

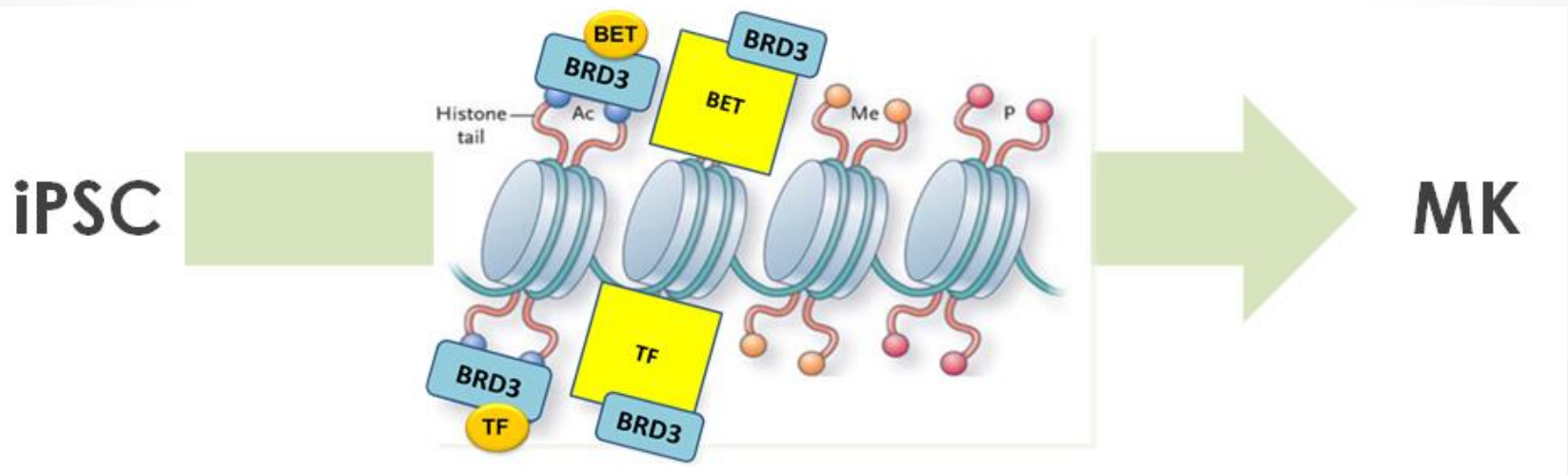
# Train Malta Summer school 2017

John Lambourne  
Isabel Rosa



# Isabel Rosa

- (2007) MSc in Biological engineering
- (2007-2009) monoclonal antibodies process development
- (2009-2011) research development of iPSC derived biologicals
- (2011-2014) pilot, pre-clinical and clinical studies in regenerative medicine
- (2014-to date) PhD in epigenetics (Haematology)



# John Lambourne

- Chronology
  - PhD (2008): Protein Science
  - Senior R&D Scientist (2008-2011): Small Biotech
  - Postdoctorate McGill University (2011-2014): Human Genetics
  - Postdoctorate Cambridge University (2014-Current): Haematology
- Wet-lab Molecular Biologist
- Primary Interest: Epigenetic of Disease and Normal State
  - Approaches like: ChIP-seq, 4C-seq, RNA-seq, genome editing, genome wide reporter assays, etc
  - Very much specialise in NGS approaches

# Isabel + John

We are very friendly people

Do not be shy to ask questions  
(it does not have to be about CRISPR)

# Practical Overview

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  - Mixture of cell culture and molecular biology

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- Surveyor Assay

- Objective is to check that you can cut the site you are interested in
- This is a fundamental QC step in CRISPR experiments,
  - a step we use to check everything works correctly before proceeding to the more expensive experiment
- These practicals will give you all the experience you need for a basic genome edit experiment

# Practicals

Cell Culture

Molecular Biology

# Practicals

Cell Culture

Monday and Tuesday

Molecular Biology

Wednesday and Friday

# Cell Culture

Monday

Objective = Transfection



# Cell Culture

Monday

Objective = Transfection

Steps: Seed cells and chemically induce endocytosis to take up DNA

# Cell Culture

## Monday

Objective = Transfection

Steps: Seed cells and chemically induce endocytosis to take up DNA

## Tuesday

Objective = Keep Cells Happy

# Cell Culture

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Objective = Transfection

Steps: Seed cells and chemically induce endocytosis to take up DNA

## Tuesday

Objective = Keep Cells Happy

Step: Media Change

# Molecular Biology

Wednesday

Objective = Harvest gDNA and PCR region of interest

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## Friday

Objective = Re-hybridize the PCR product and survey the products for nicks by enzymatic digestion

Step: Denature, hybridize, digest with the T7 Endonuclease I, and test by gel electrophoresis

# Molecular Biology

Also on Wednesday

## **sgRNA Design Computer Practical**

Remember to bring your laptops

(You can buddy up, but make sure you have a turn)

+

Think up a gene you are interested in



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(do not attempt to work 3/4 simultaneously in a hood)

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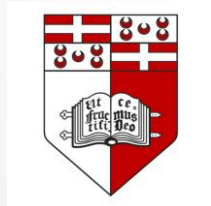
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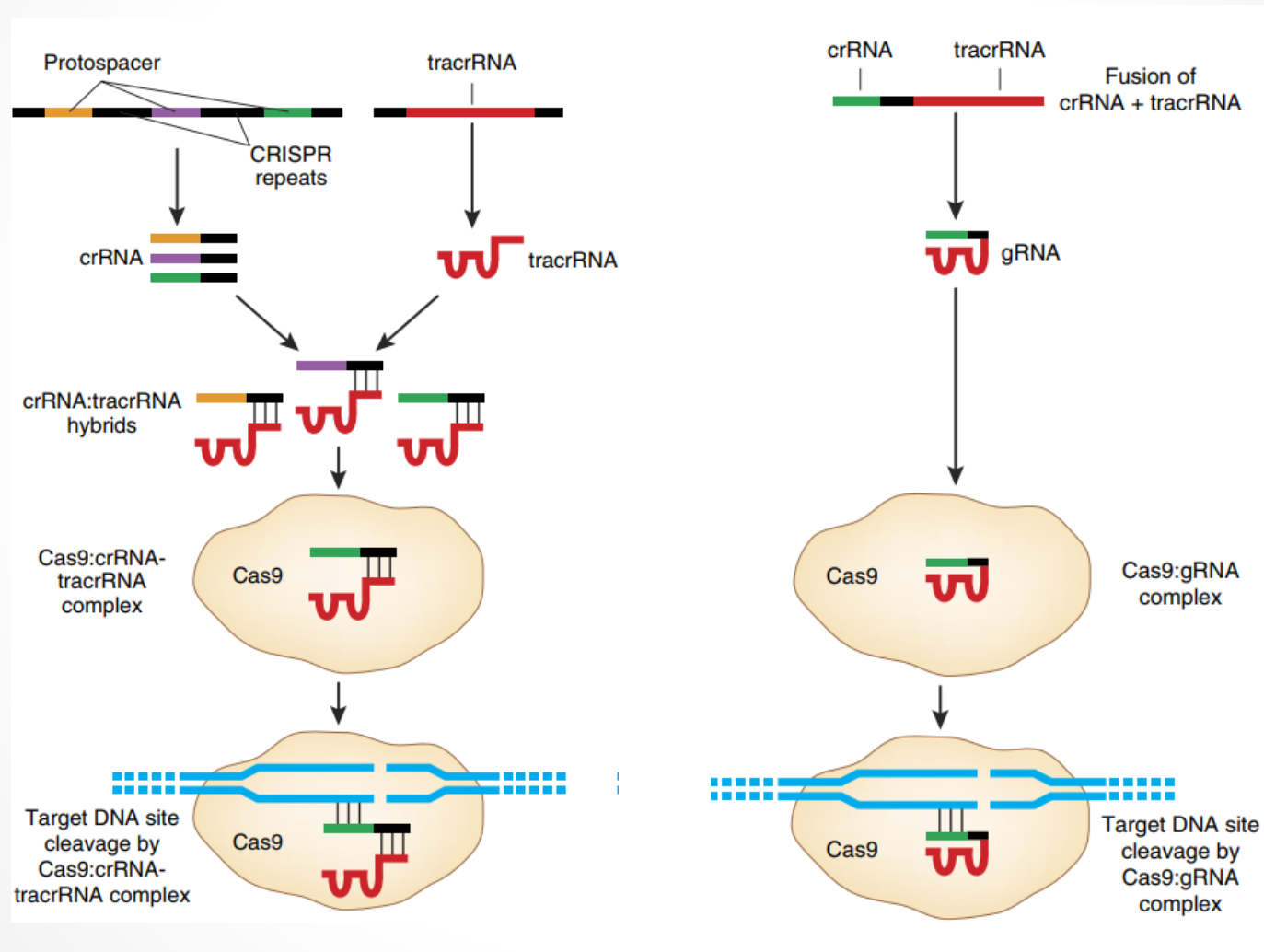
# Introduction to CRISPR

John Lambourne  
Isabel Rosa

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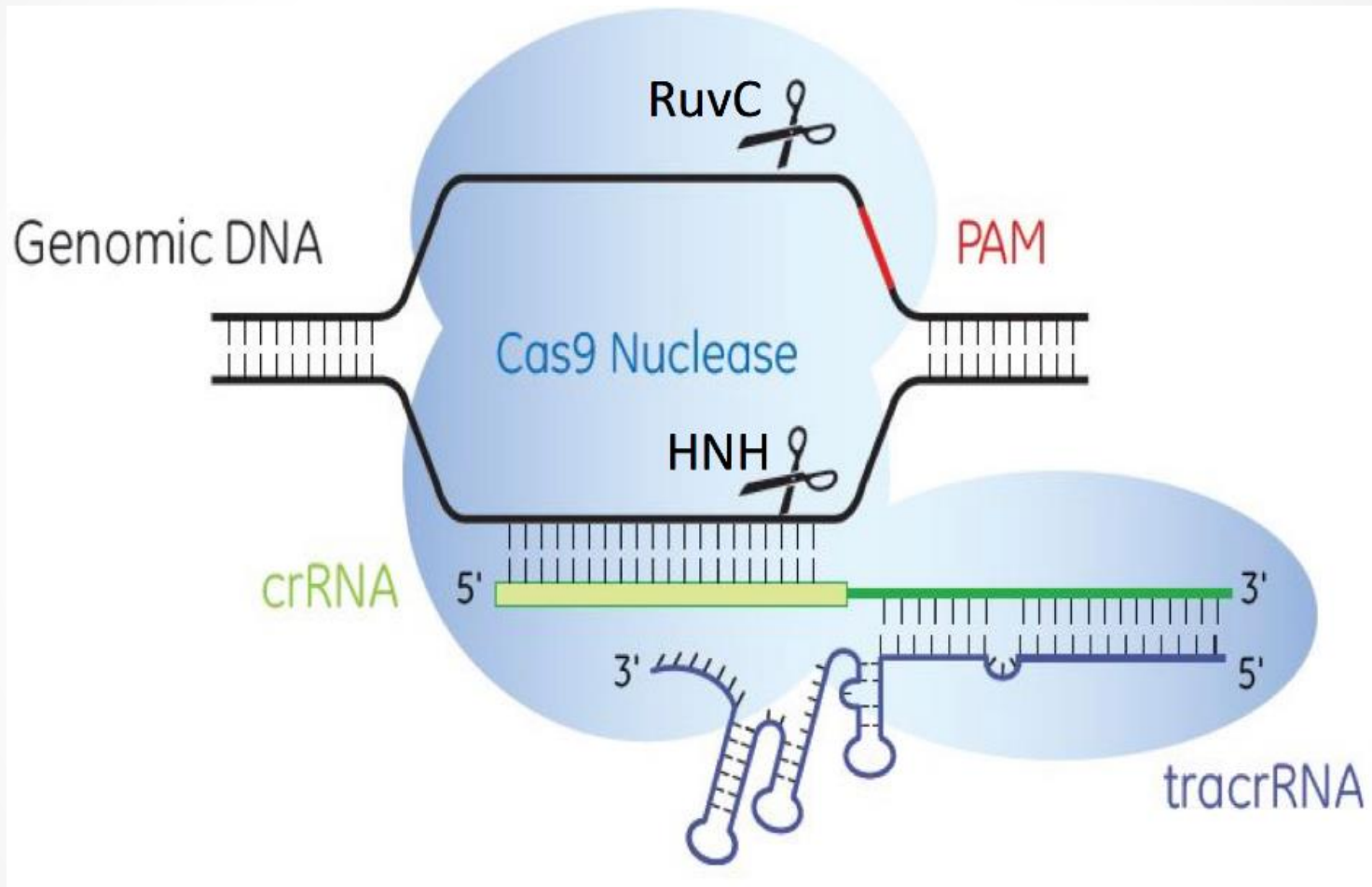


# Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)

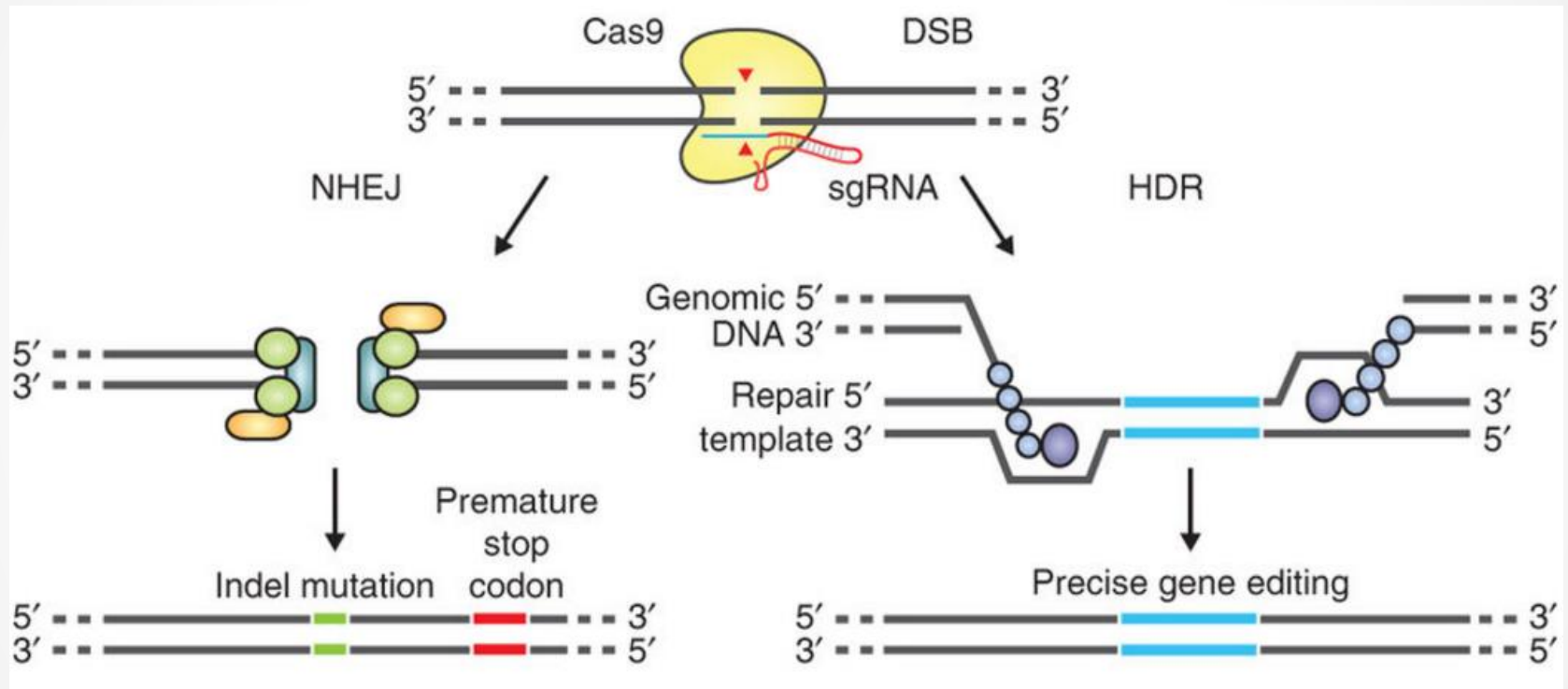




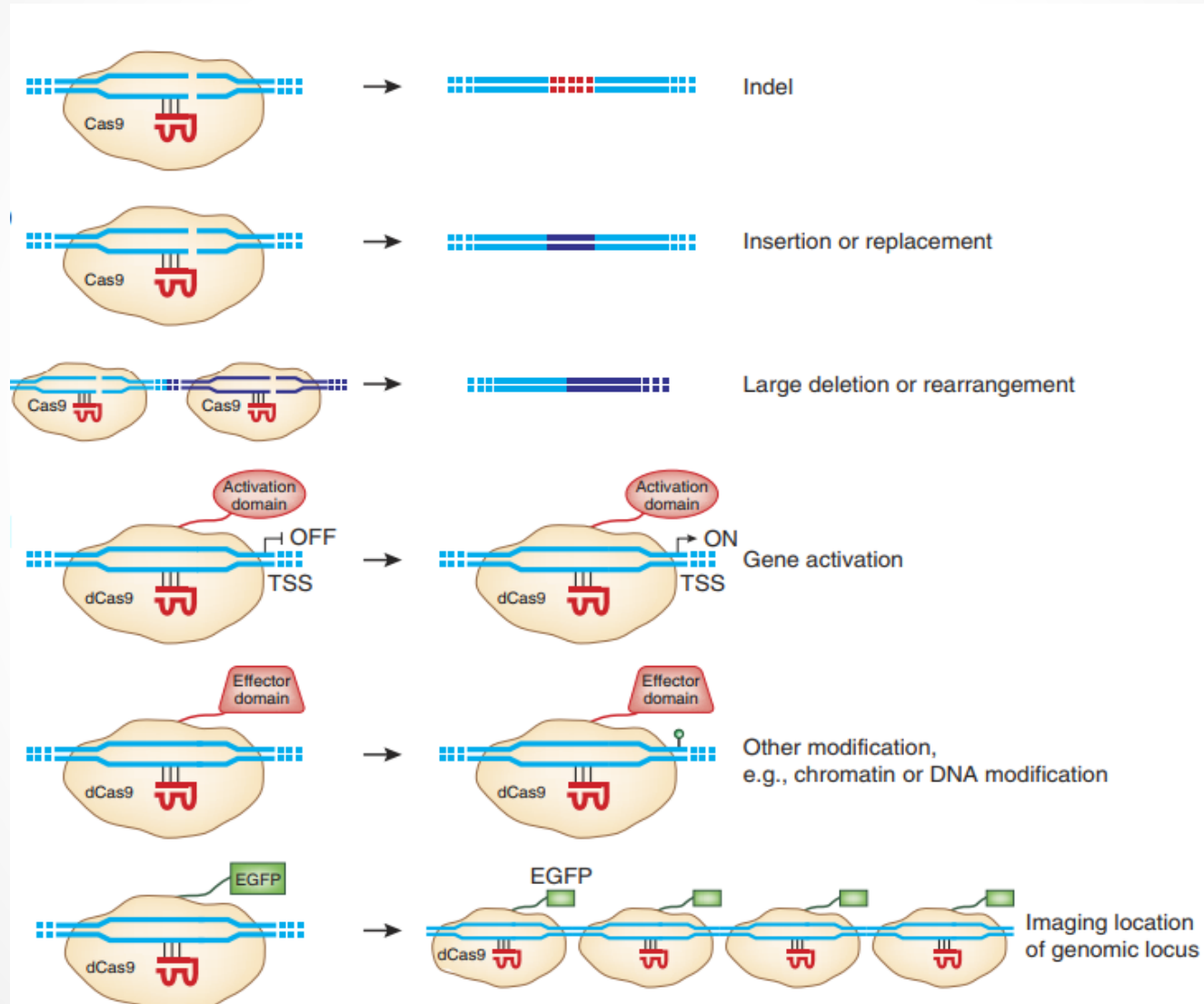
## Genome-editing with CRISPR/Cas9 - genome targeting



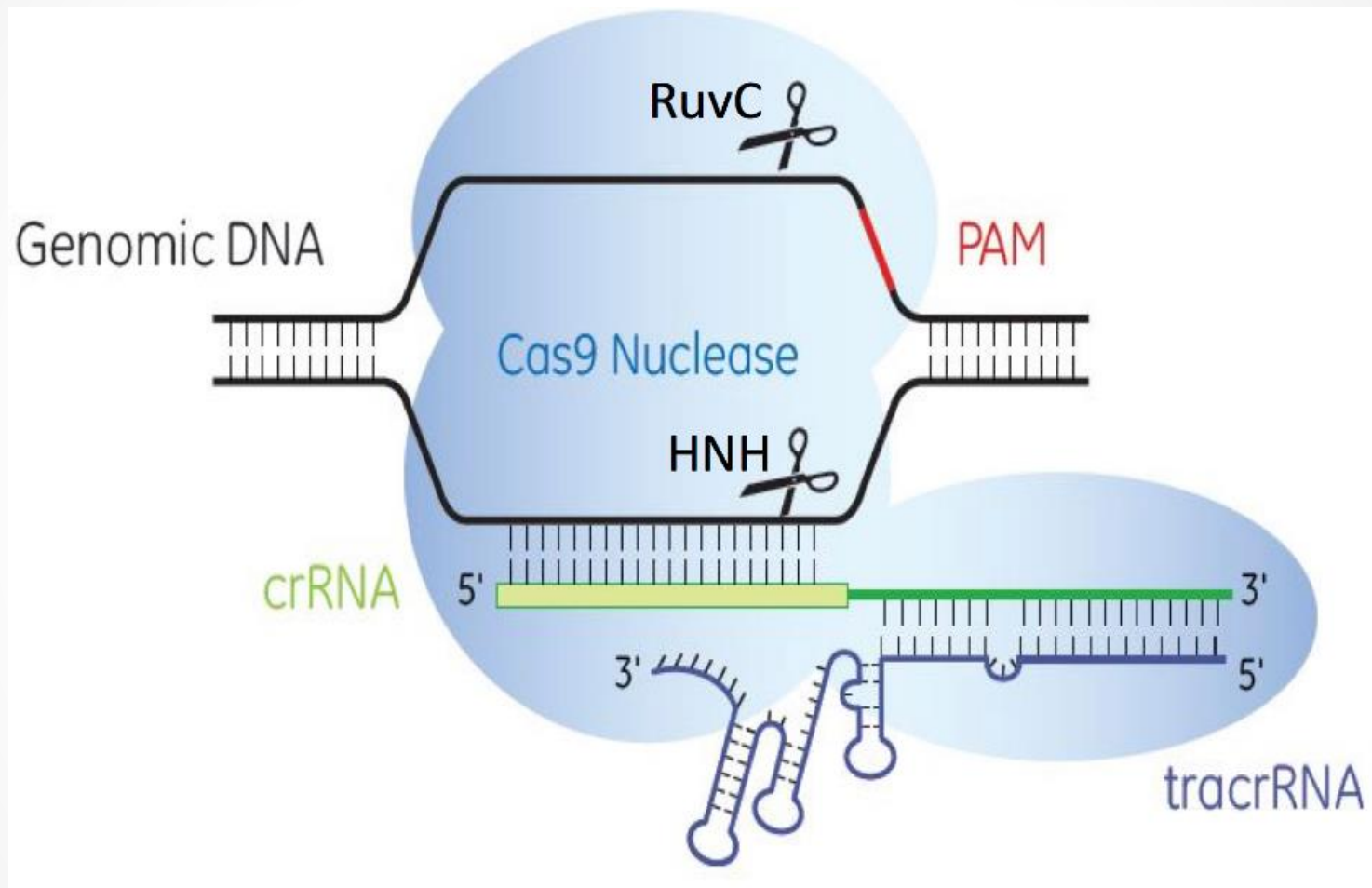
# Genome-editing with CRISPR/Cas9 – repair mechanisms



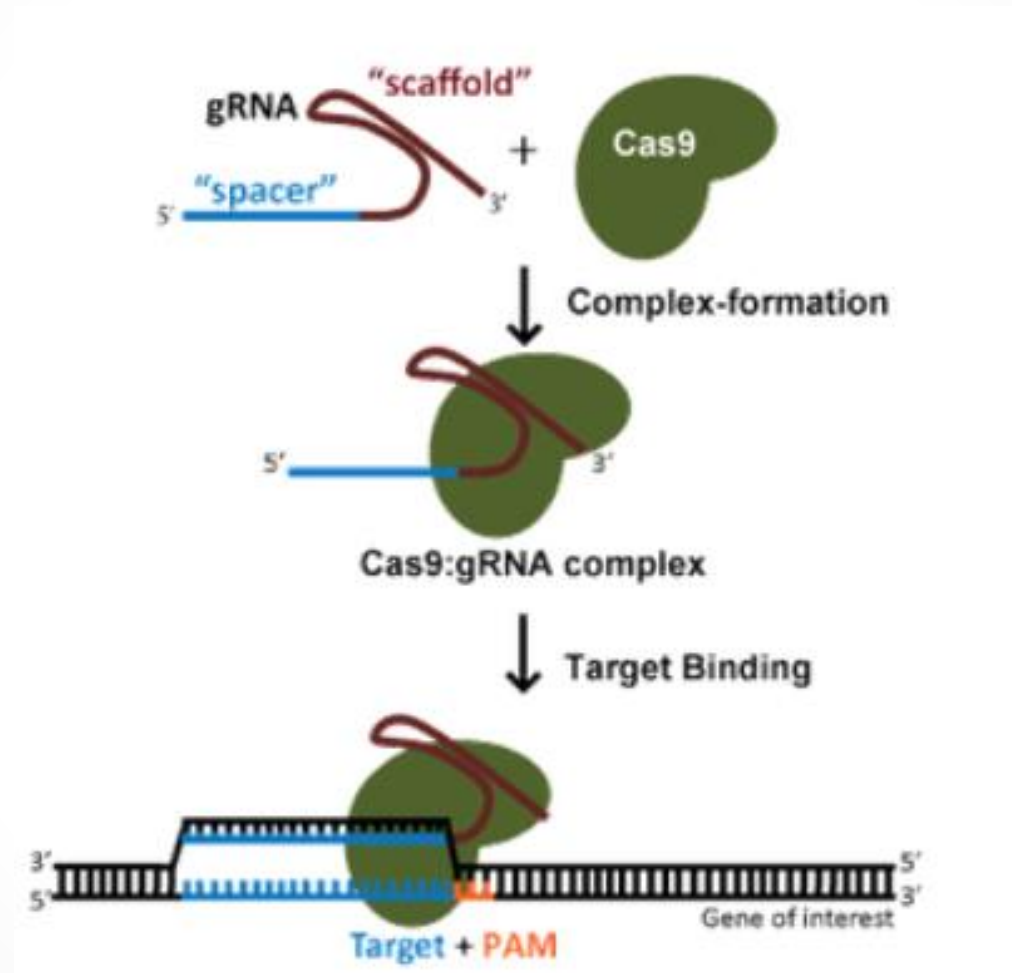
# Applications of CRISPR/Cas9 systems



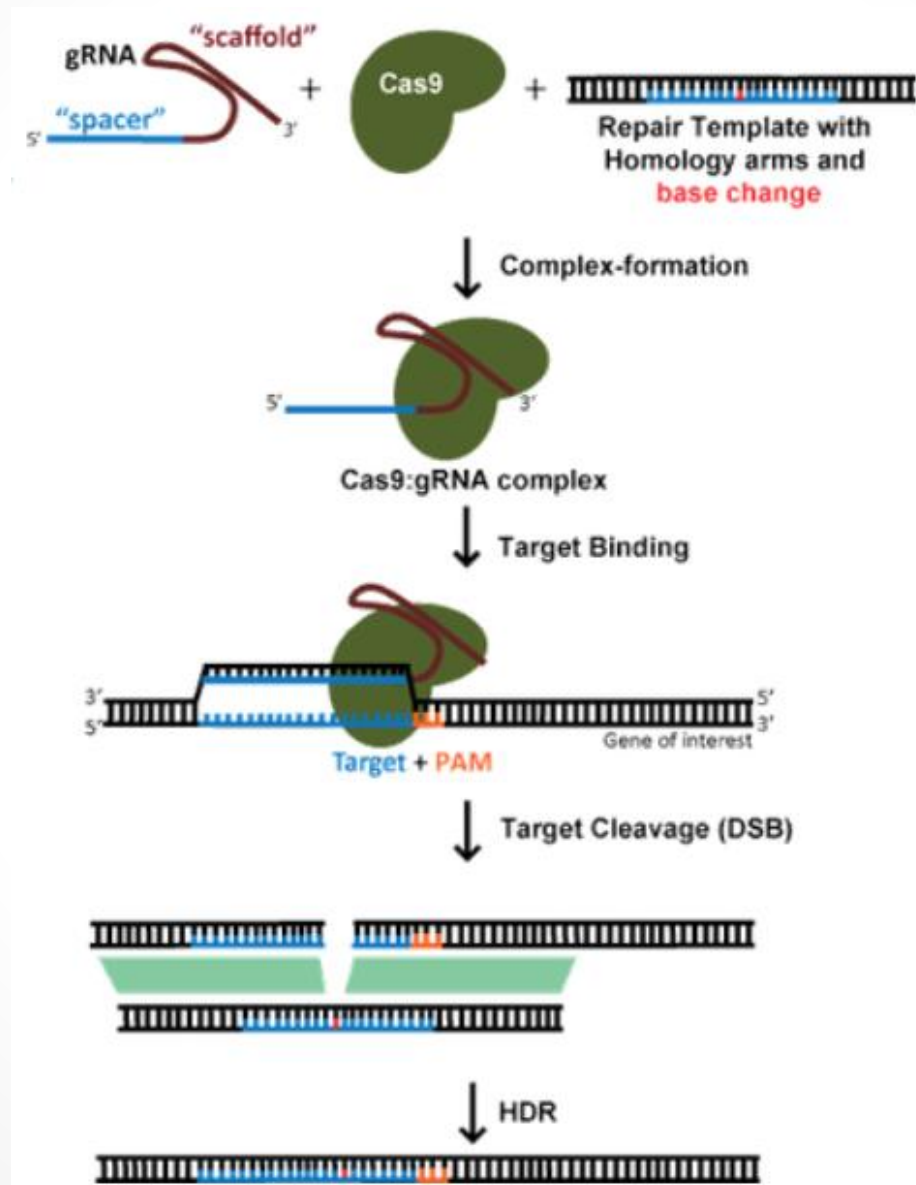
## spCas9 variants- WT Cas9



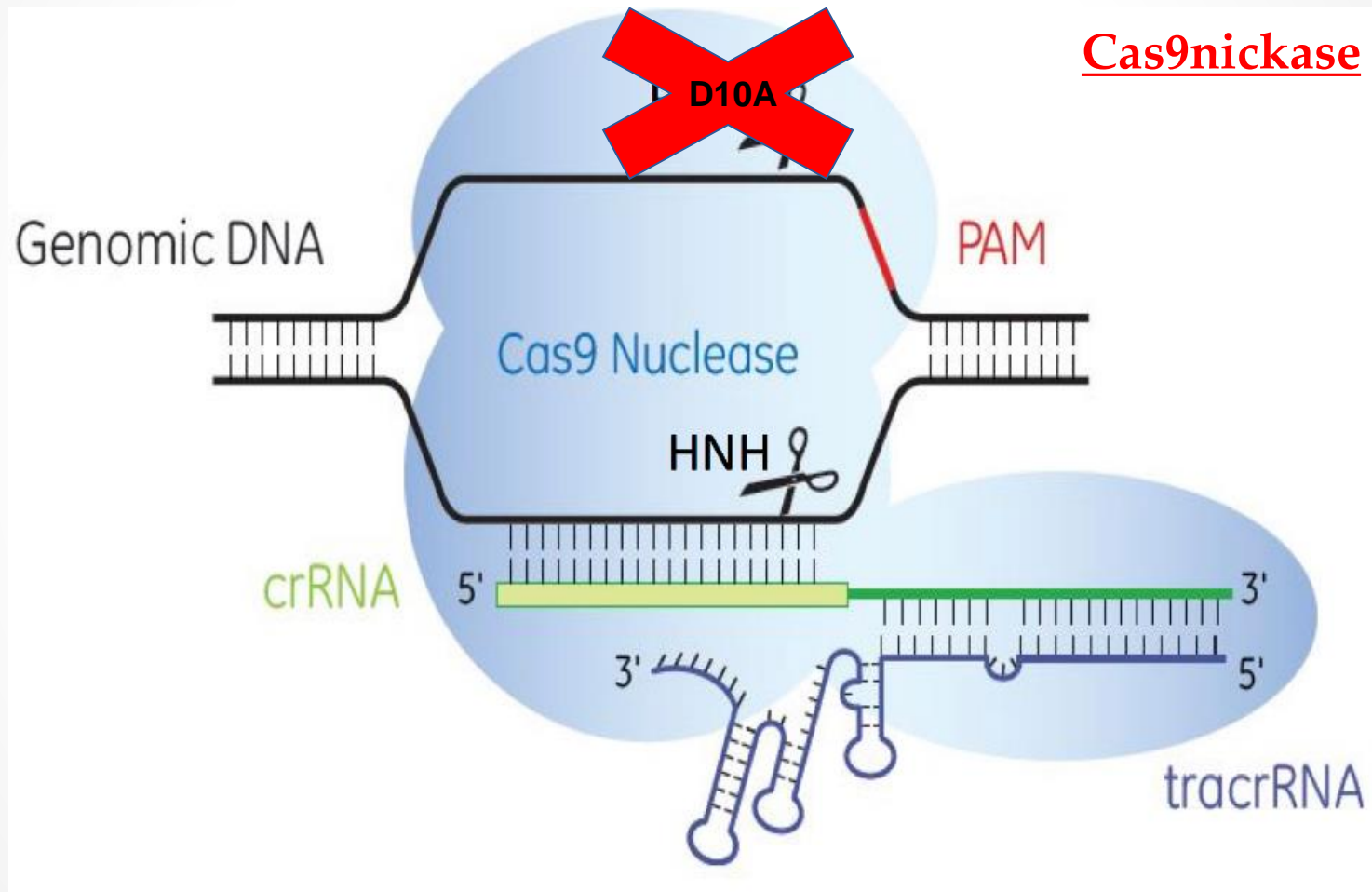
## Knockout generation- WT Cas9 nuclease



# Knock-in generation- WT Cas9 nuclease + HDR (homology directed repair)

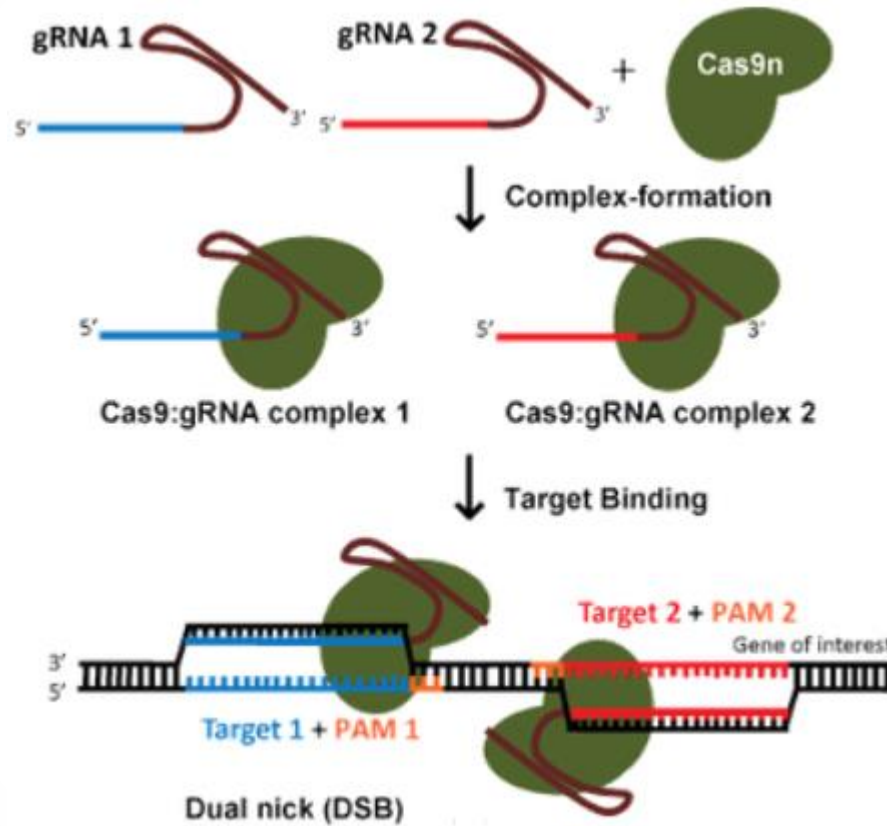


## Cas9 variants- Cas9nickase



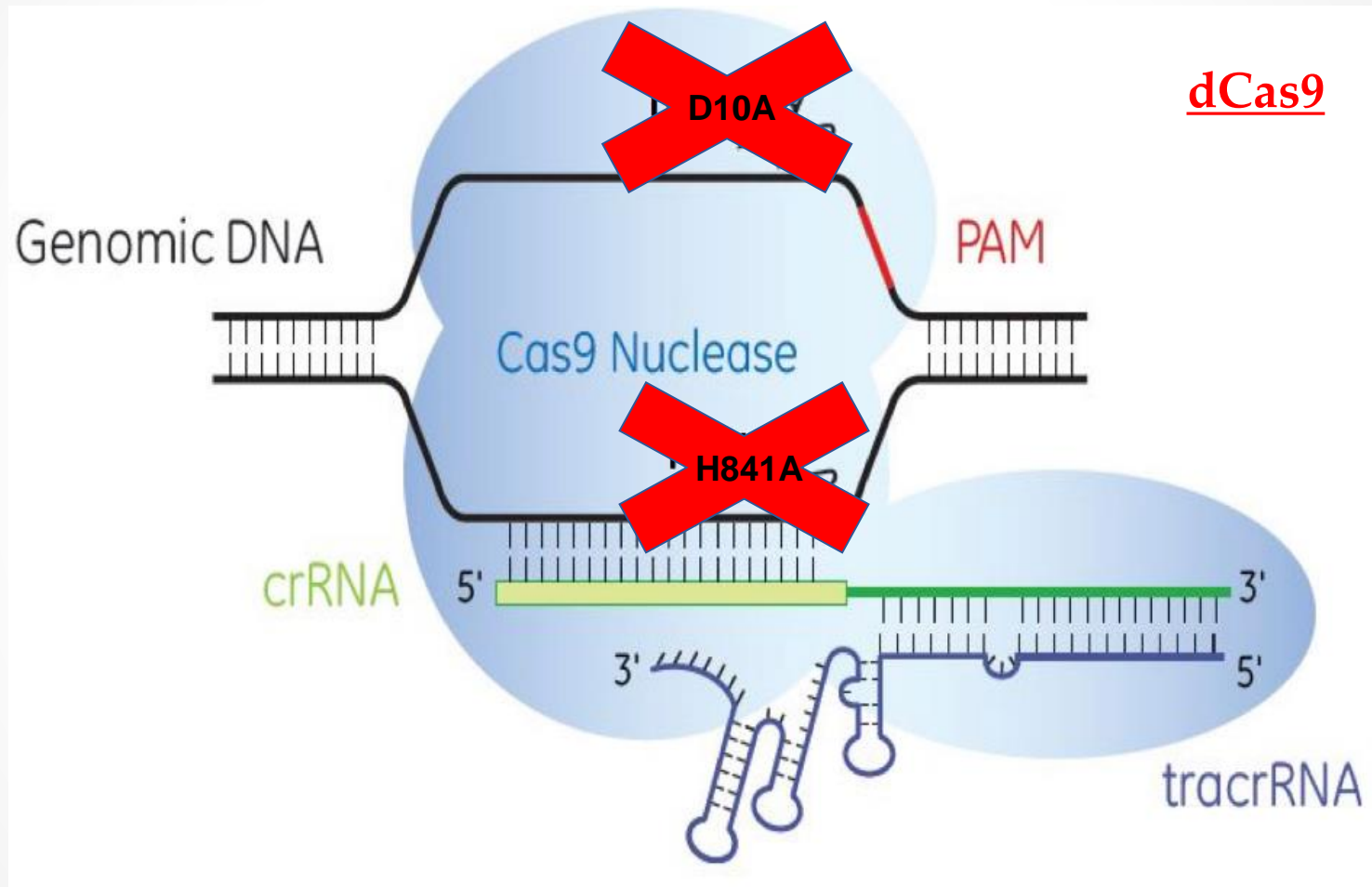


# Knockout generation- Cas9nickase nuclease



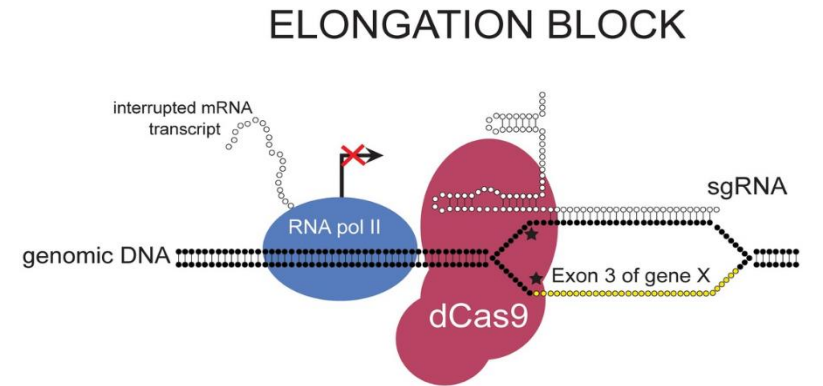
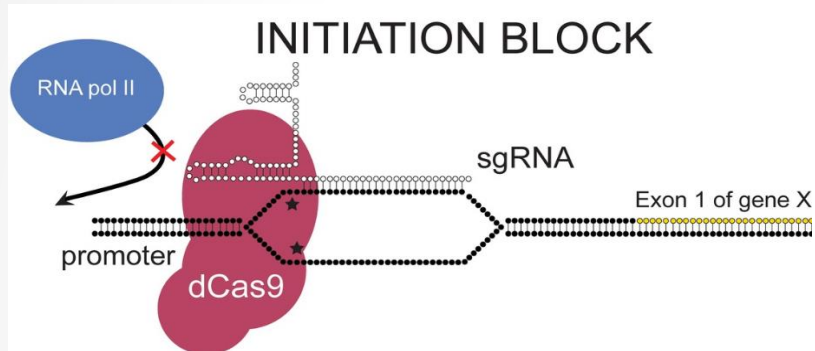


## Cas9 variants- dead Cas9

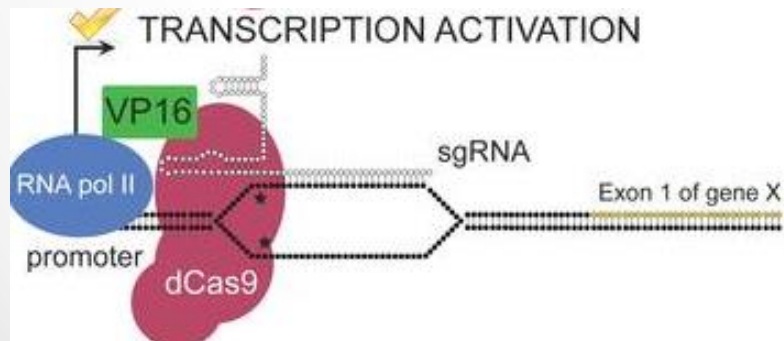


# Interference (CRISPRi) or Activation (CRISPRa) - dCas9 nuclease

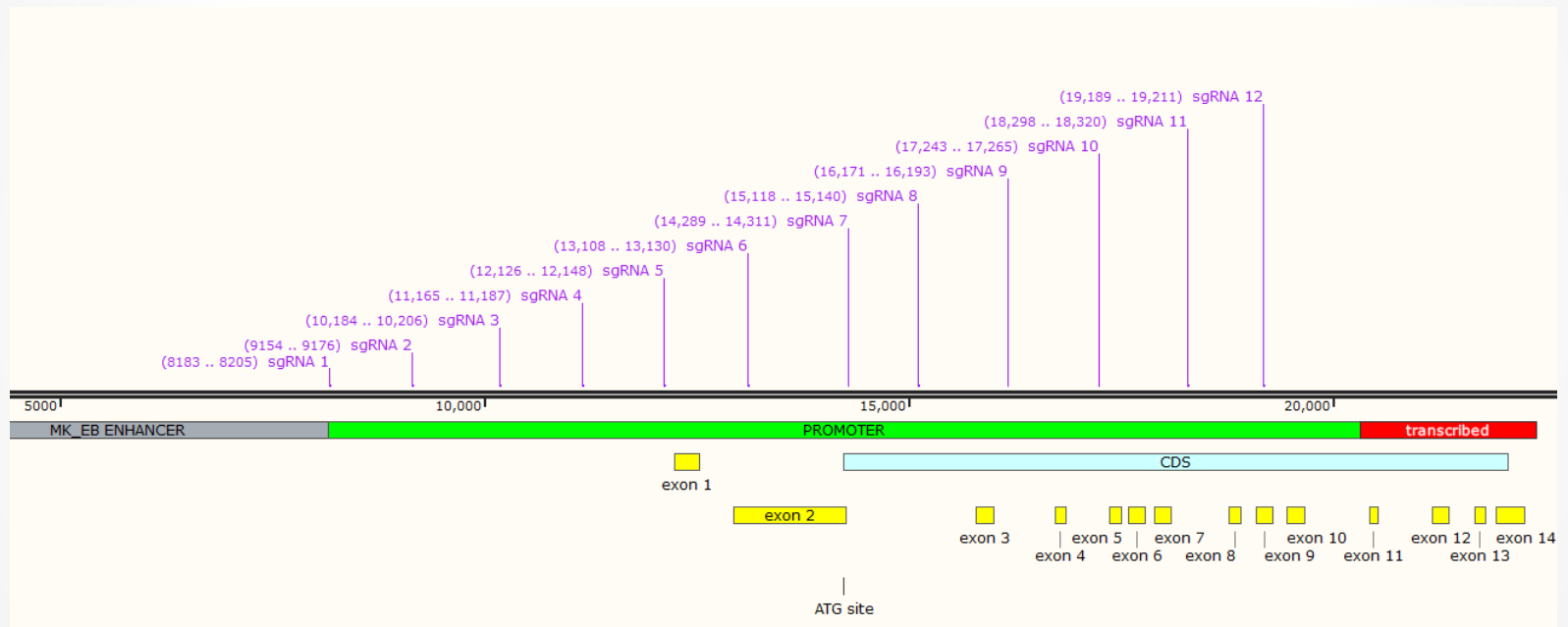
## Interference



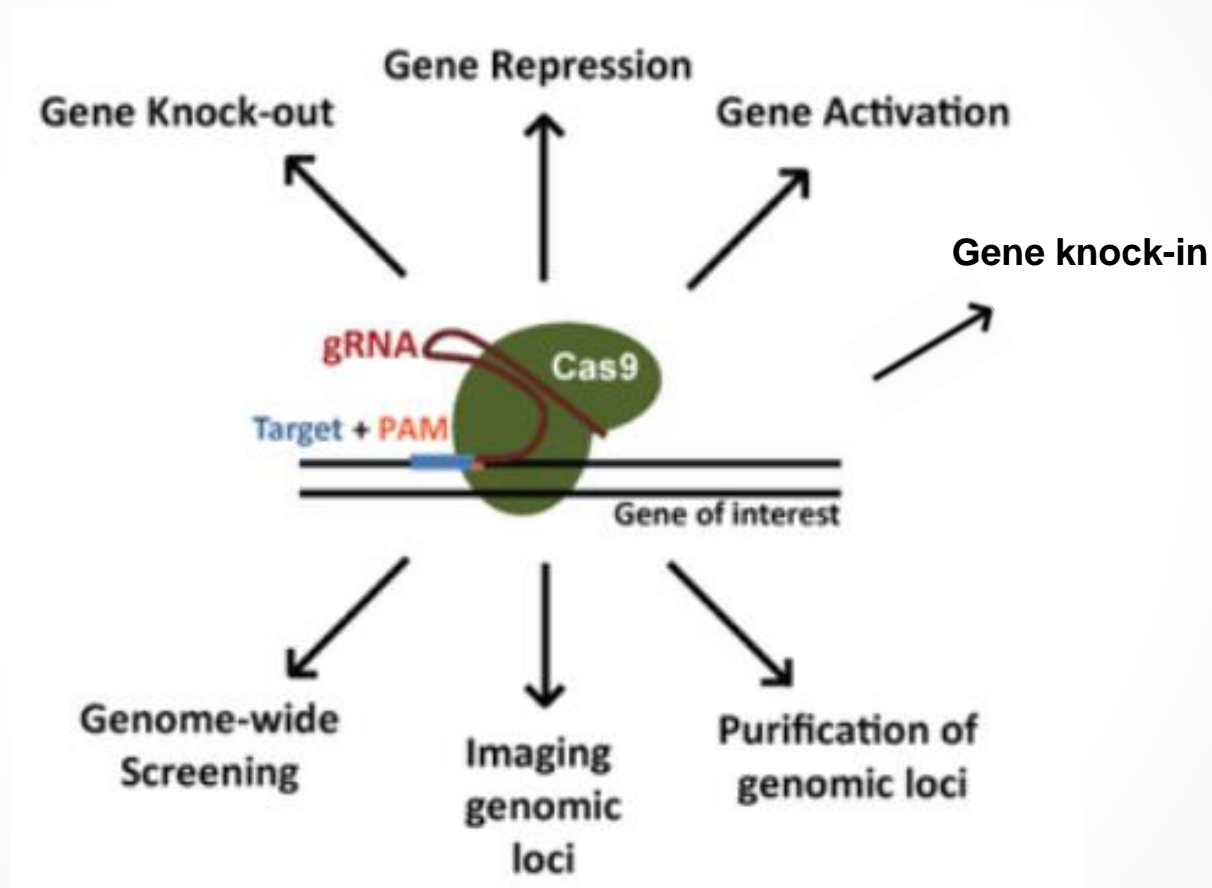
## Activation



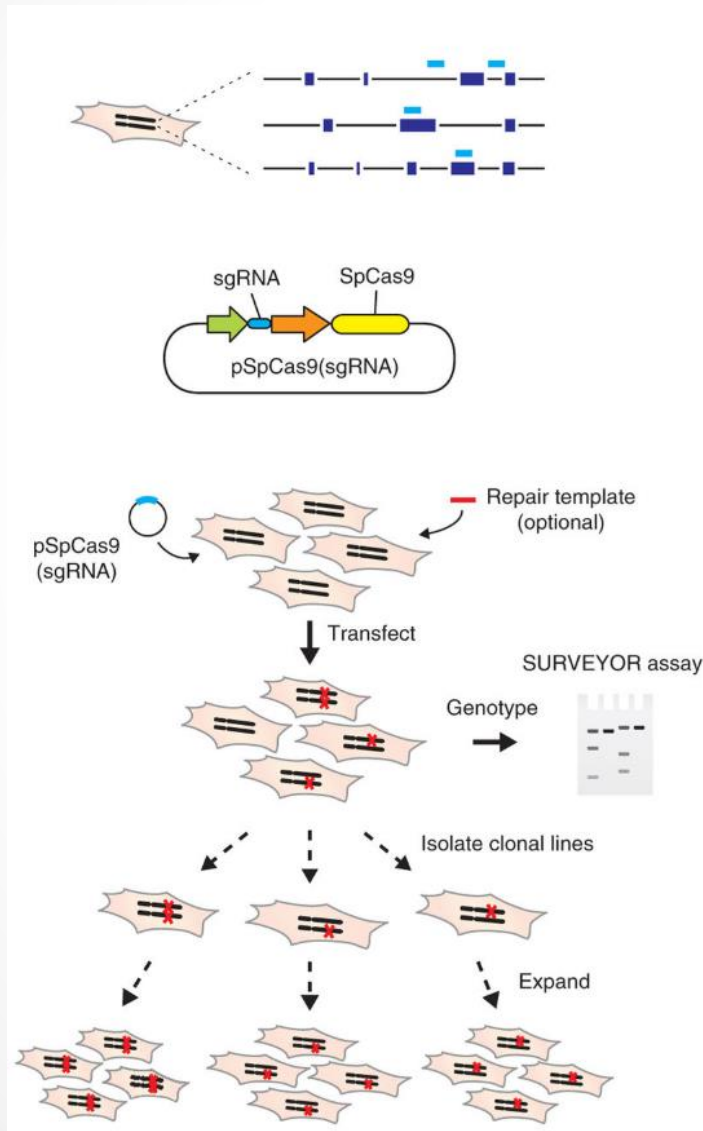
# Example of sgRNA loading for gene knockdown –promoter targeting



# Genome-editing with CRISPR/Cas9 - genome targeting



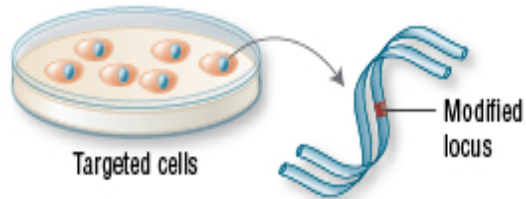
# CRISPR/Cas9 experiment design



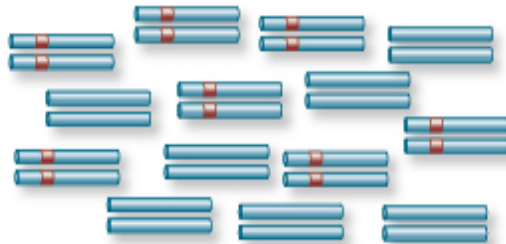
1. Selection of genetic manipulation
1. Selection of the expression system
1. Selection of target sequence and sgRNA design
1. Synthesis and cloning of sgRNAs
1. Cas9 and sgRNAs delivery
1. Validation of genetic modification
1. Clonal isolation and expansion
1. sequencing

# SURVEYOR assay

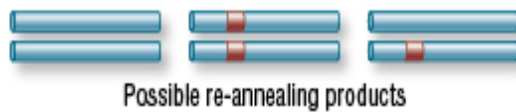
## Isolation of Genomic DNA



## PCR Amplification



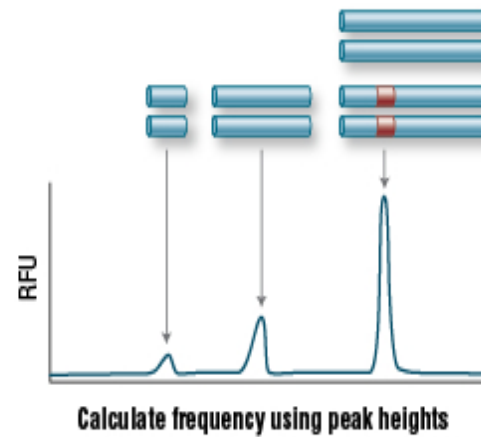
## Denaturation & Annealing



## Digestion of Mismatched Duplexes



## Fragment Analysis



## Advantages and limitations of CRISPR/Cas9 systems



**Simplicity**-a simple system to design and implement

**Editing efficiency**-Simultaneous multi-targeting with low off-target activity.

**Flexibility** - applicable to many biological systems. Cas9 variants availability.

**Customisable** - Cas9 easily retargeted to new DNA sequences.

**Scalability**- easily scaled up for genome-wide screening applications.



**PAM** requirement

**Potential off-targets**- depending on the uniqueness of the target sequence

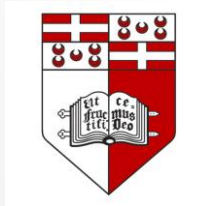




# sgRNA design

John Lambourne  
Isabel Rosa

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## sgRNA design process

### Increasing on-target activity

- Know your cell line and the genome region to be modified (sequencing)
- 20bp sequence directly upstream of PAM (protospacer associated motif)\*
- uniqueness of the sequence compared with the rest of the genome (BLAST)
  - Guanine preferred at -1 and -2 positions proximal to PAM
  - Thymine is disfavored at the -4/+4 positions close to the PAM
  - Nucleotide composition downstream of PAM contributes more significantly to sgRNA efficiency than upstream region.

### Minimising off-targeting

**Off-targets** - sequences in the genome that are partially homologous to the sgRNA sequences (targeting potential)

- Experimental planning - increase of sgRNAs on the target region (2sgRNAs + Cas9n)

# sgRNA design software - WTSI genome editing

HTGT WGE

Home

CRISPR Finder

Gibson Designer

Help

About

Contact



Login with Google

WTSI Genome Editing (WGE) is a website that provides tools to aid with genome editing of human and mouse genomes

[CRISPR Finder](#) [Ensembl for Human](#) [Ensembl for Mouse](#)

The CRISPR Finder will show CRISPR sites (paired or single) in and around genes. You can ask the finder to score the pairs for potential off-target sites, and browse individual and paired CRISPR sites using the Genverse genome browser tool. We also provide the ability to find CRISPRs in genomic sequence or by gRNA:

**Find CRISPRs in our genome browser:**



**Find CRISPRs by gene using our table:**

Exon ID	Spacer	Status	Summary
EN0803000060217	20	Complete	observed: None total pairs: 1 max. distance: 1000
3	Complete	observed: None total pairs: 1 max. distance: 1000	

**Find CRISPRs by 20bp gRNA:**

Sequence: AATAGTAGACATAAAGTCT

Species: ☐ Human (GRCh37) ☒ Human (GRCh38) ☐ Mouse (GRCm38)

Ensembl In gene In e

**Find CRISPRs in genomic sequence:**

JAAGGAATGTTCCCAATAGTAGACATAAAGTCTTGG

Crispr ID	Ensembl	In g
1106710403	13:32325087-32325109	No
1106710404	13:32325088-32325110	No
1106710405	13:32325110-32325132	No

**Find off-targets by sequence:**

☐ Mouse (GRCm38)

Orientation: ☐ PAM Right (NGG) ☒ PAM Left (CCN)

Sequence	On
GTGTCAAGTGAAGTTACTCT	par
GTGTCCAGAACTTACTCT	par

[Gibson Designer](#)

The Gibson Designer will find the oligos in either [Human](#) or [Mouse](#) genomes that can be used to create targeting vectors by Gibson assembly. The Gibson Designer matches the vector design with CRISPR sites appropriate for the creation of exon deletions.

If you use this site in your research, please cite:

WGE: A CRISPR database for genome engineering. Alex Hodgkins; Anna Farne; Sajith Perera; Tiago Grego; David J. Parry-Smith; William C. Skarnes; Vivek Iyer (Bioinformatics 2015)  
[doi:10.1093/bioinformatics/btv308](https://doi.org/10.1093/bioinformatics/btv308)

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# sgRNA design software - WTSI genome editing

## CRISPR search

### Species

- ☐ Human (GRCh38)  
☐ Mouse (GRCm38)

### Marker Symbol


### Exons


Note: the CRISPR table only shows CRISPRs that overlap the exon by at least 1 base.

To see flanking crisprs please use the genome browser

Show CRISPRs in:

 Table


 Genome Browser

 Download

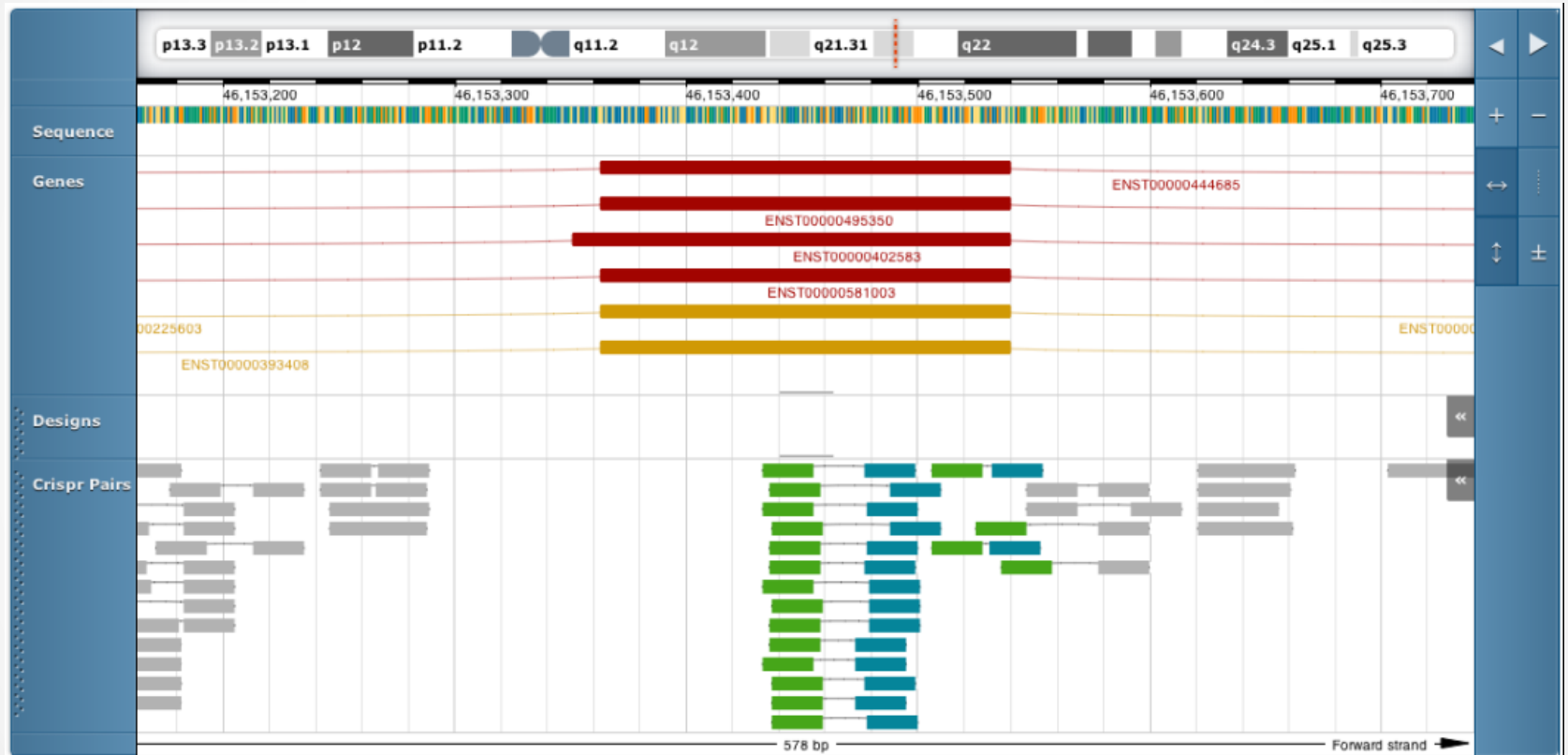
Show CRISPR pairs in:

 Table

 Genome Browser

 Download

# sgRNA design software - WTSI genome editing



# sgRNA design software - WTSI genome editing

**Single Crisprs**  
☒ Show  
☐ Hide

**Paired Crisprs**  
☐ Show  
☒ Hide

**Designs**  
☒ Show  
☐ Hide

**Crispr Filters**  
☒ Show All  
☐ Exon Only  
☐ Exon Flanking Only  
Flanking region:




**Spacer Filter**  
Crispr Pair Spacer Length  
(-10 to 30 bp)  
Min Spacer Length   
Max Spacer Length



**Variation**  
MAF threshold





[? Help](#)

**Haplotype Filters**

☒ Allele Fraction  
☒ Quality Filter  
☒ Phasing Inconsistent  
☒ Homopolymer Unphased Insertion  
☒ Rescued Molecule High Diversity

**Crispr Colour Key:**  
 Off-targets computed  
 Off-targets not computed  
 PAM site

**Crispr Pair Colour Key:**  
 Left and right off-targets computed  
 Left and right off-targets not computed

**Haplotype Colour Key:**  
 Substitution  
 Insertion  
 Deletion  
 Not on this Haplotype

[? Help](#)

**Off-Target Filters**

Maximum number of off-targets with:  
0 mismatch  no max  
1 mismatch  no max  
2 mismatch  no max  
3 mismatch  no max  
4 mismatch  no max  
   
[? Help](#)

Let's do some sgRNA designing !!!

What is your favourite gene???