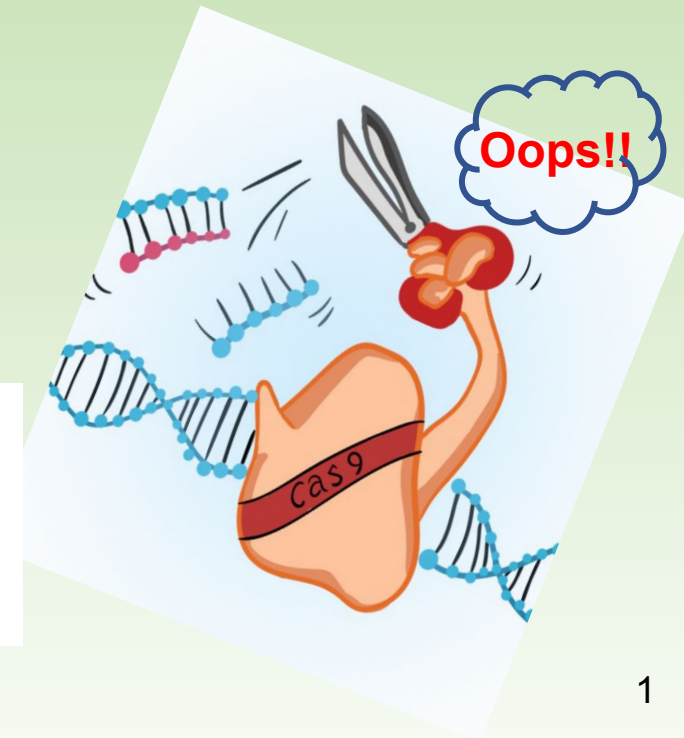# Integrated seminar on complex problems in gene editing
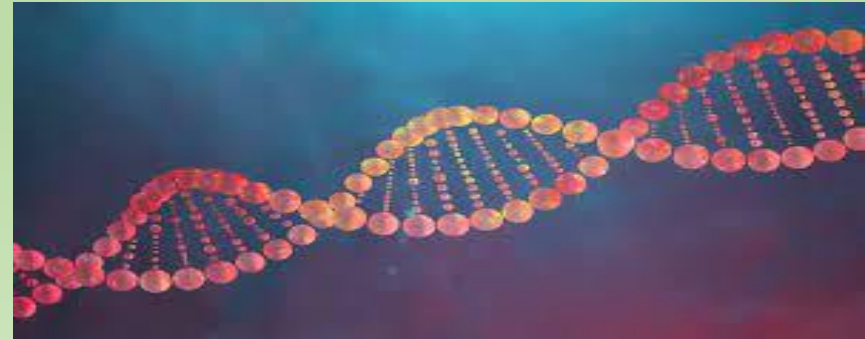
Off Target editing, a problem associated with CRISPR/Cas system

Sumedha Arora and Jasmine Randhawa
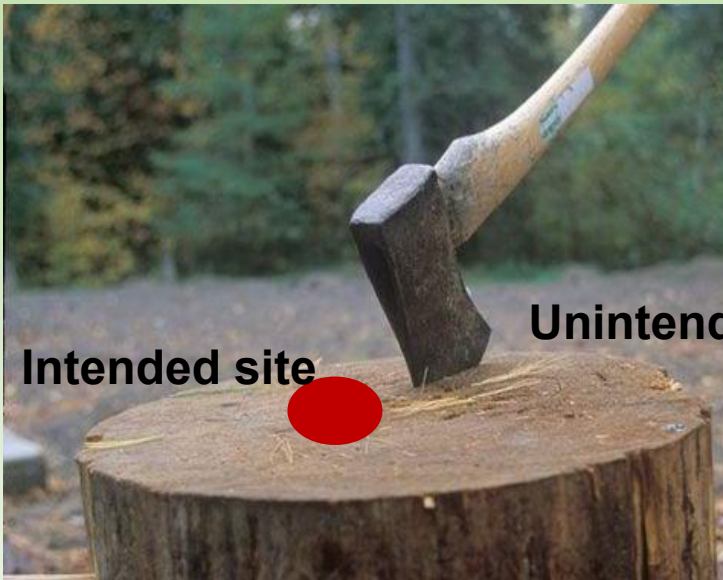(PAU and McGill University)
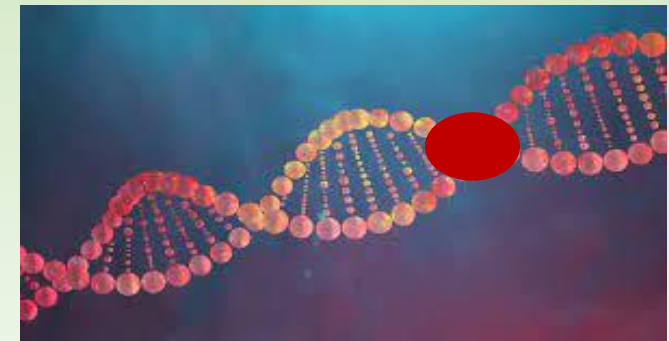Instructors: Dr. Raj Duggavathi and Dr. Jaswinder Singh
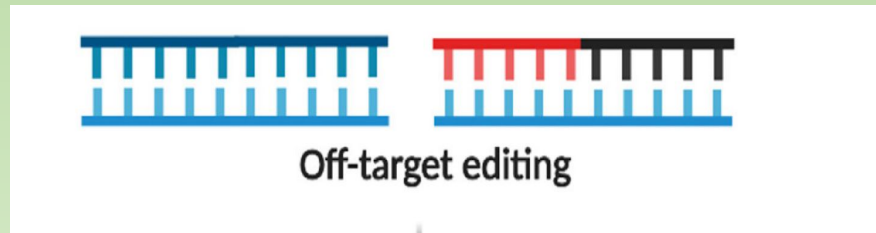
1

What Is?

DNA

Intended site

Unintended site

Unintended site

Intended site

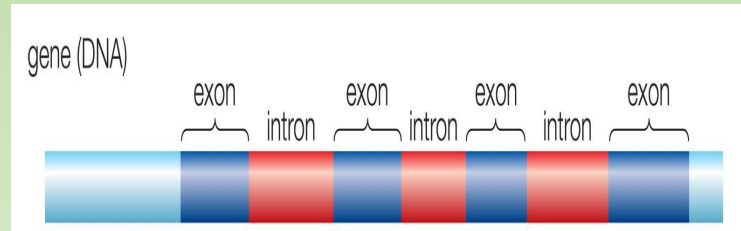https://science.howstuffworks.com/life/cellular-microscopic/dna.htm

Can go wrong!

Off-target editing

- Insertion
- Deletion

gene (DNA)

exon intron exon intron exon intron exon

Disrupted gene structure

Abnormal Phenotype

Naeem, M. et al, 2020
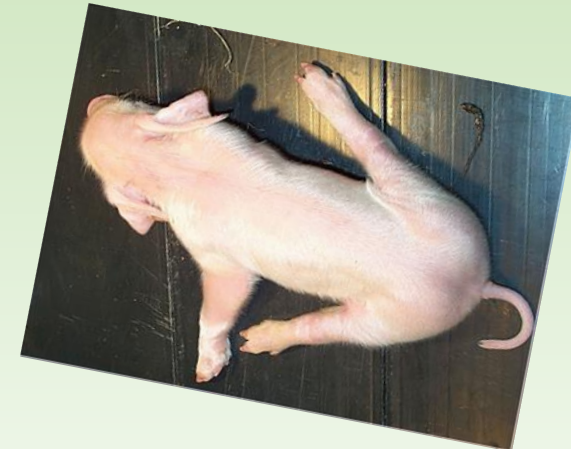
Example

Increase pig meat production
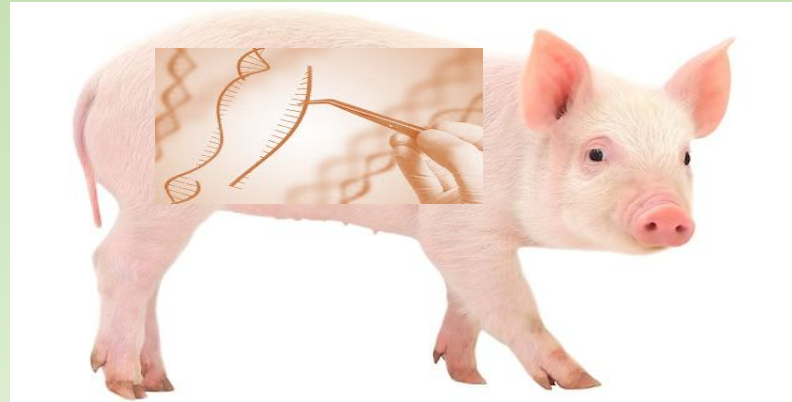
MSTN (Muscle regulatory factor)

More muscle mass

Abnormal legs

Yang. H and Wu. Z, 2018

CRISPR/Cas

Cas (CRISPR associated enzyme)

sgRNA (Single guide RNA)

3'

5' NNNNNNNNNNNNNNNNNNNNN

NNNNNNNNNNNNNNNNNNN

3'

5'

dsDNA

5'

3'

NNN

PAM (Protospacer Adjacent motif)

NNNNNNNNNNNNNNNNNNN

5

Zhang, X. et al, et al, 2015
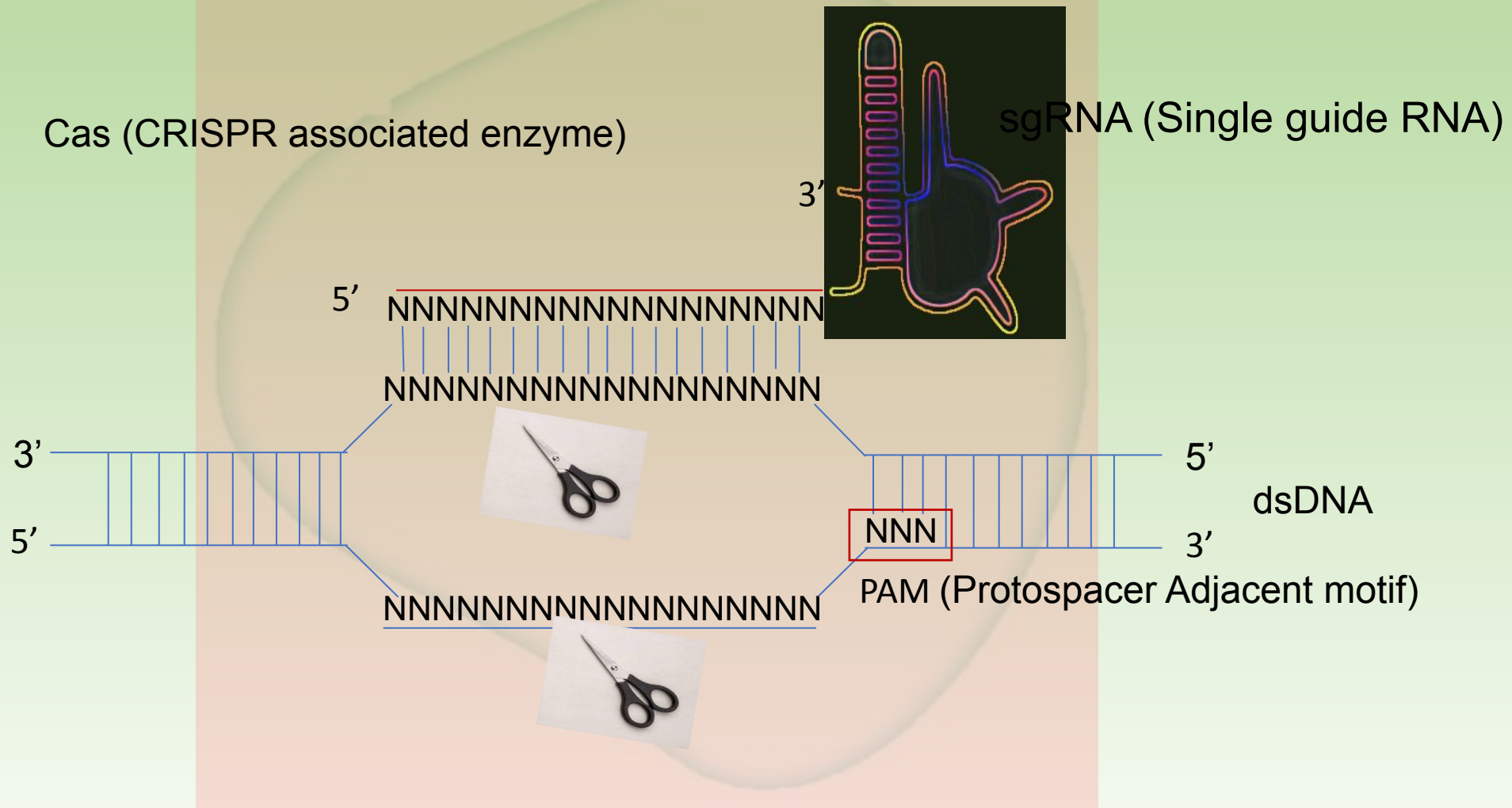
Components I: sgRNA

Initial DNA binding

Cas nuclease domain
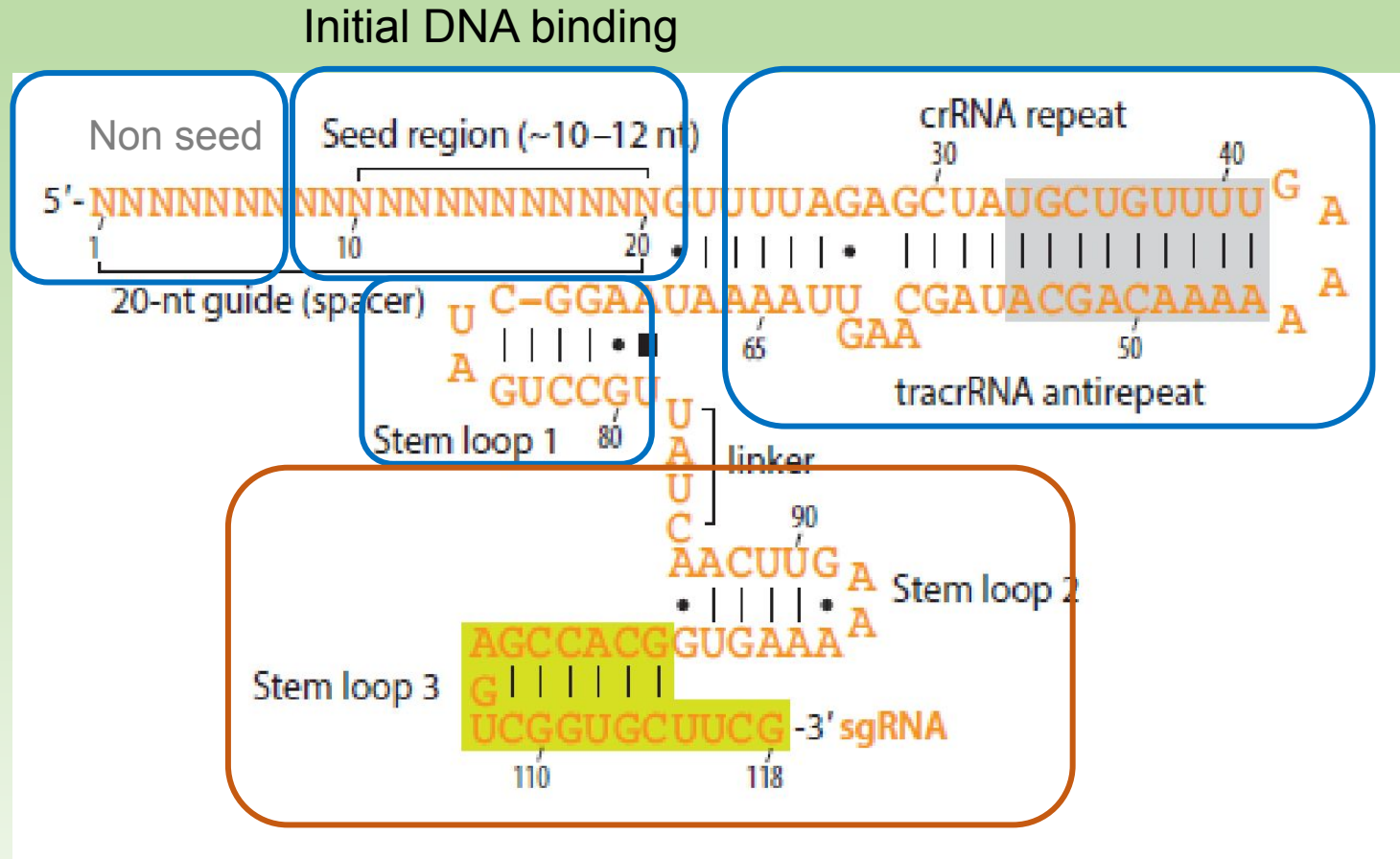
Cas binding

Stability
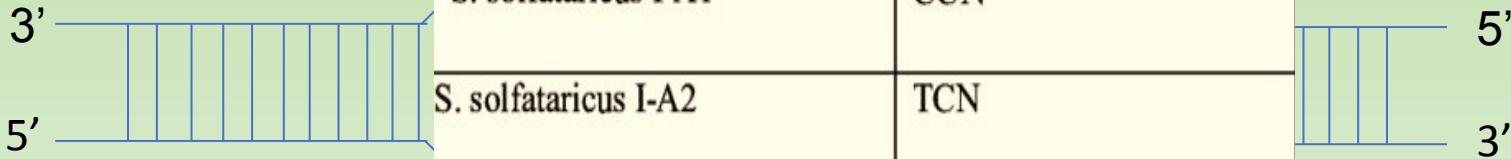
Structure of sgRNA

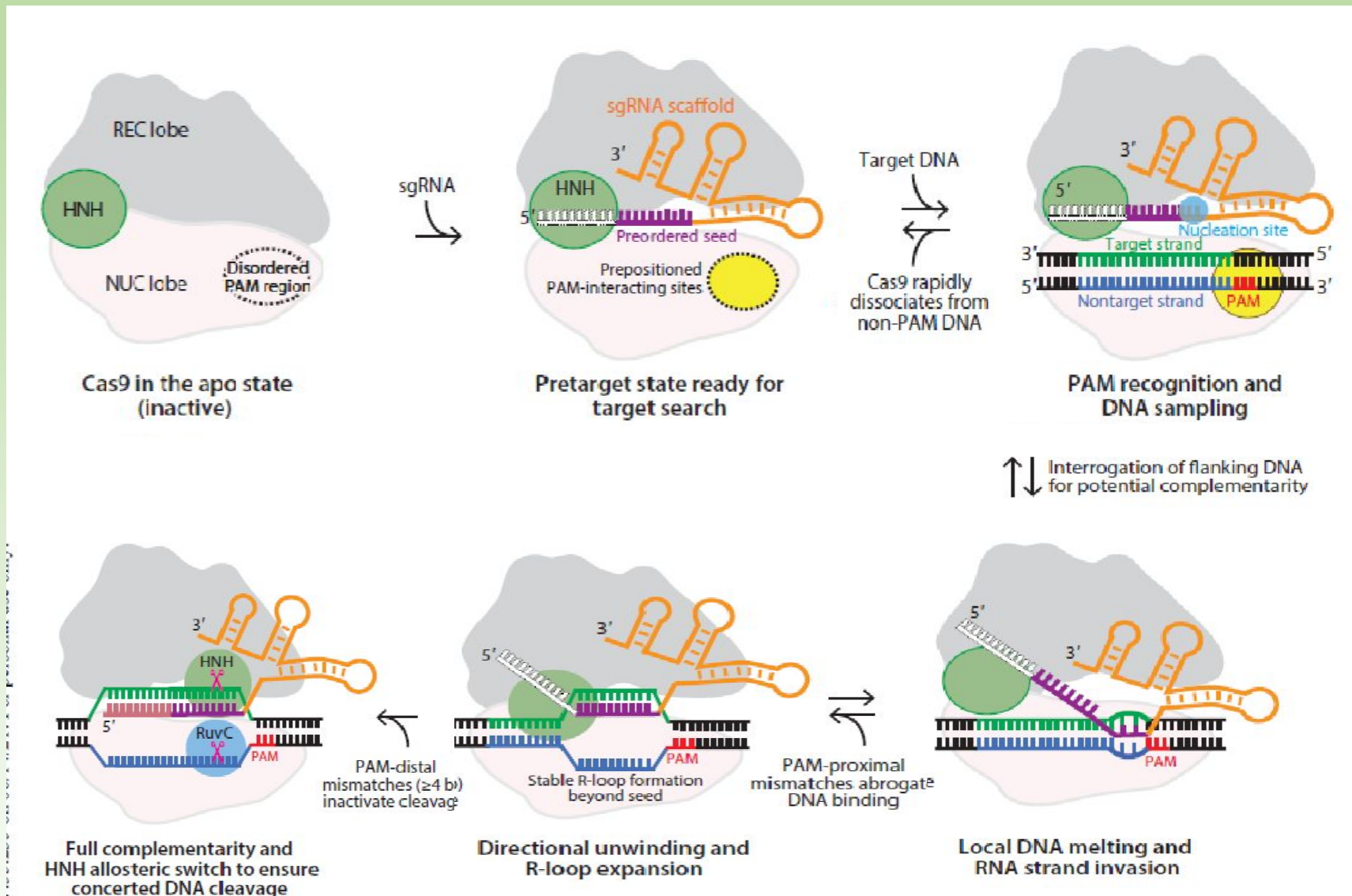# II: Cas enzyme
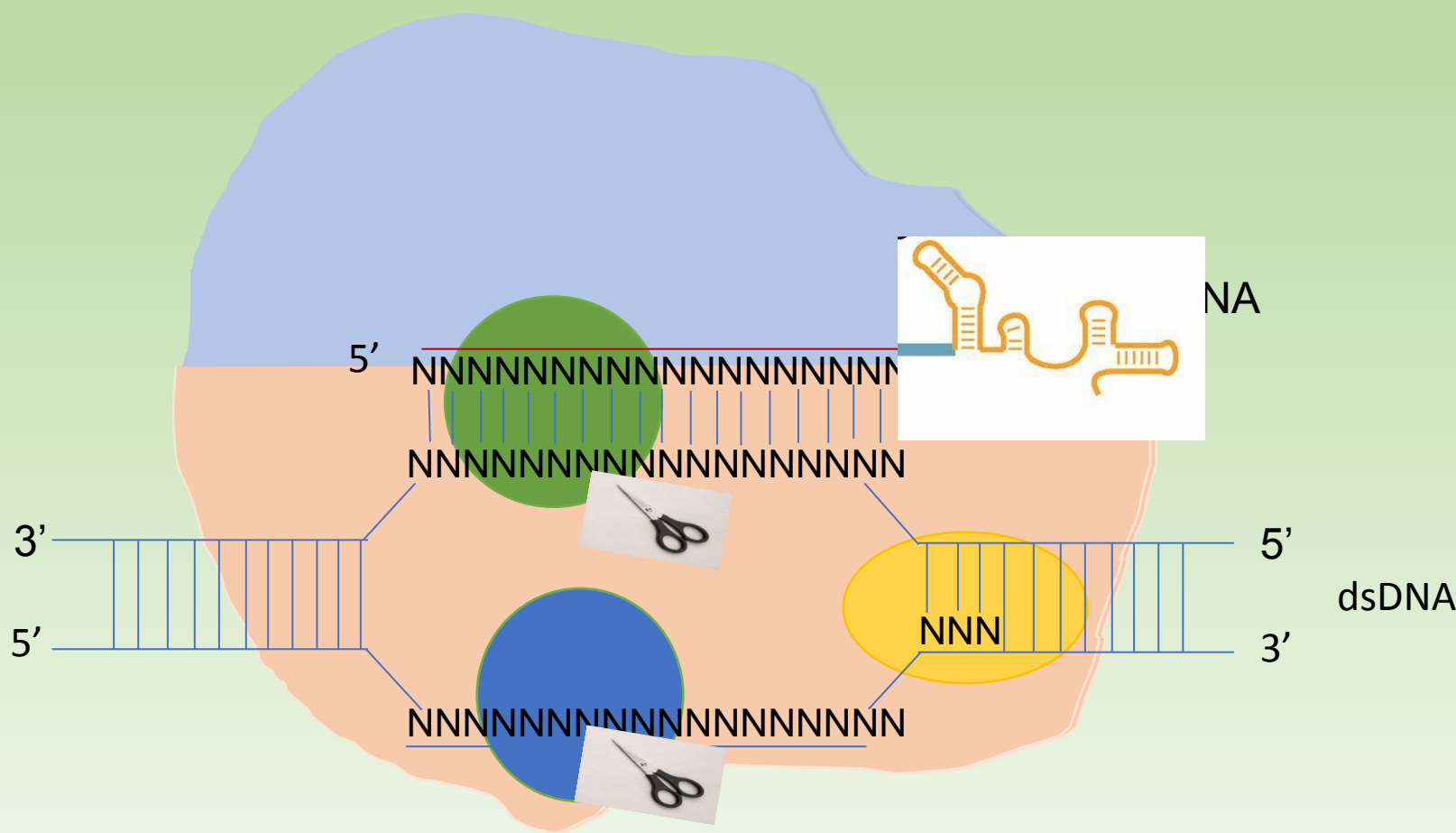
Structure of Cas enzyme

III: PAM sequence

Bacterial species	PAM sequence
Streptococcus pyogens (Sp)	NGG
S. solfataricus I-A1	CCN
S. solfataricus I-A2	TCN
Neisseria meningitidis (Nm)	NNNNGATT
Streptococcus thermophiles (St) Type II	NGGNG NNAGAAW
Streptococcus mutan (Sm)	NGG or NAAR

3'

5'

5'

3'

dsDNA

Types of PAM sequences in different bacteria

Zhang, Y. et al, et al, 2014
Jamal. M, et al, 2015

Mechanism

Jiang. F, Doudna. J, 2017

Delivered as?

Three ways:

- **Plasmid DNA**
- **mRNA**
- **RNP Complex**

Western Blot

Liang, X. et al, 2015
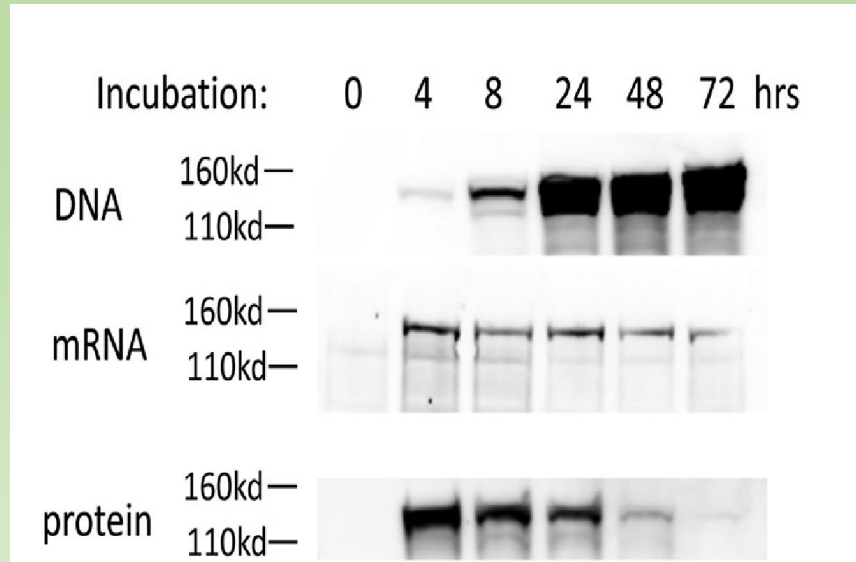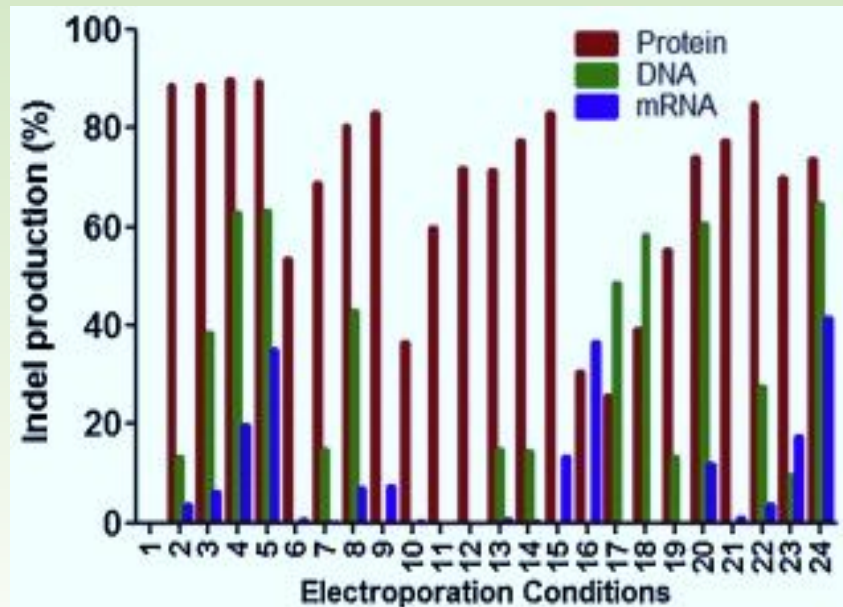
Delivered as?

Plasmid vector

Co- Transfected

Cas9   −   +   −   +

sgRNA   −   −   +   +   3ng   15ng
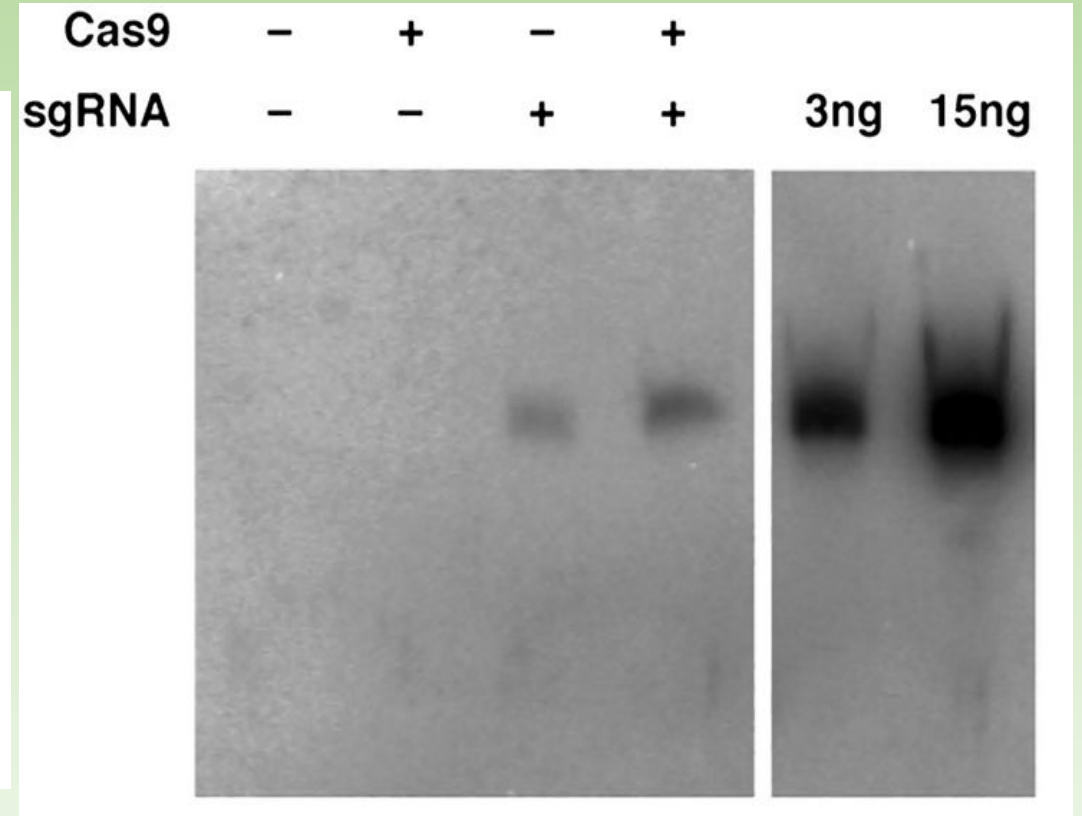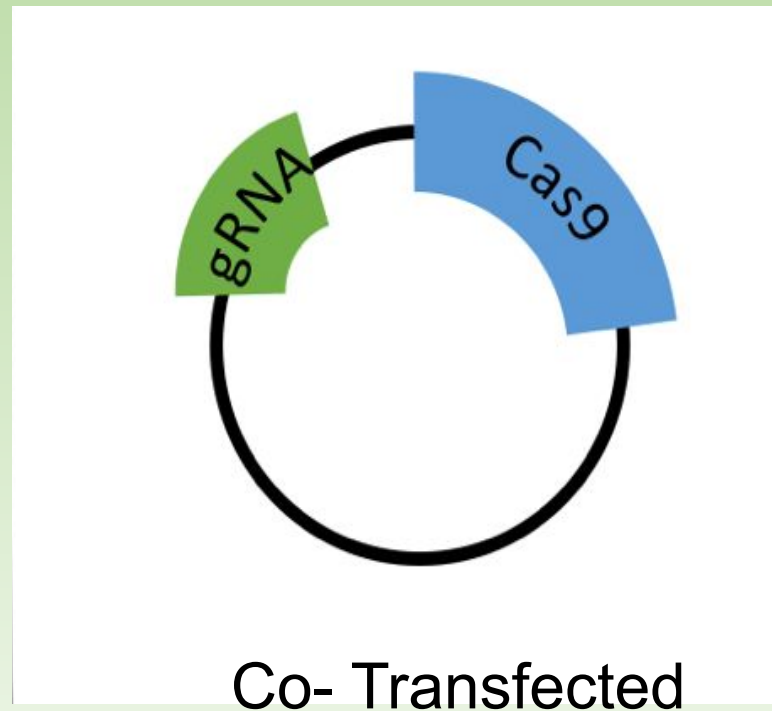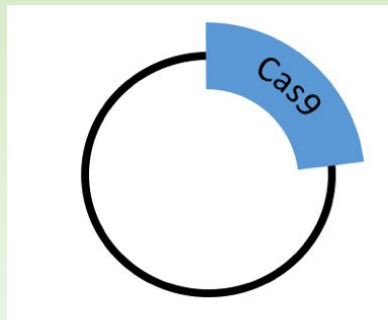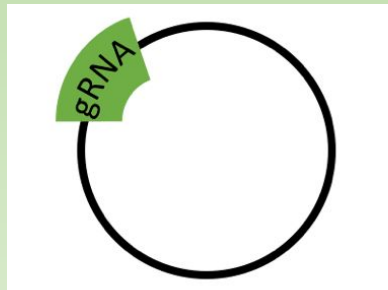
Fig: Northern Blot of sgRNA

Jinek. M. et al, 2013
https://www.benchling.com/2016/02/23/how-to-synthesize-your-grnas-for-crispr/

Methods of Off-target detection

In silico Prediction

Intended target site

Computational prediction

Predicted off-target sites

Tsai, S.Q. et al, 2016

In silico Prediction tool

CRISPR-P 2.0

GT-Scan
Finding unique targets.

E-CRISP
Design of CRISPR constructs

COSMID

CasOT

CTOP

sgRNAcas9

CRISPRdirect

CHOPCHOP

Cas-OFFinder

CRISPR-ERA
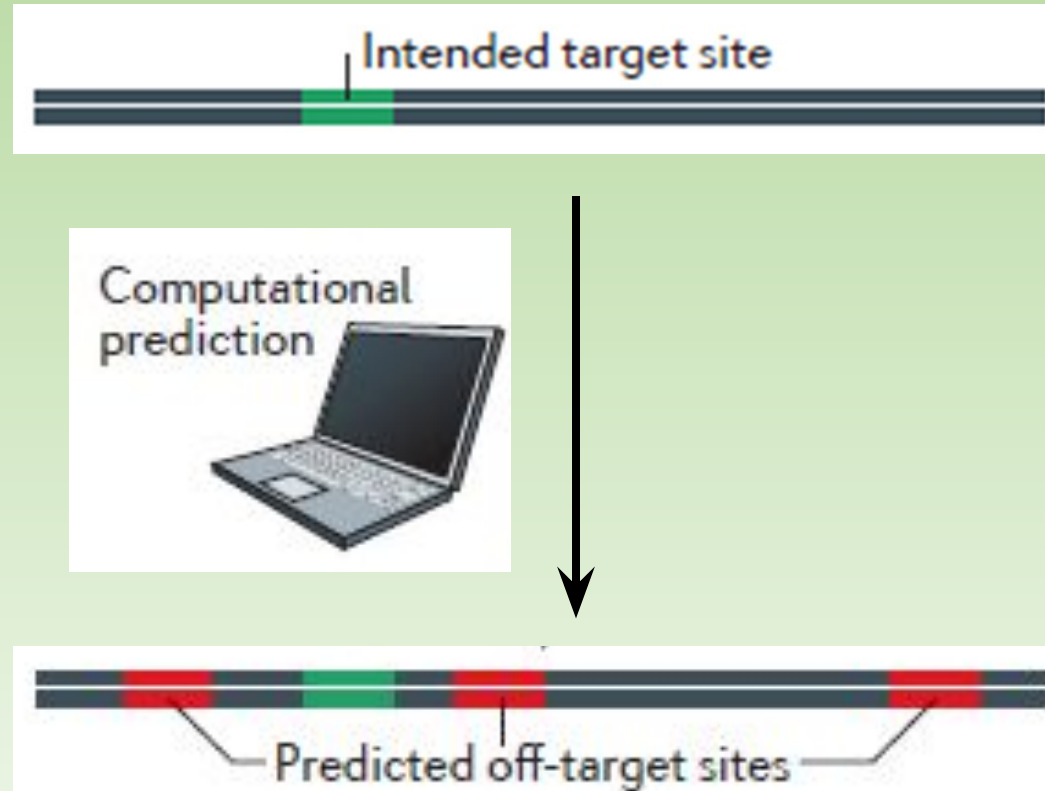
In Vitro Detection

Crispr acts on cell free system

DNA extracted

CRISPR/Cas

Ligation of adapters to cleaved sites

Amplification

Sequenced
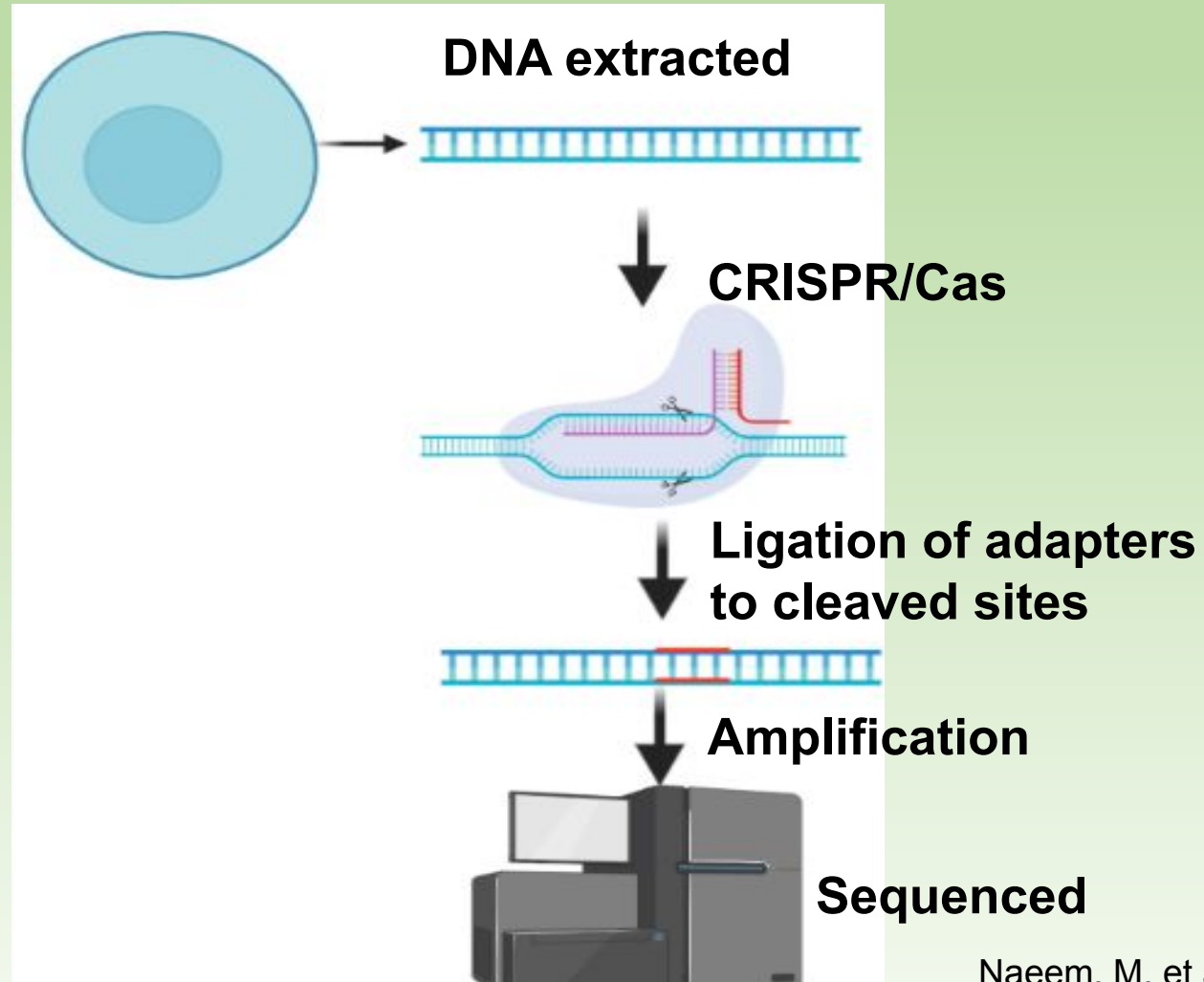
Naeem, M. et al, 2020

In Vitro Detection

CIRCLE-seq

Digenome-seq

Tsai S.Q. et al, 2017
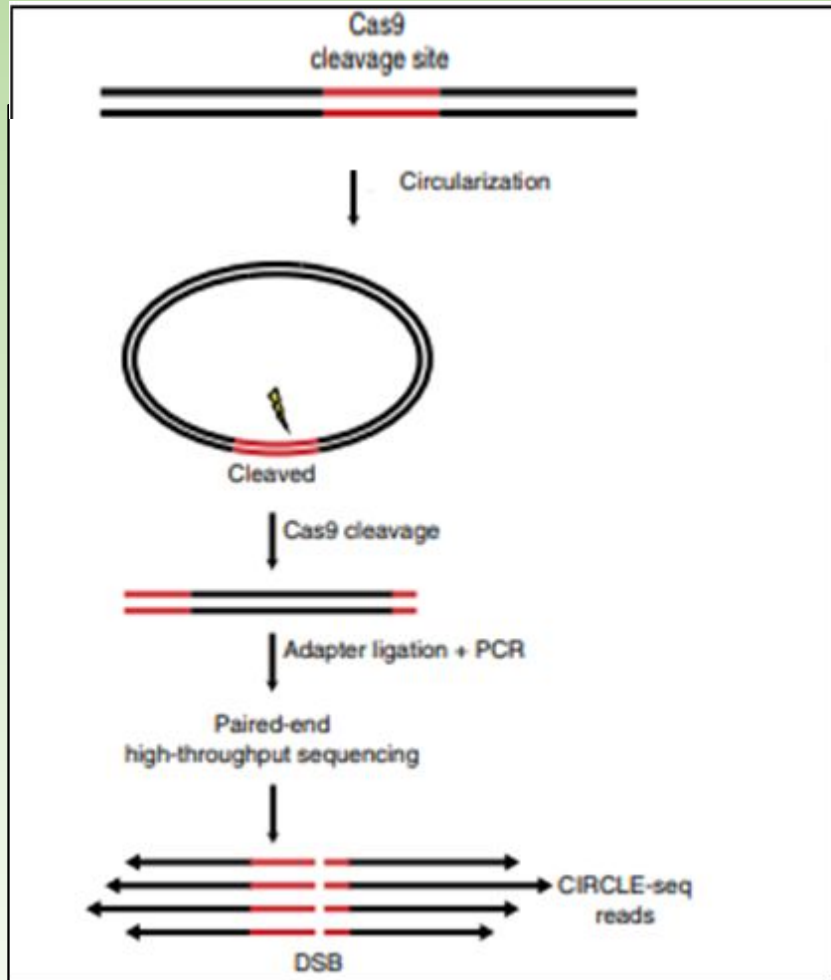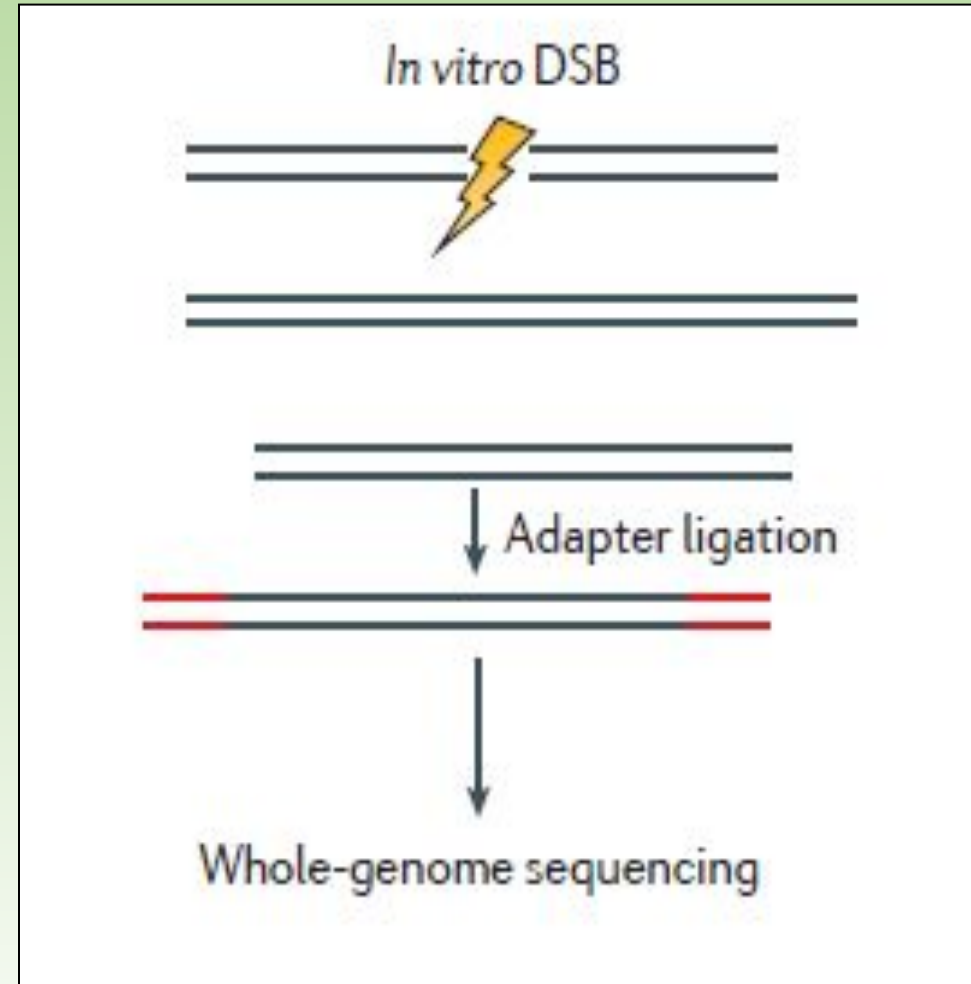
In Vivo Detection

Crispr acts within the cell

Cleaved DNA extracted

Ligation of adapters to cleaved sites

Amplification

Sequenced
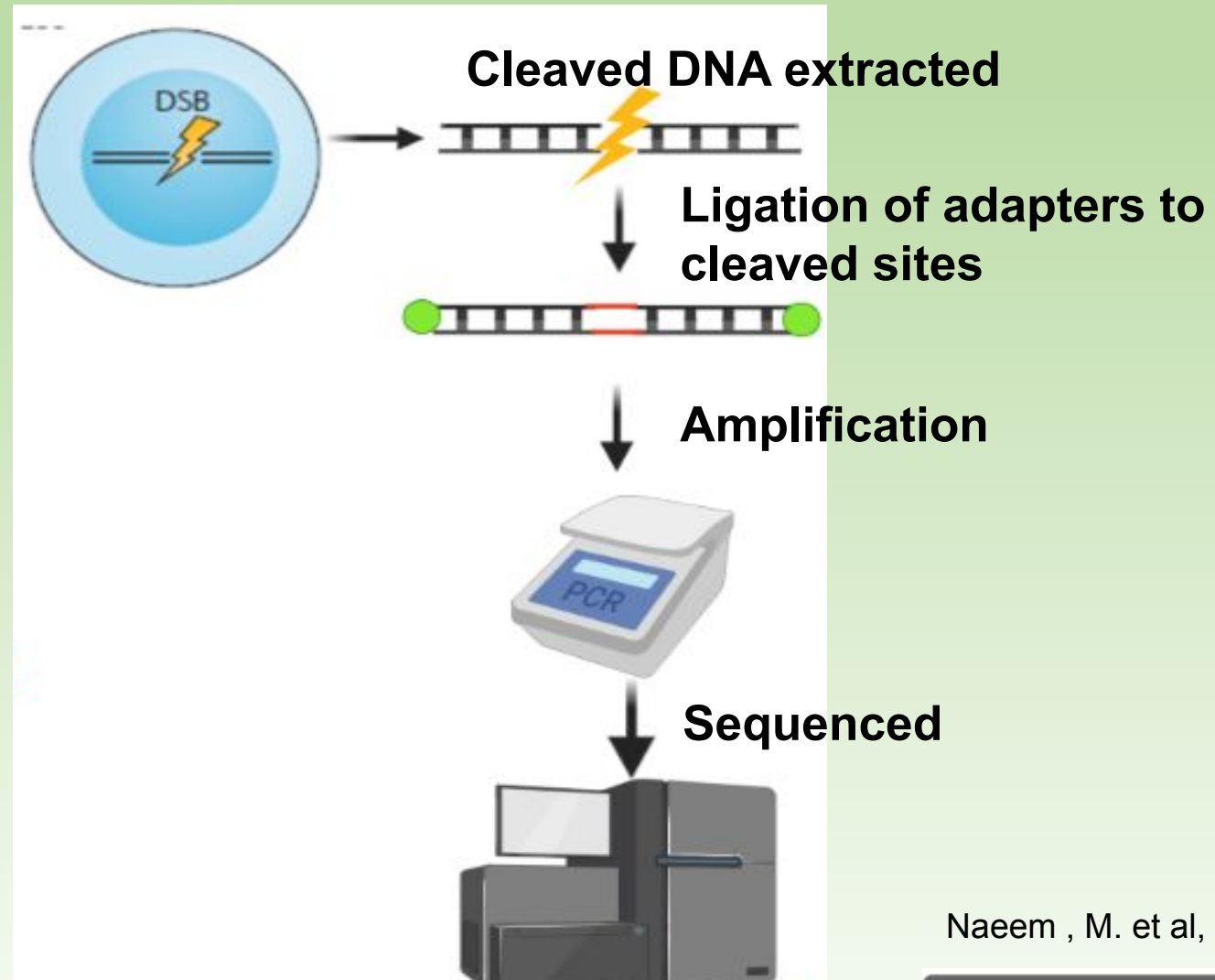
Naeem , M. et al, 2020

In Vivo Detection   I: GUIDE-seq

Tsai ,S.Q. et al, 2016

Wienert B, et al., 2020
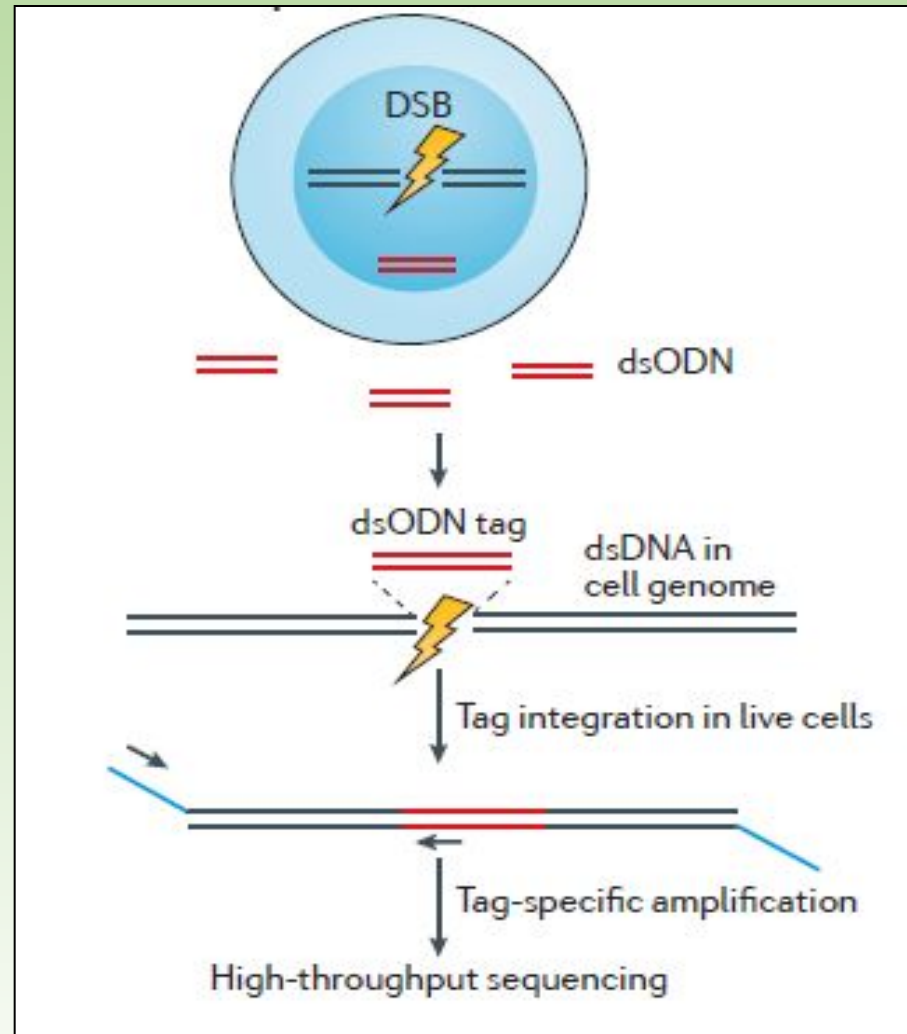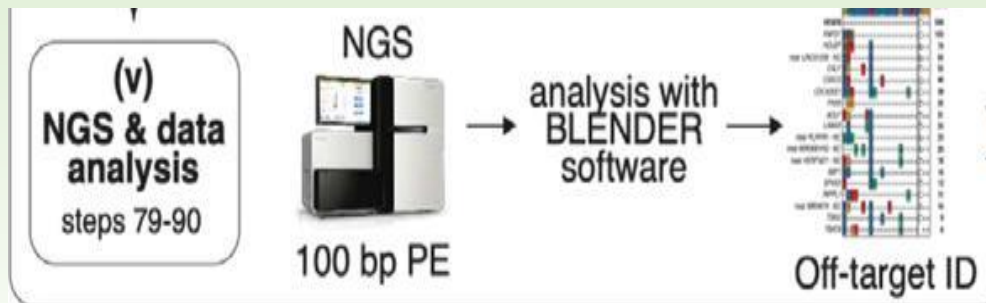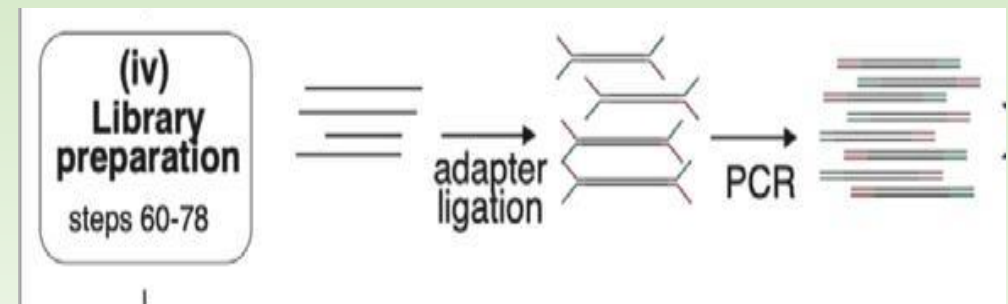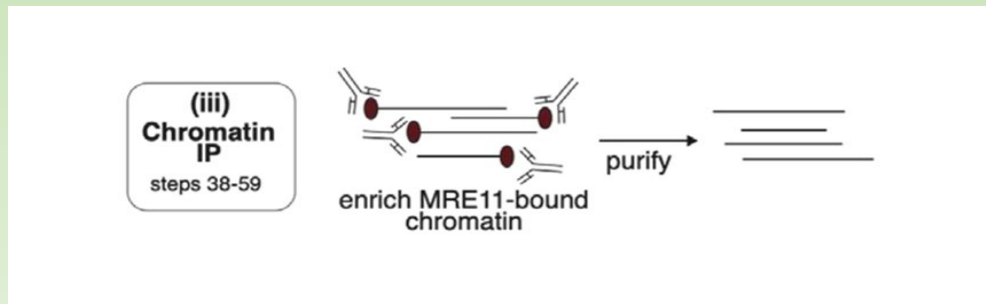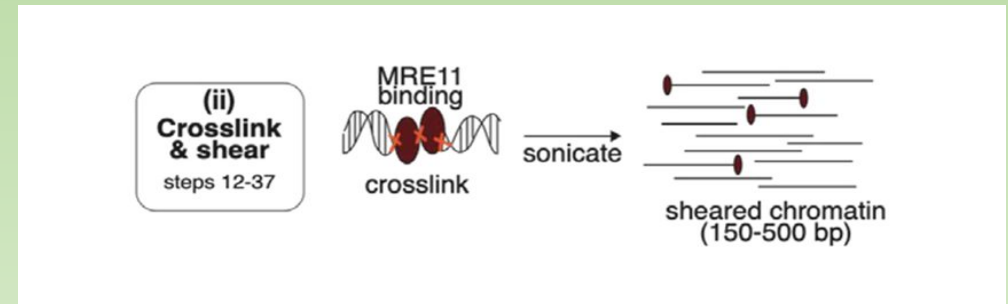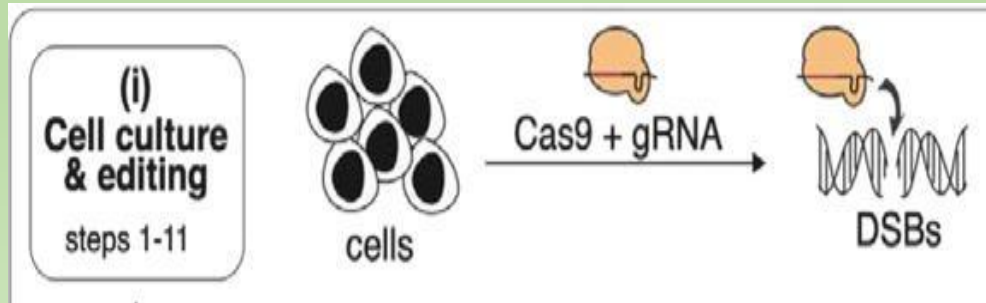
Strategies to minimize off-target effects
I: Alteration of sgRNA

20 nucleotide

17 nucleotide

GC content- 40-60%

Tsai S.Q. et al, 2016

I: Alteration of sgRNA

2'O-methyl
3'-phosphorothioate

Ryan D E. et al, 2018
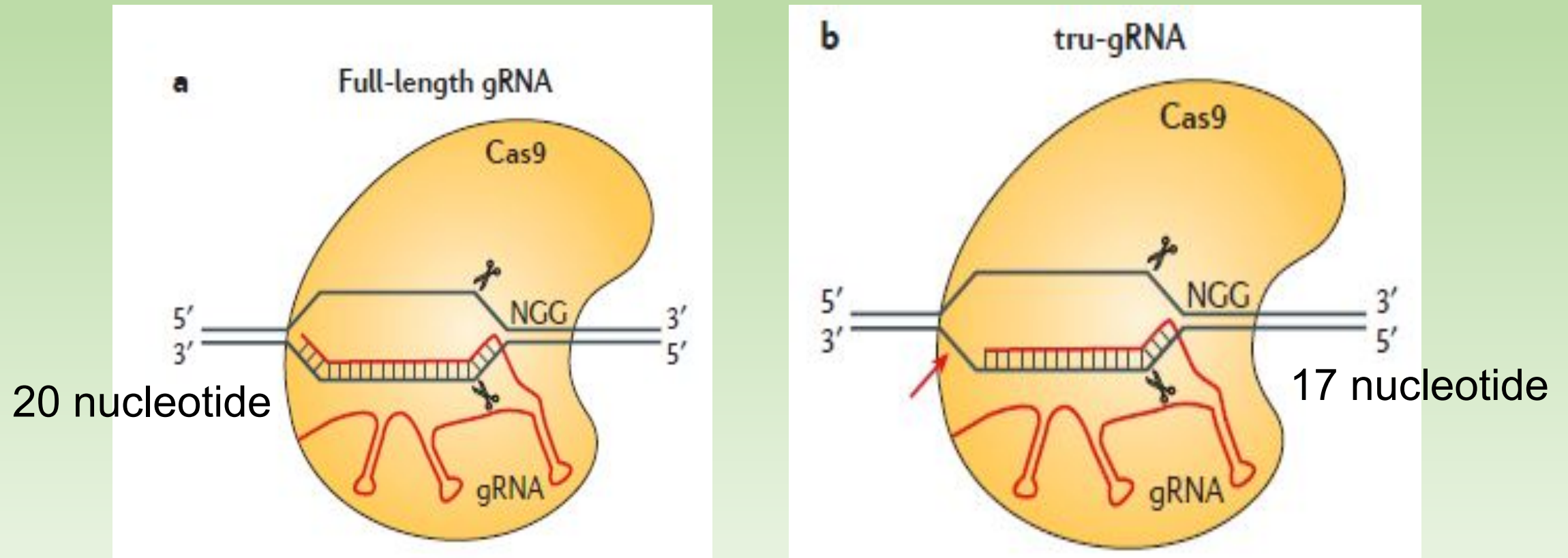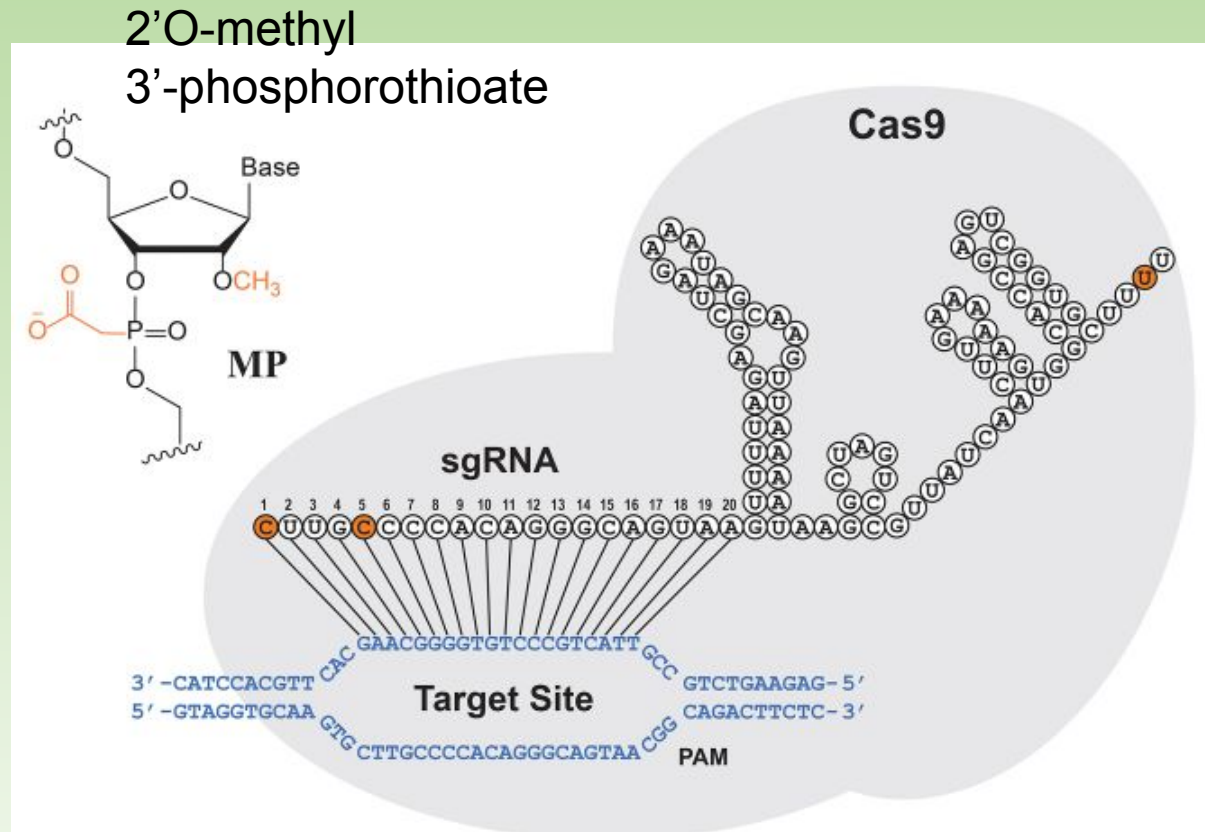
Cas9 variants

II: Improved Cas 9 Variants

SaCas9 NNGRRN (R: A or G)

St3Cas9 NGGNG

Cpf1 TTTV
(V: A,C or G)

Naeem, M. et al, 2015

III: Cytosine Base Editors

C to U conversion
C/G to T/A

https://blog.addgene.org/single-base-editing-with-crispr /
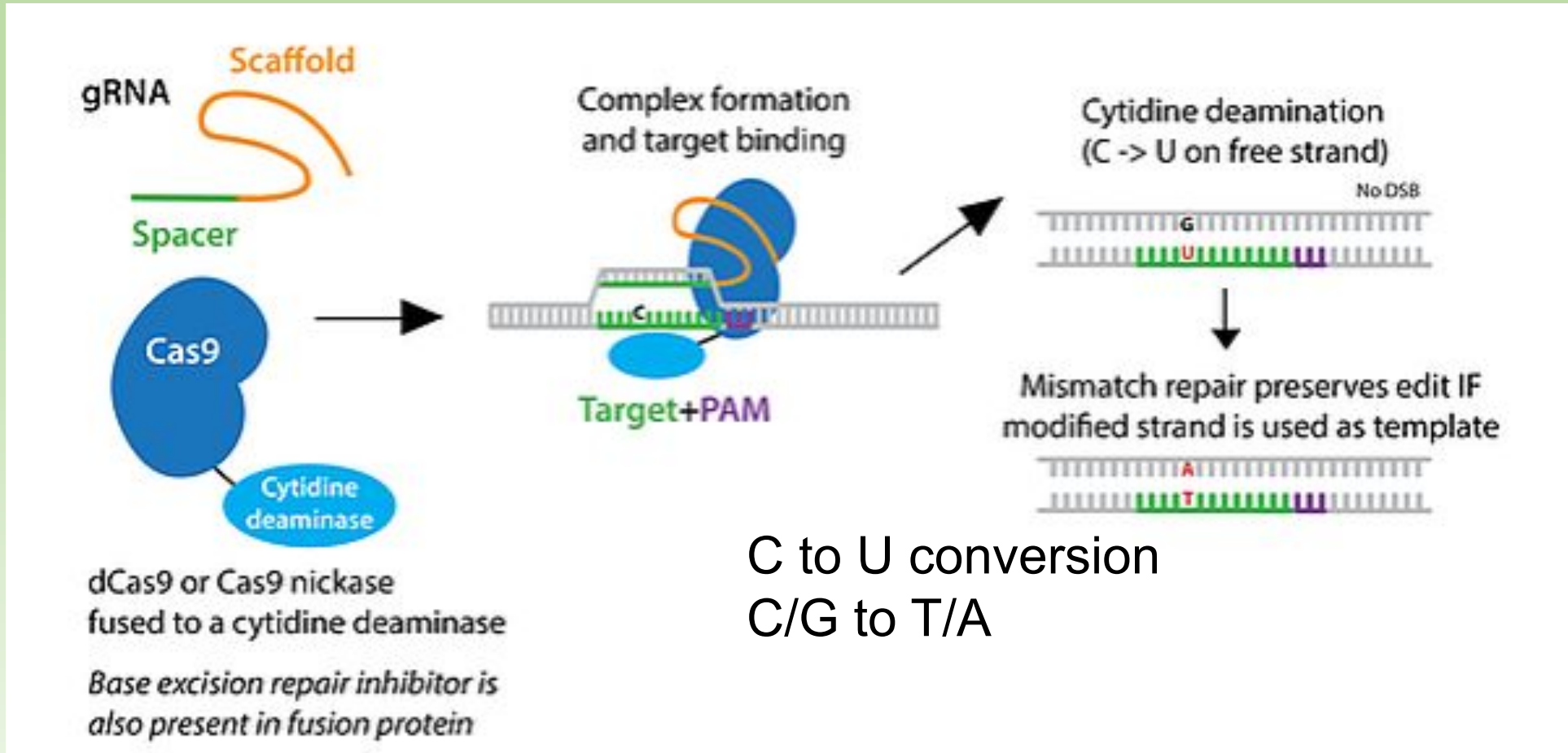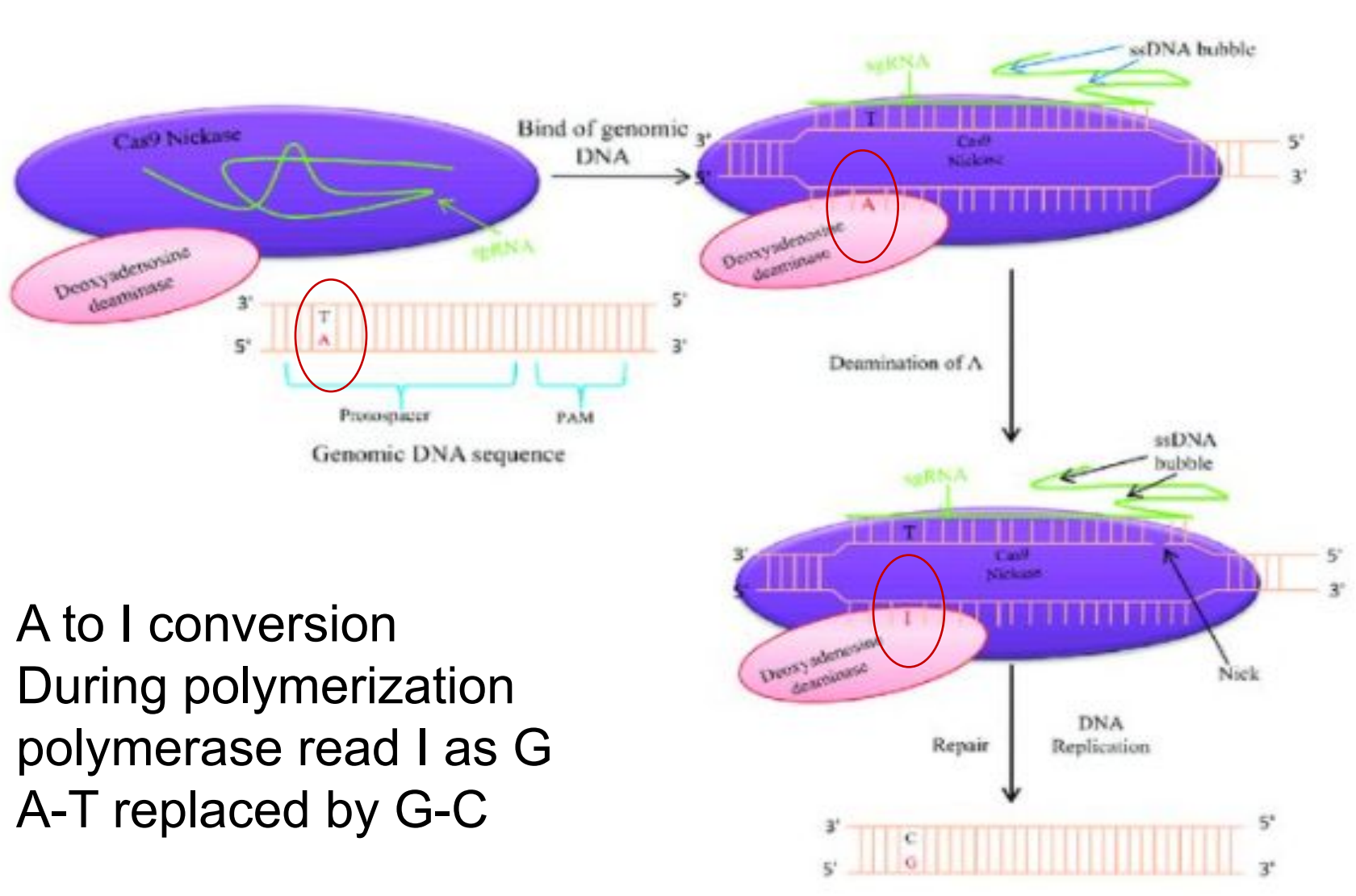
A to I conversion
During polymerization
polymerase read I as G
A-T replaced by G-C

V: Anti CRISPR proteins

AcrIIC2 inhibits crRNA loading

AcrIIA4 and AcrIIC3 inhibit DNA binding

AcrIIC1 inhibits DNA cleavage
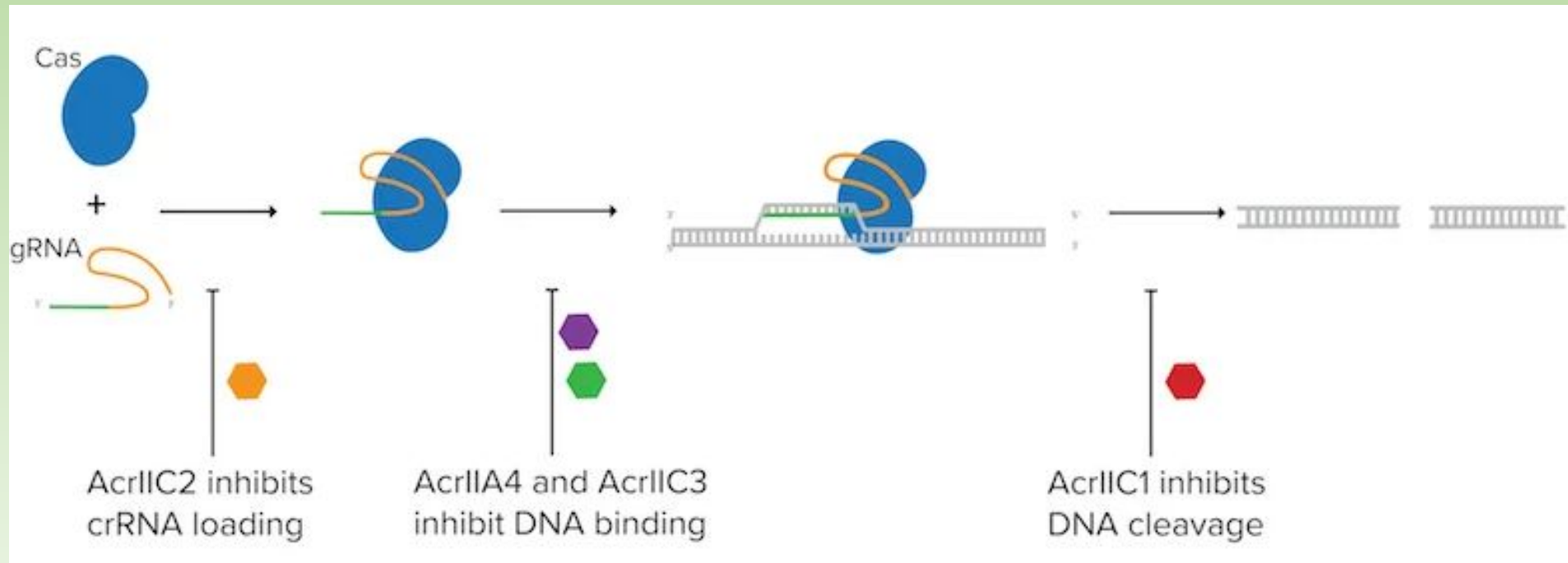
Conclusion

- Components of CRISPR/ Cas system are important for specific Cleavage

- Hence, they need to be designed to reduce the risk of off target editing.

- Different parameters can be implemented to minimize the risk possibility and detect off target mutations

Acknowledgement

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- Dr. R.S Sethi

 Program Coordinators:
- Ms. Lu Fan
- Ms. Japman Kaur Kandola

 Course members

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Thankyou!