CRISPR/Cas and Genome Editing

From Molecule to Business - Drug Discovery re-invented

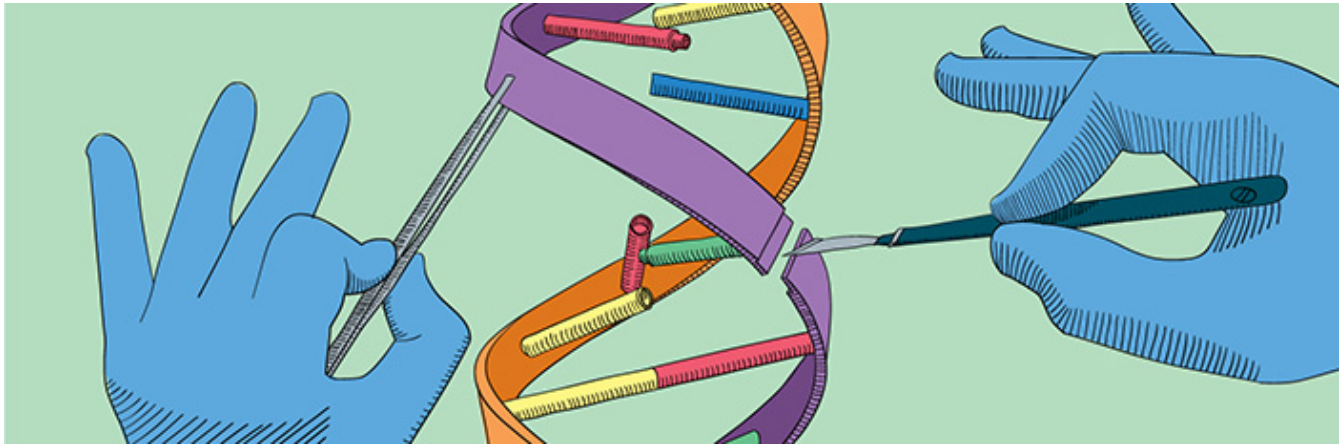
Pivot Park, Oss - Thursday 30 Sept 2021

Rick Wansink, PhD
Dept. of Cell Biology
rick.wansink@radboudumc.nl

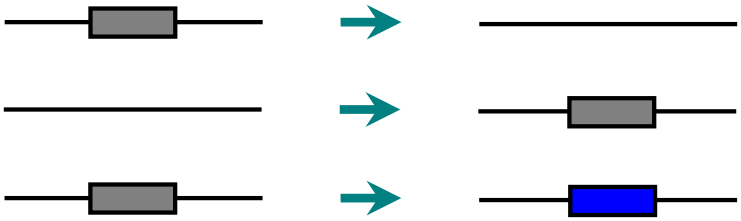
Institute for Molecular Life Sciences
Radboudumc

Genome editing – Gene editing – Genome surgery

…. is a type of genetic engineering in which DNA is inserted, deleted, modified or replaced in the genome of a living organism.

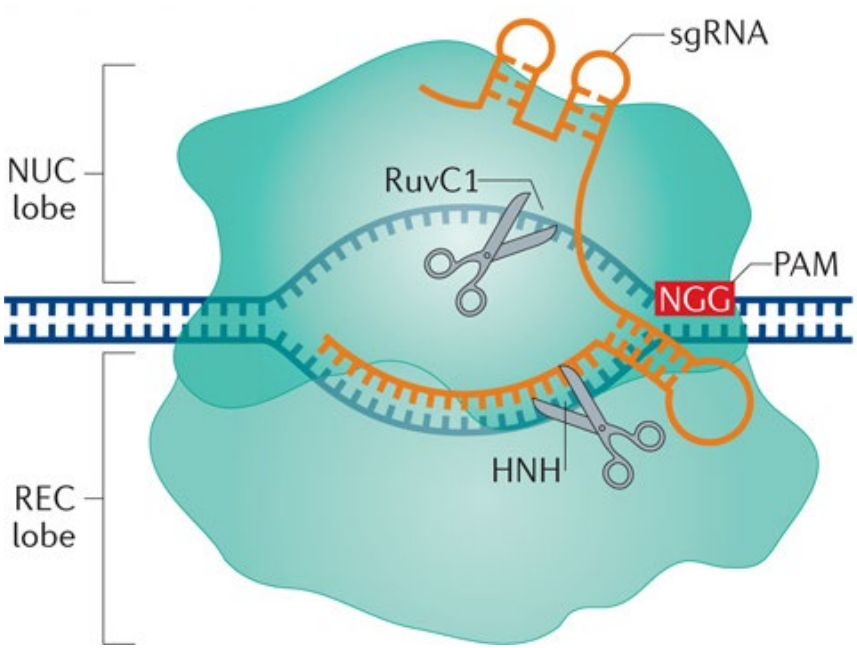


- Loss
- Gain
- Replacement



CRISPR: Clustered Regularly Interspaced Short Palindromic Repeats
Cas9: CRISPR-associated system

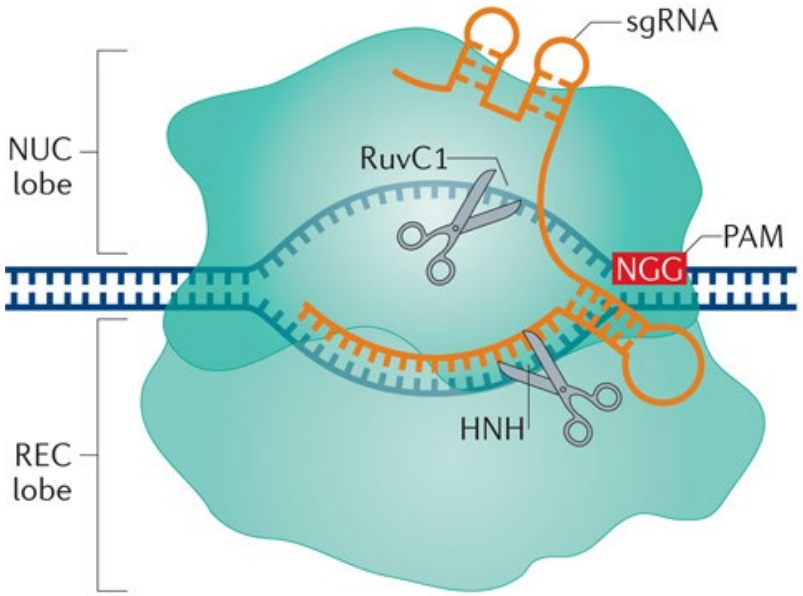
Nobel Prize in Chemistry 2020

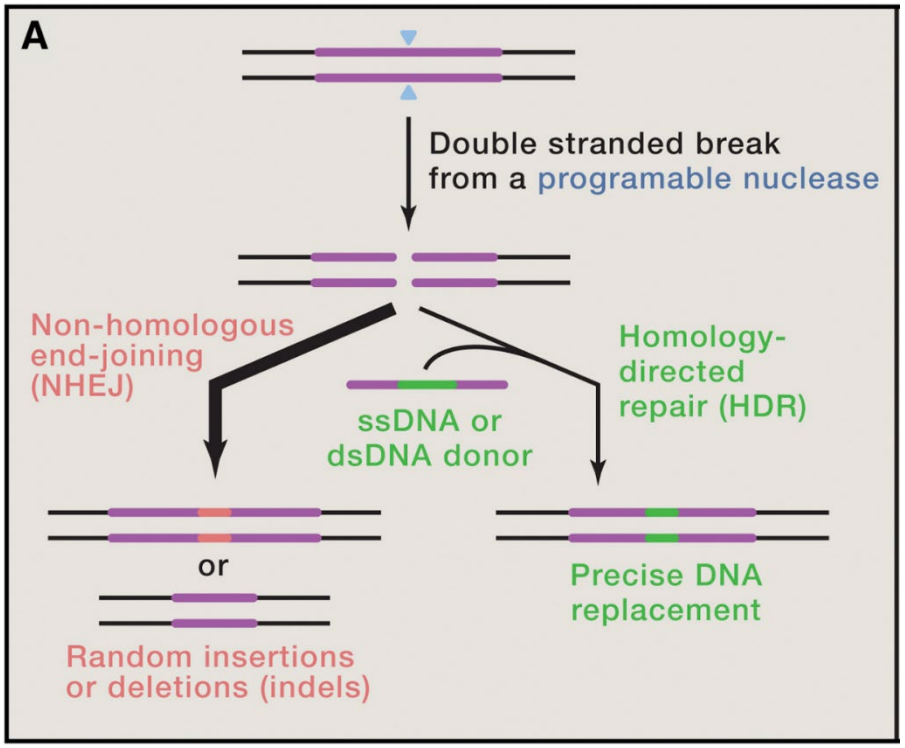


- Efficient
- Active
- Precise
- Cheap
- Reliable
- Versatile
- Easy to implement

Dominguez et al. Nat. Rev. Mol. Cell Biol. 2016

A CRISPR/Cas-induced double strand break must be repaired

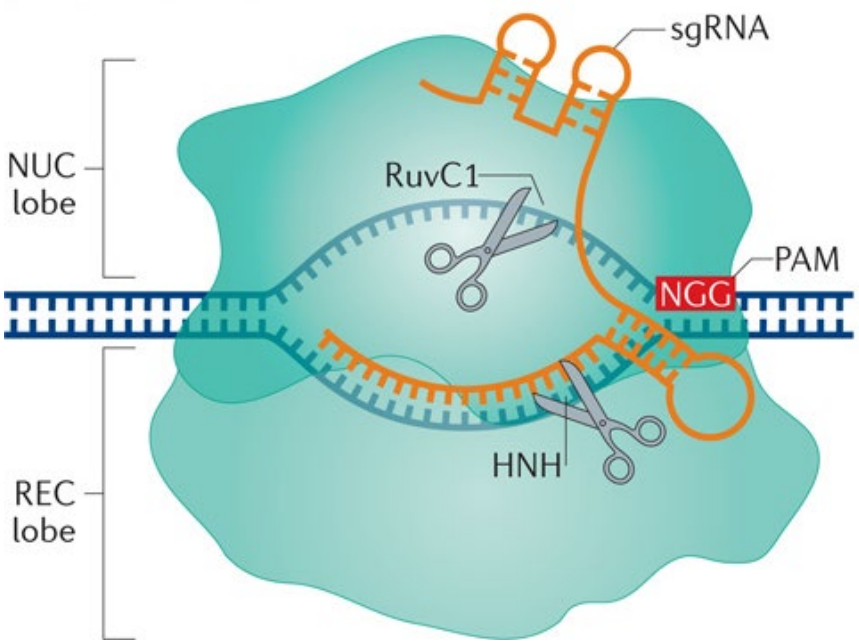




common, error prone, unpredictable	rare (only in S/G2) precise, reliable
NHEJ	**HDR**
indels	*replacement*

Dominguez et al. Nat. Rev. Mol. Cell Biol. 2016; Komor et al. Cell, 2017

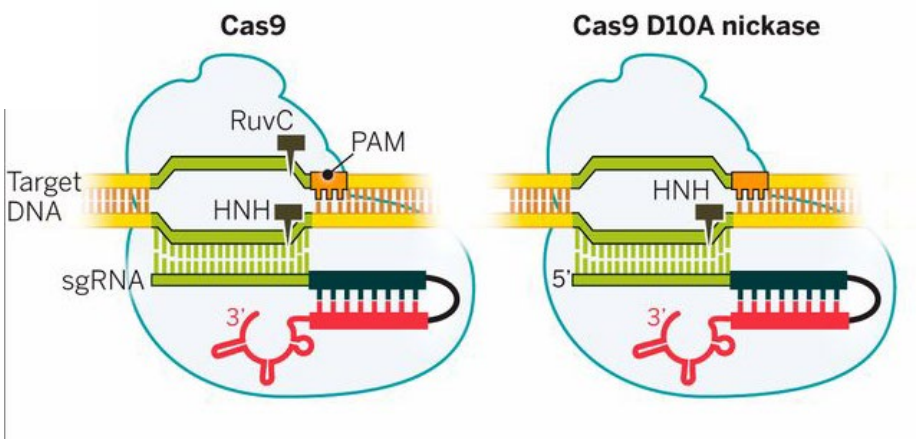
Common difficulties and pitfalls of CRISPR/Cas



- Off-target effects
- Low incidence HDR
- Delivery (plasmid, RNPs, virus, nanoparticles)
- Cutting efficiency
- GuideRNA design (PAM sequence)
- Immunogenicity of Cas9
- Risks of germline editing (ethics)

Dominguez et al. Nat. Rev. Mol. Cell Biol. 2016

An explosion of possibilities: CRISPR/Cas variations

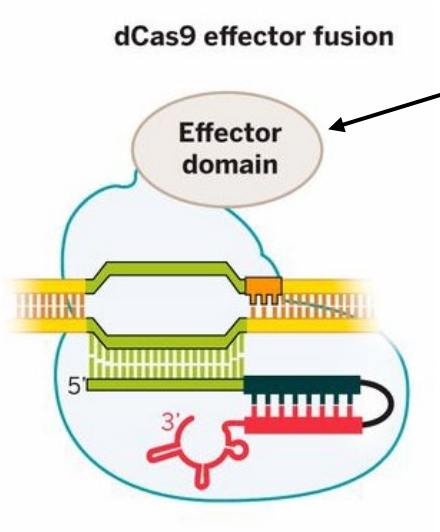


- Ds versus ss break
- Different PAM sequences
- Different cut sites
- Smaller Cas enzymes
- Cas13: targets RNA instead of DNA

- Prime editing: nickase + reverse transcriptase
- Base editing: nickase + base editor (point mutations)

Doudna and Charpentier, Science, 2014

dCas: Your guide to the genome

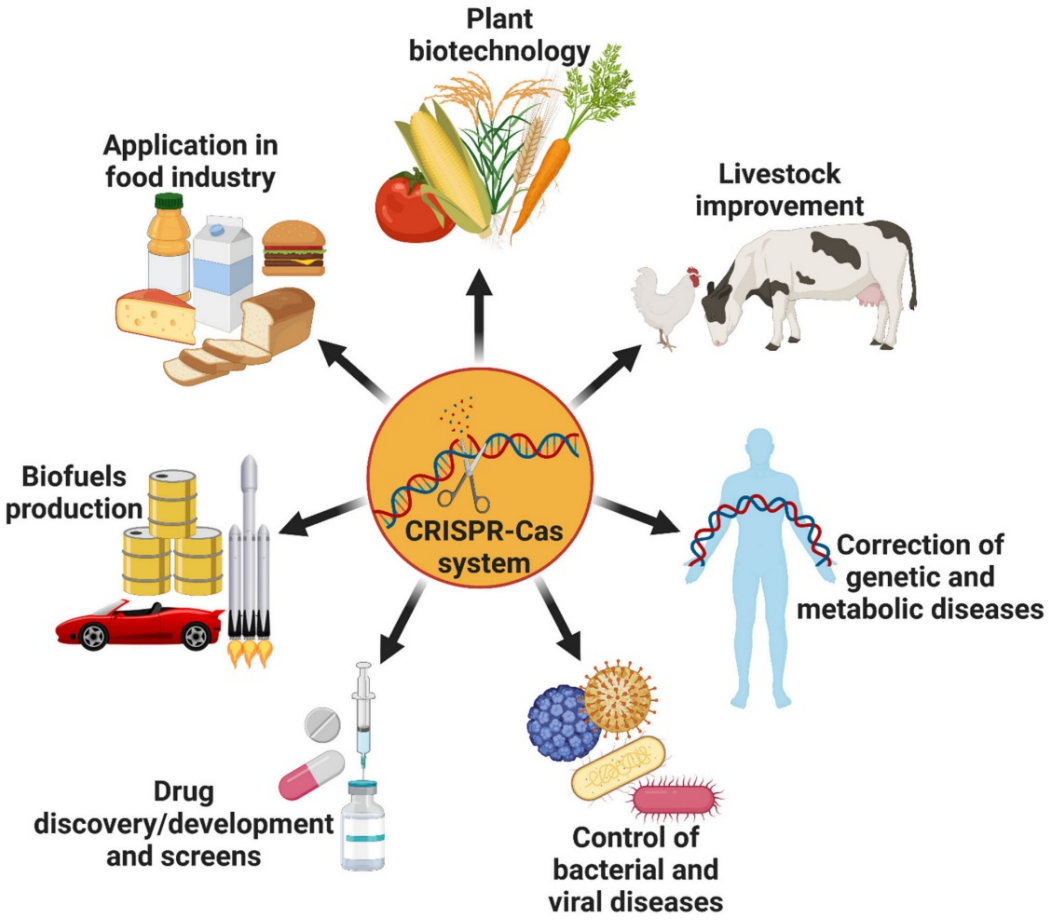


- Transcriptional activators
- Transcriptional repressors
- Epigenetic enzymes
- Fluorescent labels
- Small tags
- Etc.

- Note: permanent vs transient effects

Doudna and Charpentier, Science, 2014

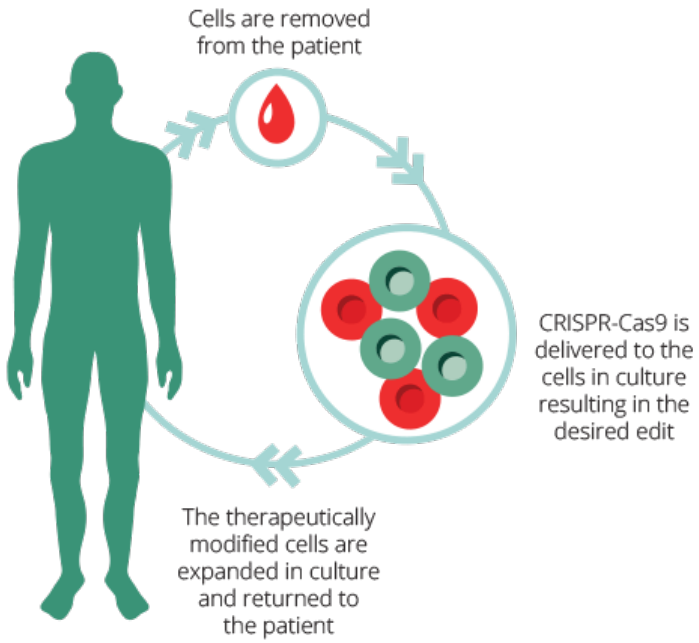
Applications



- Knockout genes in your favourite cell type
- Development of disease models (e.g. iPSCs, animals)
- Unbiased genome-scale screens
- Diagnostics (cleavage- or binding-based Cas biosensing assays)
- Therapeutic strategies *ex/in vivo*

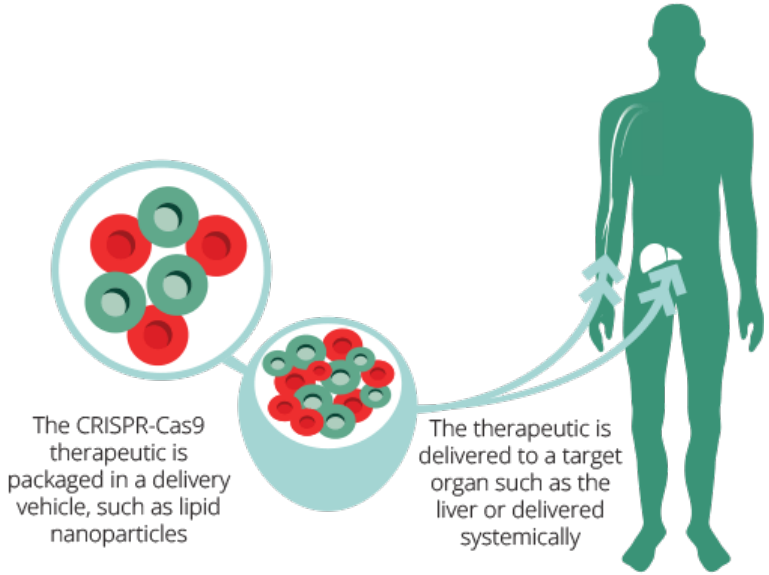
Responsible gene editing in humans

Ex vivo



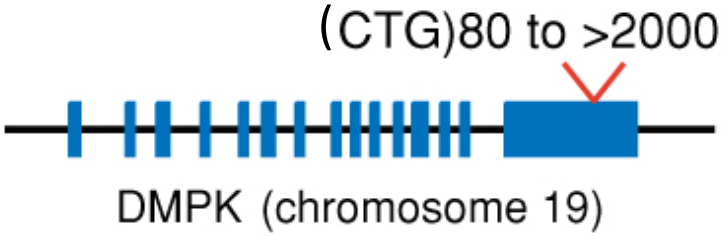
- T cells in cancer patients
  multiple trials ongoing
- β-thalassemia, sickle cell disease
  trials ongoing

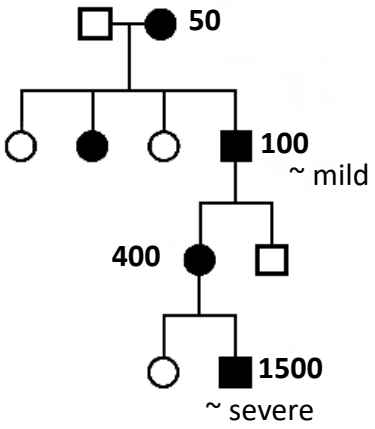
In vivo



- Transthyretin amyloidosis (*NEJM 2021)*
- Leber's congenital amaurosis (eye disease)
  removal of a point mutation in *CEP290*)
  first patient treated in April 2020
- Mucopolysaccharidosis type II
  trial ongoing?

Own research: RNA toxicity in myotonic dystrophy type 1



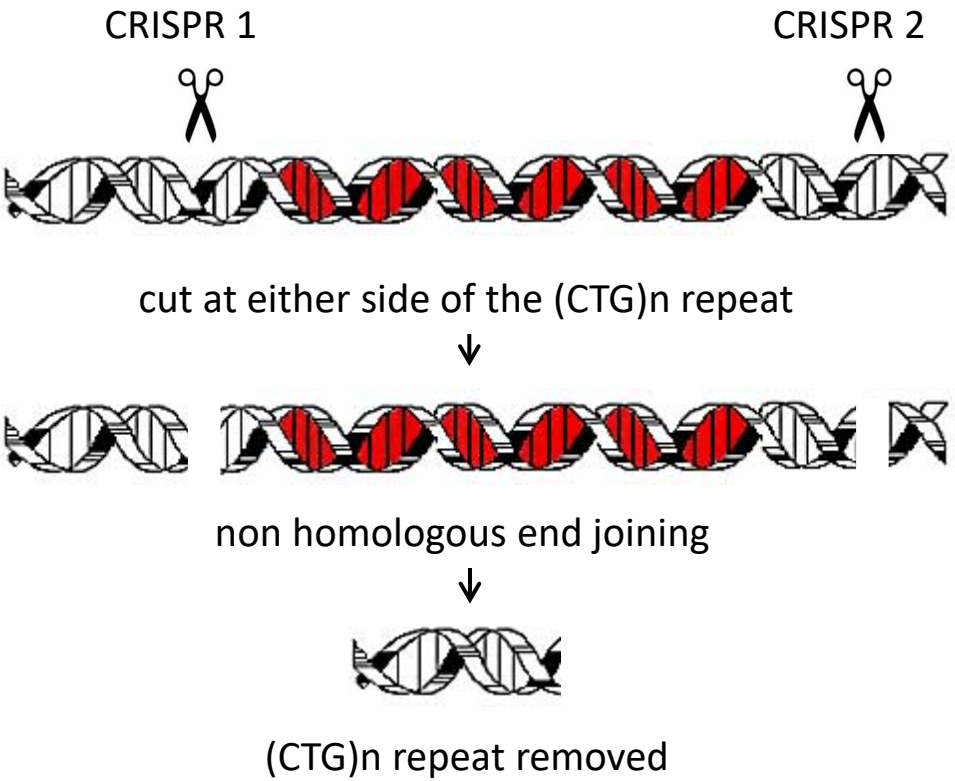


anticipation

I = normal
II = pre-mutation/late onset
III = adult onset/childhood onset
IV = congenital

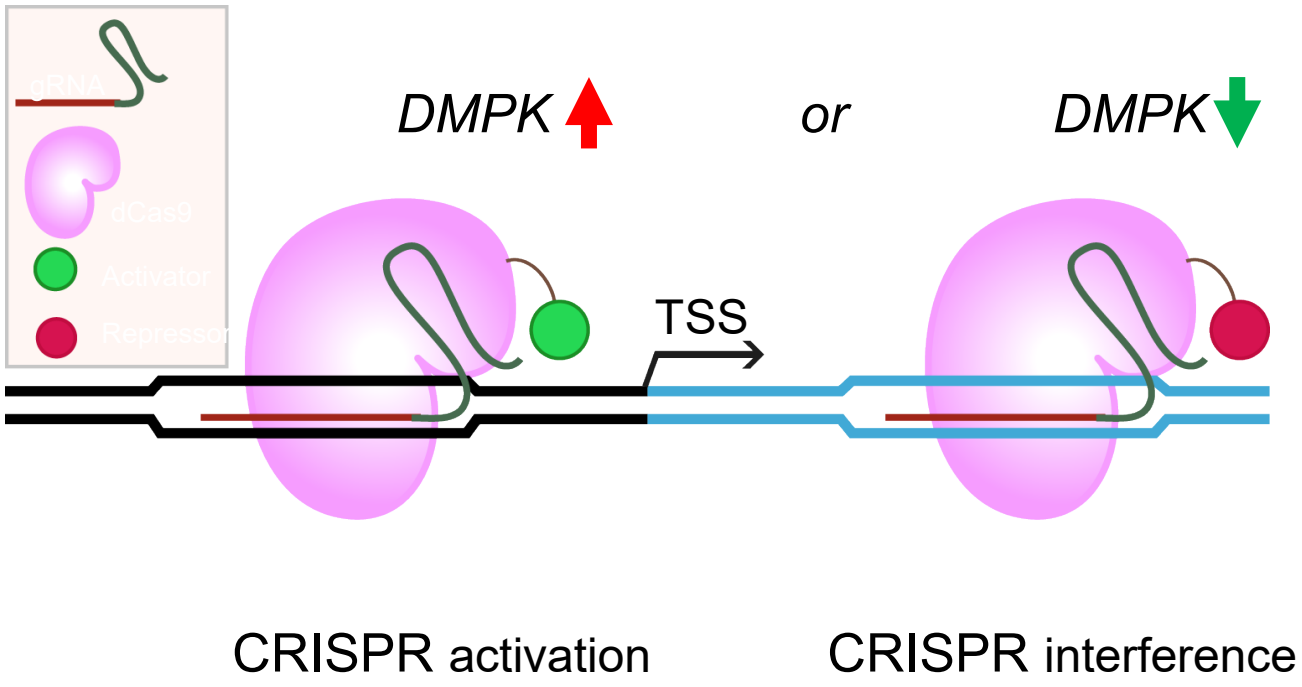
- Repeat in 3' UTR, so does **not** affect *DMPK* protein-coding information
- RNA-mediated disease mechanisms -> **expanded (CUG)n RNA is toxic to cells**

(1) Remove expanded (CTG)n repeat through dual CRISPR/Cas9 cleavage

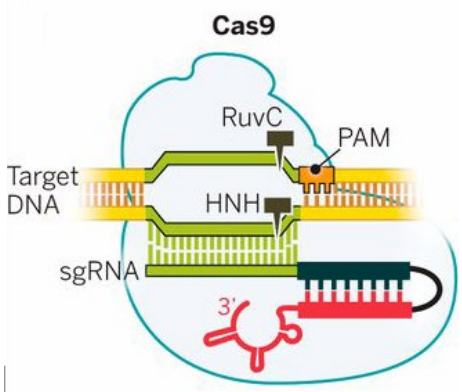


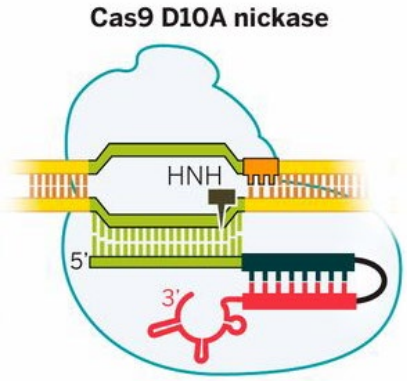
Van Agtmaal et al. Mol Therapy, 2017; André et al. IJMS, 2019

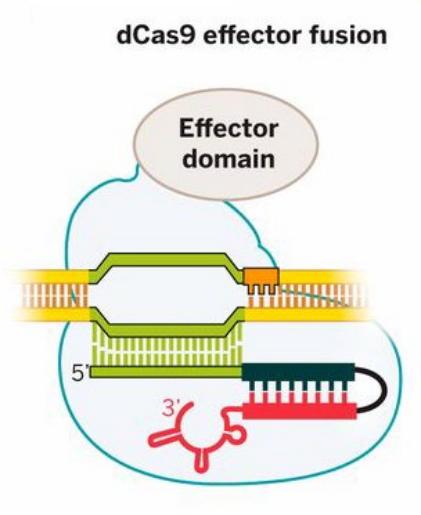
(2) Silence *DMPK* transcription initiation by dCas9-repressor fusion protein

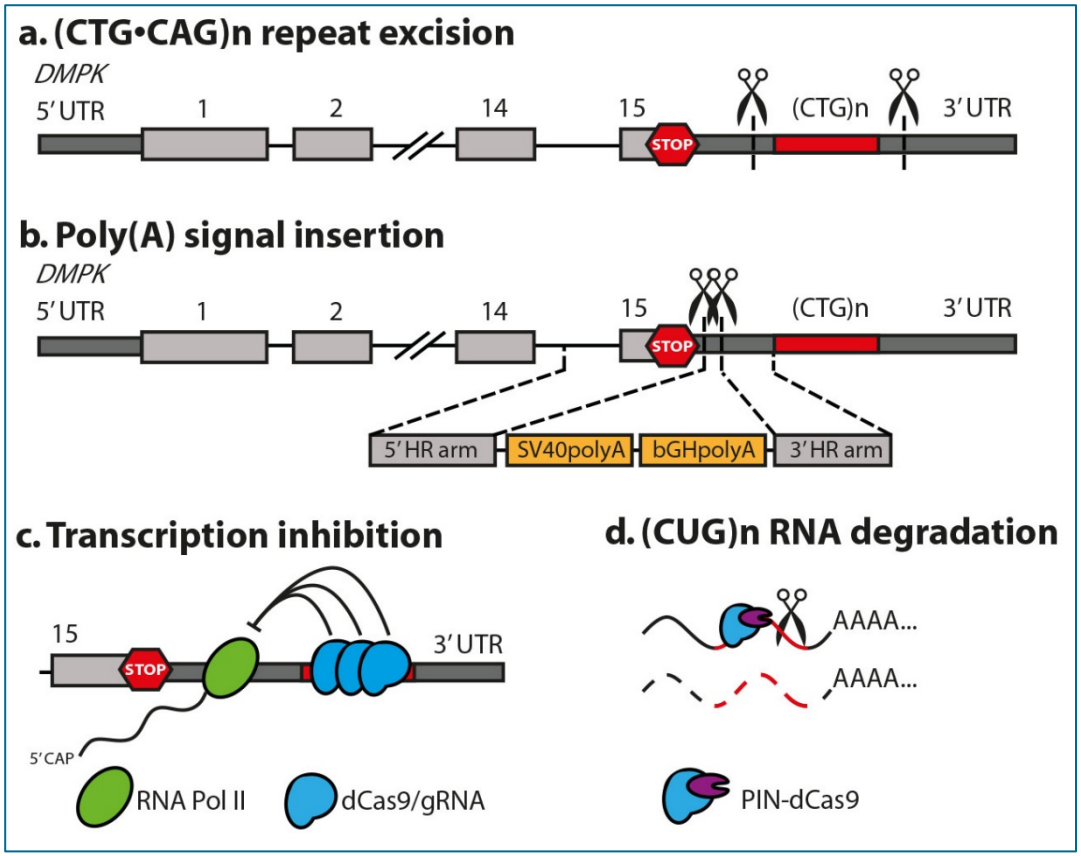


Ripken et al. unpublished









Raaijmakers et al. IJMS, 2019

Rick Wansink, PhD
Dept. of Cell Biology
rick.wansink@radboudumc.nl

Institute for Molecular Life Sciences
Radboudumc