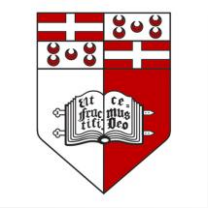
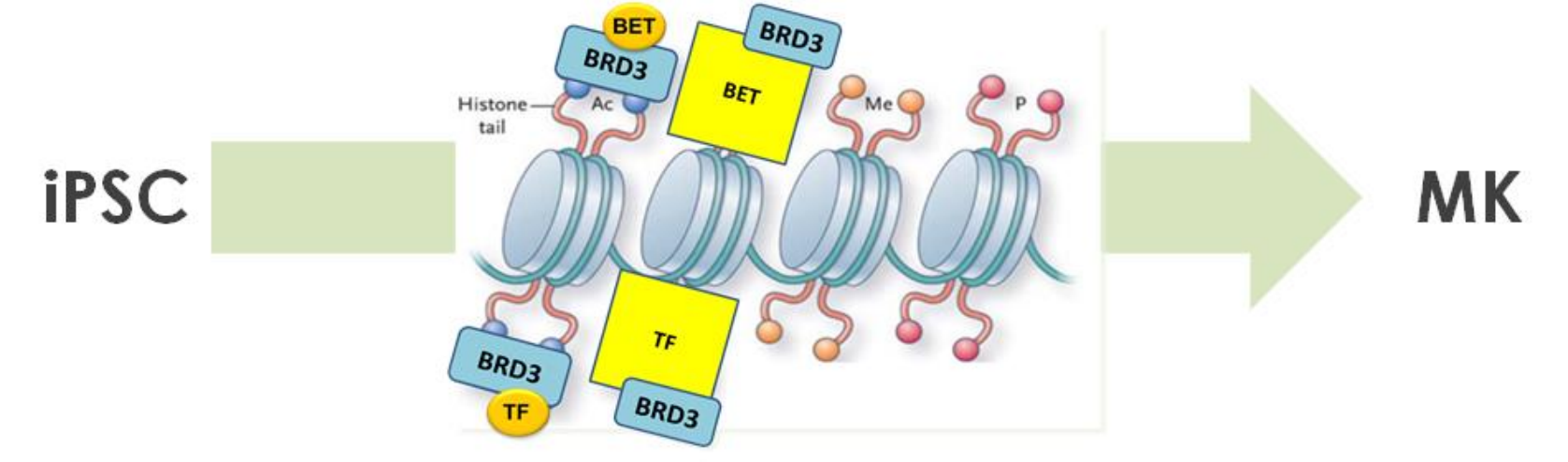
Train Malta Summer school 2017

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

- Multi-discipline experiment
  - Mixture of cell culture and molecular biology

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  - 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 endoocytosis to take up DNA

Cell Culture

Monday

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

Tuesday

Objective = Keep Cells Happy

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Step: Media Change

Molecular Biology

Wednesday

Objective = Harvest gDNA and PCR region of interest

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Steps: Determine transfection success, harvest cells, extract gDNA, quantification of gDNA, PCR amplification of the target sequence, check by gel electrophoresis, and then recover the PCR product

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

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Friday

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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

Let's Get Started

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  - Two medium hoods (4 people per hood – two pairs) and Four little hoods (3 people per hood)
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Murghalina!

Introduction to CRISPR

John Lambourne
Isabel Rosa

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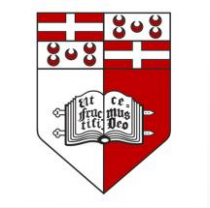
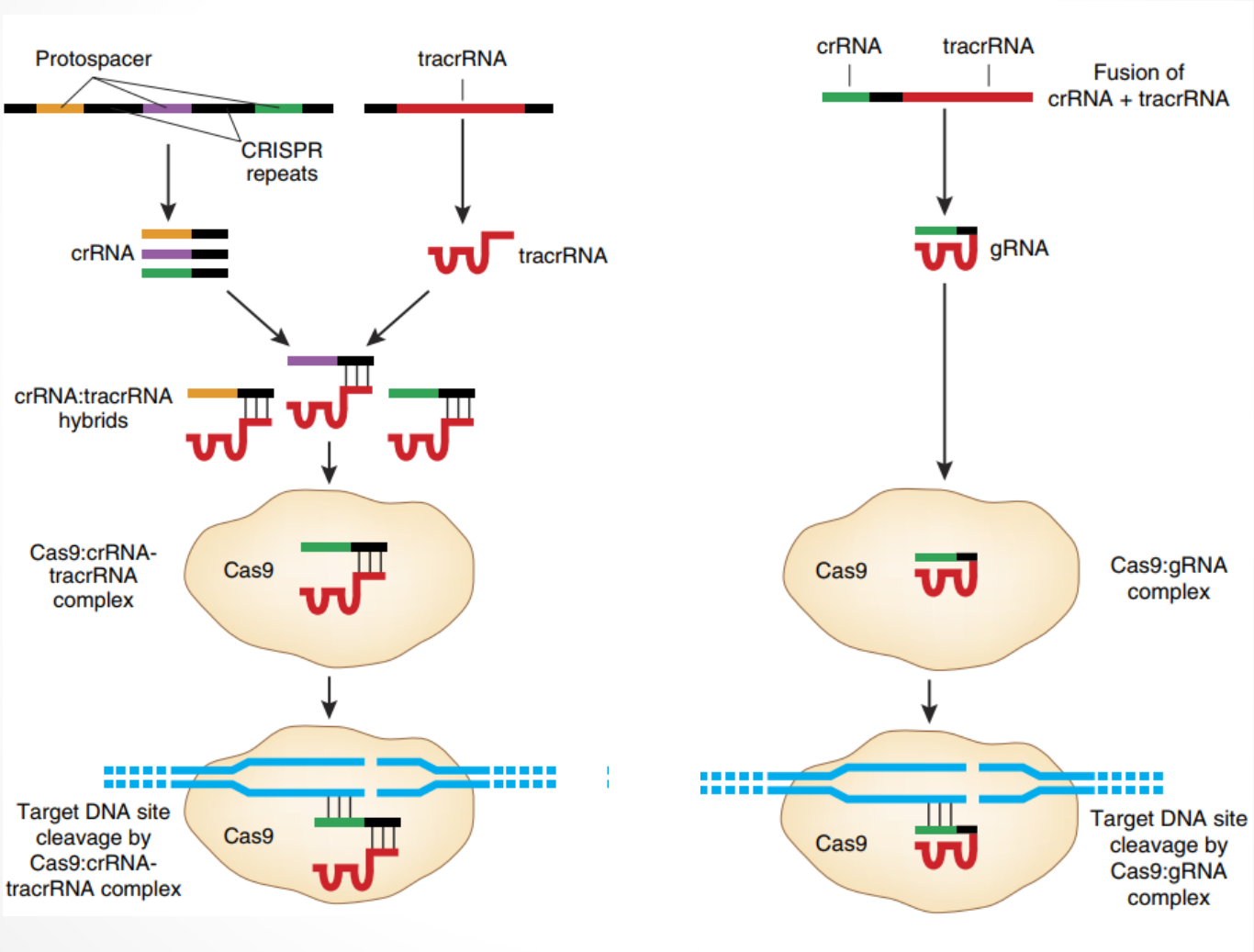
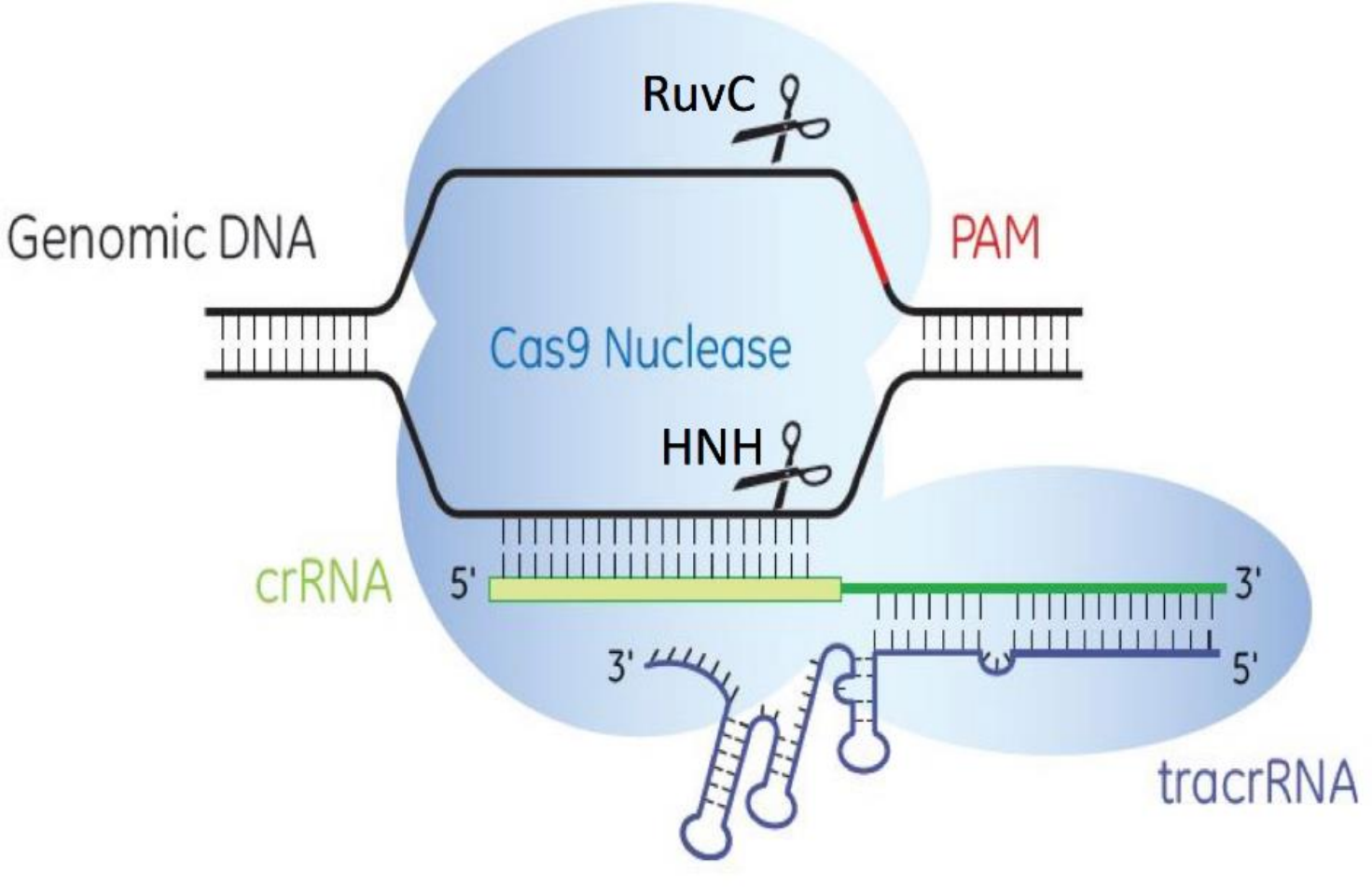


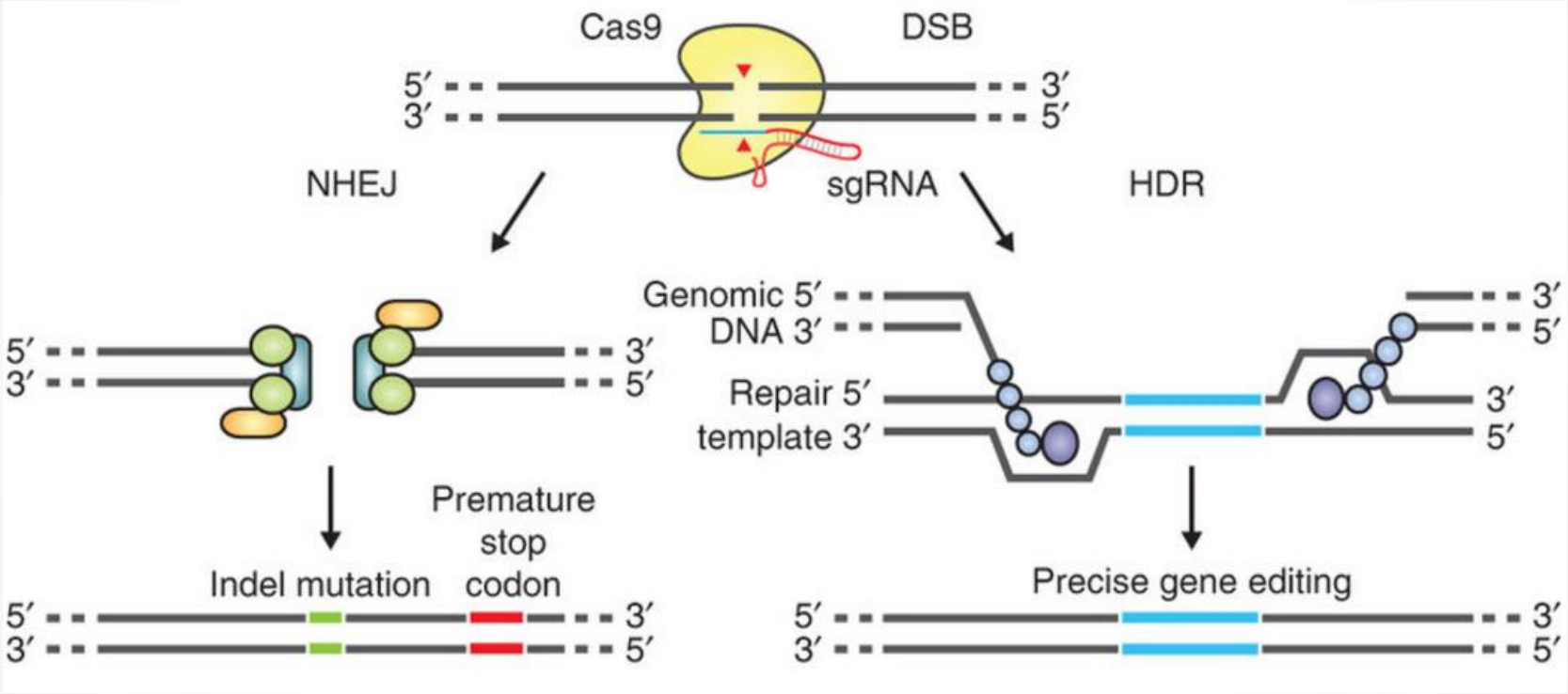
Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)



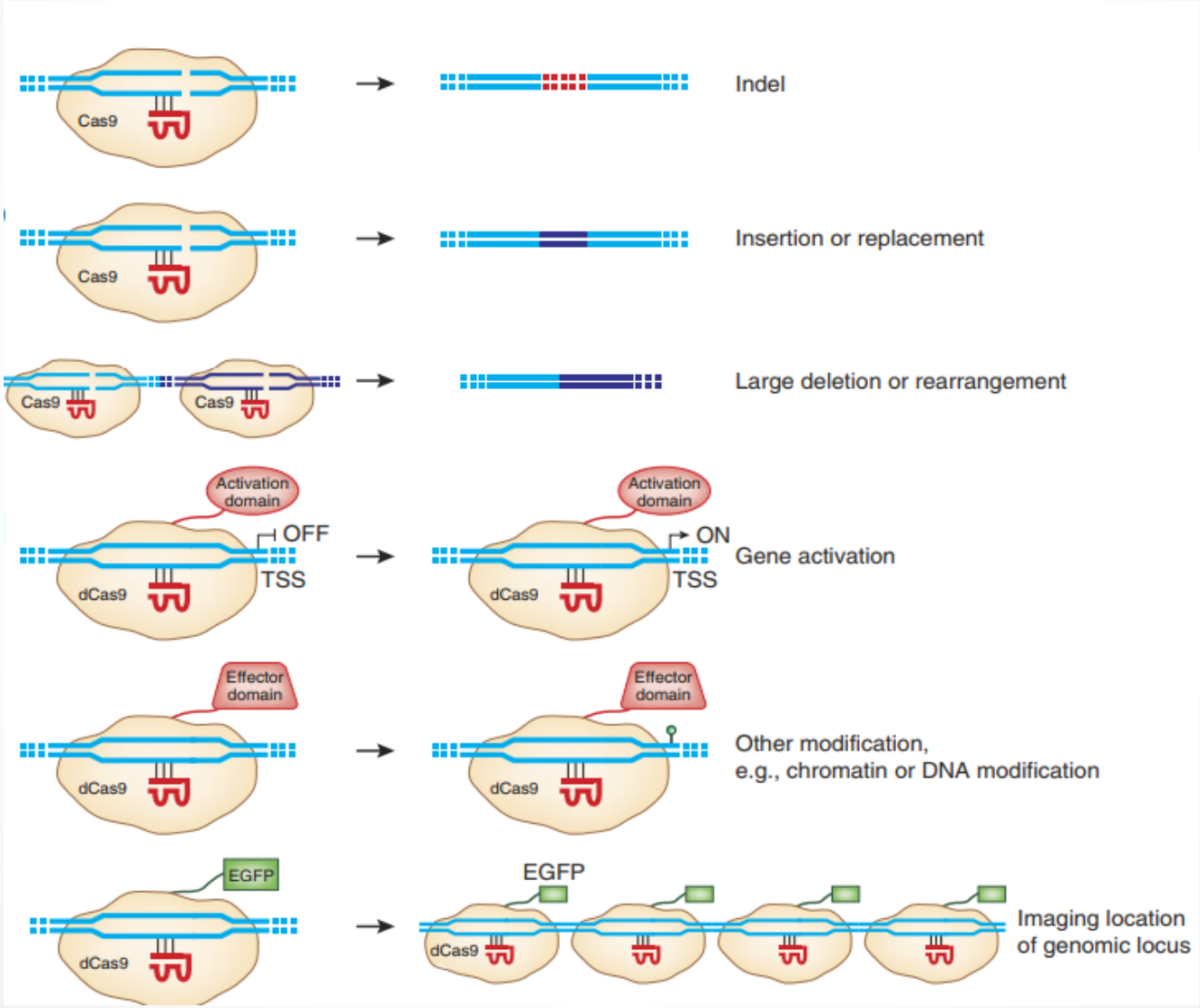
Genome-editing with CRISPR/Cas9 - genome targeting



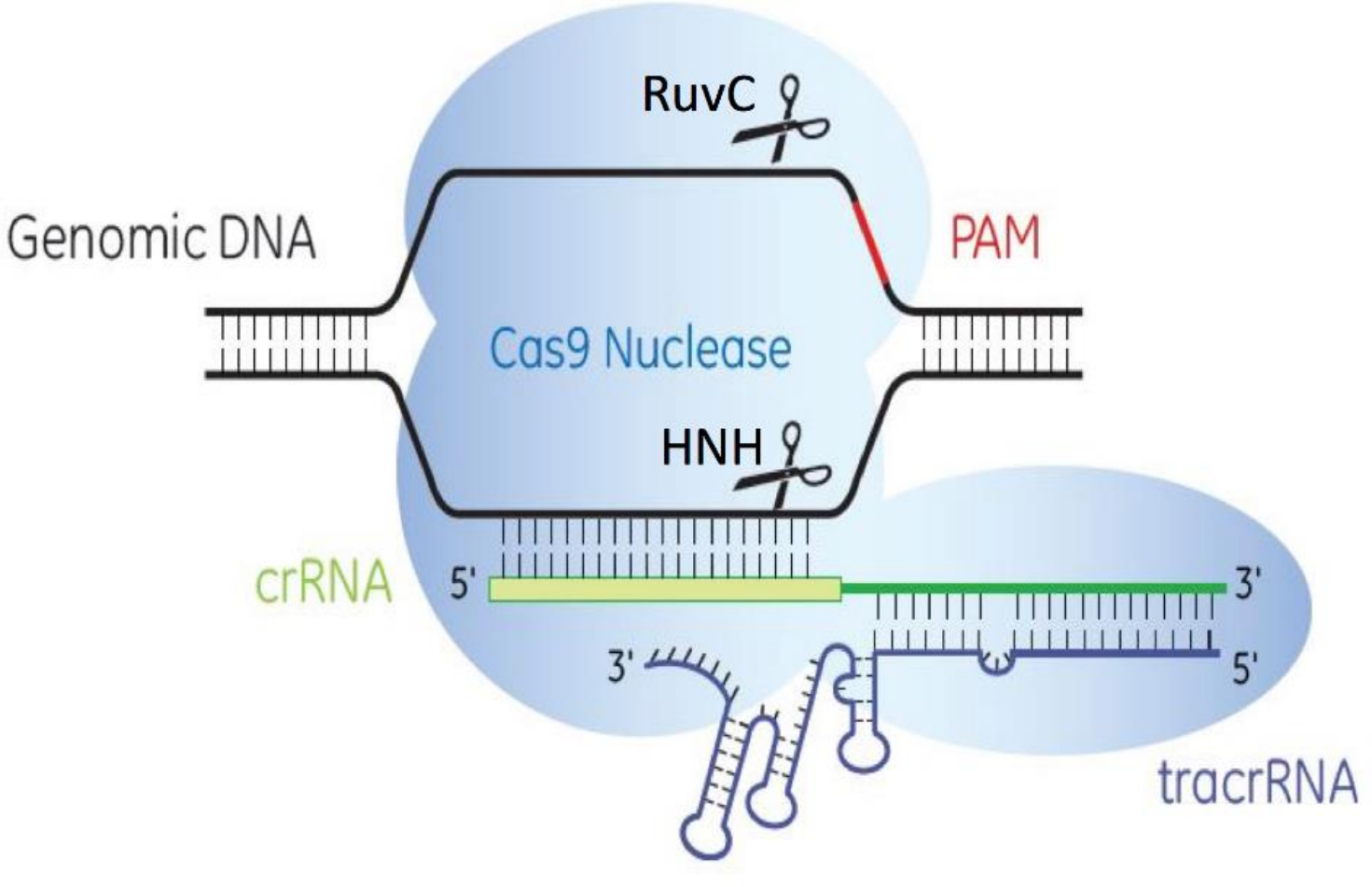
Genome-editing with CRISPR/Cas9 – repair mechanisms



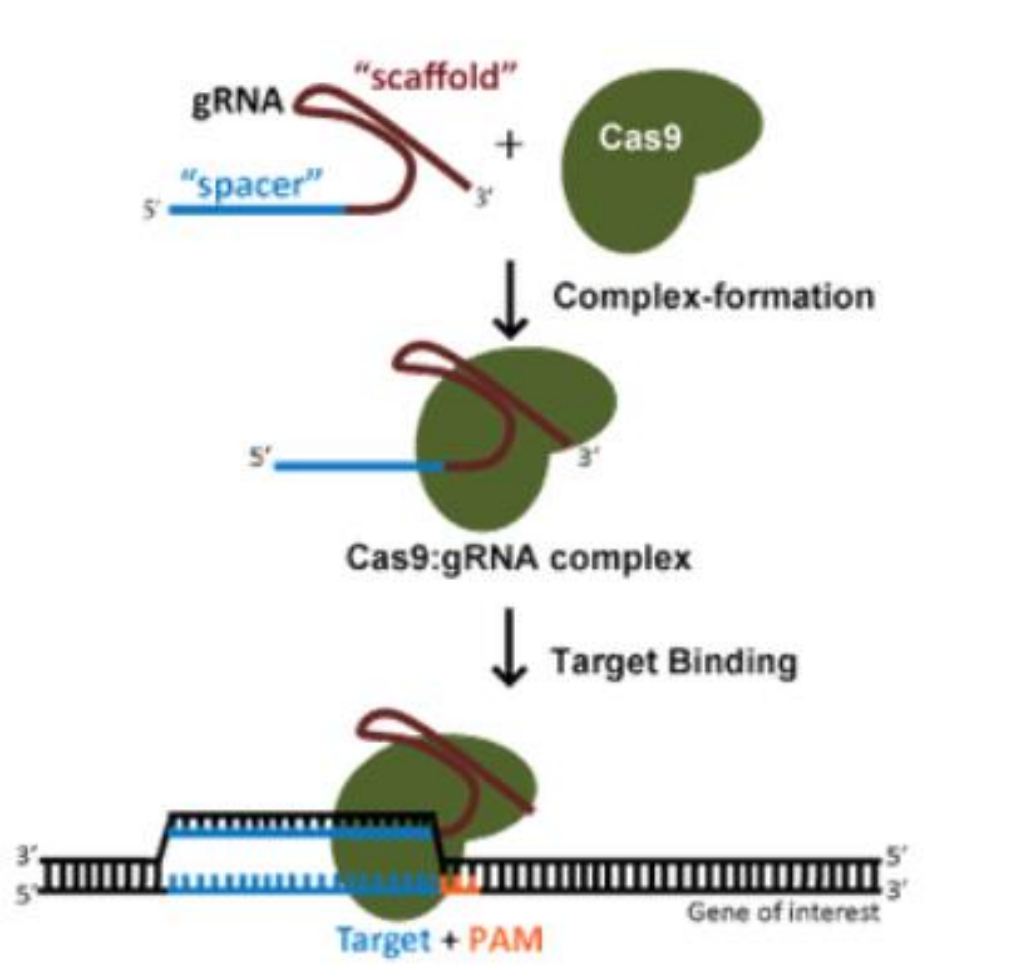
Applications of CRISPR/Cas9 systems



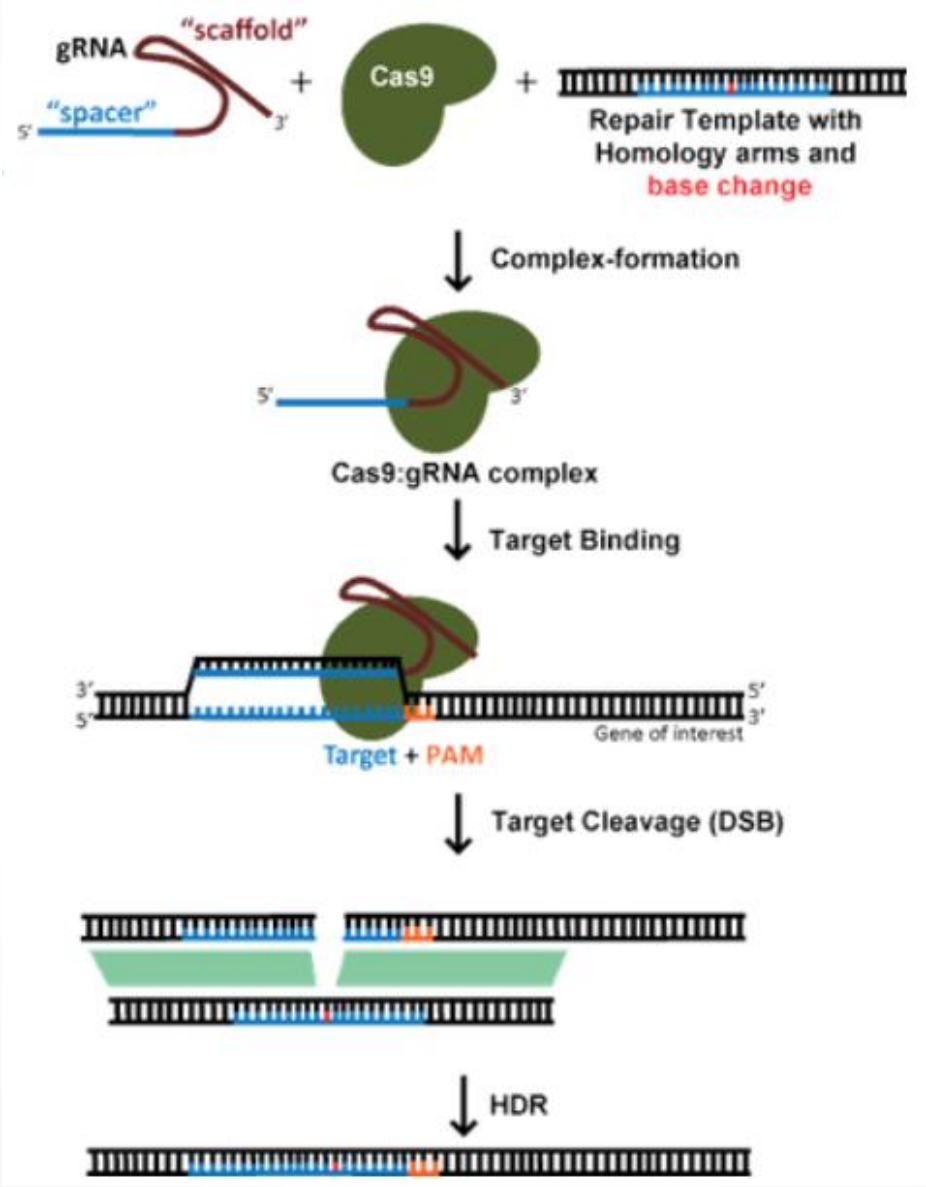
spCas9 variants- WT Cas9



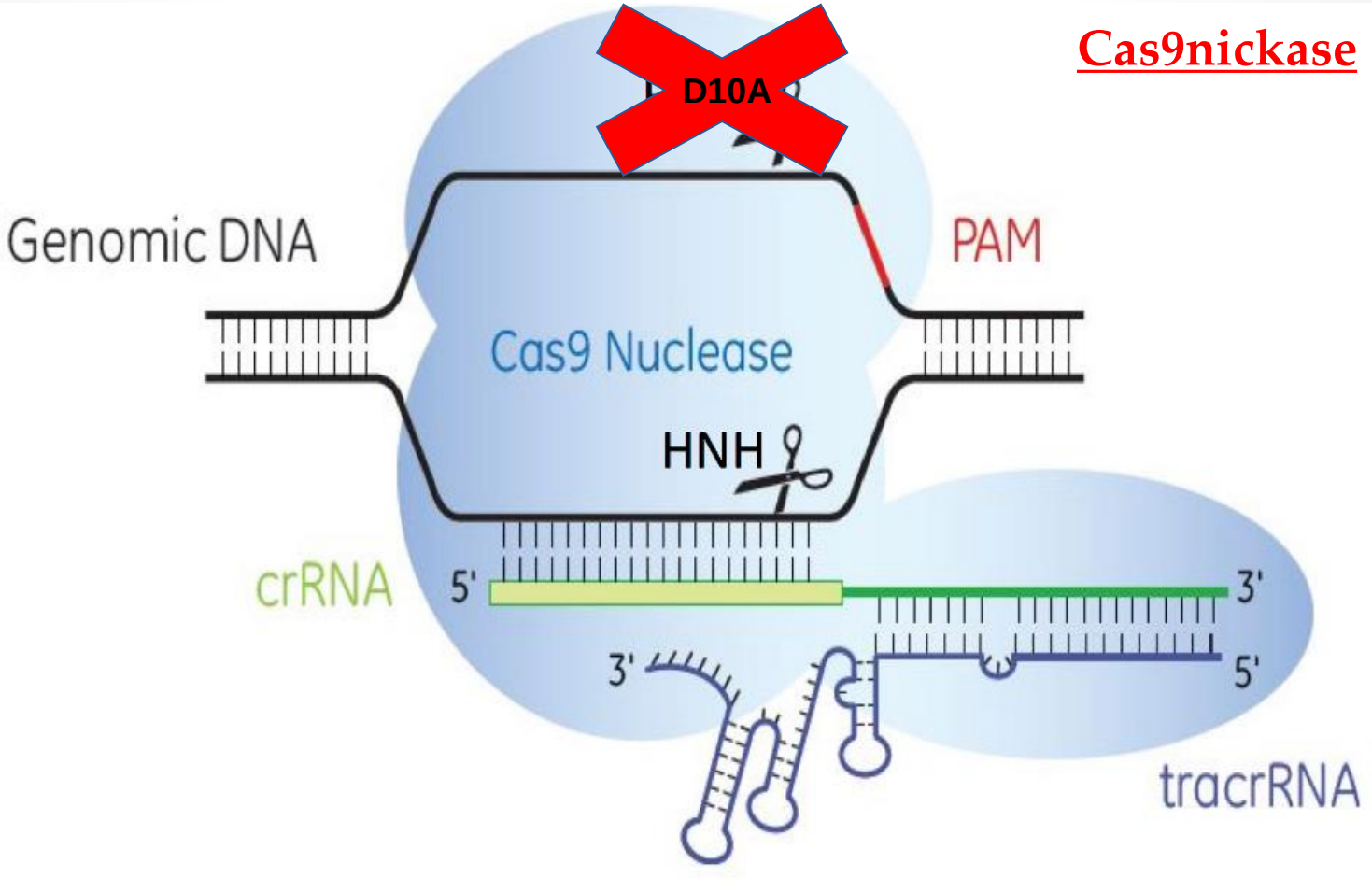
Knockout generation- WT Cas9 nuclease



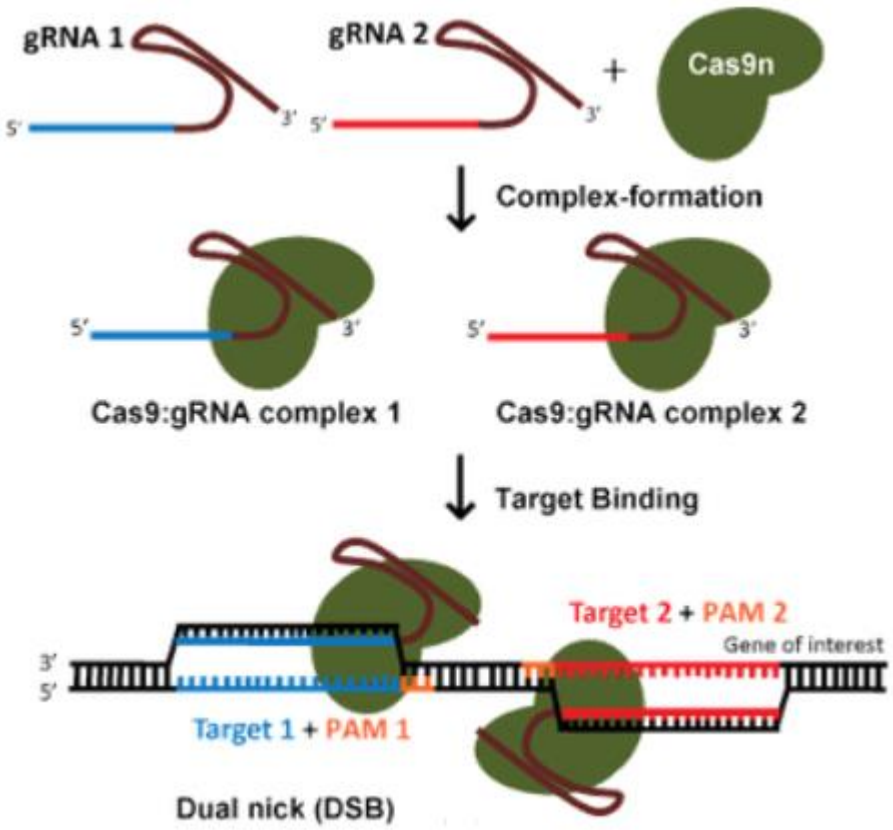
Knock-in generation- WT Cas9 nuclease + HDR (homology directed repair)



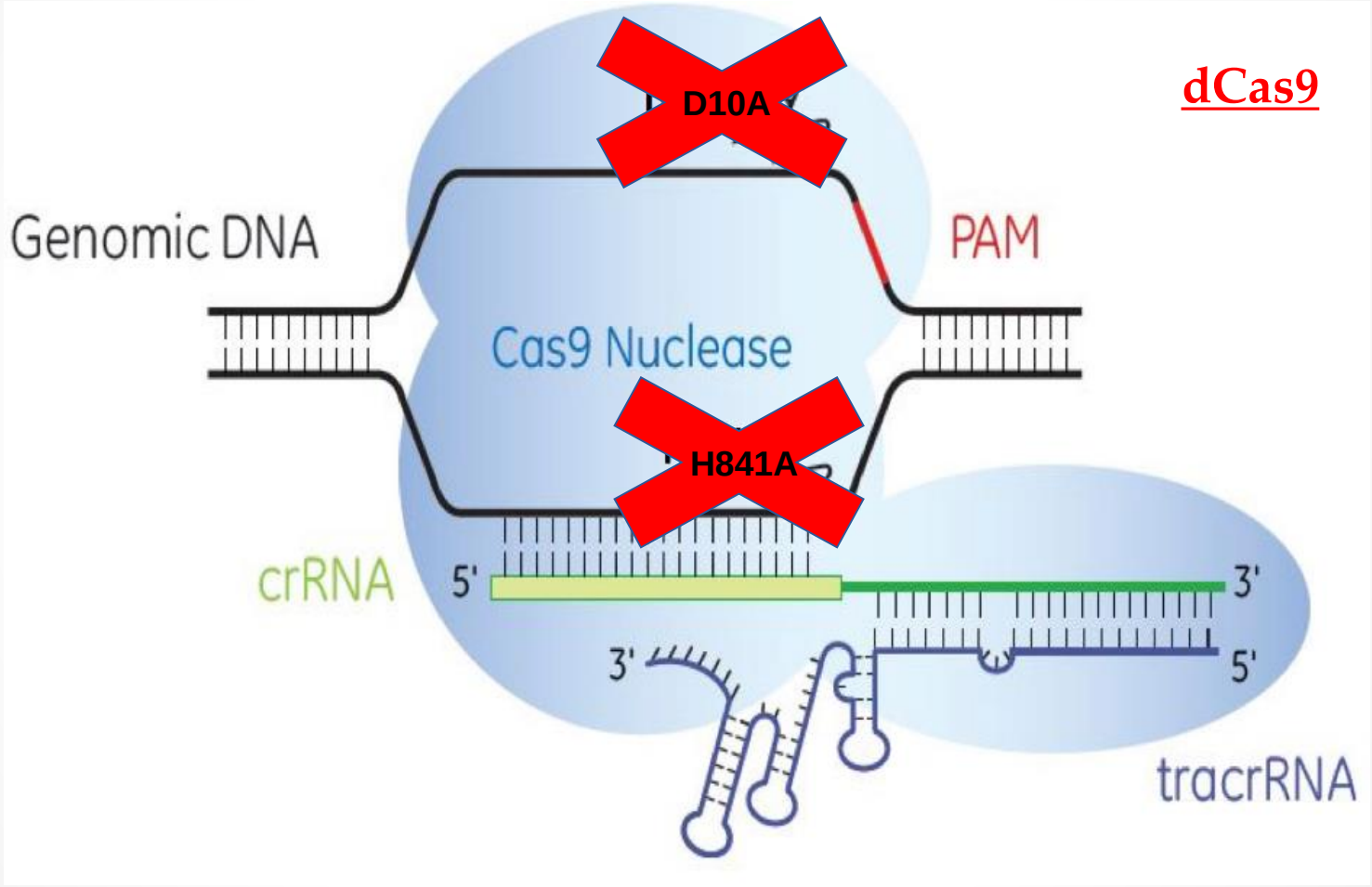
Cas9 variants- Cas9nickase



Knockout generation- Cas9nickase nuclease

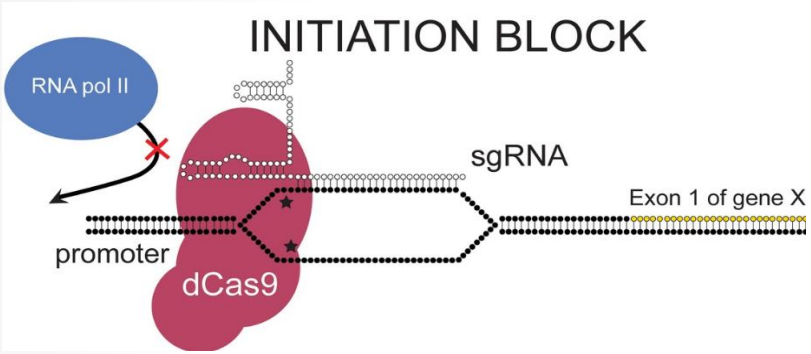


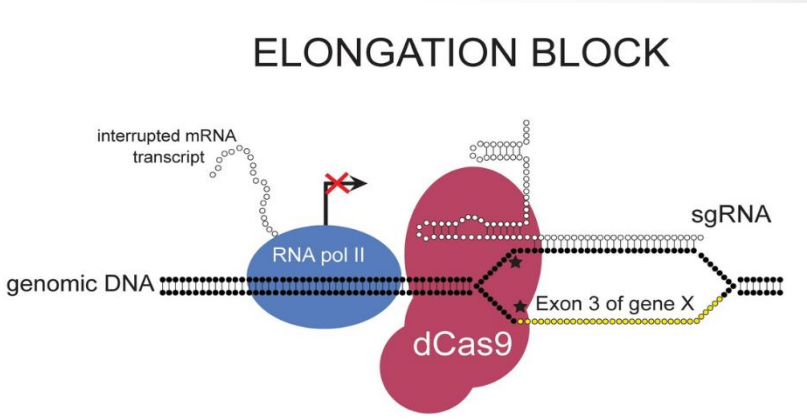
Cas9 variants- dead Cas9



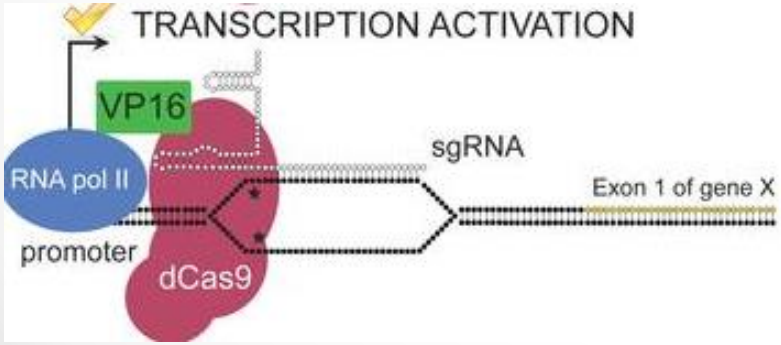
Interference (CRISPRi) or Activation (CRISPRa) - dCas9 nuclease

Interference

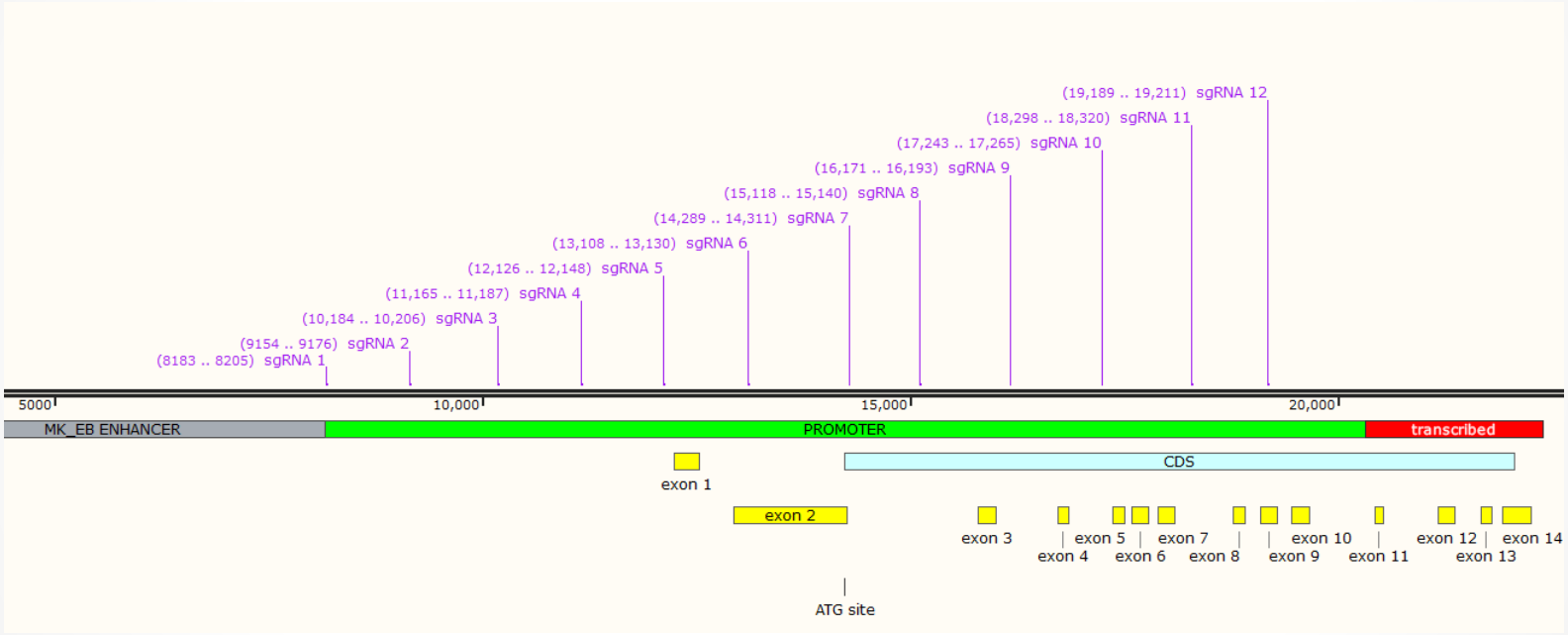




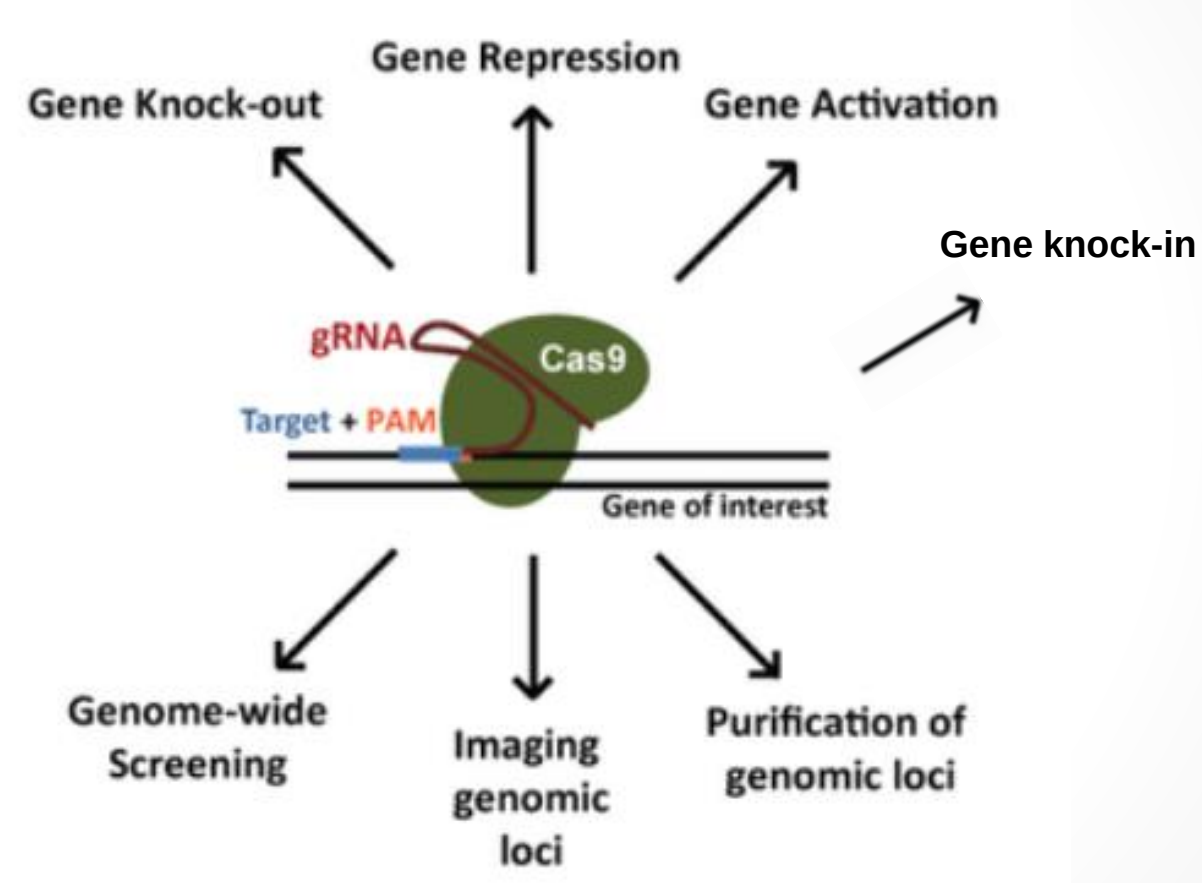
Activation



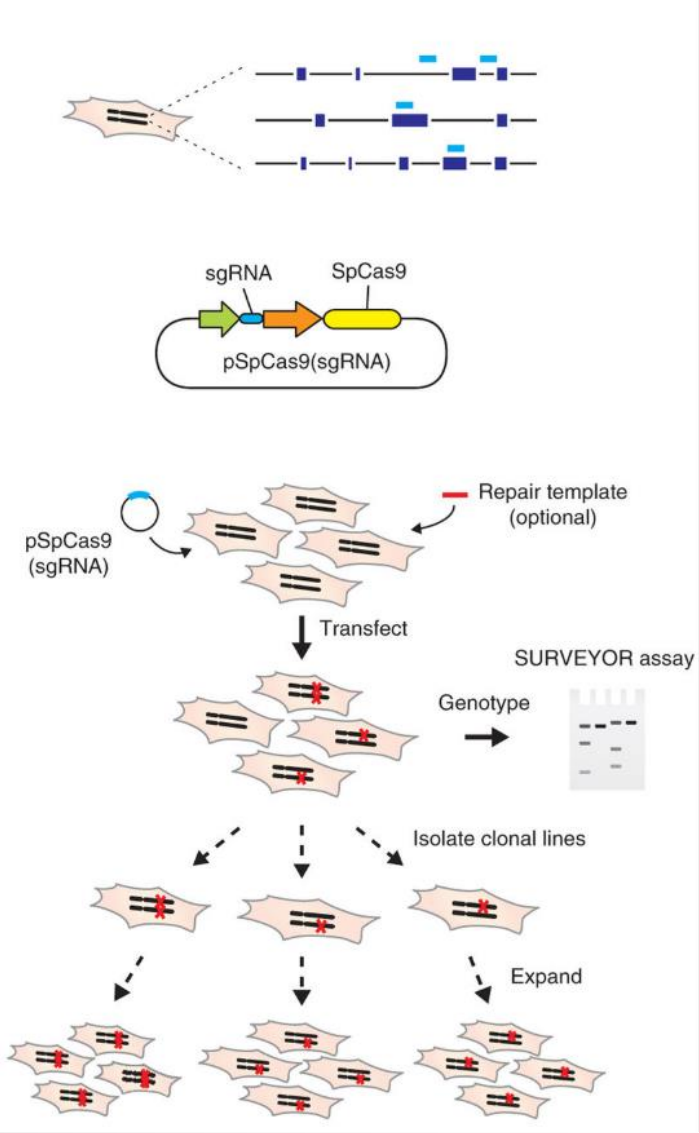
Example of sgRNA loading for gene knockdown –promoter targeting



Genome-editing with CRISPR/Cas9 - genome targeting

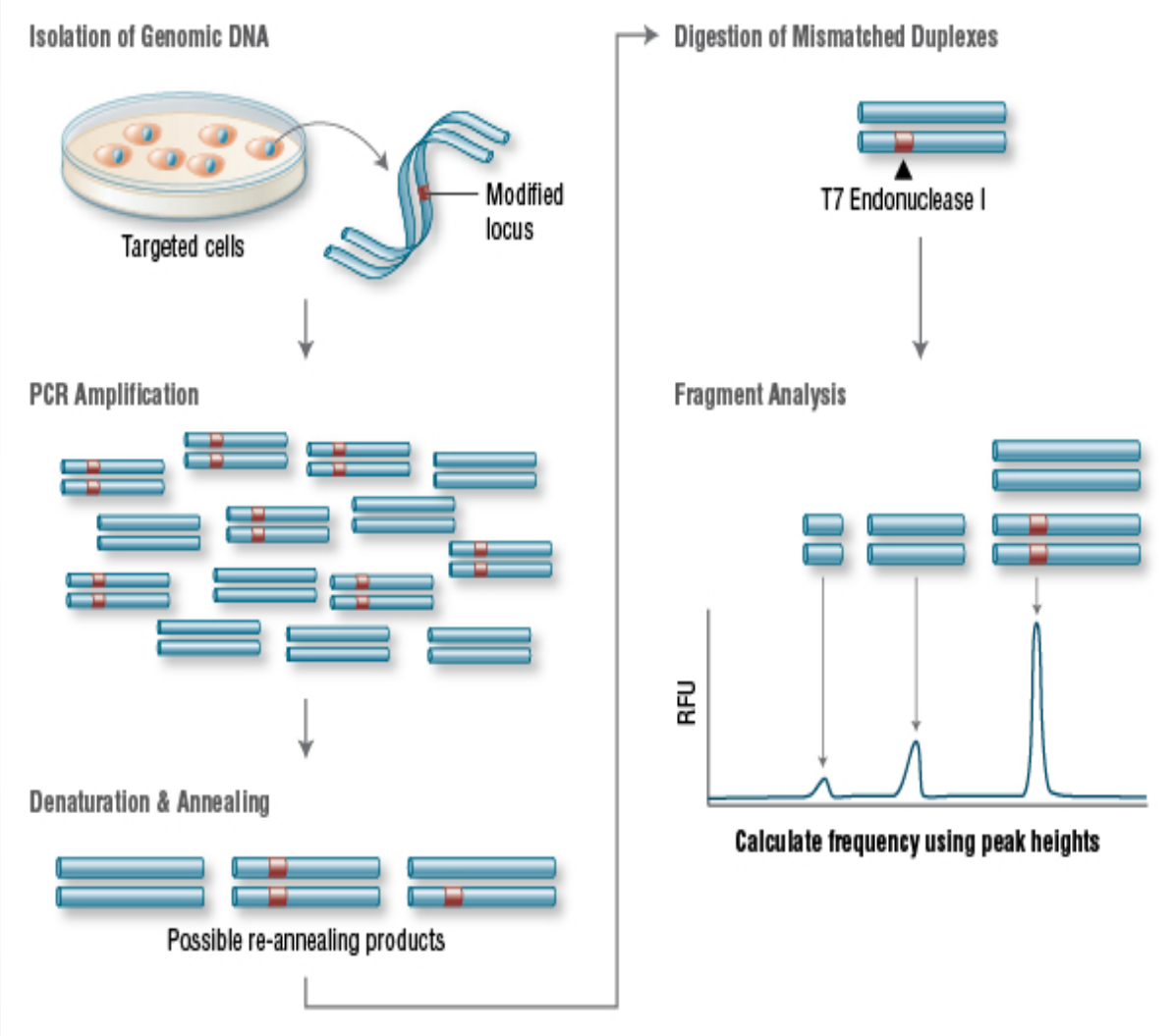


CRISPR/Cas9 experiment design



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



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
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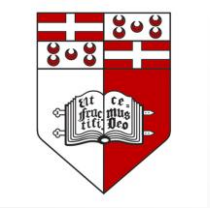

sgRNA design process

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)

  - Guanines preferred at -1 and -2 positions proximal to PAM
  - Thymines are disfavored at the -4/+4 positions close to the PAM
  - Nucleotides 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 Genoverse 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:

Find CRISPRs by 20bp gRNA:

Find CRISPRs in genomic sequence:

Find off-targets by sequence:

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

wellcome trust sanger institute

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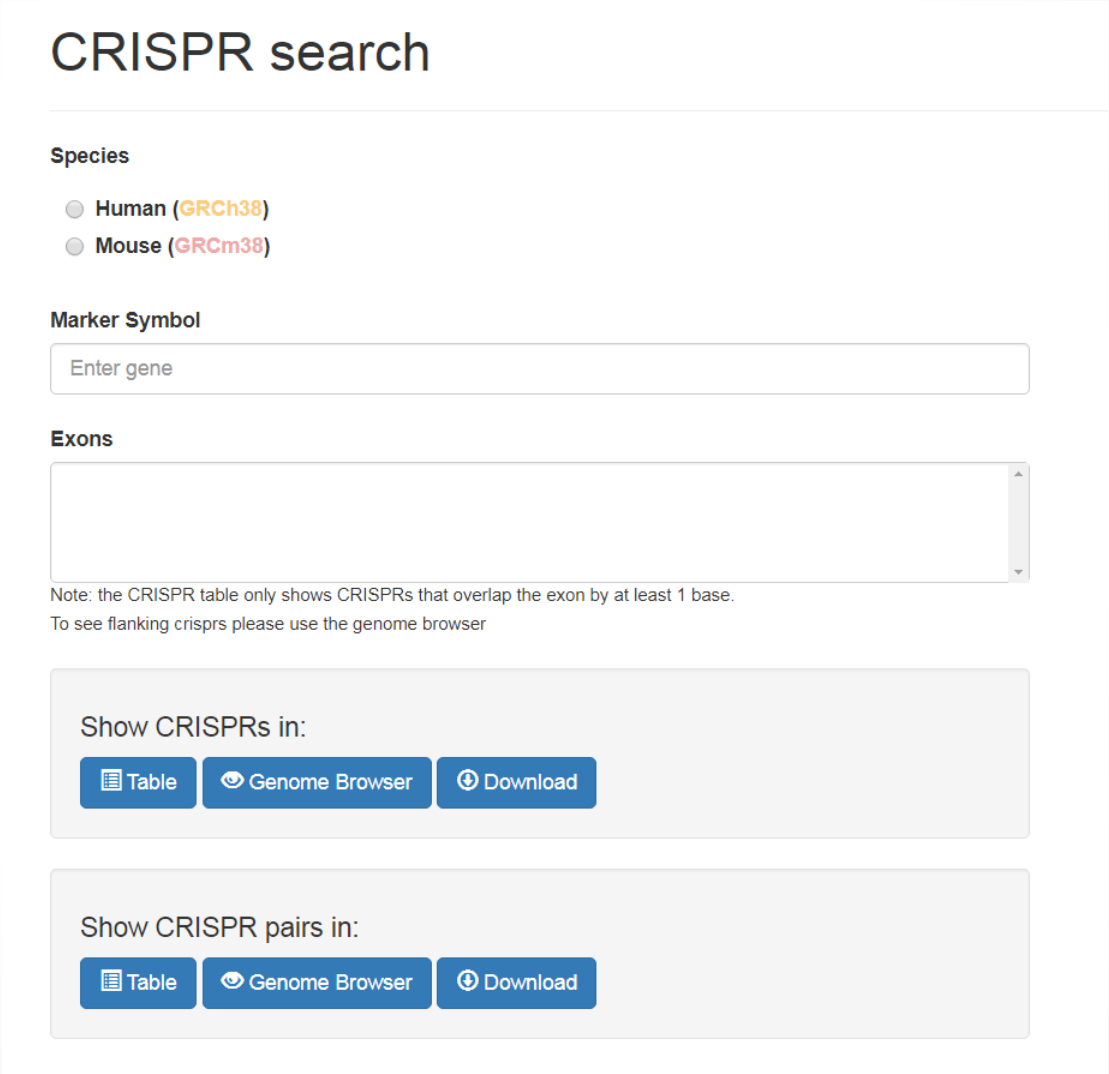

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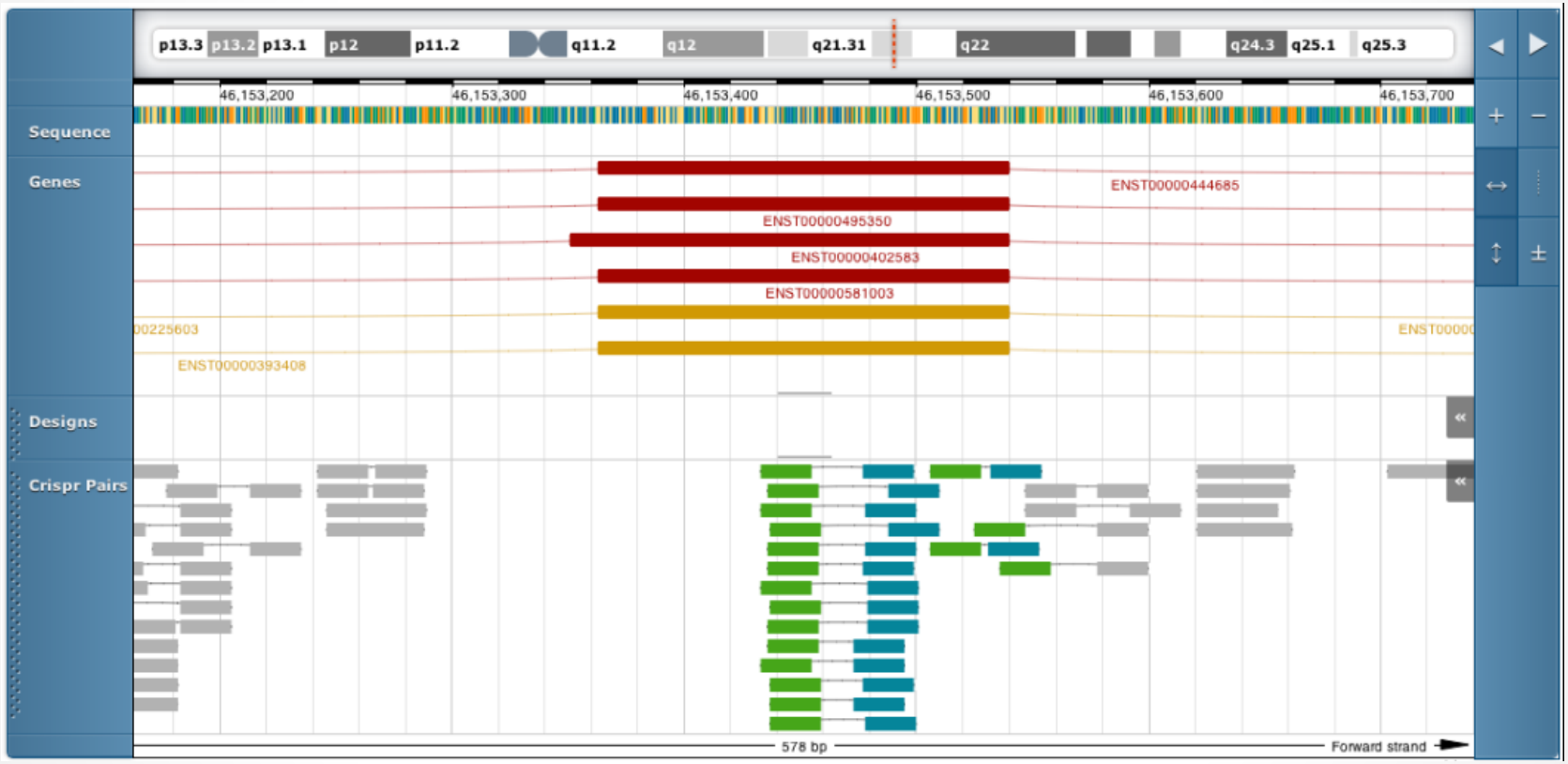

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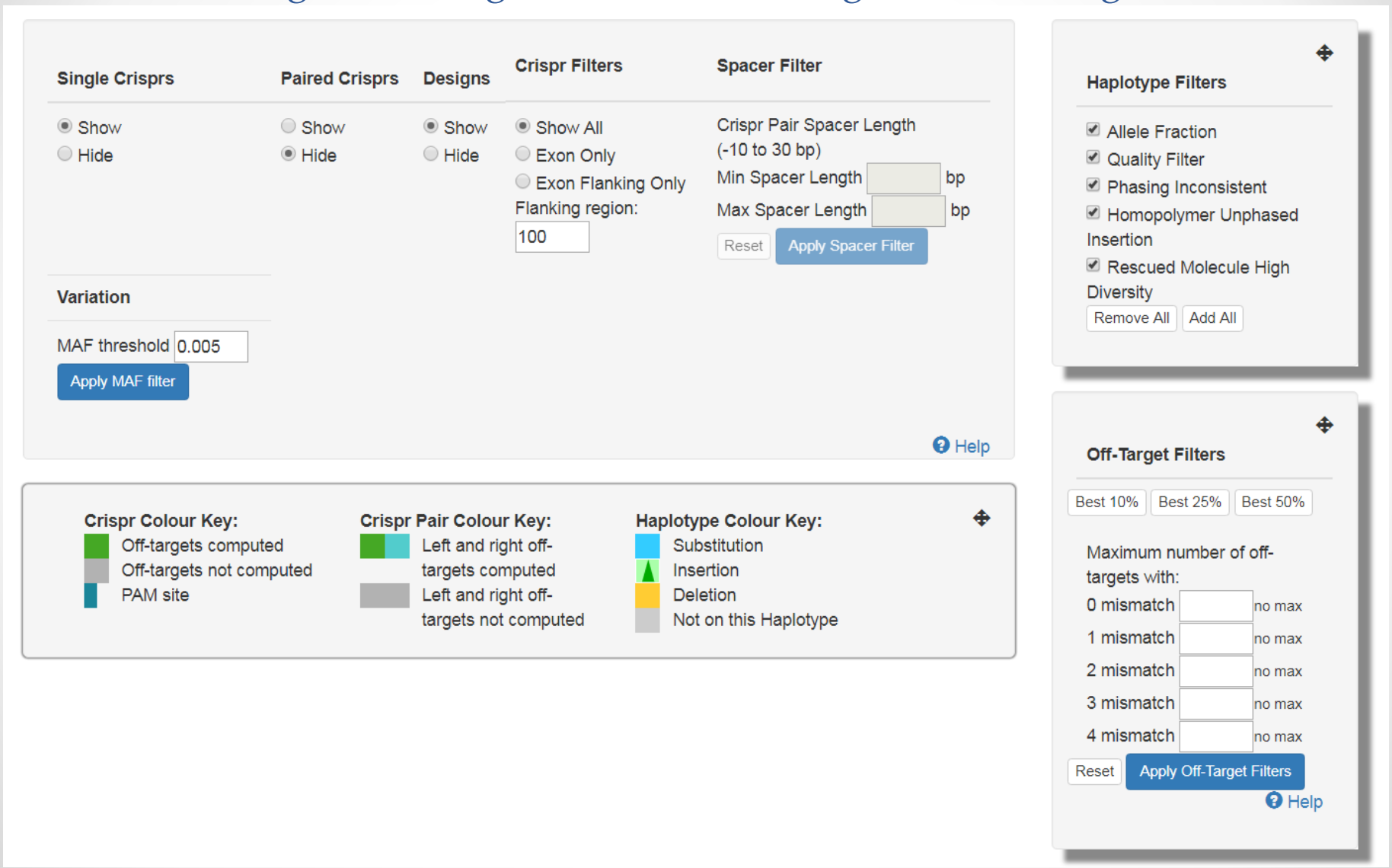

Let's do some sgRNA designing !!!

What is your favourite gene???