Erasmus MC
Universitair Medisch Centrum Rotterdam

cgec.erasmusmc.nl

A basic introduction in the CRISPR/Cas9 genome editing technique

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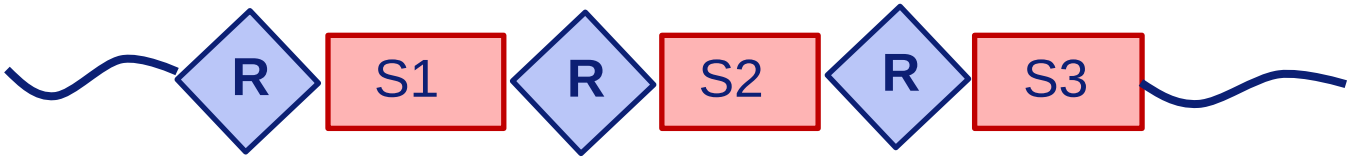
CGEC

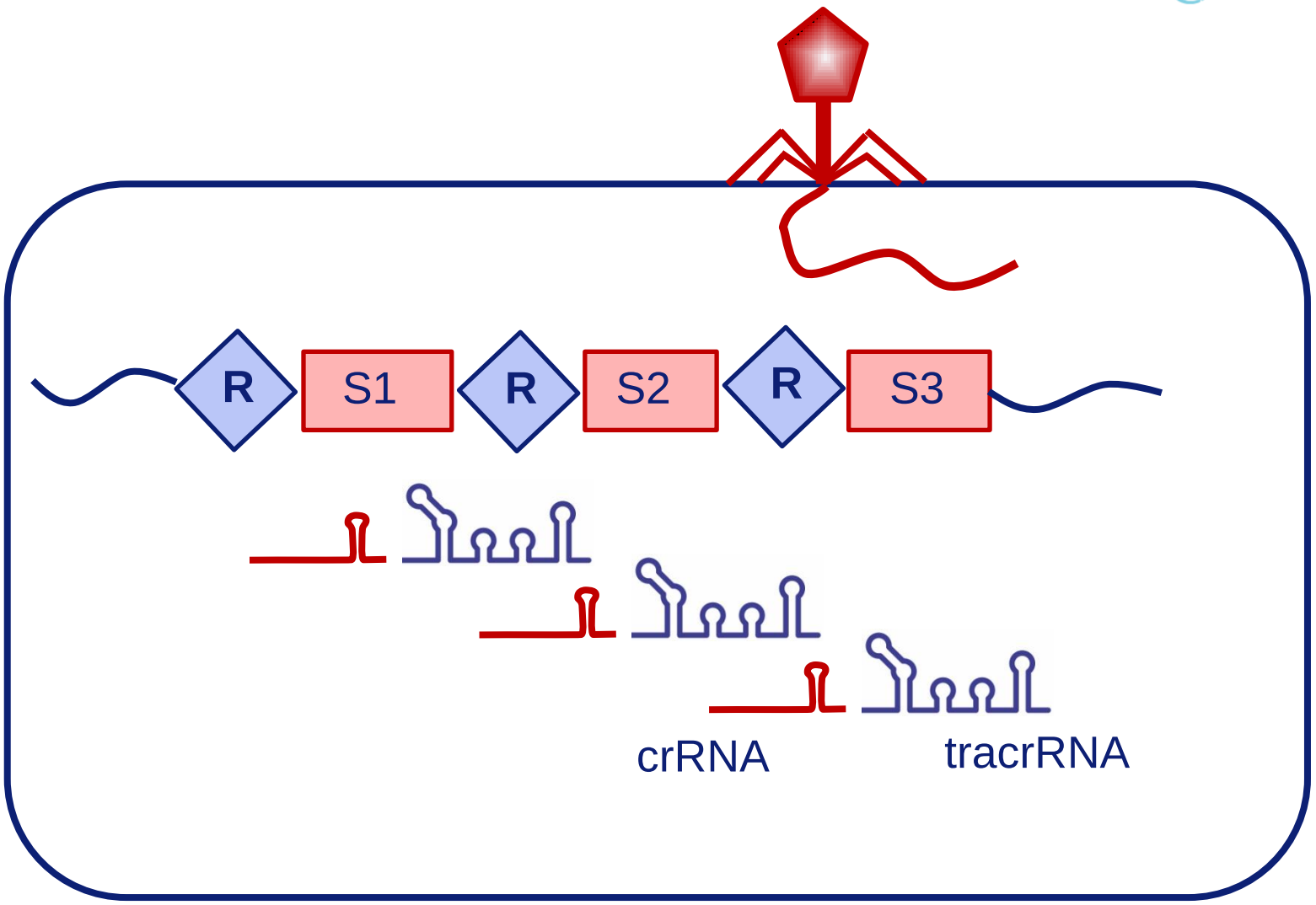
Cancer Genome Editing Center

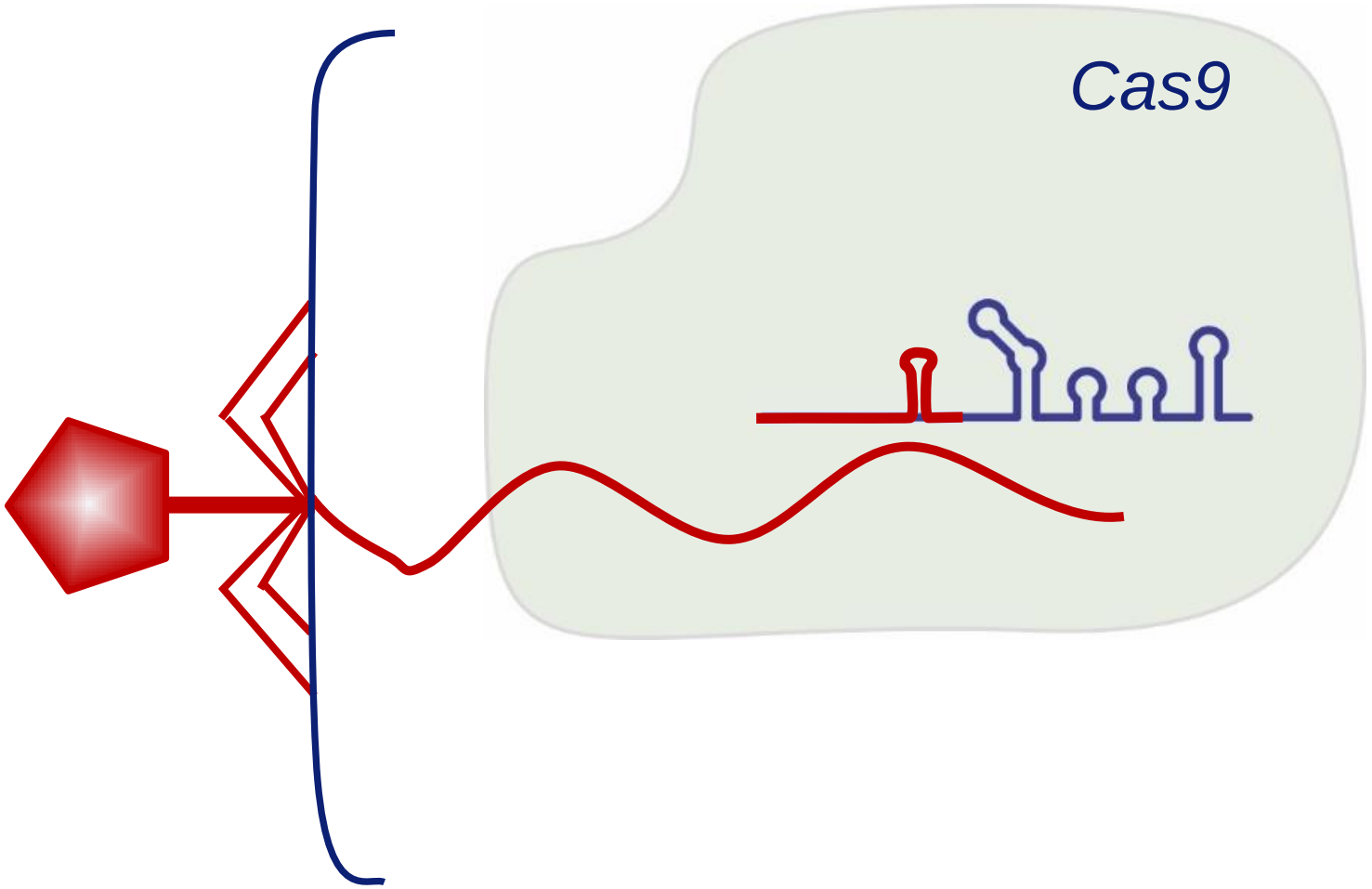
CRISPR/Cas9

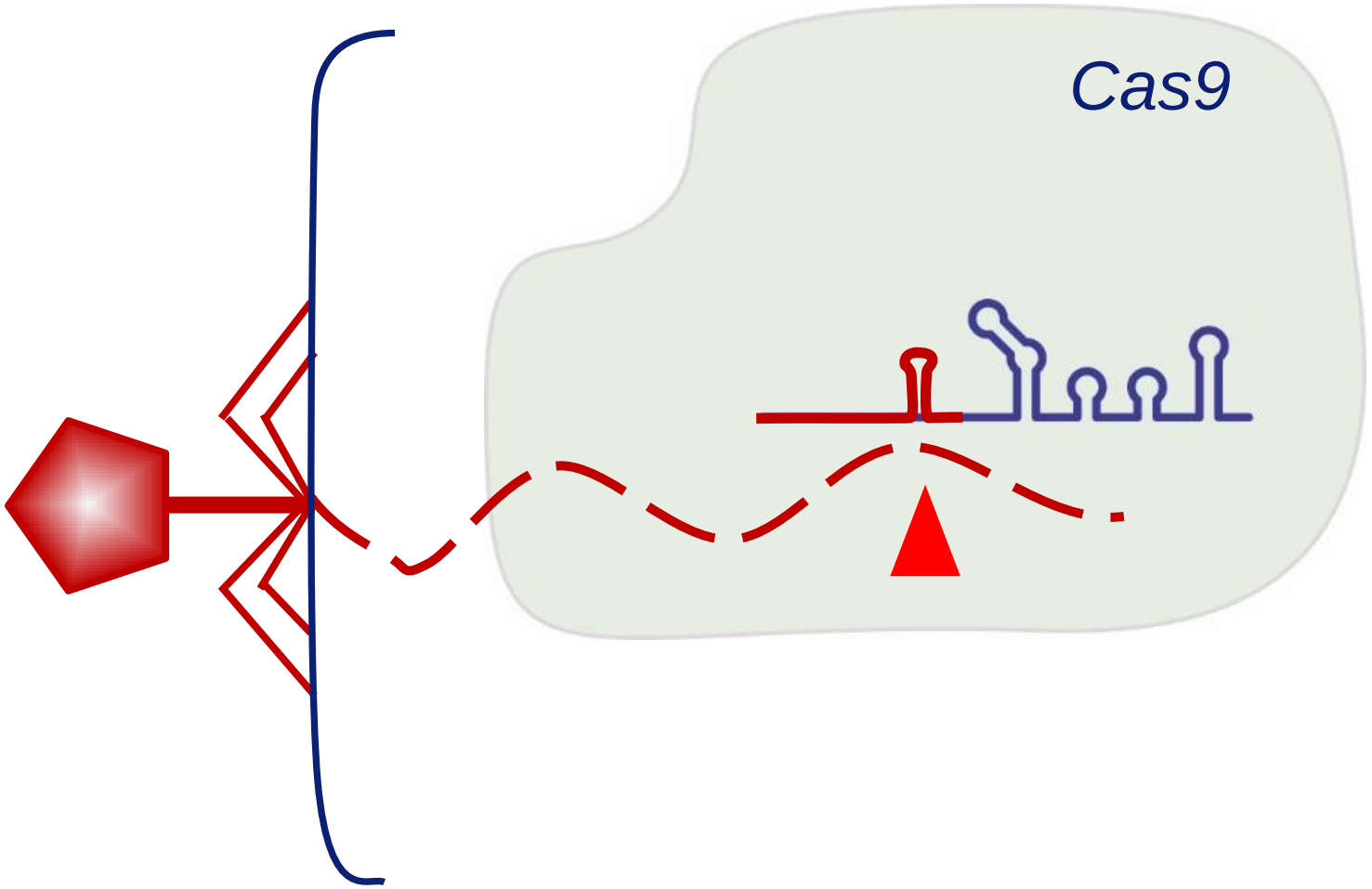
- CRISPR/Cas9 – The immune system of bacteria
- CRISPR/Cas9 – as a biomedical tool
- What to think of when you design your experiment
- Cas9 Variants
- CRISPR in the lab



Clustered Regularly Interspaced Short Palindromic Repeats


R
S1
R
S2
R
S3
crRNA
tracrRNA


Cas9


Cas9

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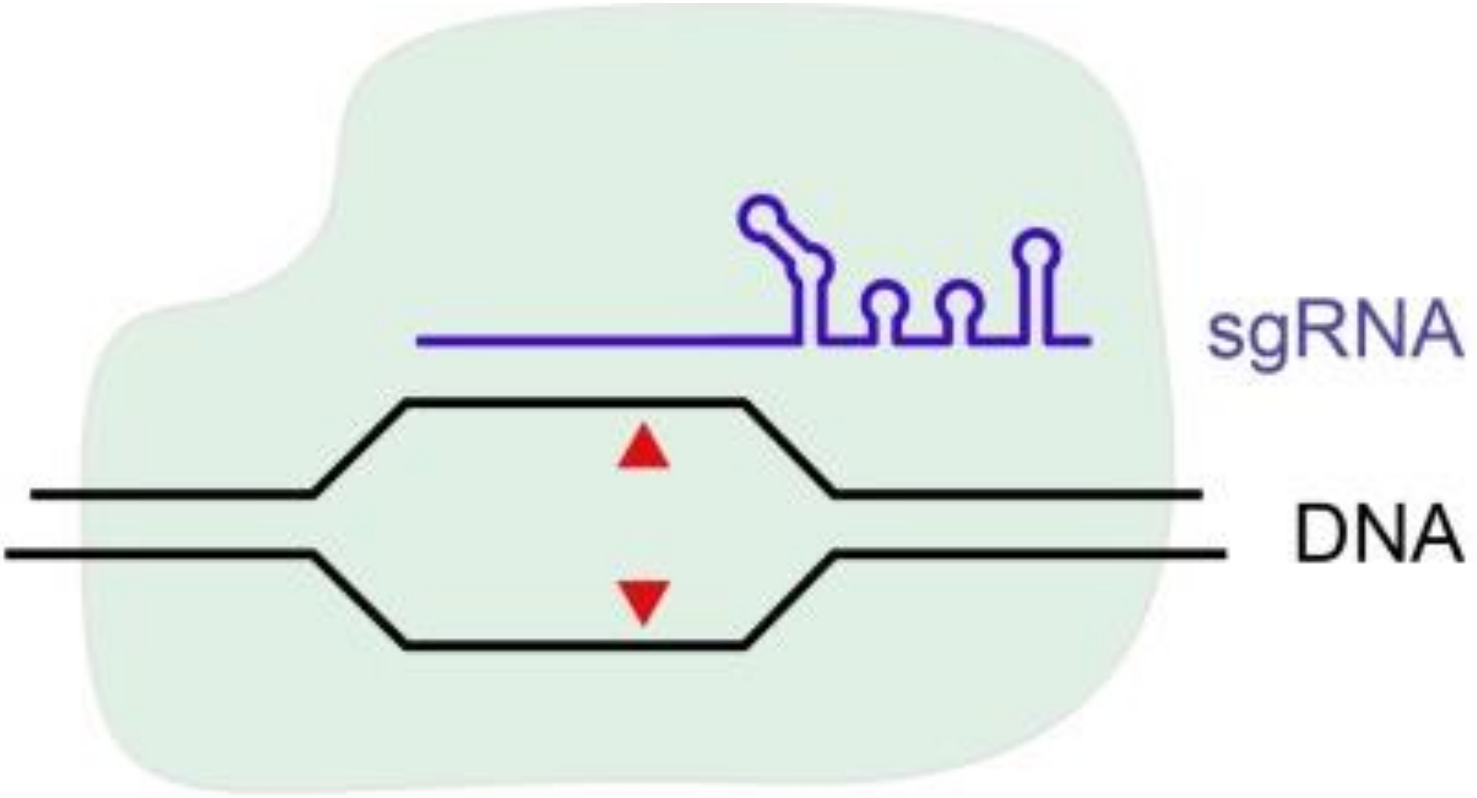
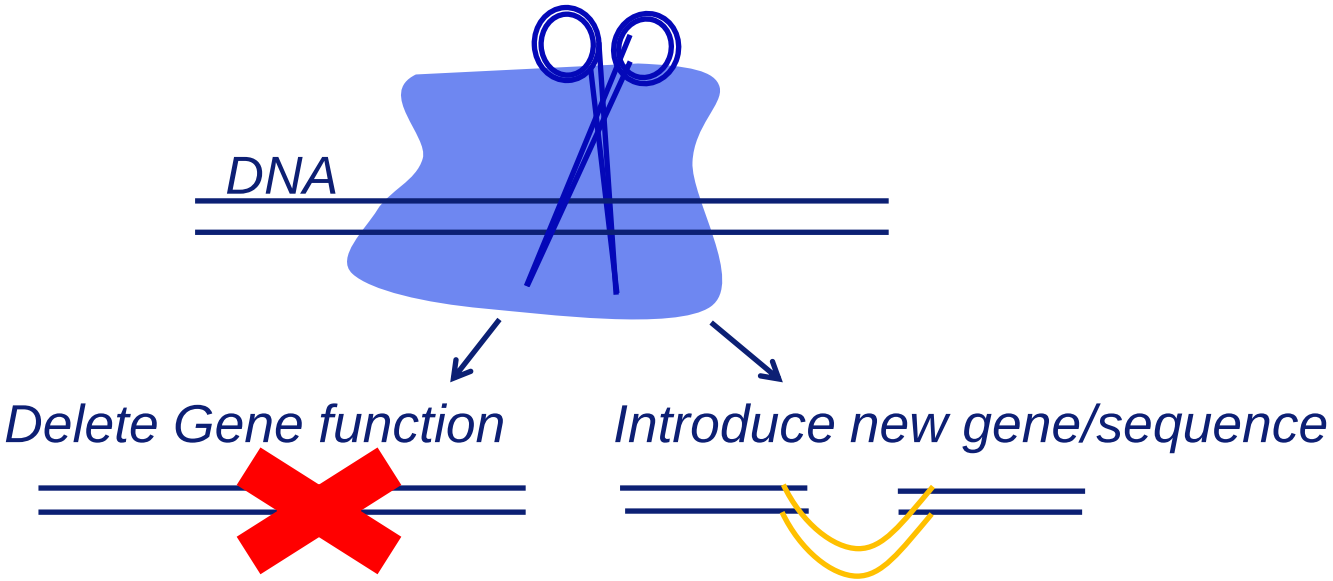
CRISPR/Cas9 as a tool for biomedical research

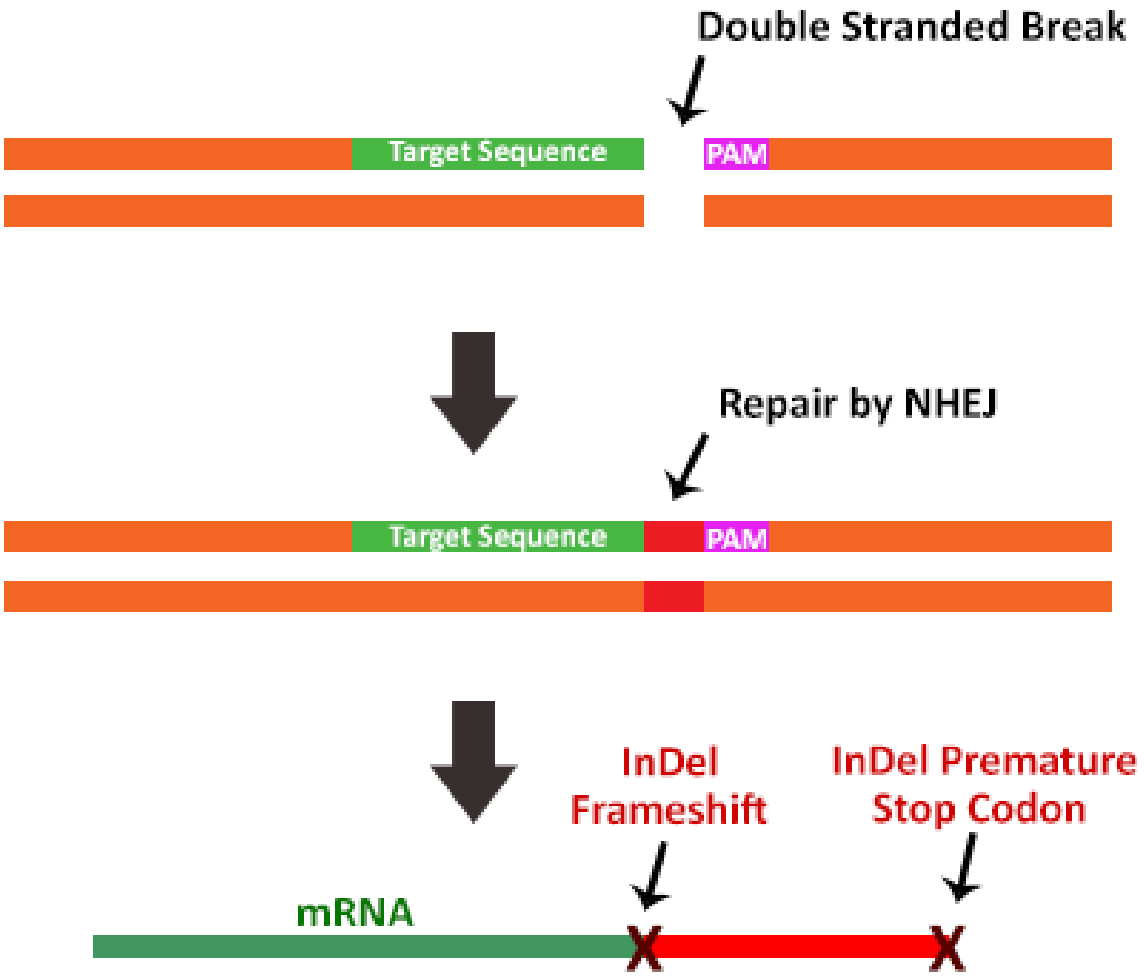


Genome editing options for CRISPR/Cas9

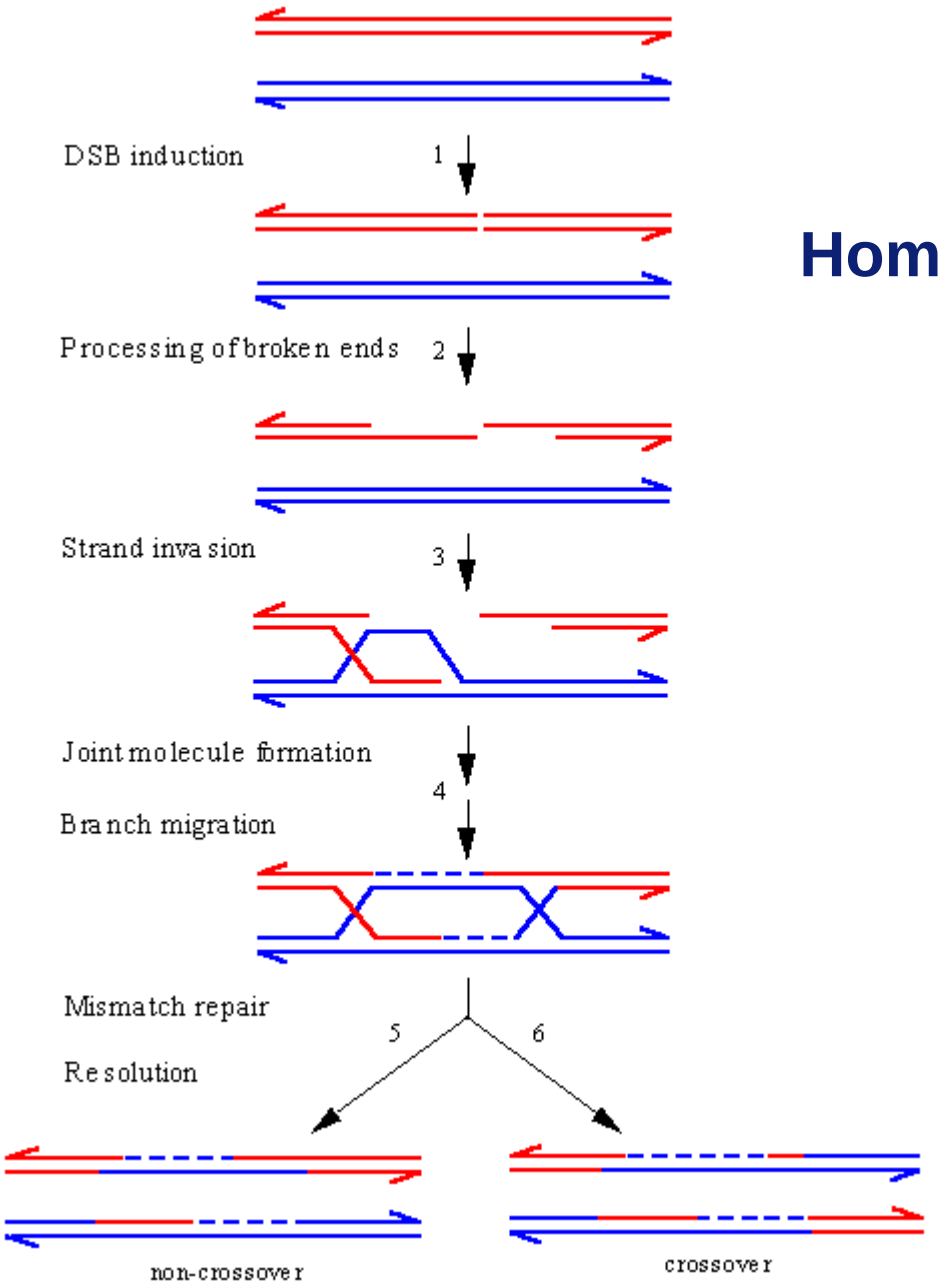
- Generation of:
  - Mutations
  - (large) deletions
  - Integrations (reporters, tags)
  - Activation/repression of transcription



Non homologous end joining (NHEJ)

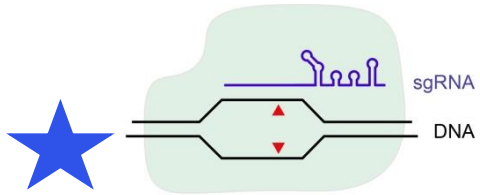


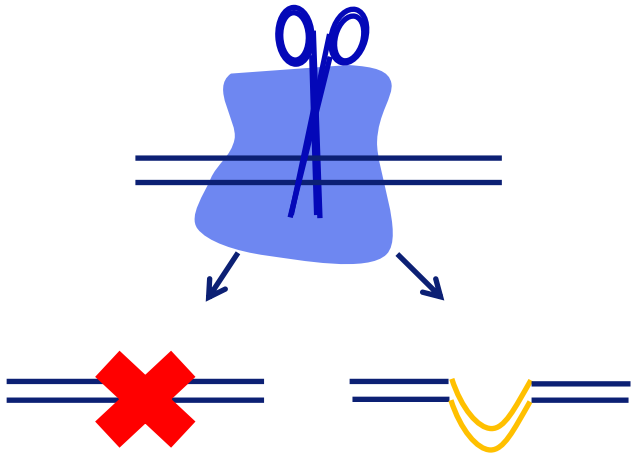
Homology directed repair (HDR)

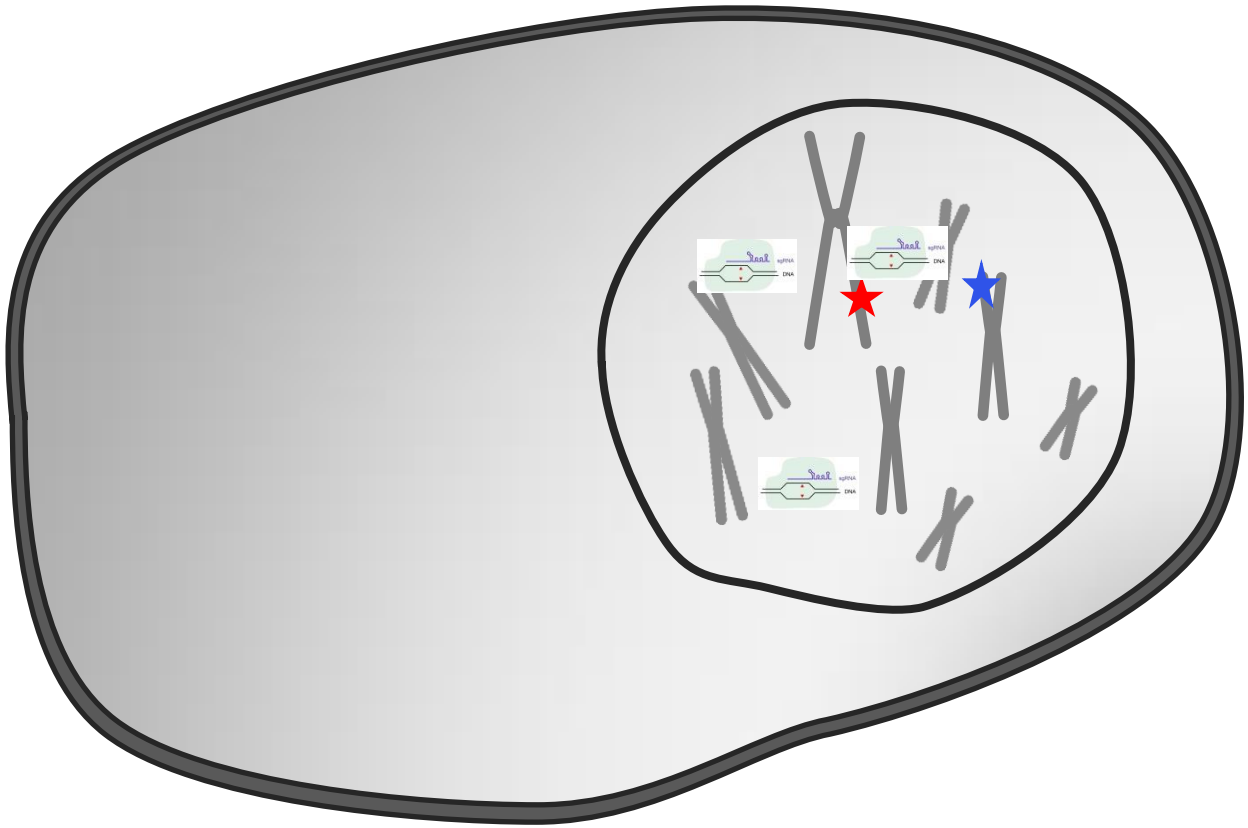


What to think of when you design your experiment

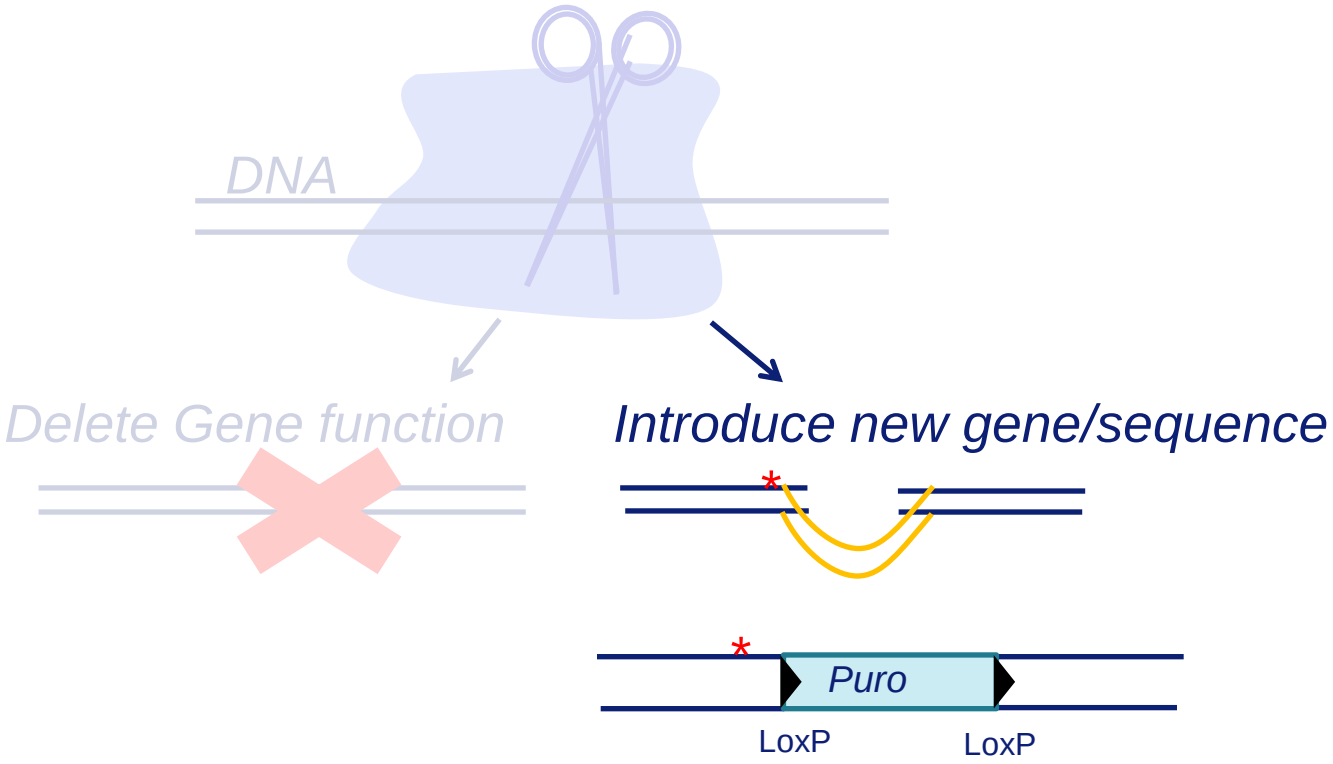
- Cas9 delivery
- Off target effects
- Repairable cell
- Editing efficiency



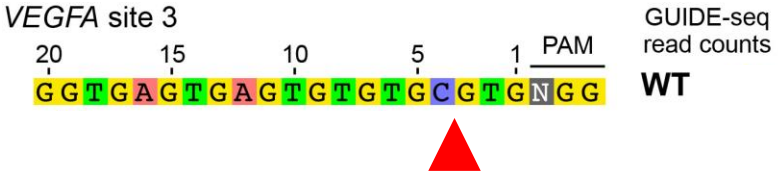




Generating a patiënt specific mutation



Off target effects



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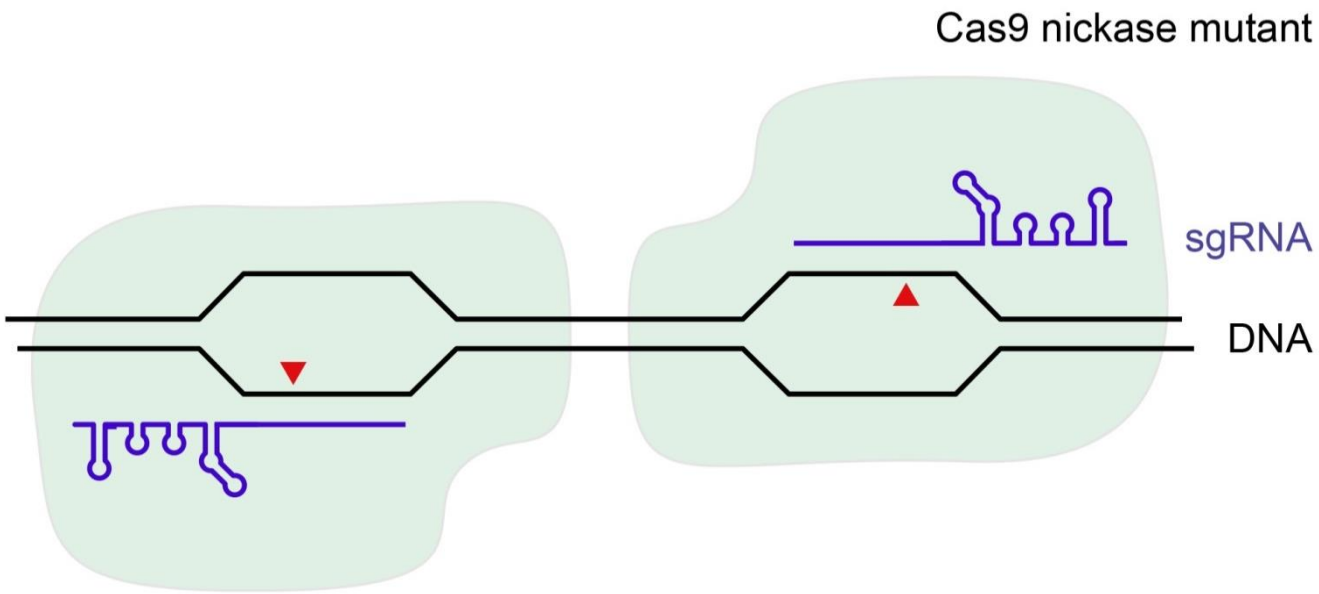
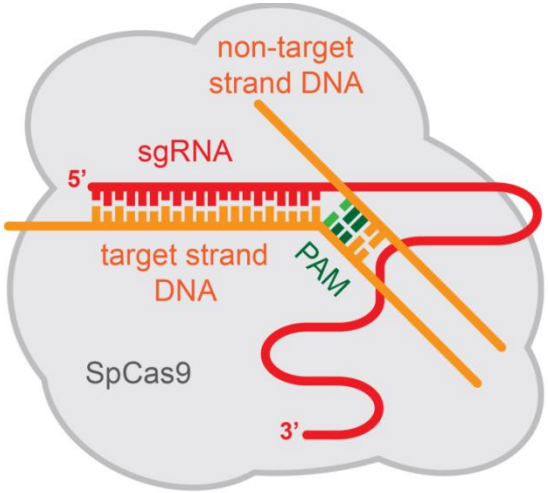
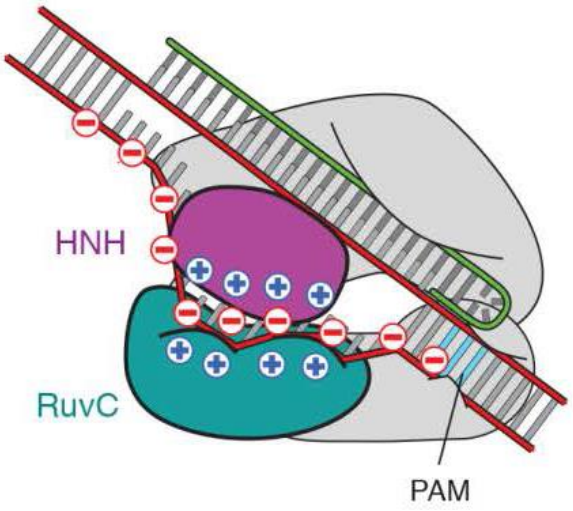
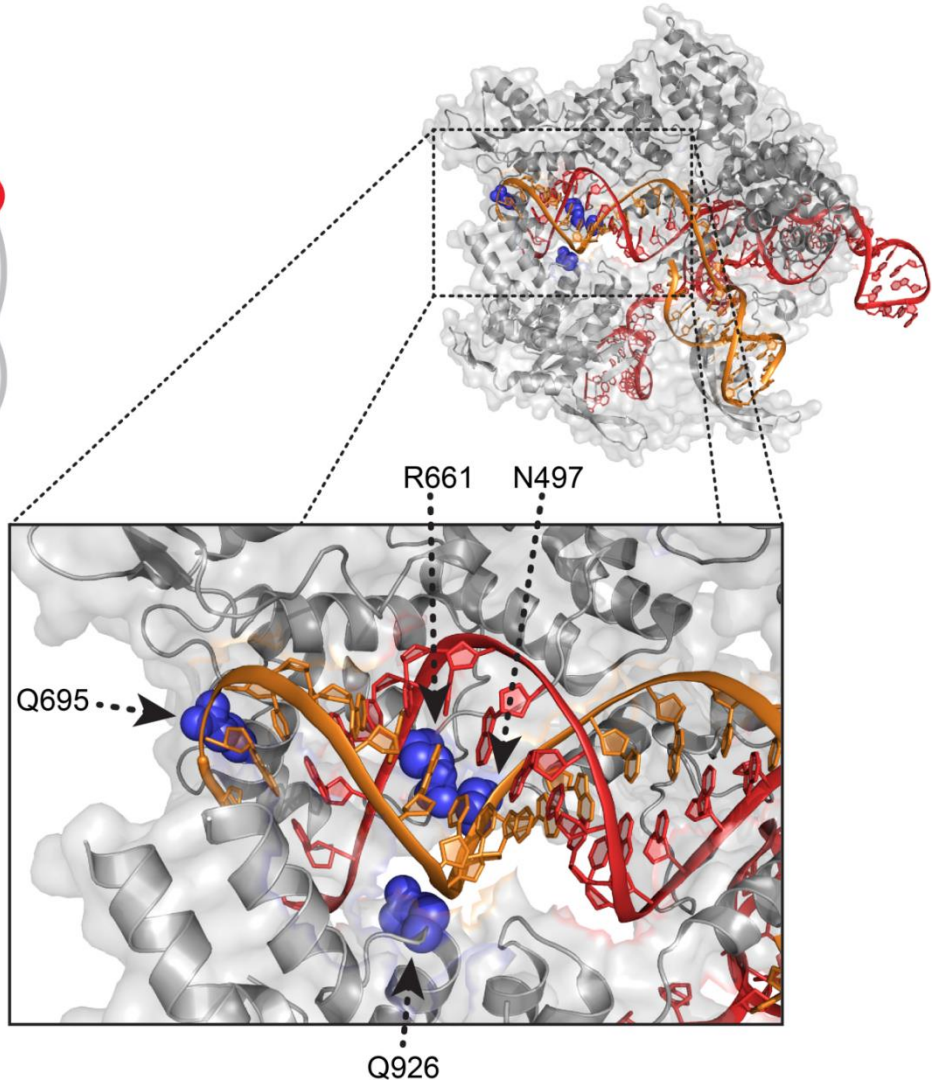
How to make CRISPR/Cas9 more specific?





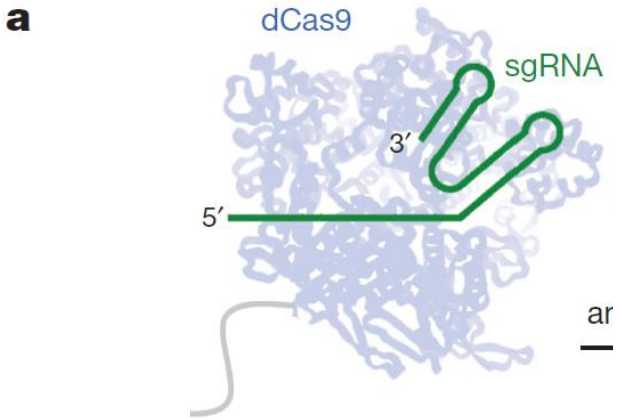


Slaymaker, Science, 2015 (eSpCas9)



Kleinstiver, Nature, 2016 (HF-Cas9)

Base editing



Komor et al., Nature, 2016 (Cytidine deaminase) C>T
Gaudelli et al., Nature in press (deoxyadenosine deaminase) A > G

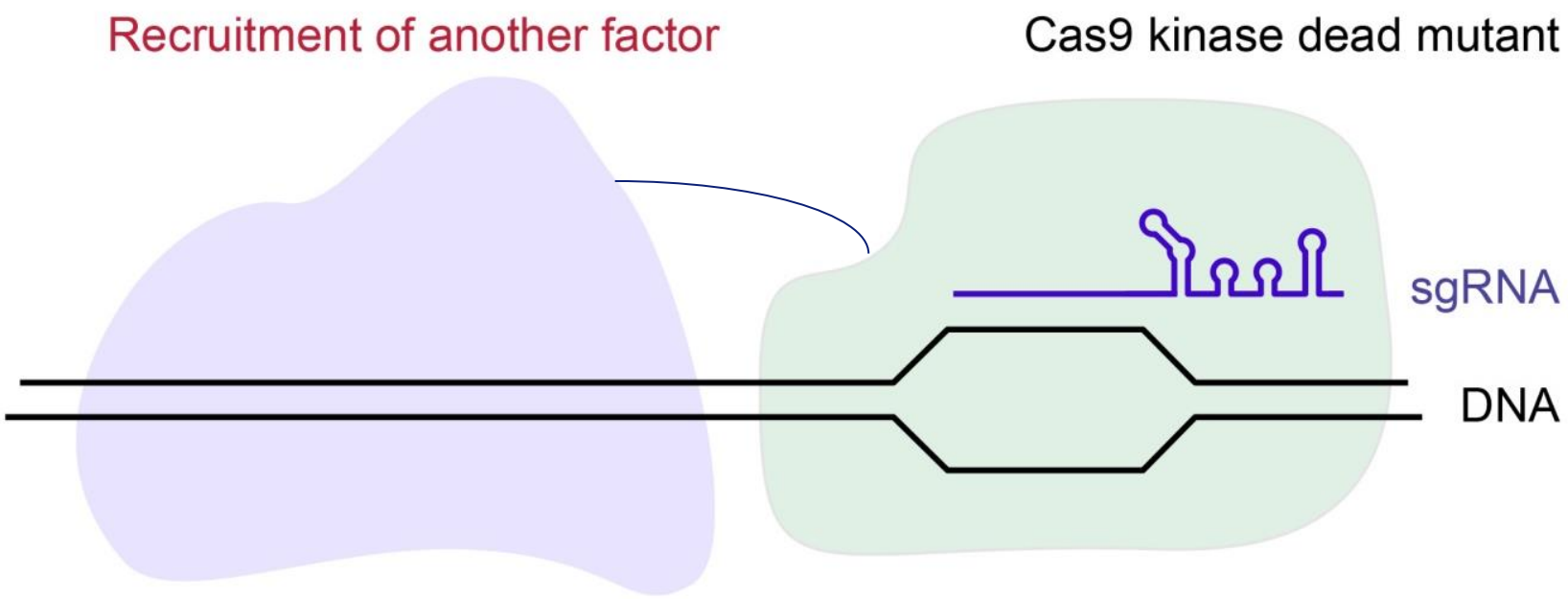
How to deal with off-target effects

- Check your clone with NGS
- Redesign your guide
- HF or eCas9
- Nickase Cas9

- Use multiple clones or multiple guides
- Use a hit and run method (ribonuclearprotein transfection)
- Backcross your mouse line

- When Cas9 is loaded, fewer off-targets!

- Modification of gene expression
  - Activation (CRISPRa)
  - Repression (CRISPRi)



How it works in the lab

Visit cgec.erasmusmc.nl for a detailed protocol

- Make sure your target sequence is what you think
  - www.ensembl.org (and sequence verify)
- Design your guide (GG-18N-NGG)
  - crispr.mit.edu/
  - chopchop.rc.fas.harvard.edu/
- Clone your guide into proper Cas9 expression vector
- Transfect your cells
  - Cas9 is large, make sure you get ~90% efficiency with a GFP control vector
- Pick and screen clones by PCR/sequence verification