNA-Gui Engineer

Prashant Mali,<sup>1,5</sup> L  
James E. DiCarlo,<sup>1</sup>

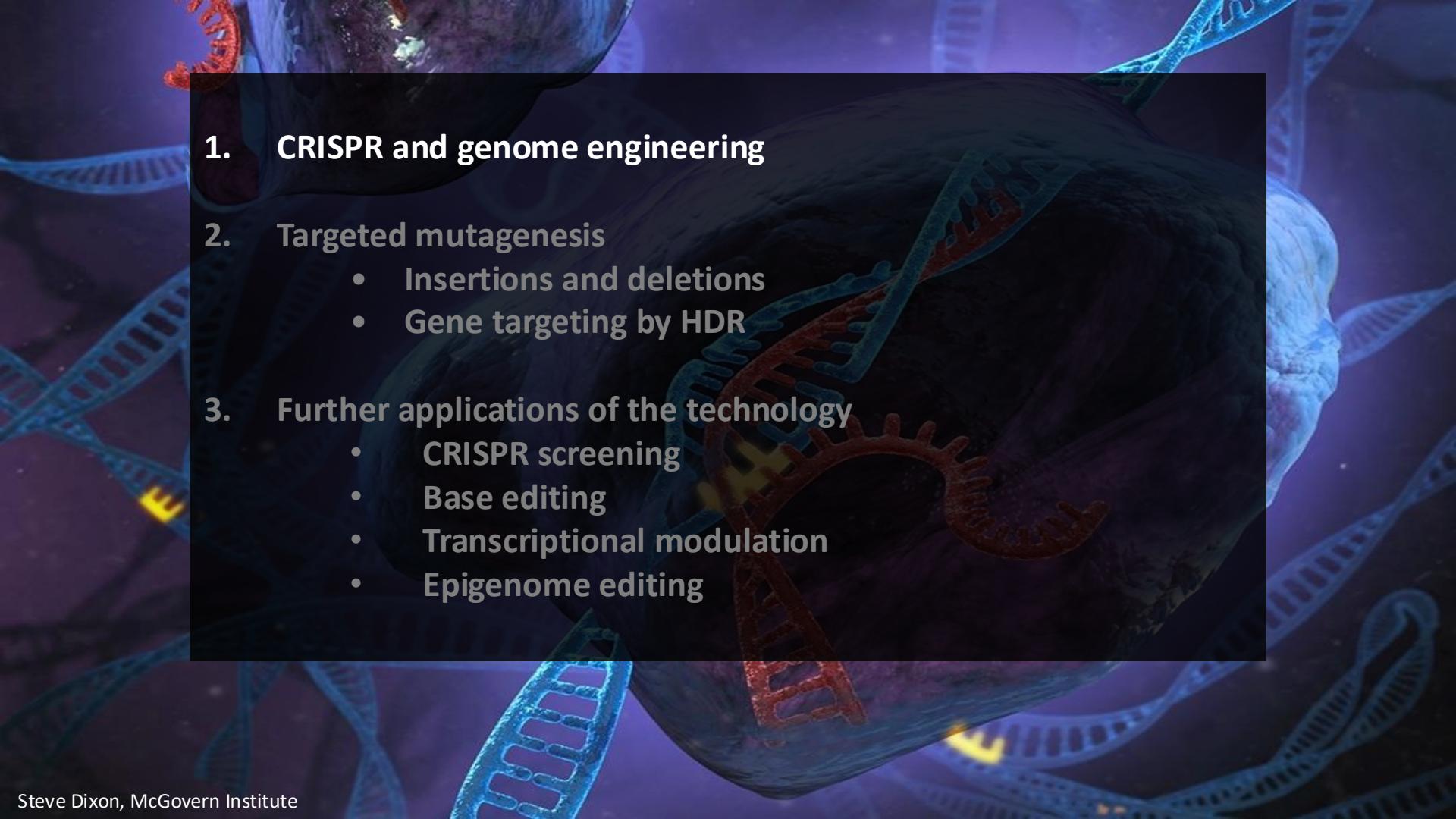


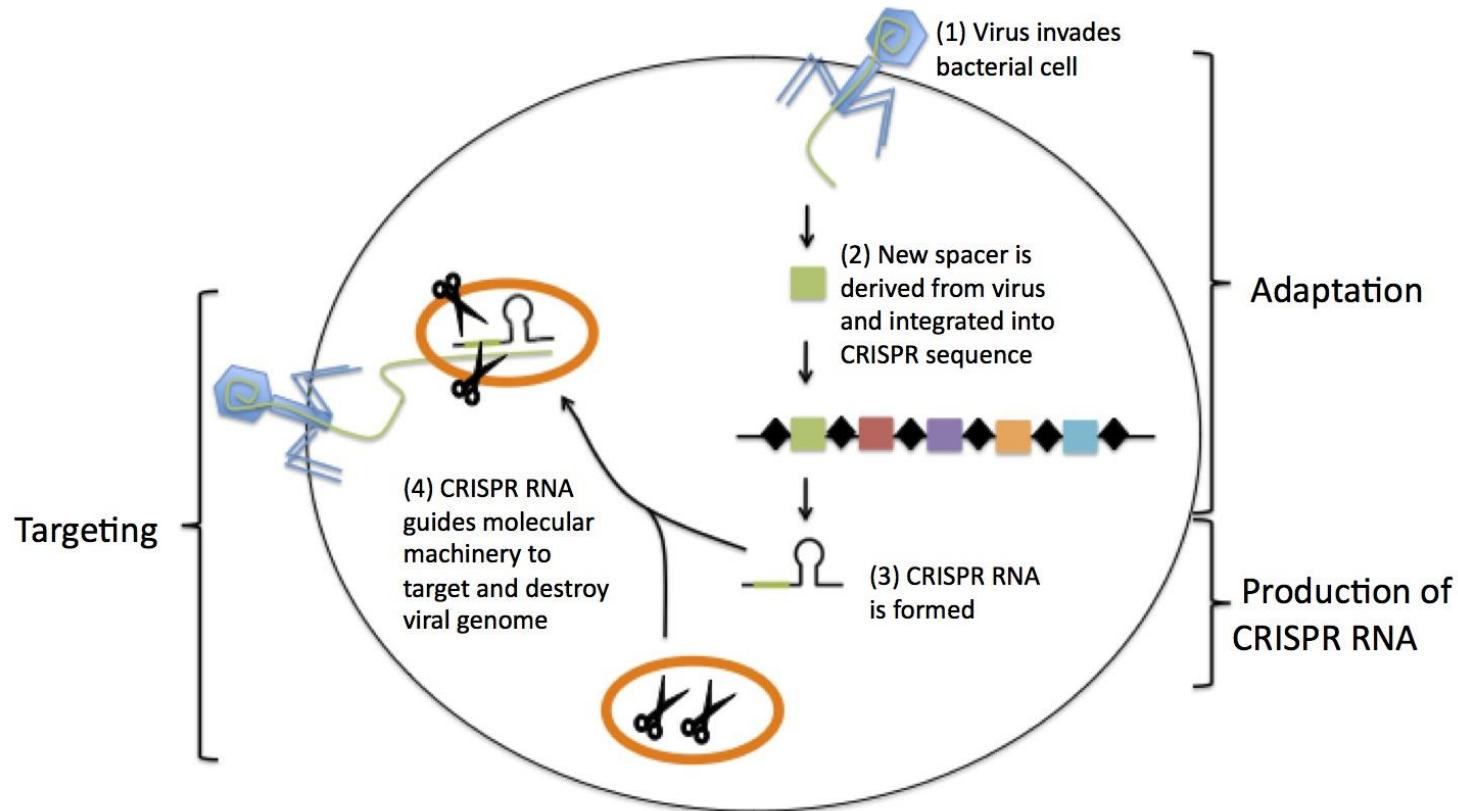
[Engineering Using

Lin,<sup>1,5</sup> Robert Barreto,<sup>6</sup>  
Jian Jiang,<sup>6</sup> Luciano A.

system 35 papers per day!

Nearly 1% of all PubMed citations<sup>3</sup>,  
Woong Il Kwang<sup>1</sup>, Tianshu Fu<sup>1</sup>, Deepak Roychowdhury<sup>3</sup>,  
Morgan L Maeder<sup>2,4</sup>, Shengdar Q Tsai<sup>2,3</sup>,  
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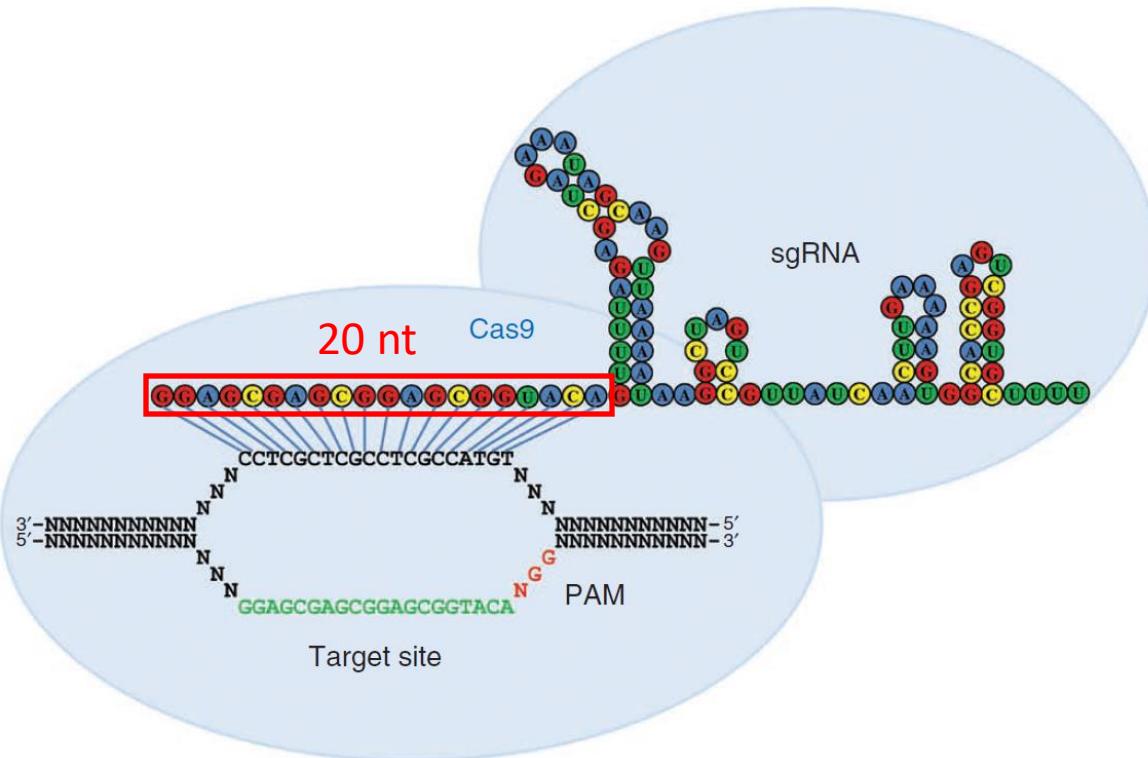
- 
- 1. CRISPR and genome engineering**
  - 2. Targeted mutagenesis**
    - Insertions and deletions
    - Gene targeting by HDR
  - 3. Further applications of the technology**
    - CRISPR screening
    - Base editing
    - Transcriptional modulation
    - Epigenome editing



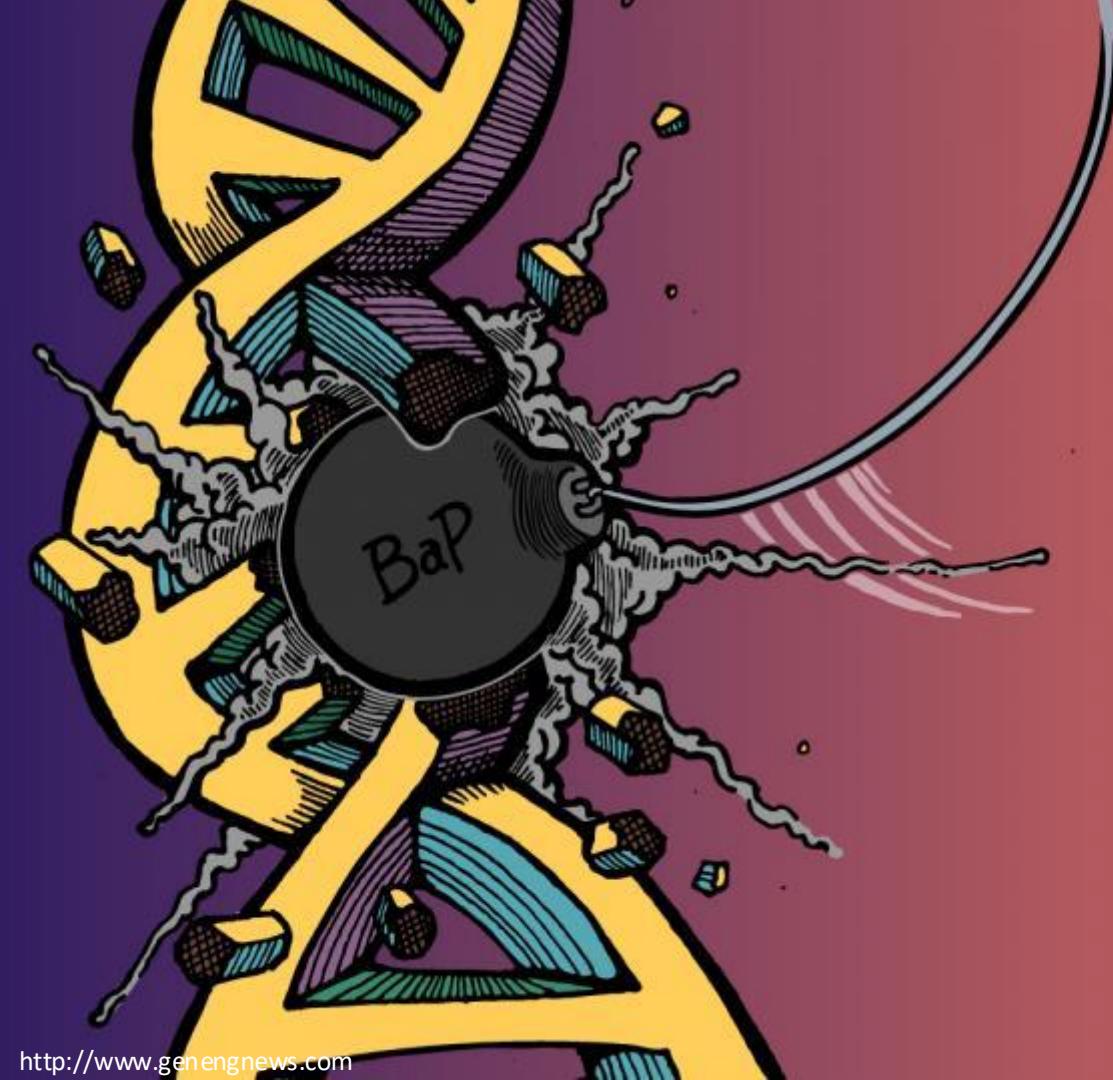
<http://sitn.hms.harvard.edu>

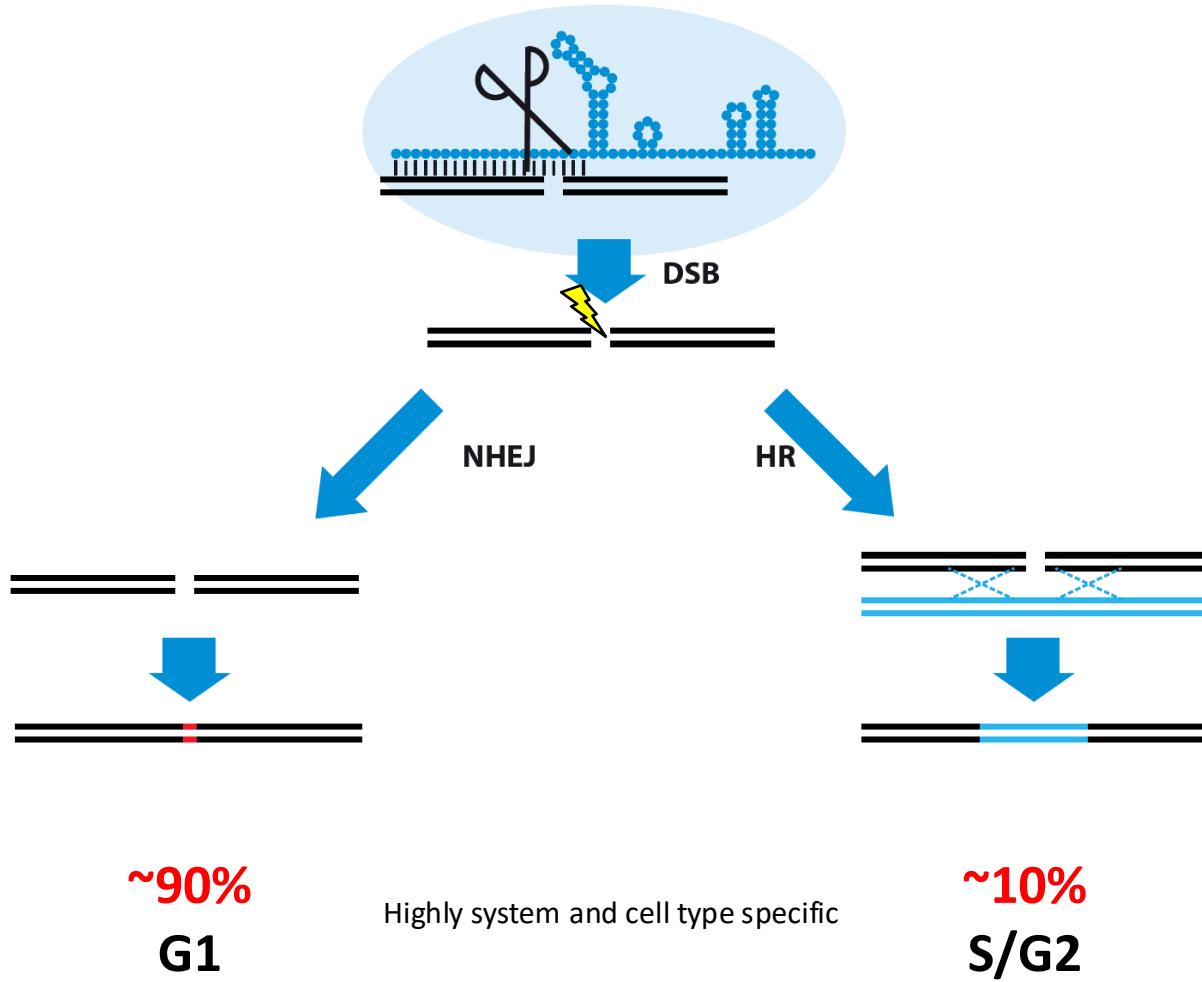
CRISPR = clustered, regularly interspaced, short palindromic repeats

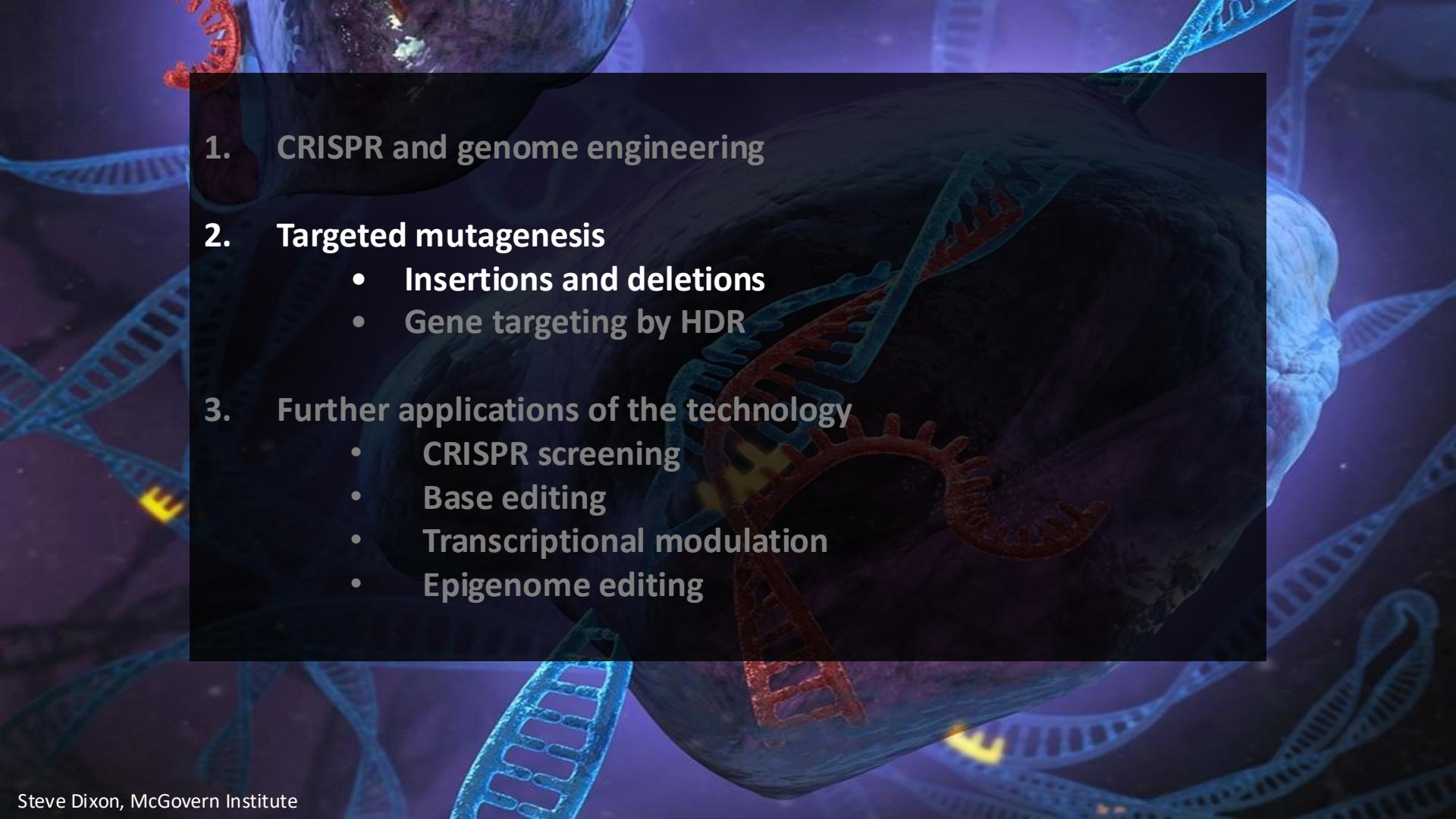
# RNA-guided site-specific DNA cleavage Gasiunas et al. PNAS 2012, Jinek et al. Science 2012

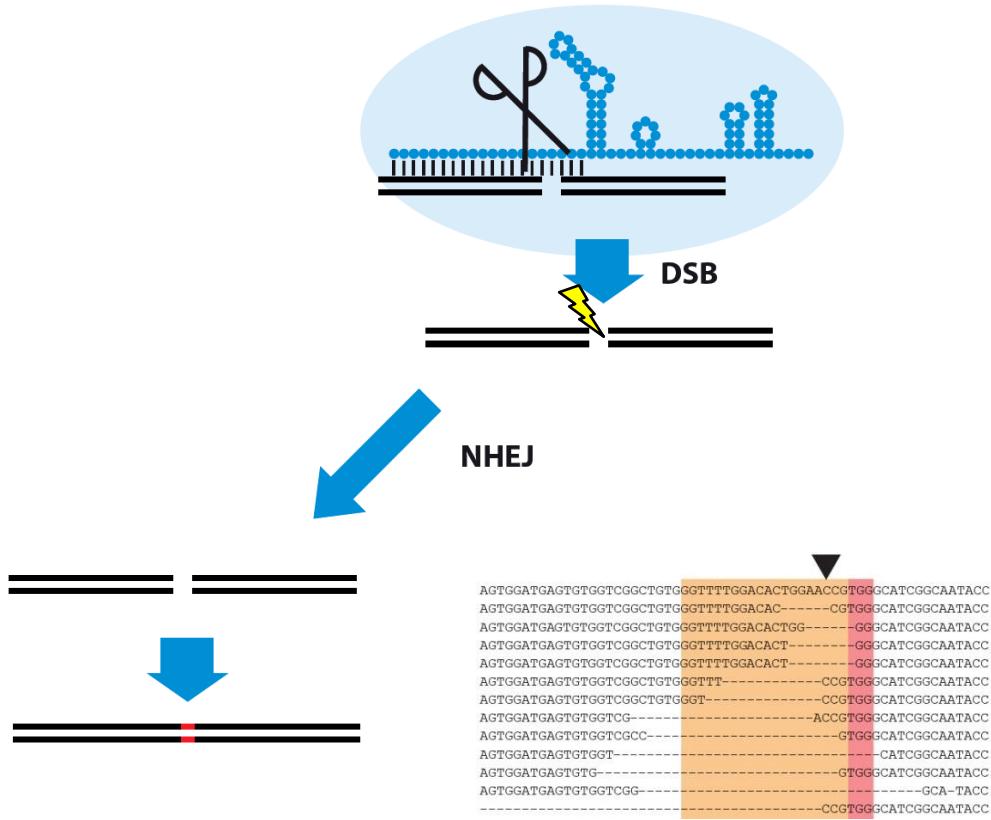






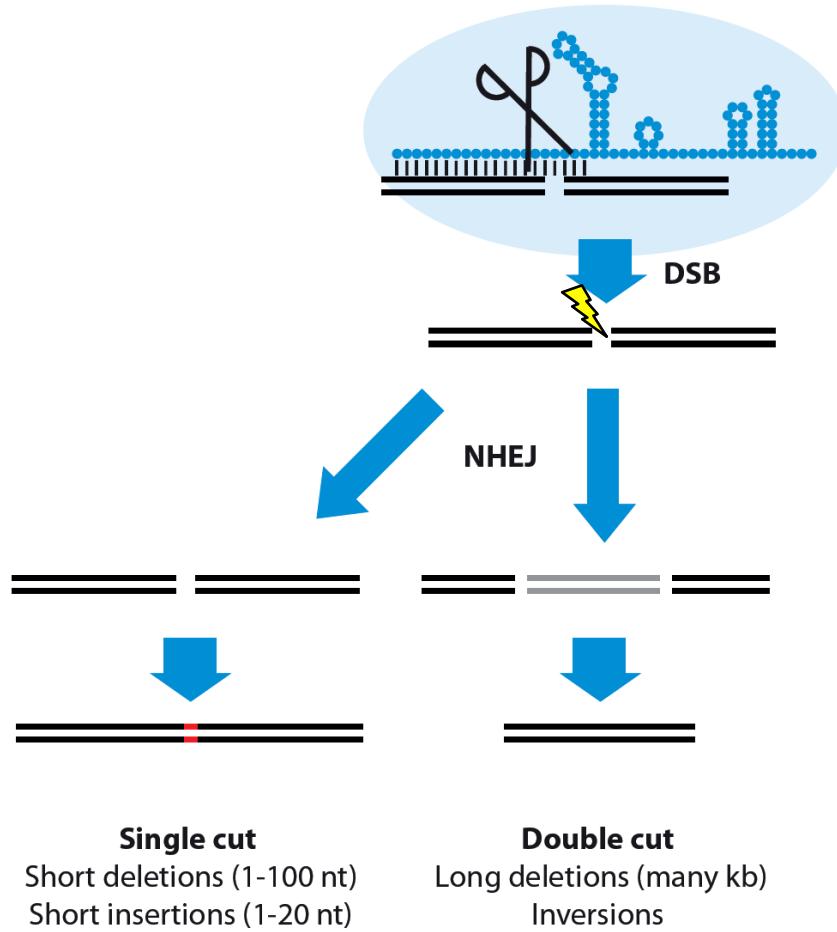


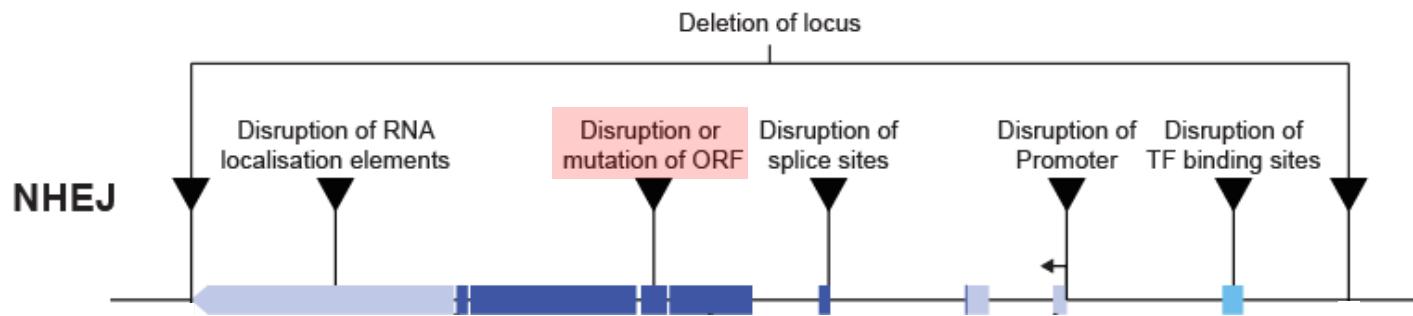
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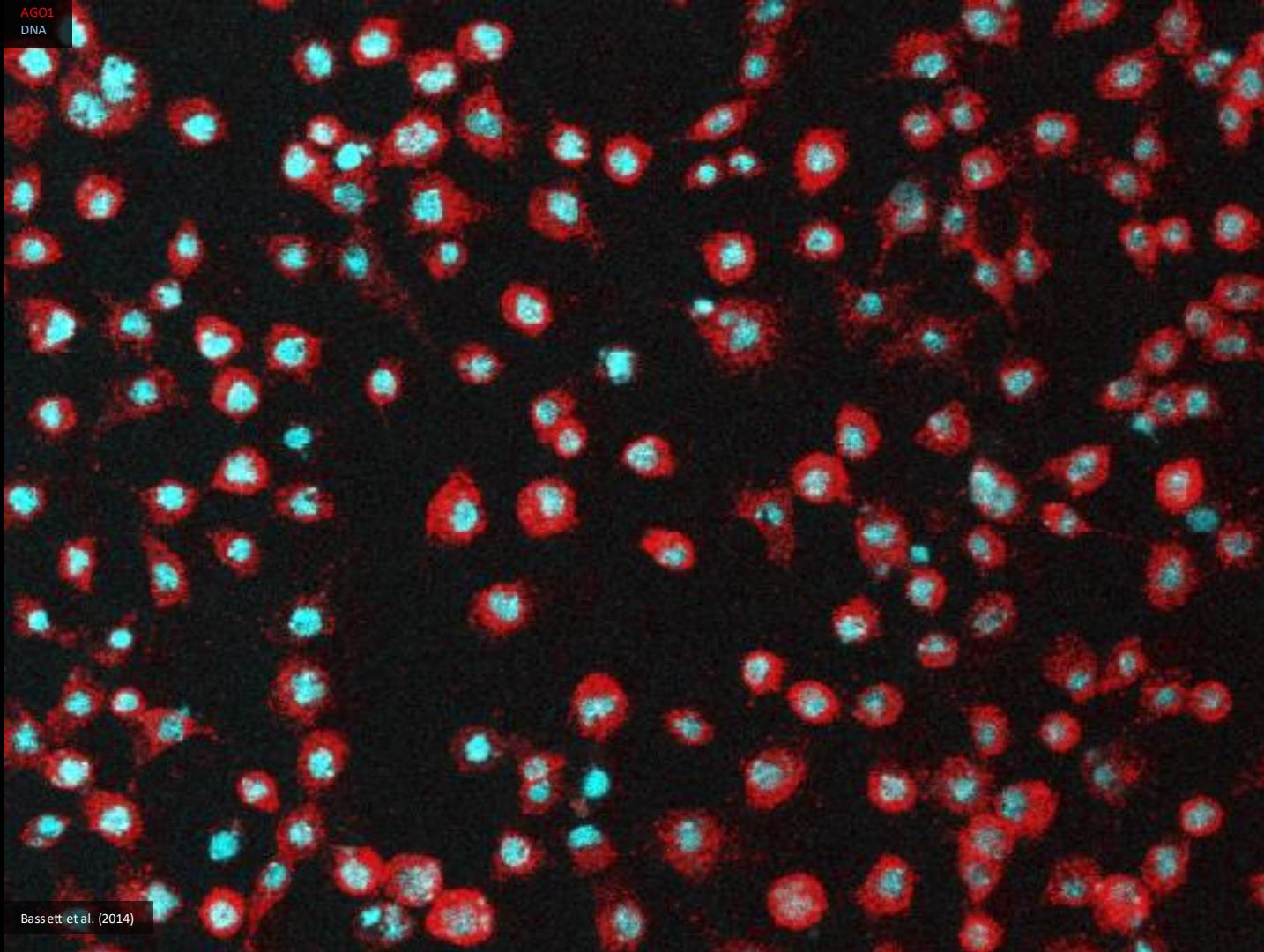
### Single cut

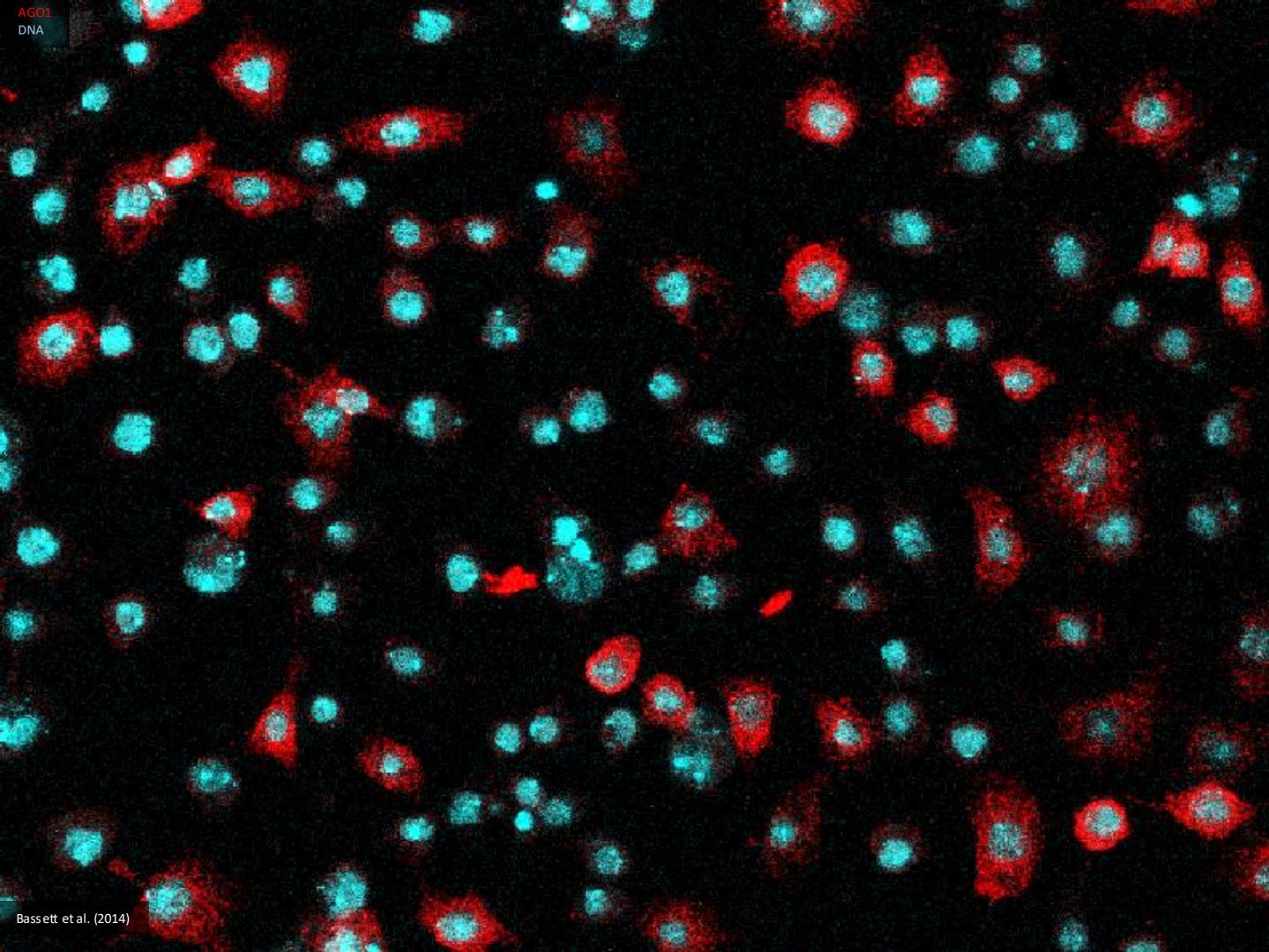
Short deletions (1-100 nt)  
Short insertions (1-20 nt)





AGO1  
DNA

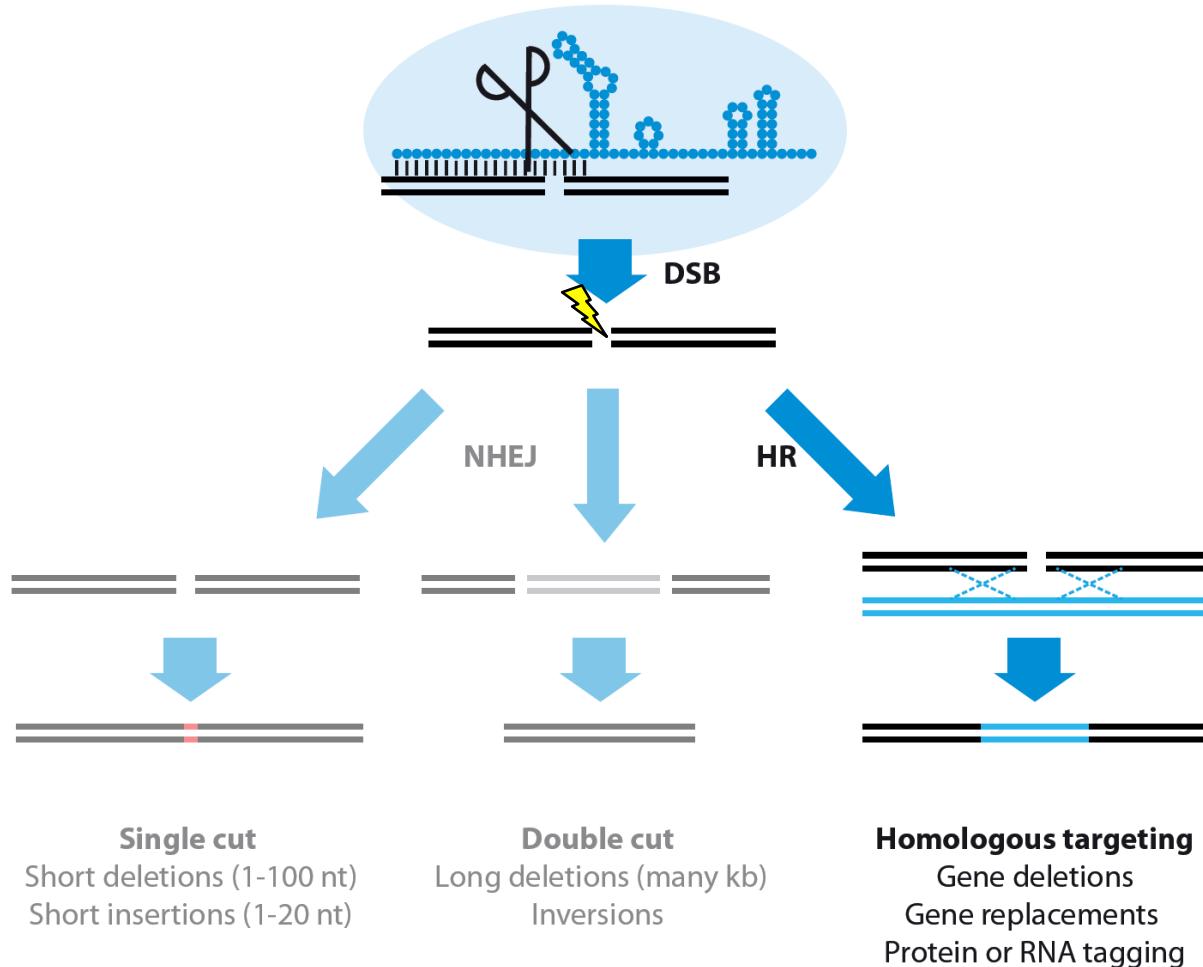


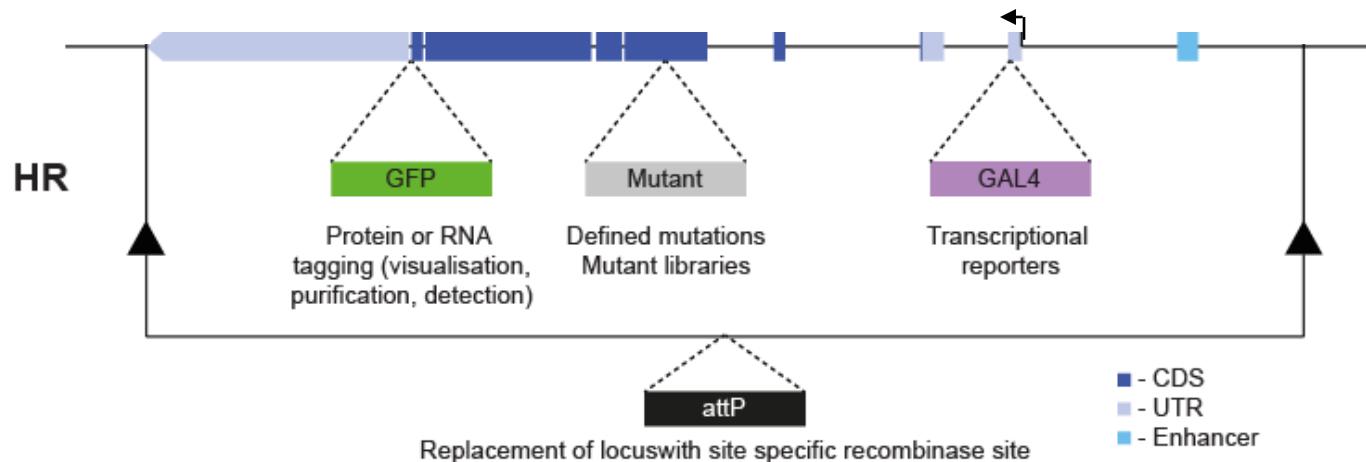


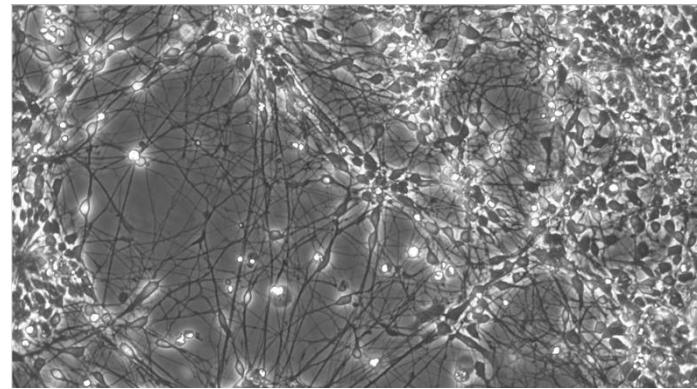
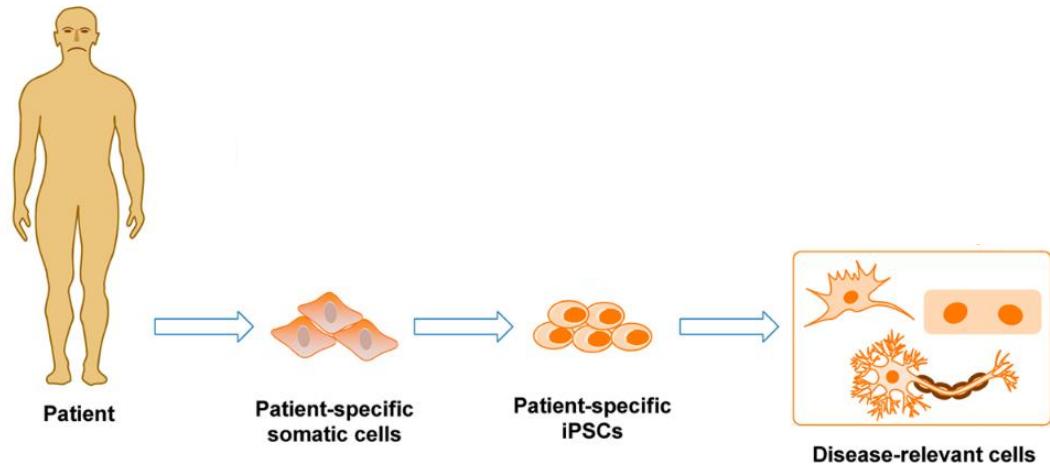


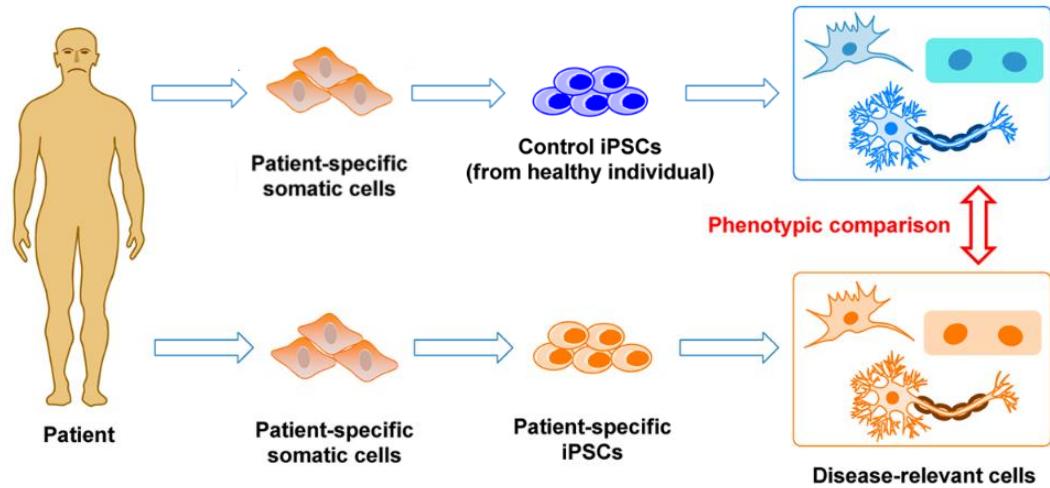
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CCATTGAGCAGTCGATCCCGATGGCGATACTTGGATGCCCaGC GGCGATCGAAAGGCAA  
CCATTGAGCAGTCGATCCCGATGGCGATACTTGGAT-----GGGGCGATCGAAAGGCAA  
CCATTGAGCAGTCGATCCCGATGGCGATACTTGGAT-----GGGGCGATCGAAAGGCAA  
CCATTGAGCAGTCGATCCCGATGGCGATACTTGGAT-----GGGGCGATCGAAAGGCAA  
CCATTGAGCAGTCGATCCCGATGGCGATACTTGGAT-----GGGGCGATCGAAAGGCAA  
CCATTGAGCAGTCGATCCCGATGGCGATACTTGGAT-----GGGGCGATCGAAAGGCAA

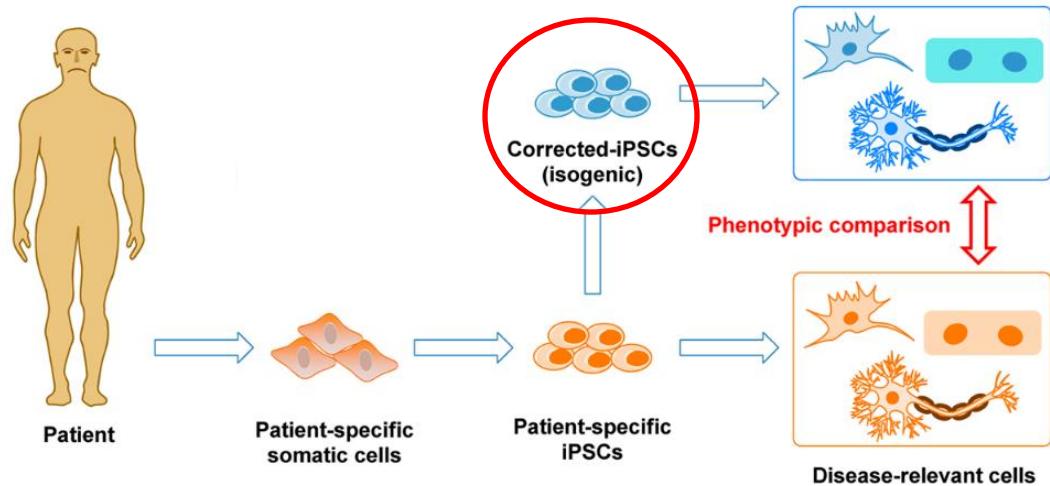
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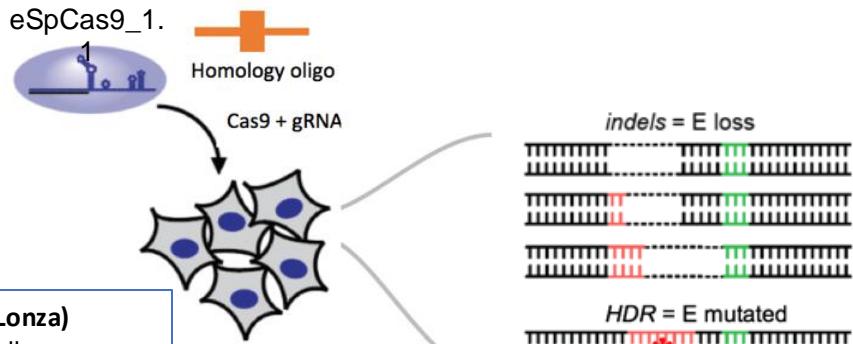




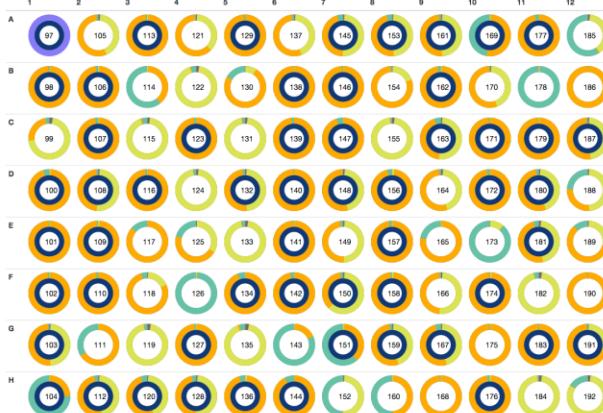
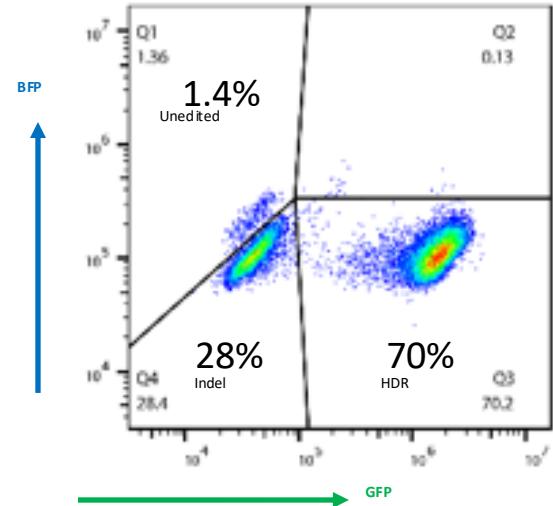


- Eliminate genetic background variability between “WT” control lines
- Enables more subtle changes to be seen
- Establish true causative lesion

# Isogenic hiPSCs

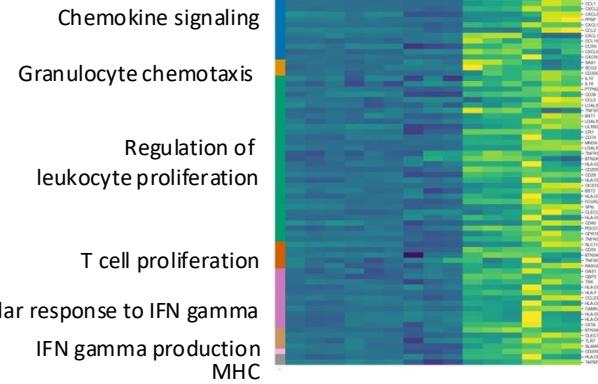
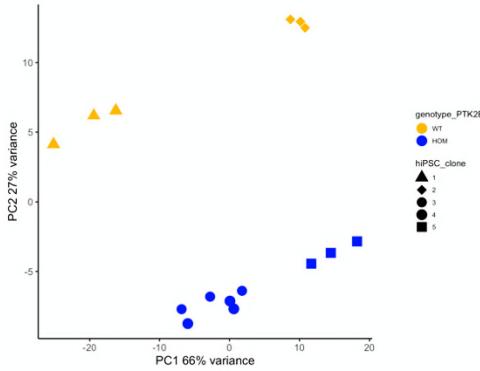


**Amaca 4D (Lonza)**  
1 x 10<sup>6</sup> cells  
120 pmol Cas9 protein  
250 pmol Synthetic sgRNA  
500 pmol 100 nt ssDNA oligo  
AZD DNAPKi, cold shock

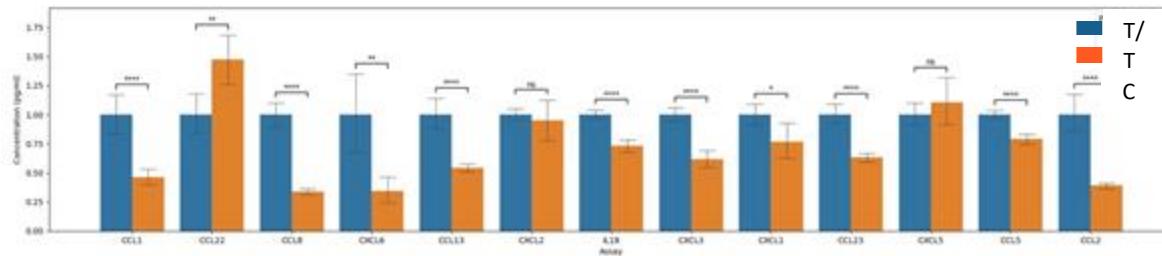


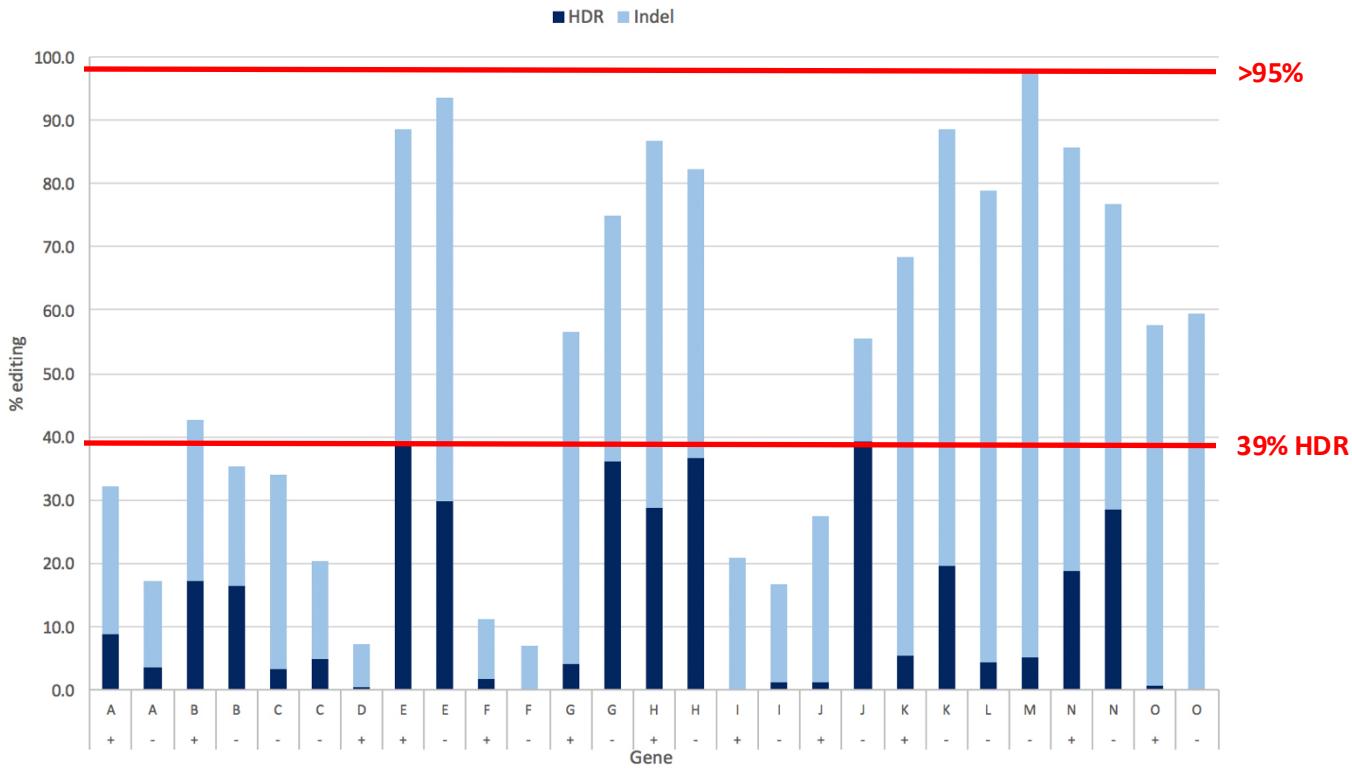
# PTK2B intronic variant

RNAseq

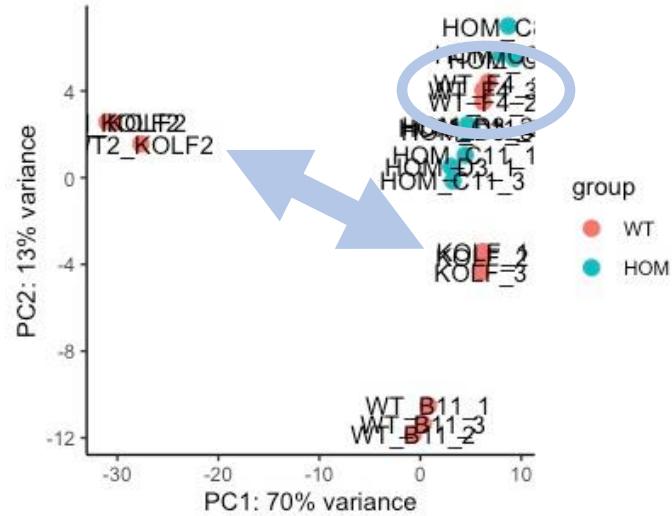
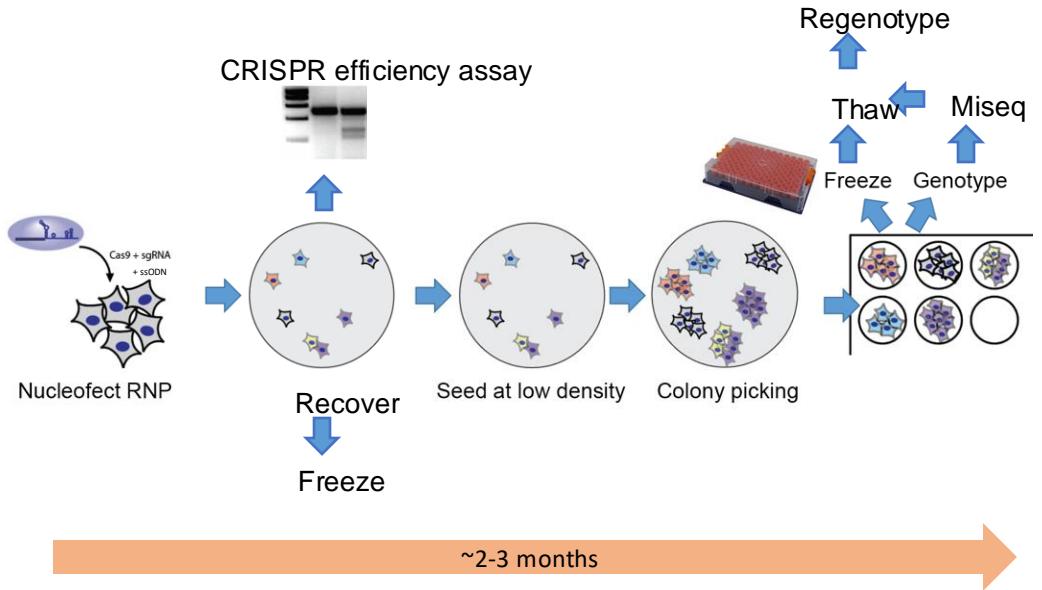


Chemokine release

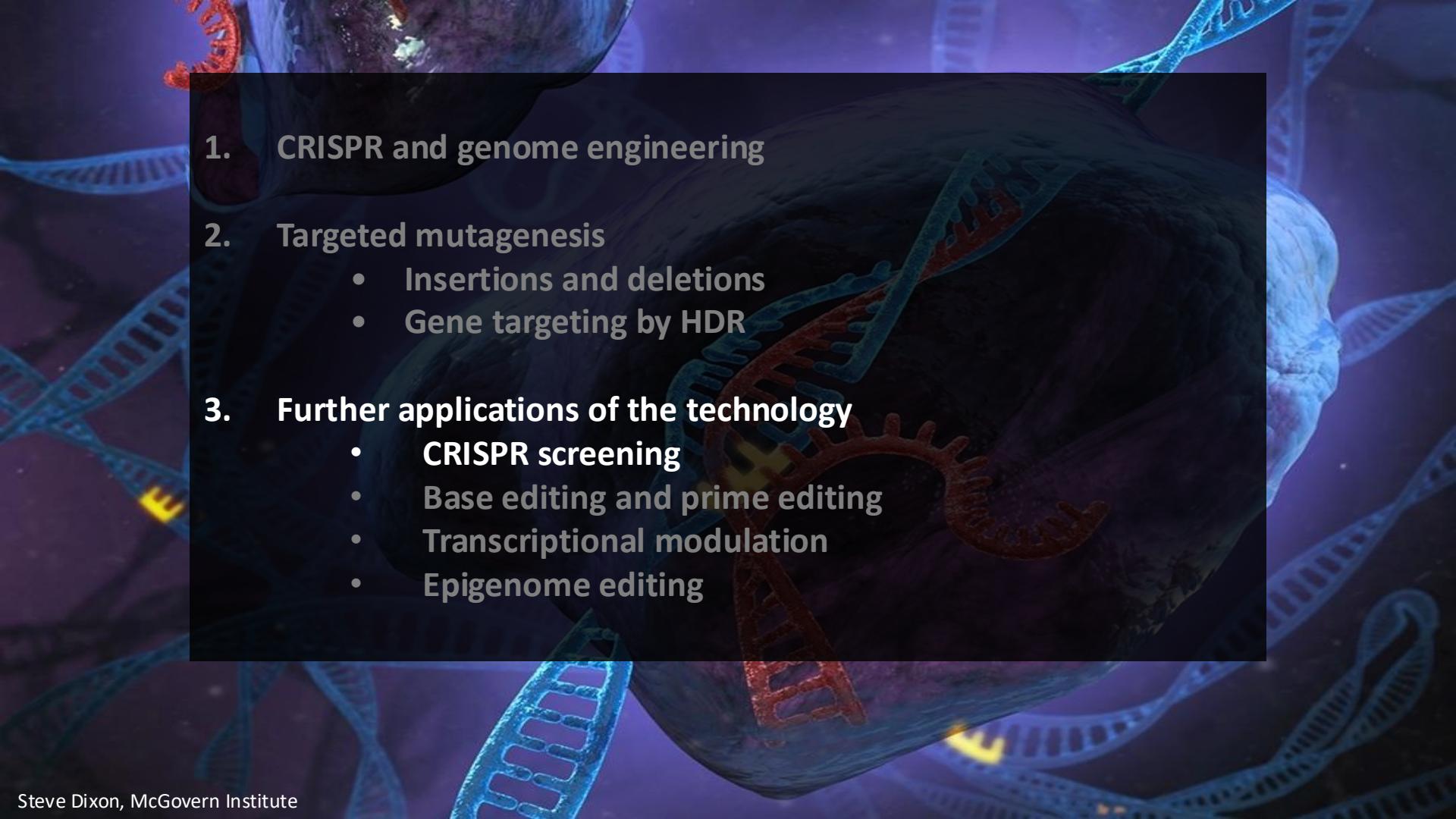




- Efficiency down to <1% in worst cases

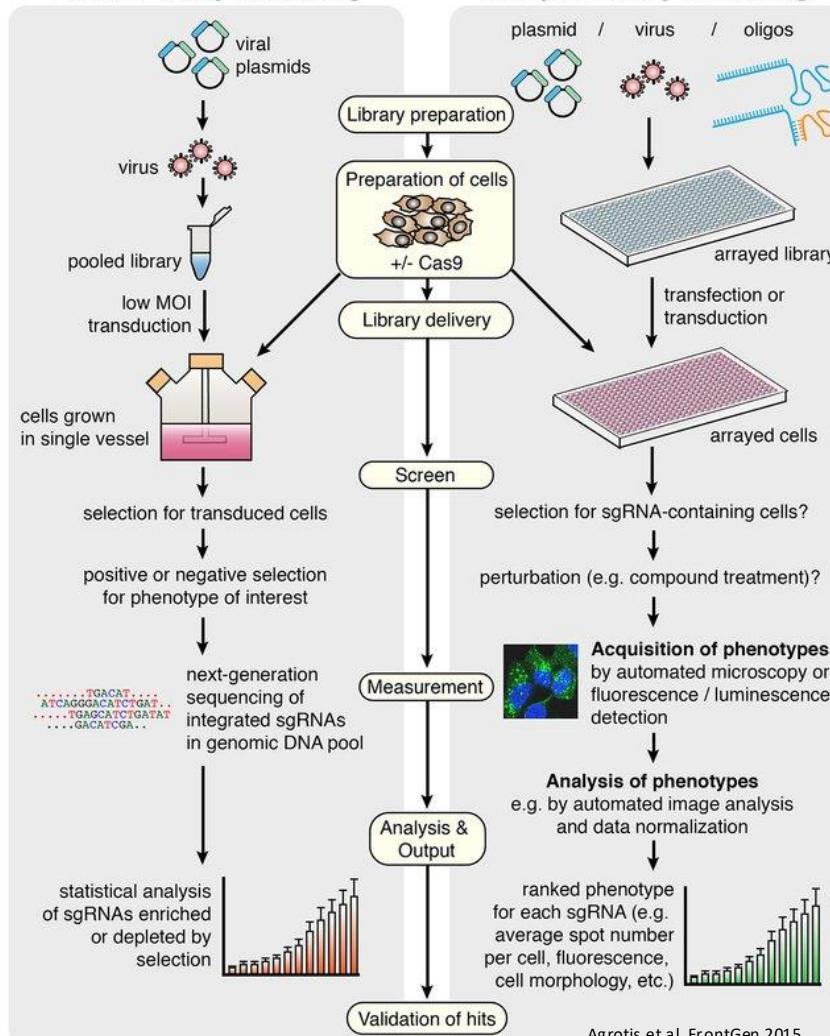


- Time and cost
- Multiple clones
- Variability between clones
- Variability in differentiation
- ... Not feasible for screening

- 
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## Pooled library screening

## **Arrayed library screening**

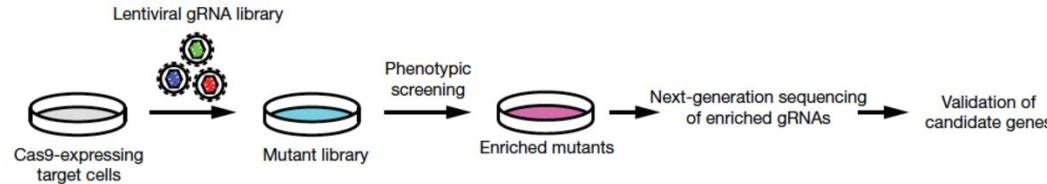


- Does not require robotics
  - Limited to single cell assays  
(e.g. life/death, FACS,  
scRNASeq, OPS)

- Complex and multidimensional readouts  
e.g. high content imaging
  - Limited number of genes, or robotics required for larger screens

# Genome-wide recessive genetic screening in mammalian cells with a lentiviral CRISPR-guide RNA library

Hiroko Koike-Yusa<sup>1,2</sup>, Yilong Li<sup>1,2</sup>, E-Pien Tan<sup>1</sup>, Martin Del Castillo Velasco-Herrera<sup>1</sup> & Kosuke Yusa<sup>1</sup>



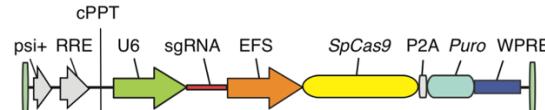
## Genetic Screens in Human Cells Using the CRISPR/Cas9 System

Tim Wang,<sup>1,2,3,4</sup> Jenny J. Wei,<sup>1,2</sup> David M. Sabatini,<sup>1,2,3,4,5\*</sup> † Eric S. Lander<sup>1,3,6\*</sup> †



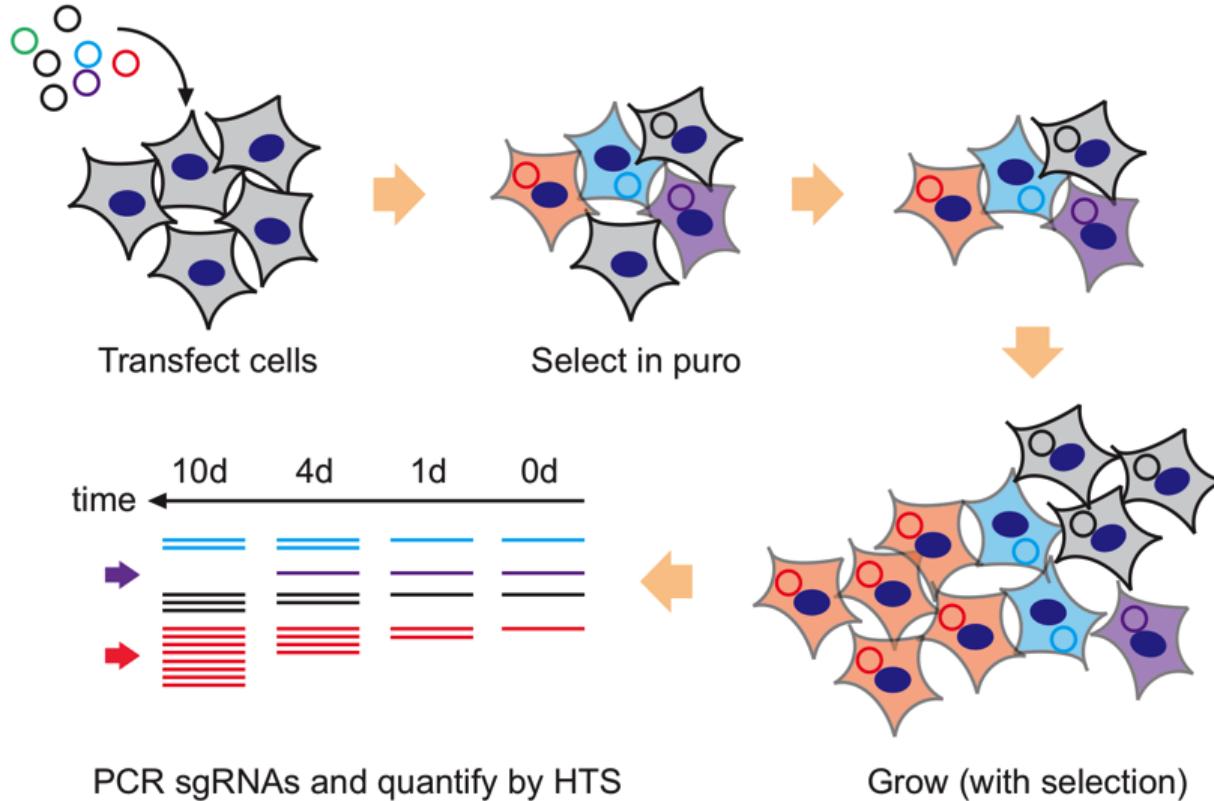
## Genome-Scale CRISPR-Cas9 Knockout Screening in Human Cells

Ophir Shalem,<sup>1,2,\*</sup> Neville E. Sanjana,<sup>1,2,\*</sup> Ella Hartenian,<sup>1</sup> Xi Shi,<sup>1,3</sup> David A. Scott,<sup>1,2</sup> Tarjei Mikkelsen,<sup>1,2,†</sup> Dirk Heckl,<sup>4</sup> Benjamin L. Ebert,<sup>4</sup> David E. Root,<sup>1</sup> John G. Doench,<sup>1</sup> Feng Zhang<sup>1</sup>



... other better libraries available now e.g. MinLib (Sanger), Brie/Brunello (Broad), Weissman CRISPRa/i  
<https://www.addgene.org/pooled-library/>







# Cancer Dependency Map

Identifying all dependencies in every cancer cell

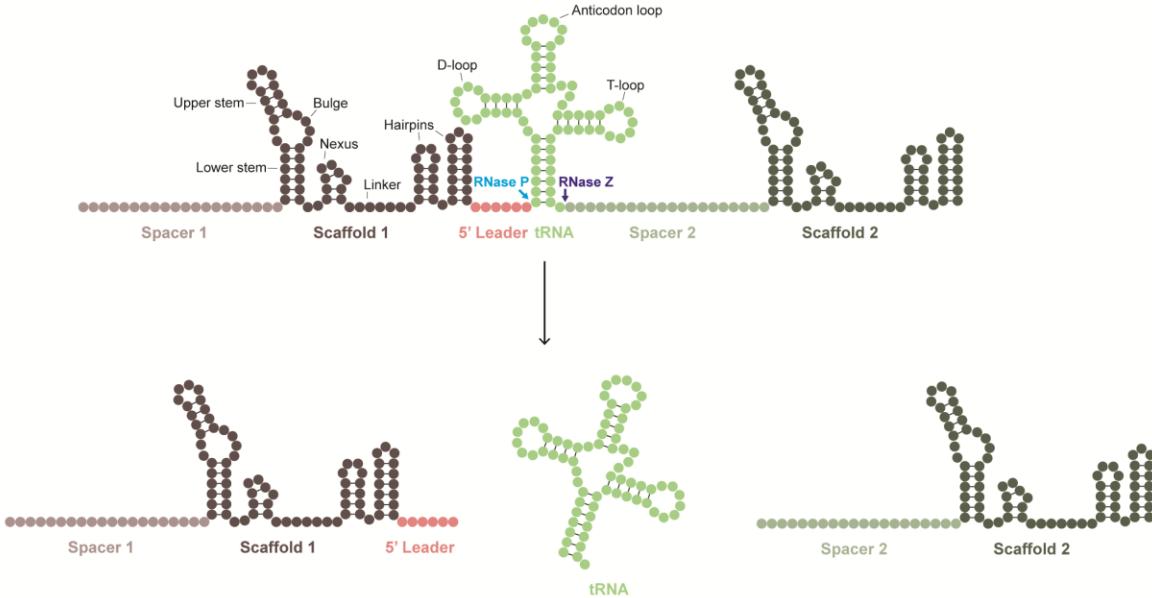


# project score

[www.depmap.sanger.ac.uk](http://www.depmap.sanger.ac.uk)  
[depmap.org/portal/](http://depmap.org/portal/)

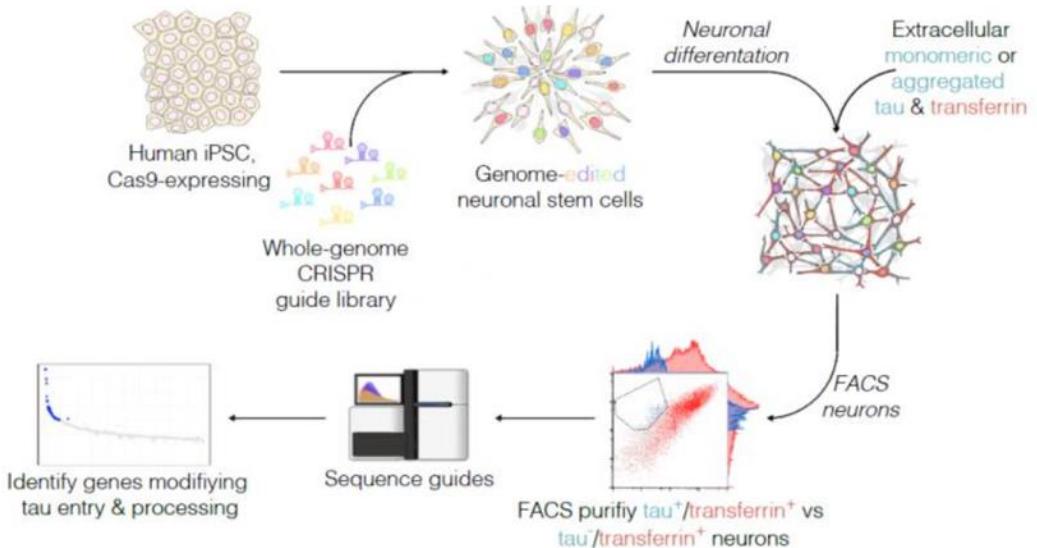
789 cancer cell lines screened

# Dual perturbation screening



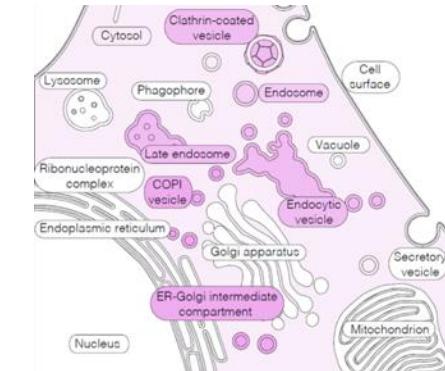
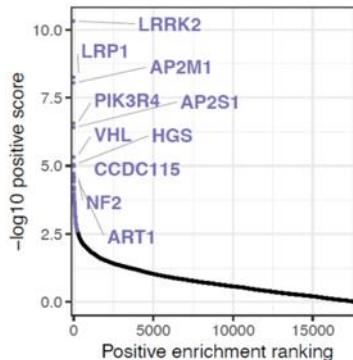
- Synthetic lethal interactions
- Genetic interaction mapping
- Smaller genome-wide libraries

# Neuronal uptake of tau

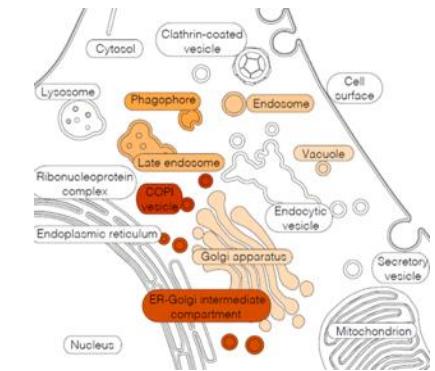
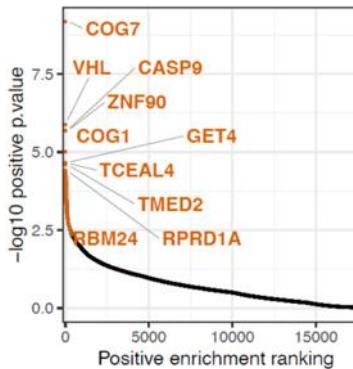


- Top 2 hits: recently discovered tau receptor, the low density lipoprotein receptor LRP1, and PD-associated kinase LRRK2
- Overall similarity with receptor-mediated viral entry screens suggests tau spreading is a quasi-infectious process

## Monomeric tau

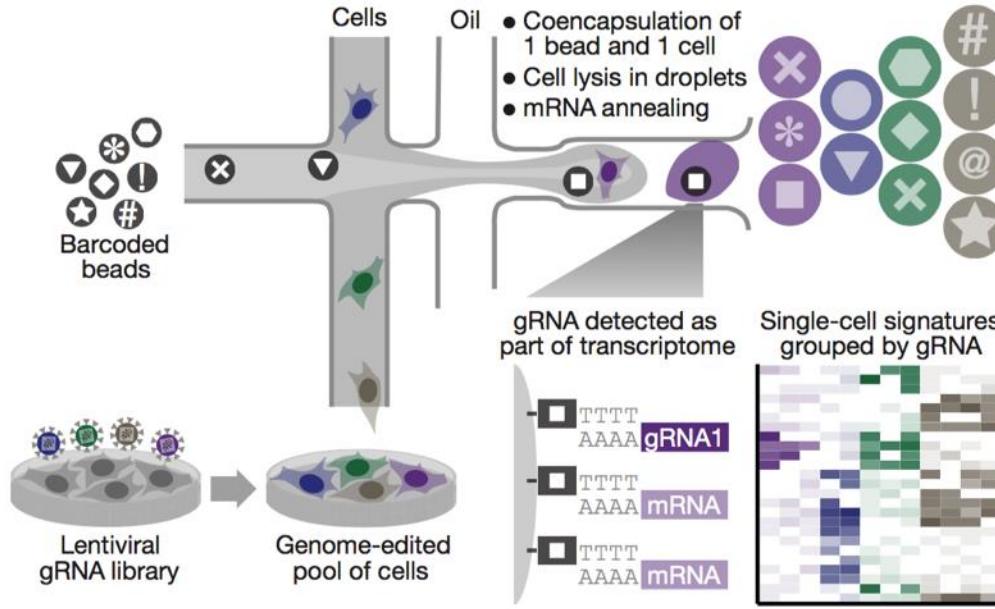


## Aggregated tau



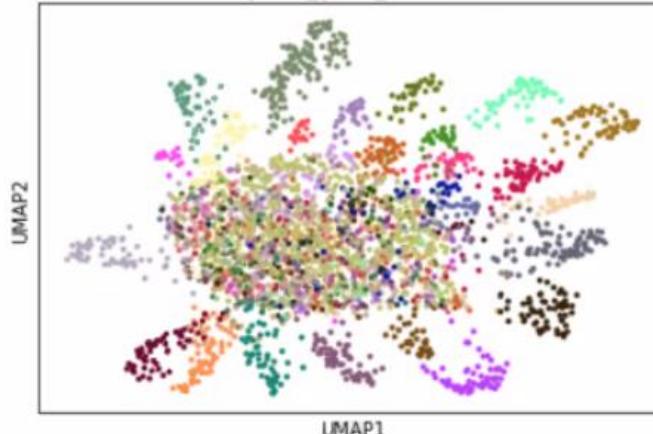
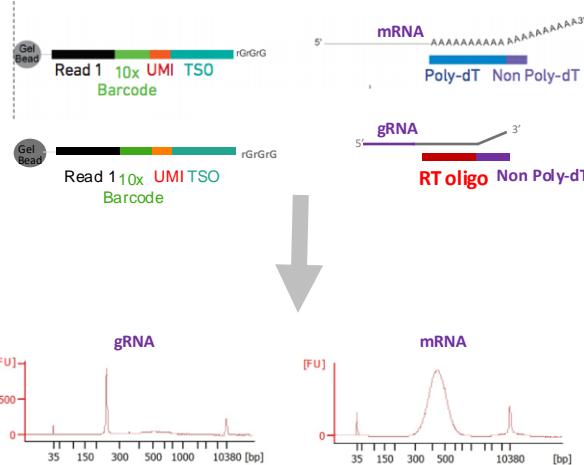
# Single cell CRISPR screening

(CROPseq, dc-perturbSeq)



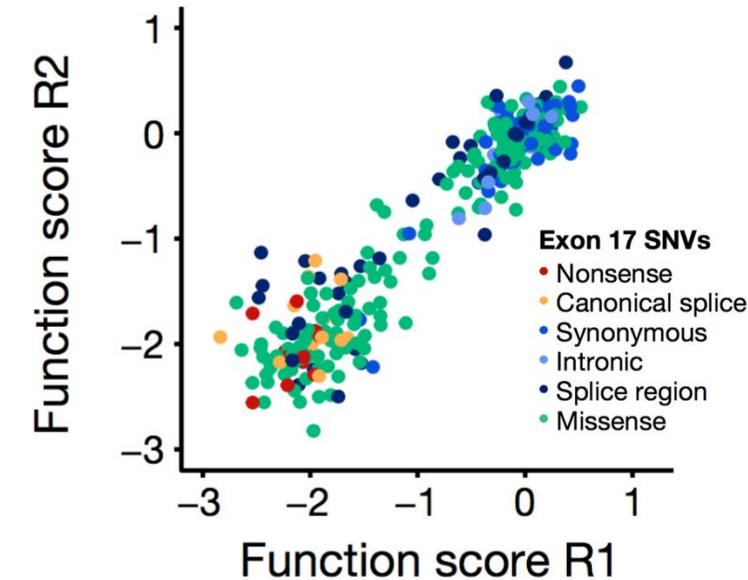
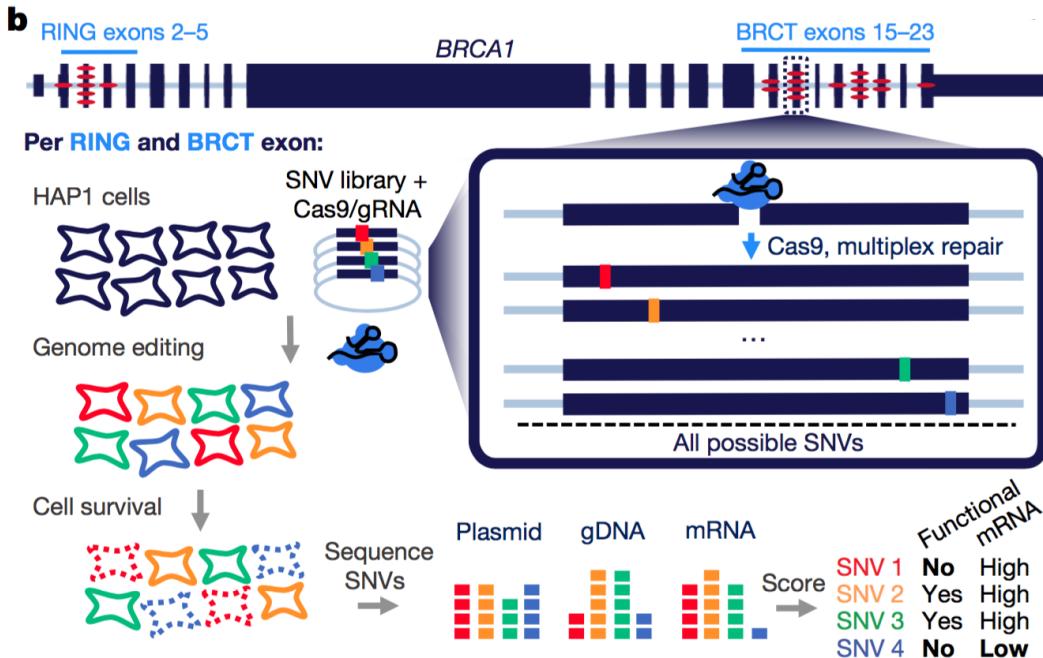
- Complex transcriptomic readout, can be analysed for any gene expression signature
- Can analyse cell-specific effects in mixed populations
- Limited to ~100's of gene knockouts

# Single cell CRISPR screening



- AHNAK
- ANGPTL8
- BRINP2
- CAND2
- CCDC60
- DNMT3L
- FADS3
- FAT2
- GCNA
- IFIT3
- IL17RE
- LAMA3
- LGI1
- NLRP11
- NPY2R
- Non-Targeting
- PILRA
- PROM2
- RAB37
- SLC16A5
- STK32B
- SULT1A2
- TADA3
- TIAM2
- TMEM86B
- TMEM265
- TNNT2

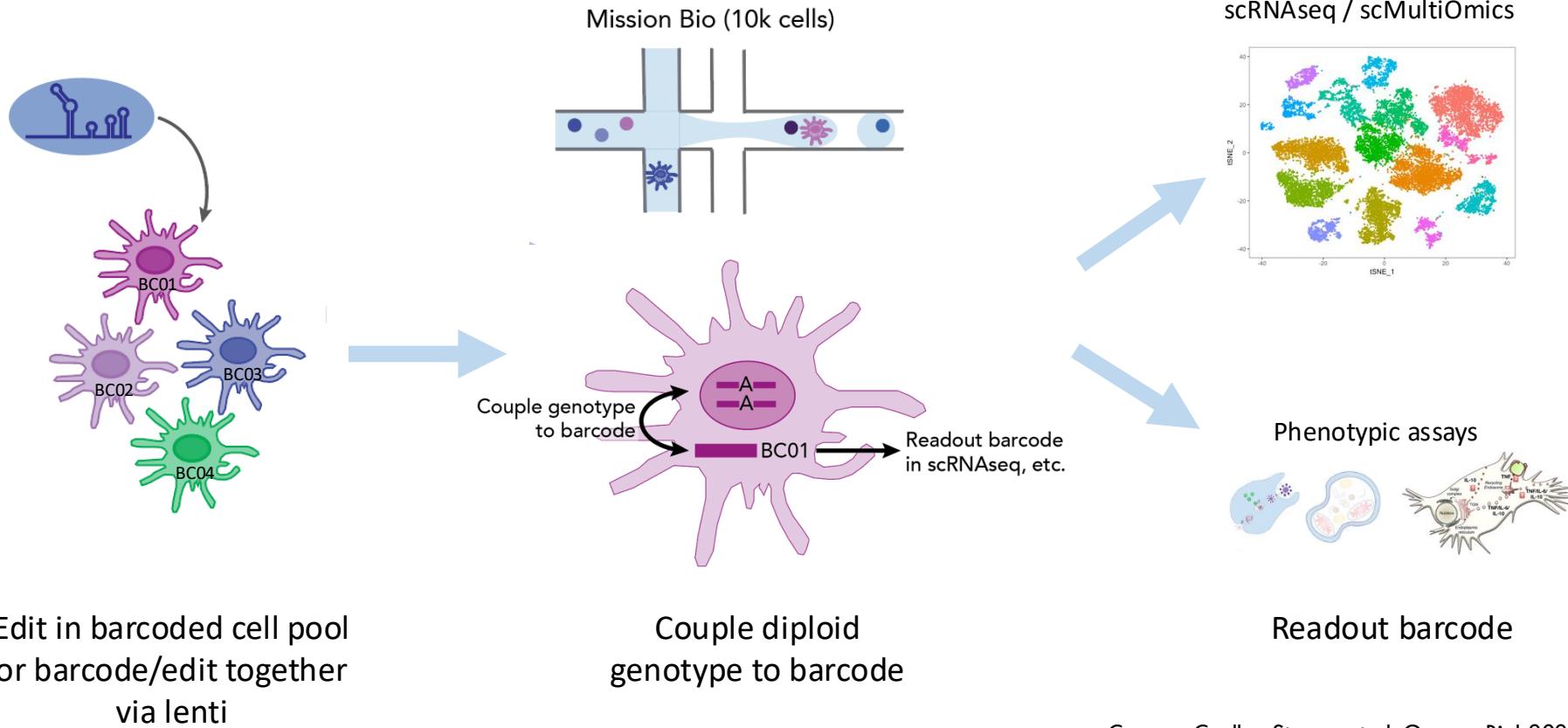
# Saturation genome editing



Atlas of Variant Effects

Findlay et al. Nature 2018  
Waters et al. NatGenet 2024

# scSNVseq - coupling scGenotype and scRNAseq



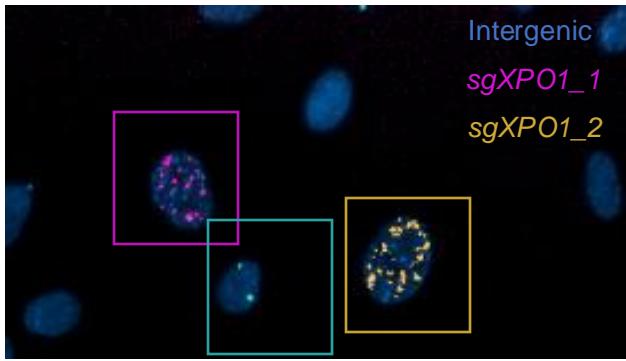
Edit in barcoded cell pool  
or barcode/edit together  
via lenti

## Couple diploid genotype to barcode

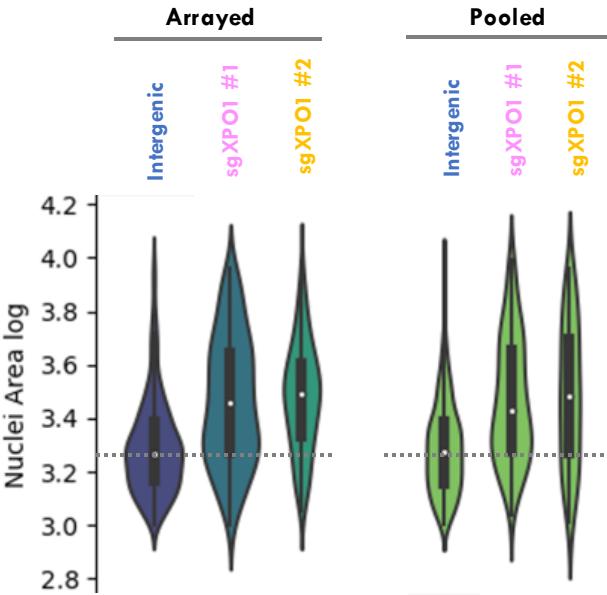
## Readout barcode

Cooper, Coelho, Strauss et al. GenomeBiol 2024  
<https://doi.org/10.1186/s13059-024-03169-y>

# Optical Pooled Screening



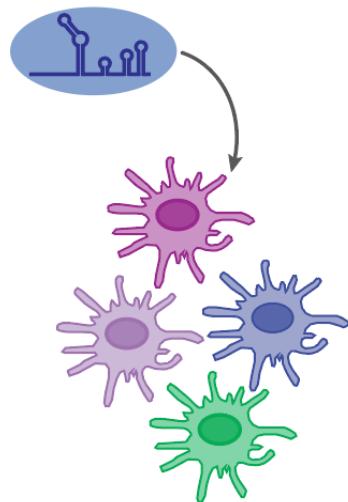
- KO of XPO1 causes increase in nuclear size



# Pooled screening

**Perturbations**

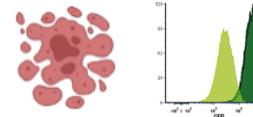
- RMCE OE
- CRISPRa
- Dual guide
- SNPs



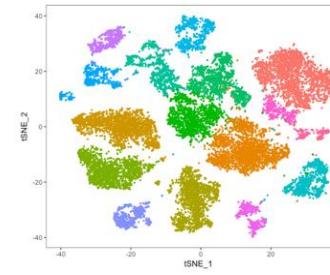
## Perturbations

## Readouts

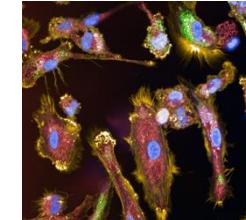
Enrichment phenotypic assays

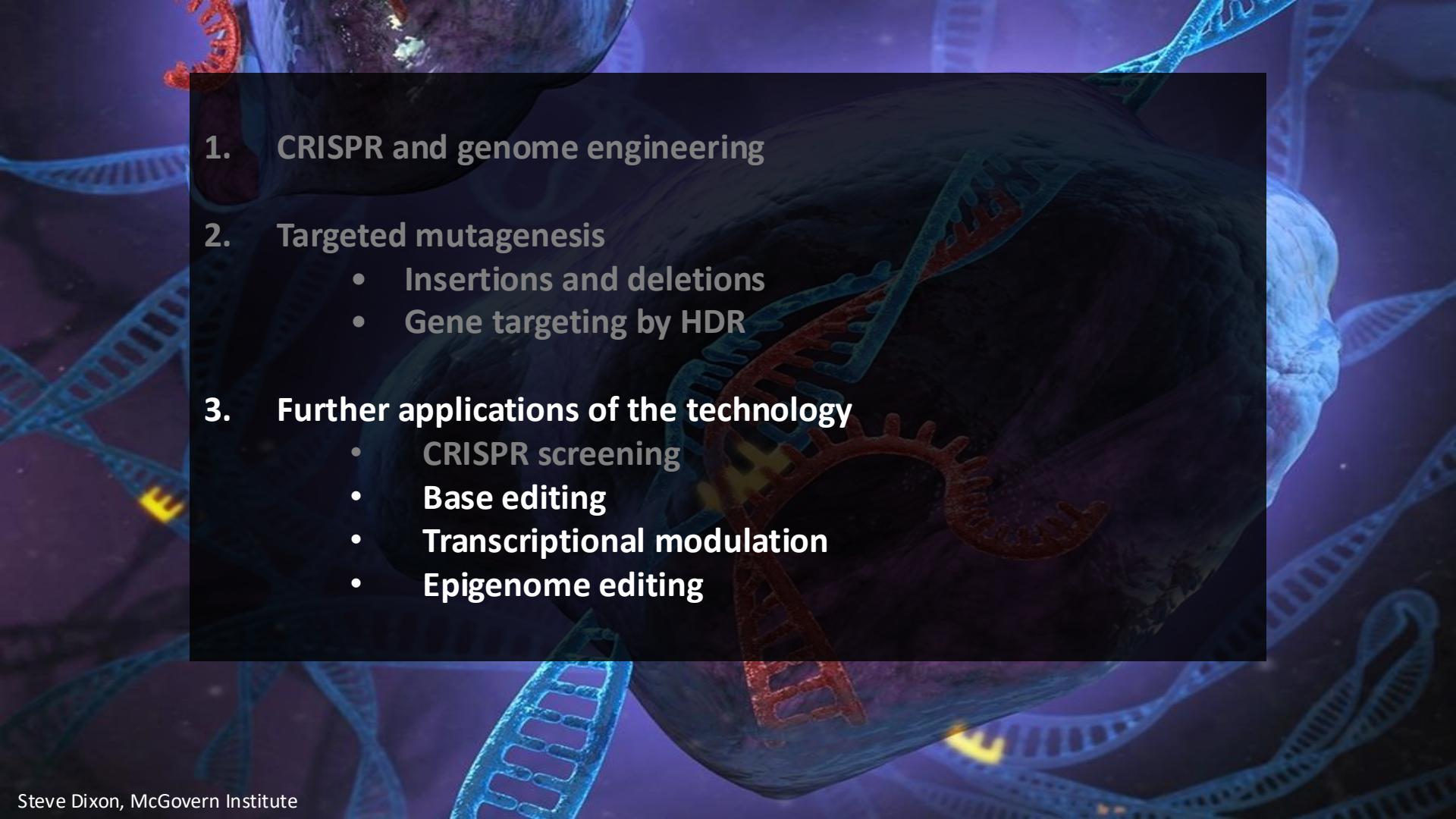


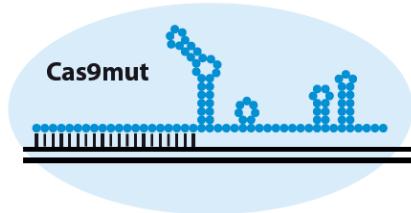
Single cell



Imaging-based assays

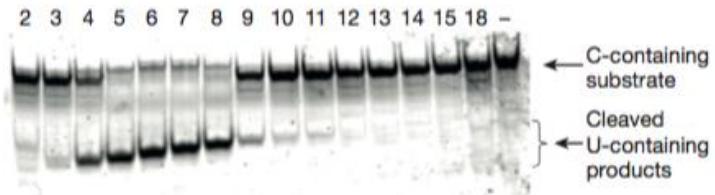
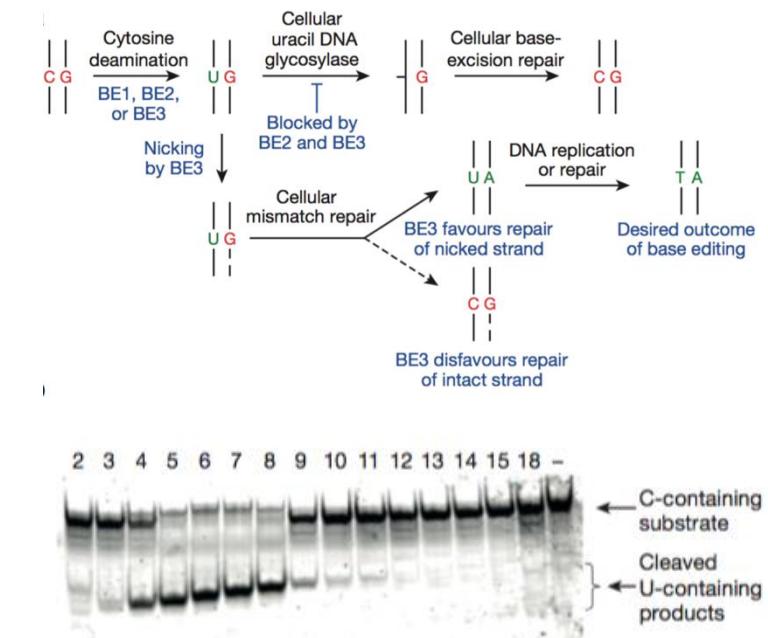
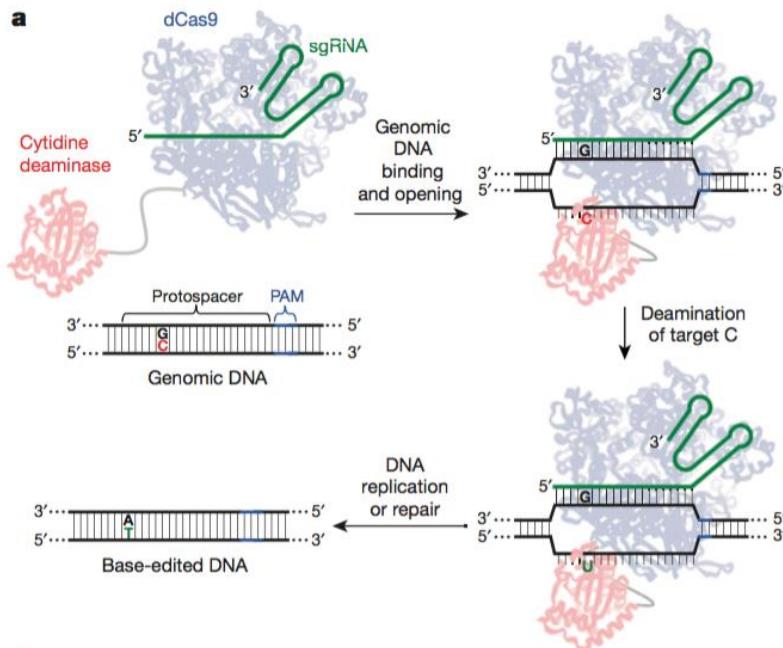


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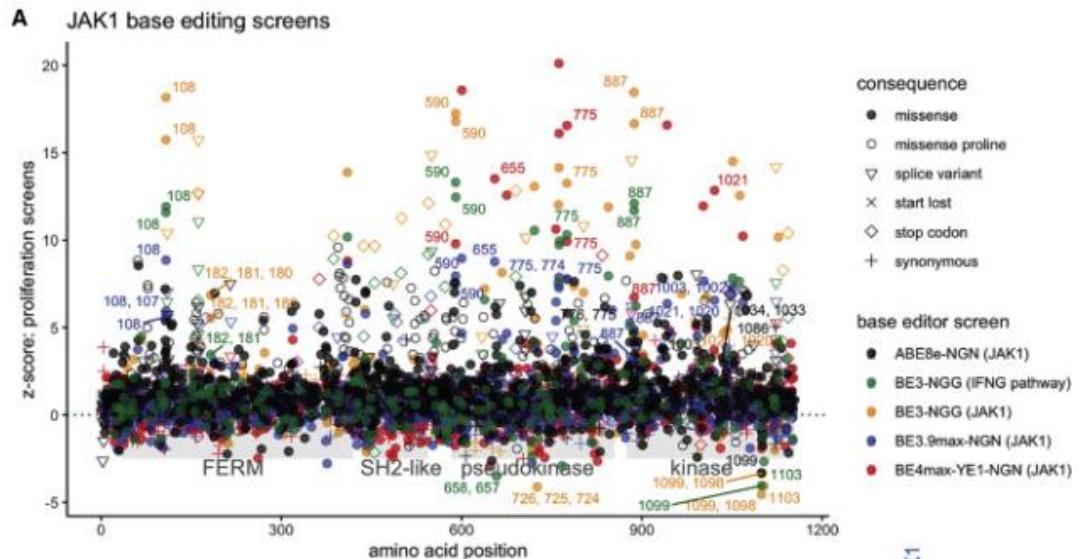
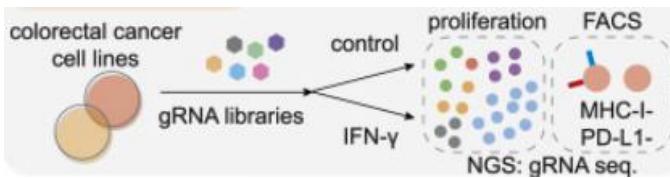
**Sequence-specific DNA binding factor**

# Base Editing



- Targeting range limited by PAM (but variant e.g. Cas9-NG enzymes)
- Several nucleotides can be modified
- A-G editors also described (Gaudelli et al. Nature 2017, NatBiotech 2020)
- Also C-G editors (Kurt et al. NatBiotech 2020)

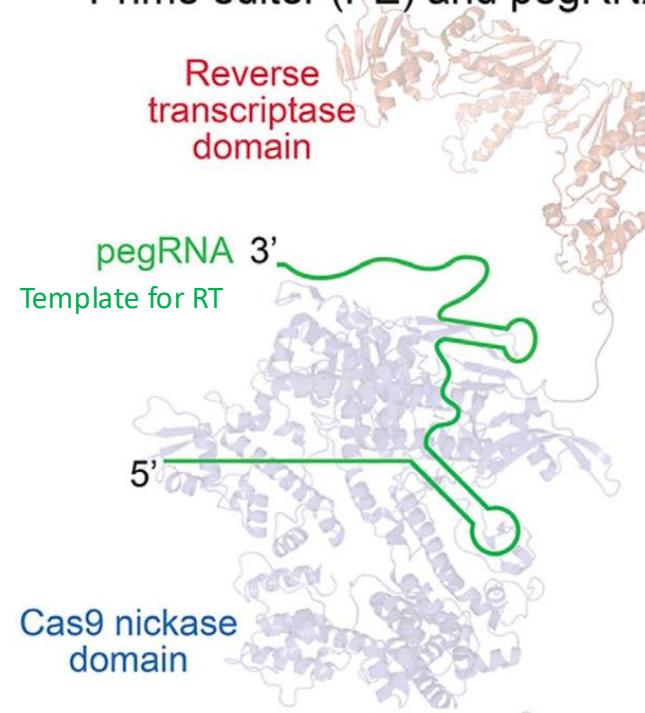
# JAK1 tiling BE screen

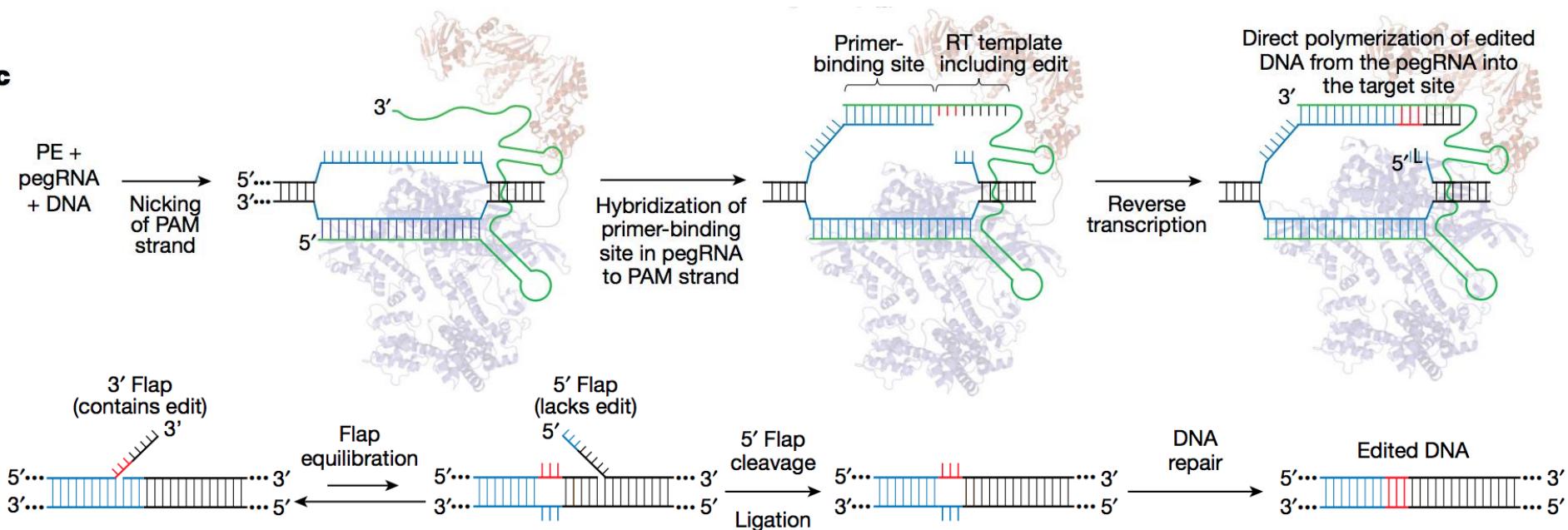


- Can screen across hundreds of thousands of variants at diverse sites
- Somewhat limited in targeting range
- Multiple edits within and sometimes outside window

# Prime Editing

Prime editor (PE) and pegRNA



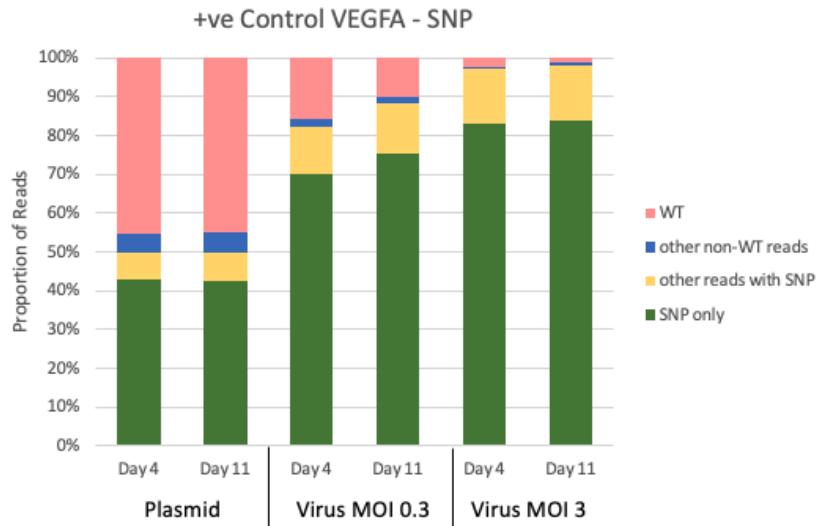
**c**

- Allows ANY base substitution in the genome... but efficiency still needs work

# Prime Editing

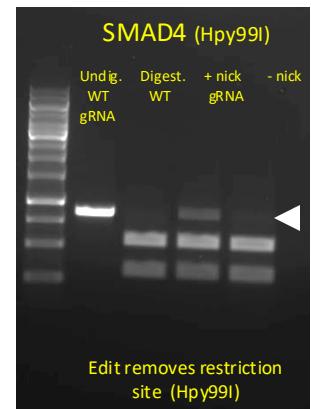
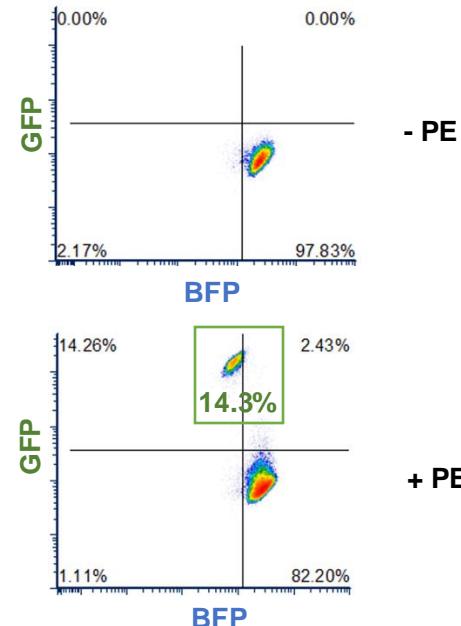
## HAP1

$\Delta$ MLH1 (mismatch repair pathway)  
PiggyBac inducible PE clonal line

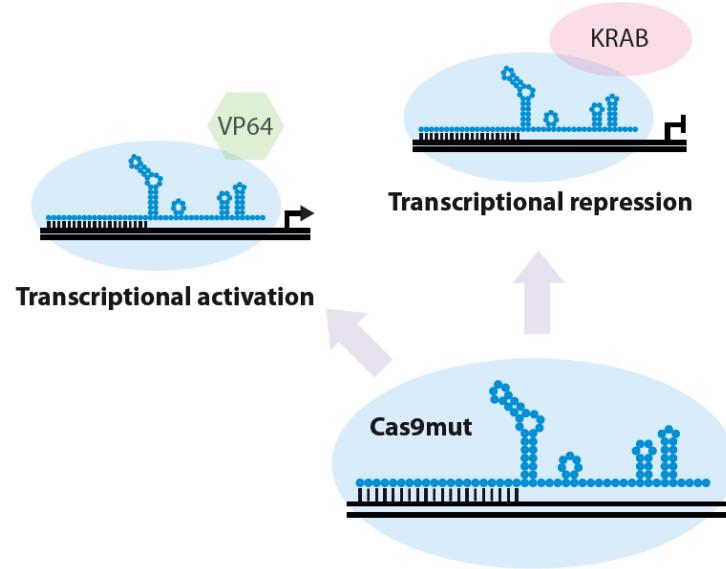


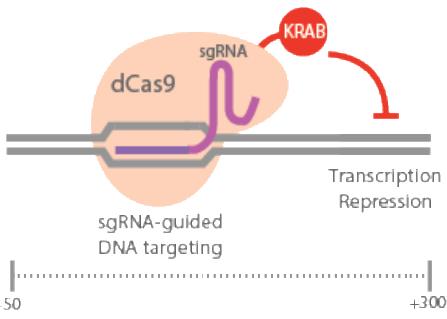
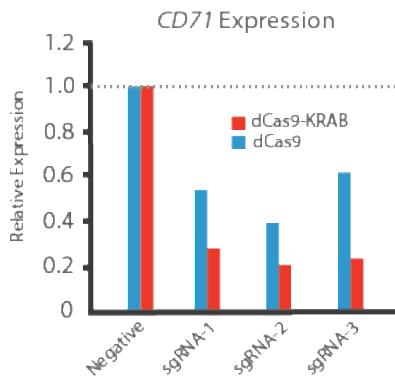
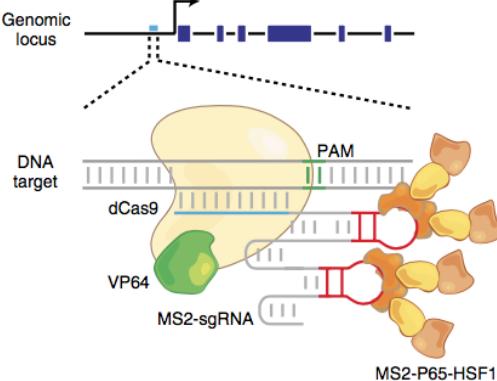
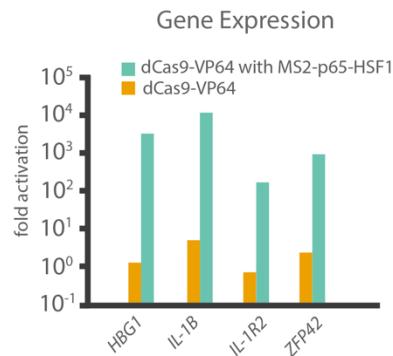
## hiPSC

PE + dnMLH1 mRNA + sgRNA delivery

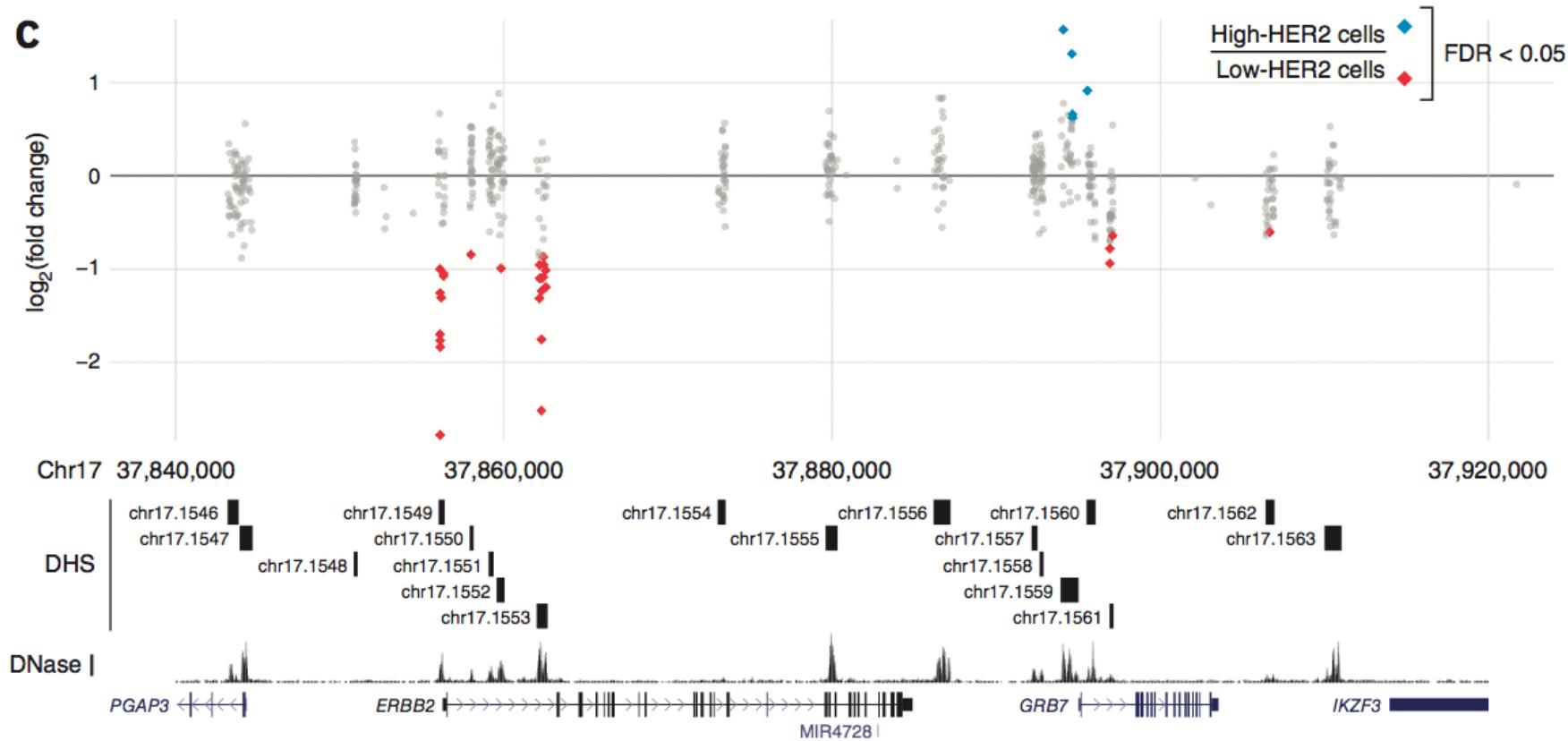


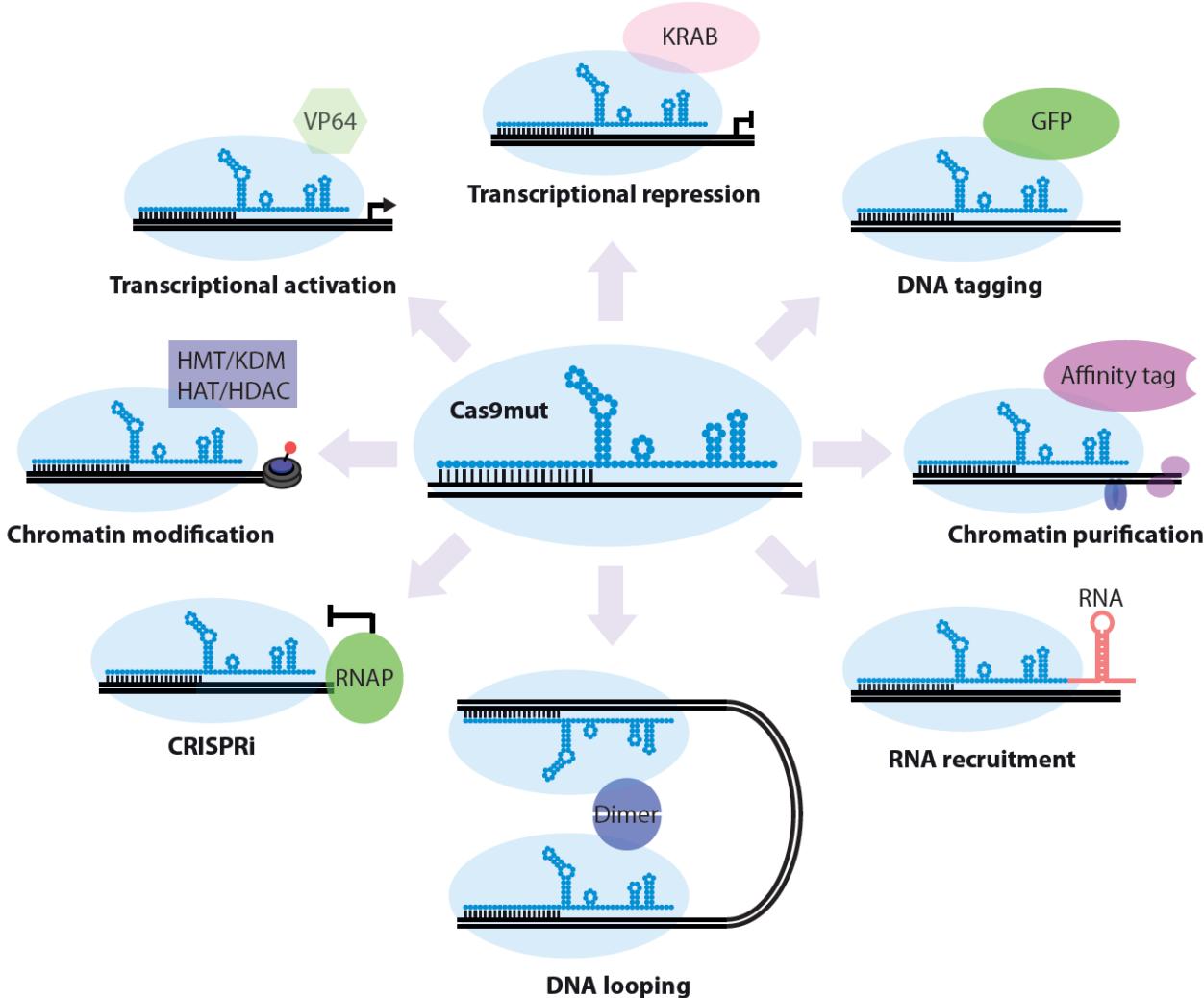
- Highly variable between sites and pegRNAs



**a****b****a****b**

Genome-wide CRISPRi and CRISPRa libraries e.g. Gilbert et al. 2015, Konermann et al. 2015, Horlbeck et al. 2016, Weissman 2021  
<https://www.addgene.org/pooled-library/>

**C**





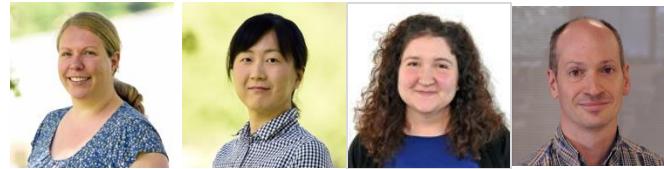
Henry Scowcroft  
@oh\_henry

If you genetically edit the lettuce genome, you can make it CRISPR  
#crisprfacts



Andrew Maynard via Twitter

## Cellular and Gene Editing Research



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