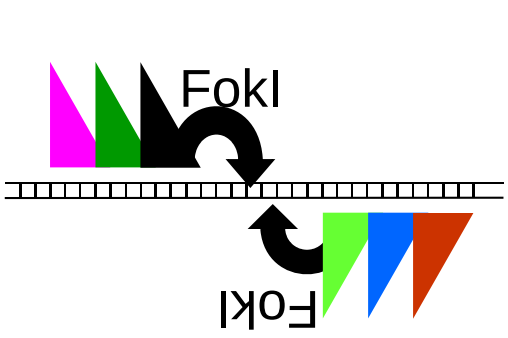
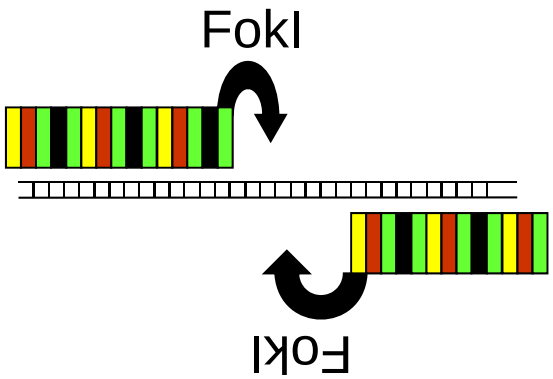
# Programmable Nucleases for Genome Editing



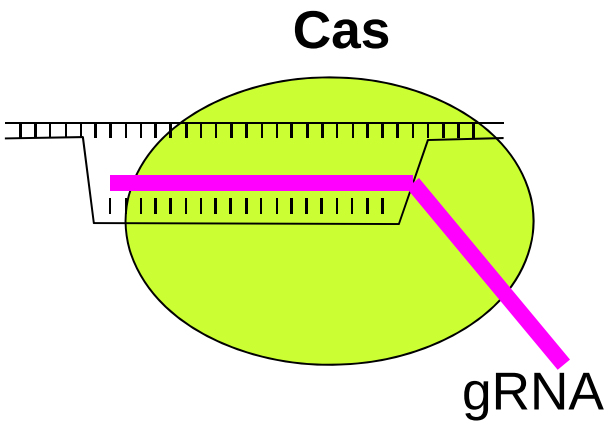

ZFN (1996)
**Code: 1 ZF motif / 3bp**

**Dow AgroSciences**
**Exzact Precision Technology**




TALEN (2010)
**Code: 1 RVD motif / 1bp**

**Science Breakthroughs 2012**



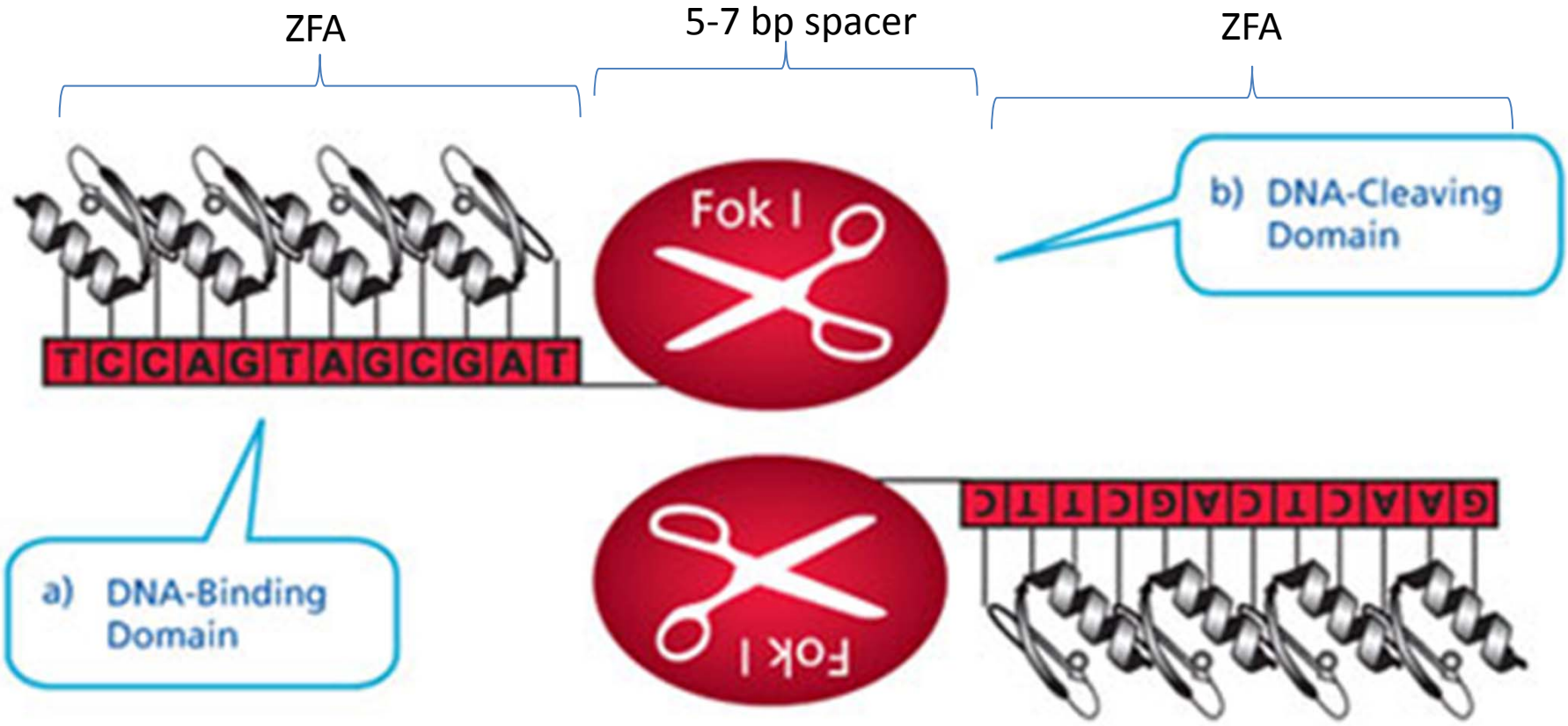

RGN (2012)
**Code: RNA-DNA, 1base / 1bp**

**Science Breakthroughs 2013**

**Program nuclease based on DNA binding specificity of zinc fingers and TAL effectors**

**Program nuclease according to RNA:DNA base pairing**

--Slide courtesy Dr. Yinong Yang




---From Sigma catalog

ZFN (1996)

**Code: 1 ZF motif / 3bp**

**In practice, need 4-6 ZF motifs in tandem array (ZFA) for good site specificity on each side of cut site.**

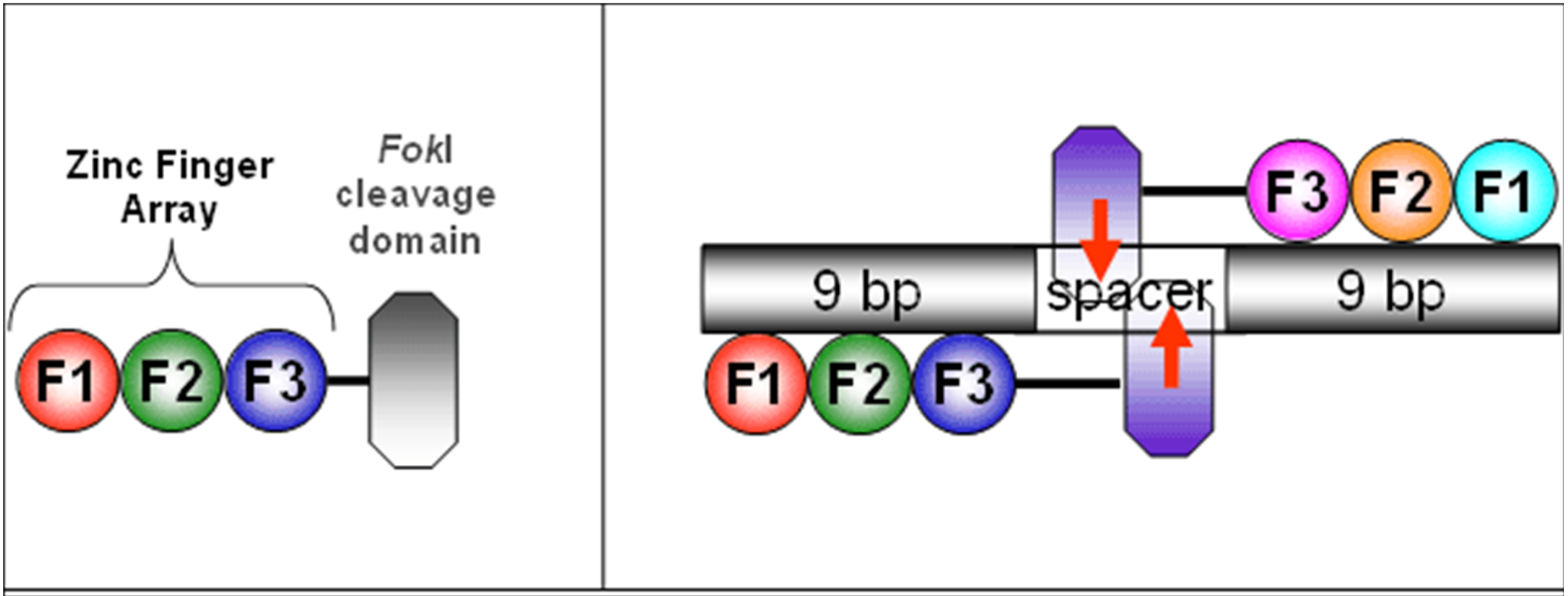
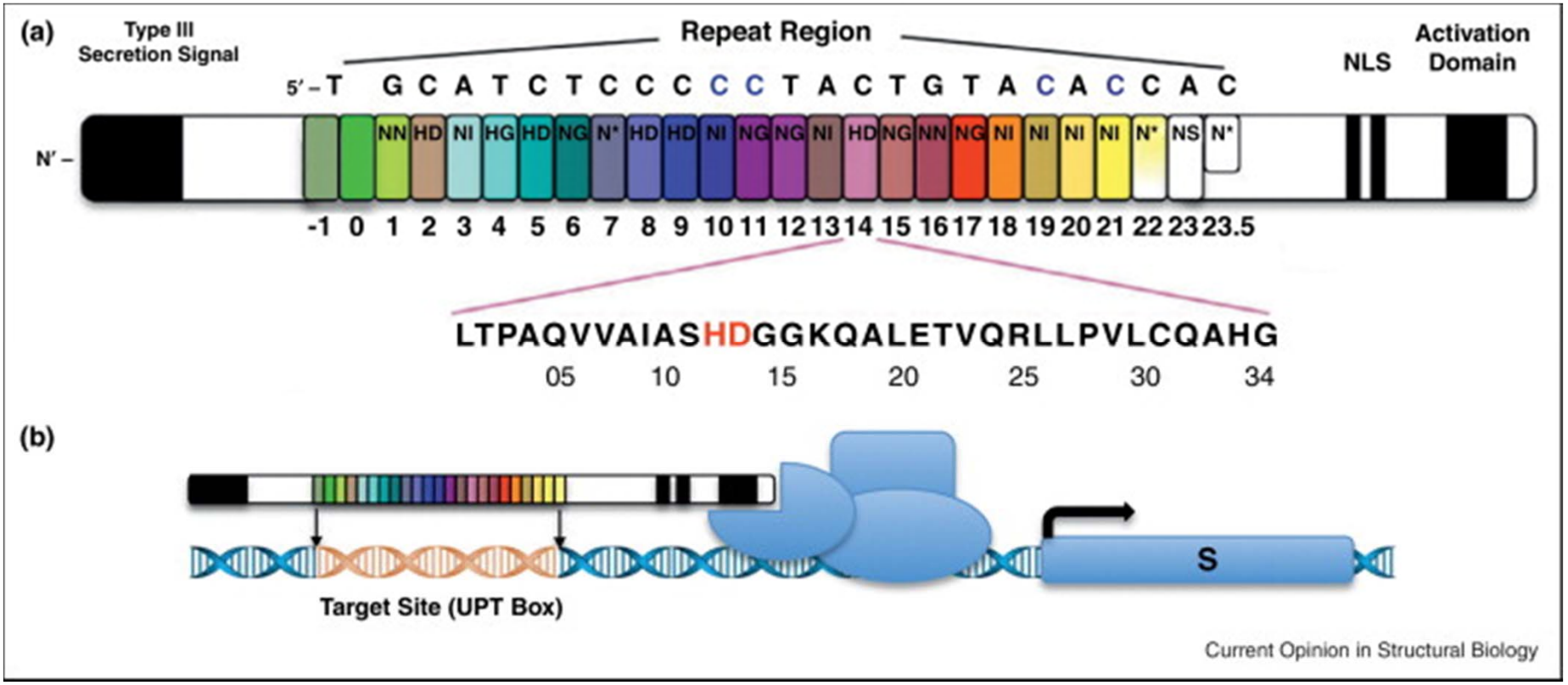
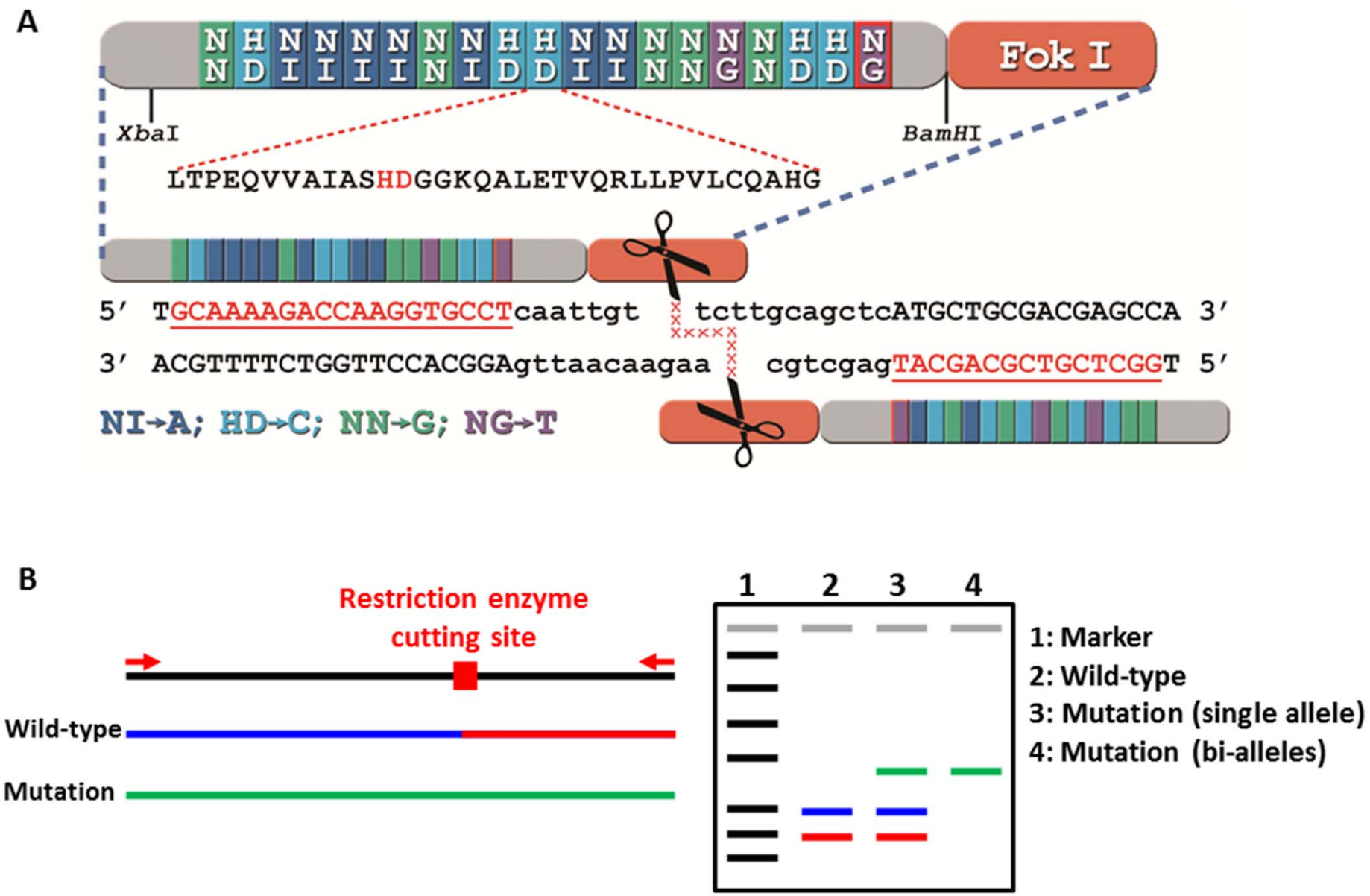



Figure 2 Schematic of zinc finger nucleases. Left panel shows structure of a three-finger ZFN. Right panel shows a dimer of three-finger ZFNs bound to their target cleavage site. Note that the "spacer" sequence shown is typically 5 to 7 base pairs in length. Red arrows indicate cleavage points on the DNA.

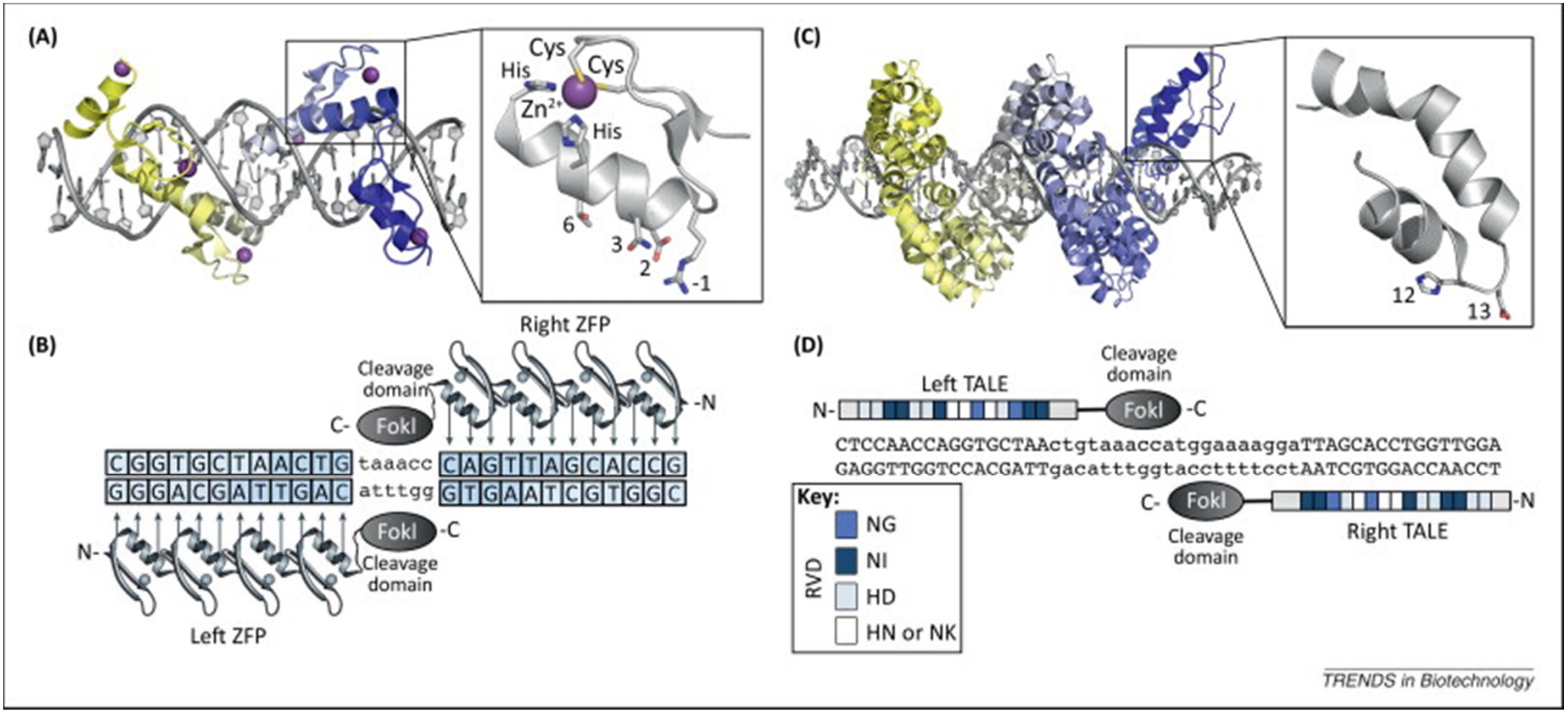
**---http://www.addgene.org/**




--Mak et al. 2013. Cur. Op. Struct. Bio. 23:93–99




--Shan et al. 2013. *Mol. Plant 6 : 1365-1368.*



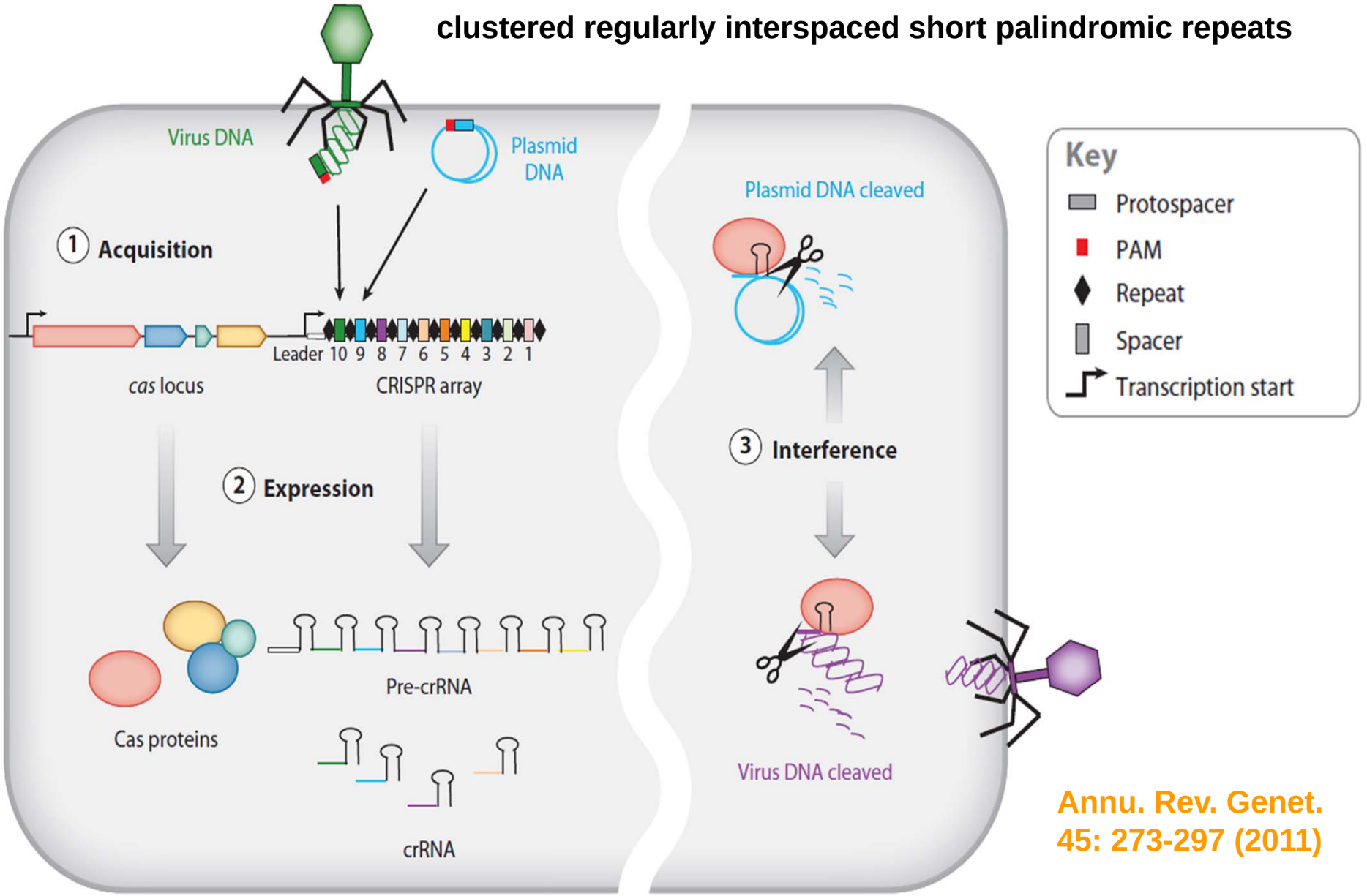

**Need to choose a target in a stretch of DNA?**

**Use TAL-Effector Nucleotide Targetter 2.0**

**---https://tale-nt.cac.cornell.edu**

# Bacterial CRISPR-Cas Adaptive Immune System

**clustered regularly interspaced short palindromic repeats**




Annu. Rev. Genet.
45: 273-297 (2011)

# First Publication on CRISPR/Cas9 Genome Editing

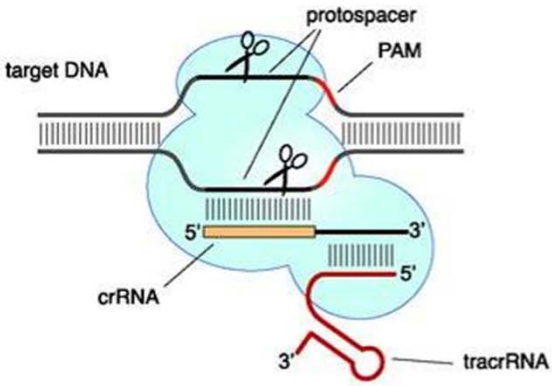
## A Programmable Dual-RNA–Guided DNA Endonuclease in Adaptive Bacterial Immunity

Martin Jinek,[1,2]* Krzysztof Chylinski,[3,4]* Ines Fonfara,[4] Michael Hauer,[2]†
Jennifer A. Doudna,[1,2,5,6]‡ Emmanuelle Charpentier[4]‡

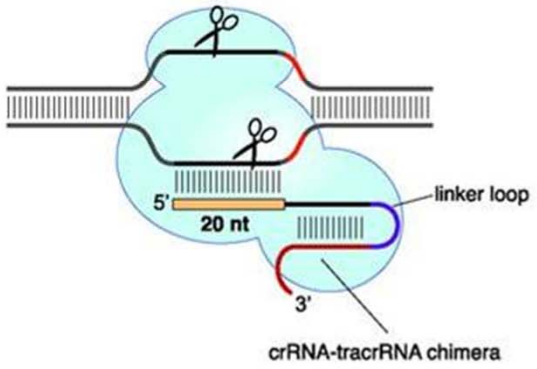
Clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) systems provide bacteria and archaea with adaptive immunity against viruses and plasmids by using CRISPR RNAs (crRNAs) to guide the silencing of invading nucleic acids. We show here that in a subset of these systems, the mature crRNA that is base-paired to trans-activating crRNA (tracrRNA) forms a two-RNA structure that directs the CRISPR-associated protein Cas9 to introduce double-stranded (ds) breaks in target DNA. At sites complementary to the crRNA-guide sequence, the Cas9 HNH nuclease domain cleaves the complementary strand, whereas the Cas9 RuvC-like domain cleaves the noncomplementary strand. The dual-tracrRNA:crRNA, when engineered as a single RNA chimera, also directs sequence-specific Cas9 dsDNA cleavage. Our study reveals a family of endonucleases that use dual-RNAs for site-specific DNA cleavage and highlights the potential to exploit the system for RNA-programmable genome editing.

SCIENCE VOL 337 17 AUGUST 2012

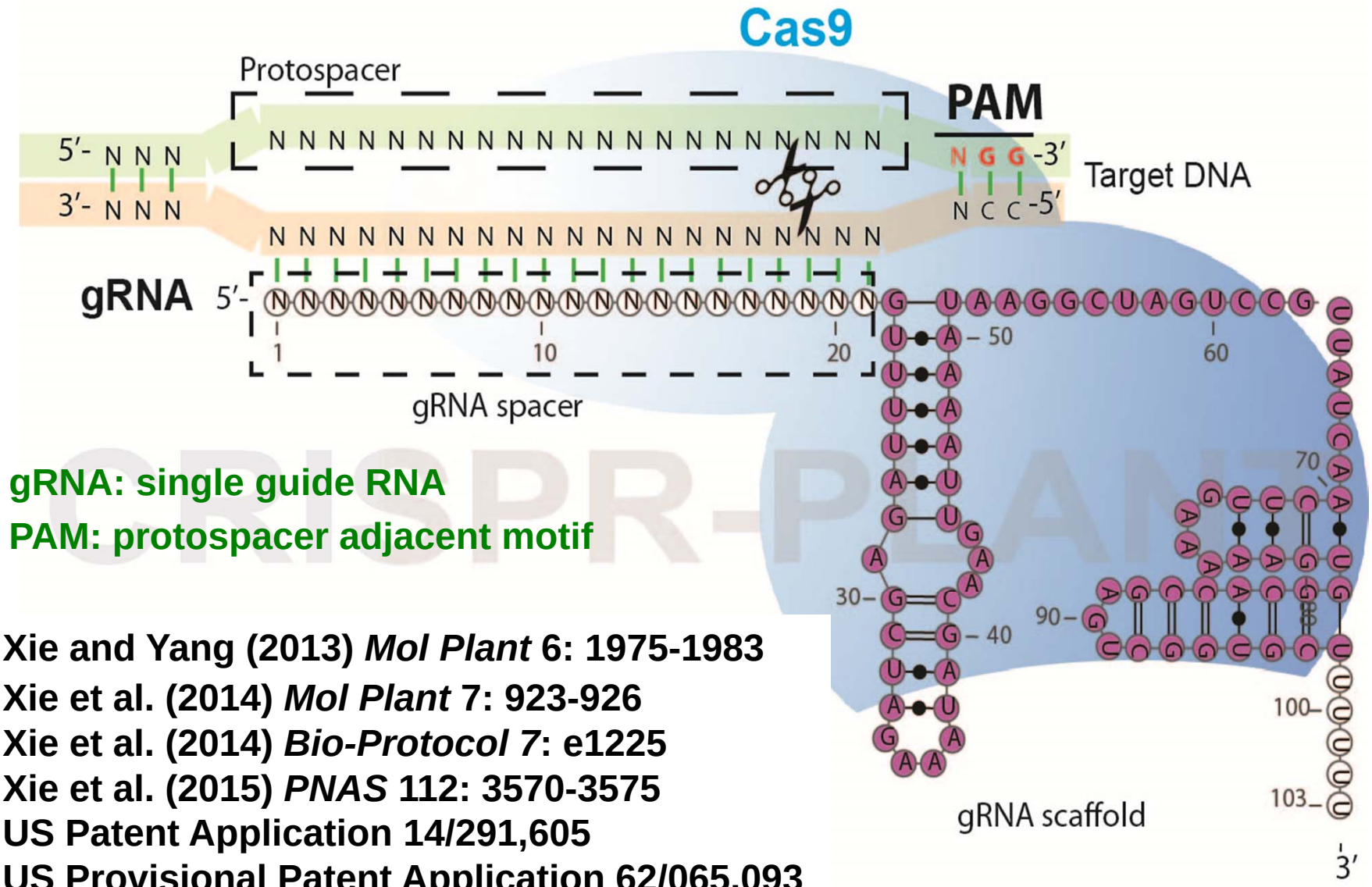
Cas9 programmed by crRNA:tracrRNA duplex




Cas9 programmed by single chimeric RNA



# Engineering CRISPR/Cas9 for Plant Genome Editing




**gRNA: single guide RNA**

**PAM: protospacer adjacent motif**


**Xie and Yang (2013) *Mol Plant* 6: 1975-1983**

**Xie et al. (2014) *Mol Plant* 7: 923-926**

**Xie et al. (2014) *Bio-Protocol 7*: e1225**

**Xie et al. (2015) *PNAS* 112: 3570-3575**

**US Patent Application 14/291,605**

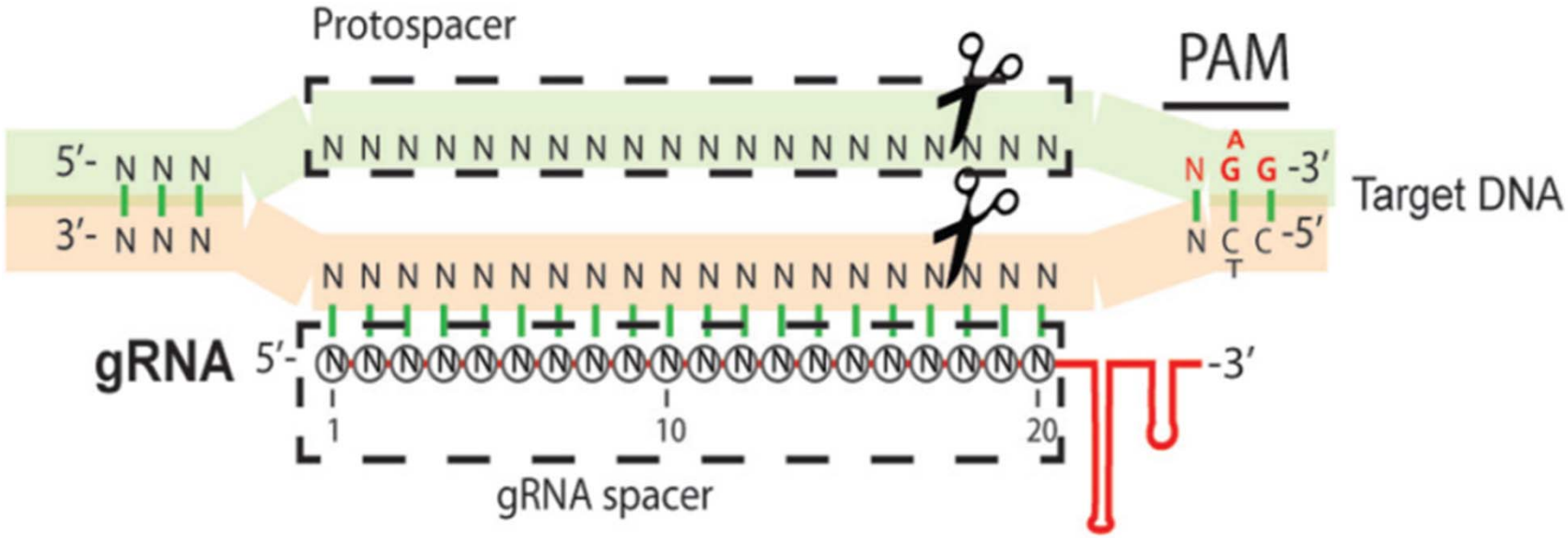
**US Provisional Patent Application 62/065,093**

# CRISPR/Cas9 Editing Specificity

## Specificity/off-target determinants:

1. Cas9 protein (some variants may be more specific)
2. Cas9 concentration in the cell
3. Cas9 exposure time or duration (transient vs. stable)
4. DNA sequences of genomic sites

## Selection of specific gRNA spacer



Note: If provide no repair template, get mutation via NHEJ (non homologous end joining).

Note: If provide repair template, do **not** get double CO, as shown. Instead, likely get SDSA, initiated at the DSB on the cut chromosome.

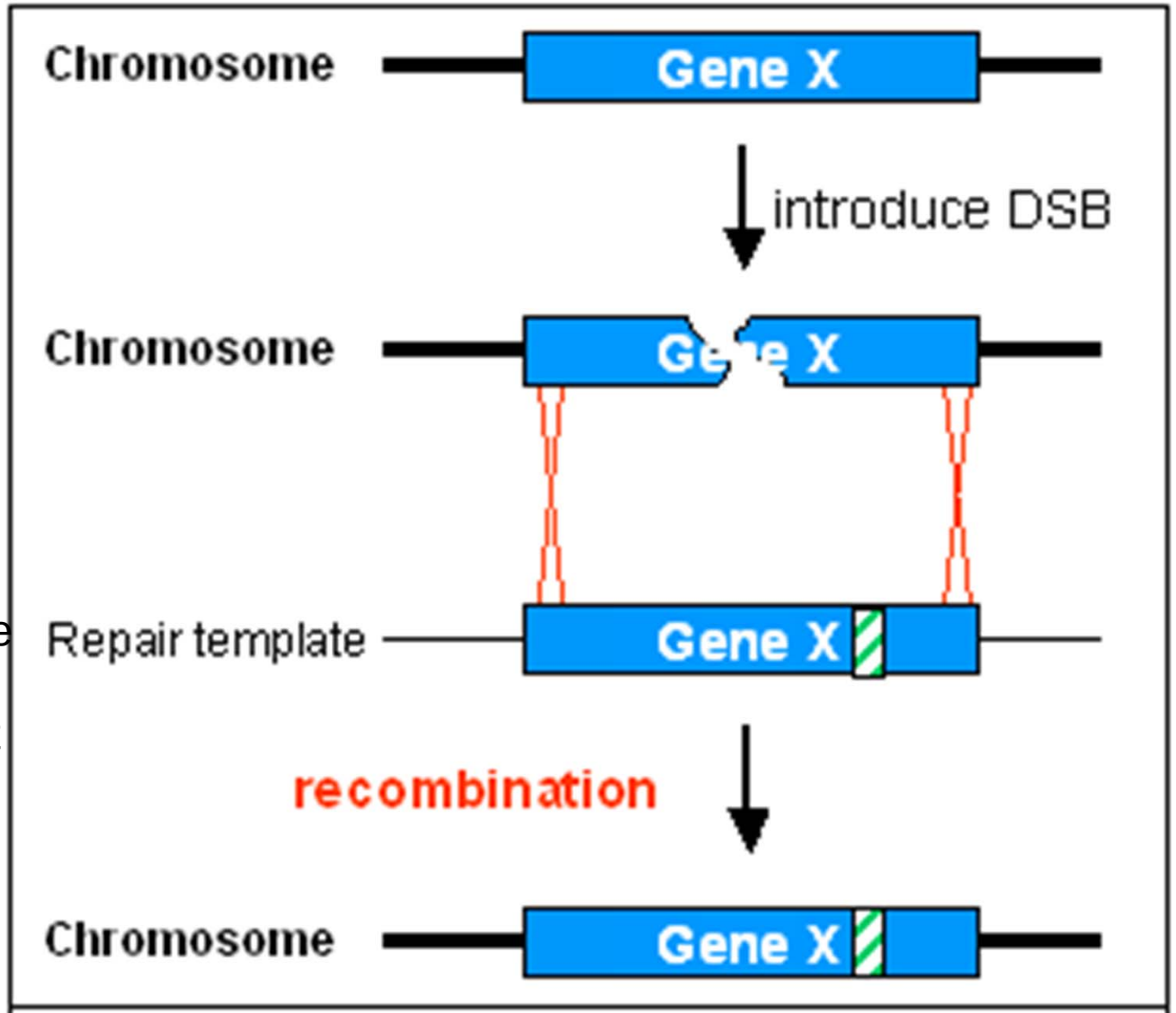
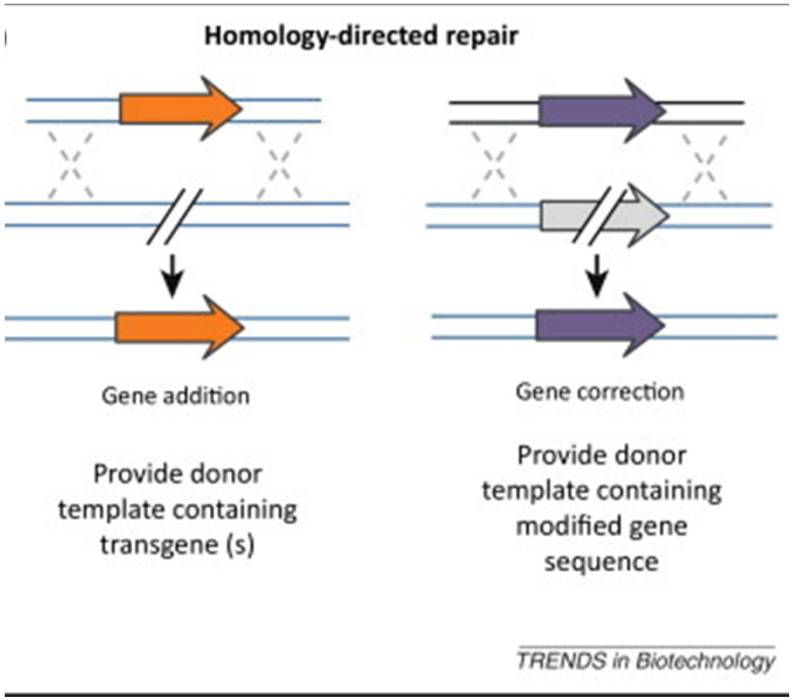
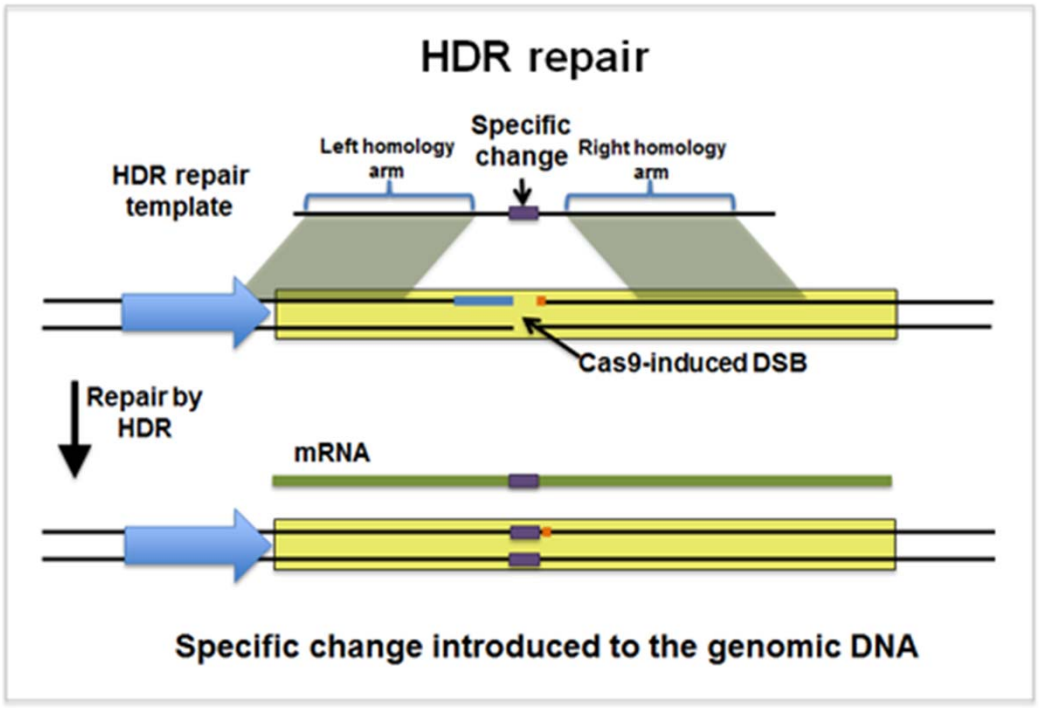



**Figure 1: DSB-enhanced gene targeting**




--Gaj. 2013. TrendsBiotech. 31:397-405




--- www.addgene.org

DNA repair template containing the desired sequence can be added into the cell along with the gRNA/Cas9. For HDR, a high degree of homology both upstream & downstream of the DSB must be present. As might expect, CRISPR-Cas9 will keep working in the cell, even on repaired area, so it is useful to design the repair template such that it is not cut by your CRISPR-Cas9 after repair is complete.