

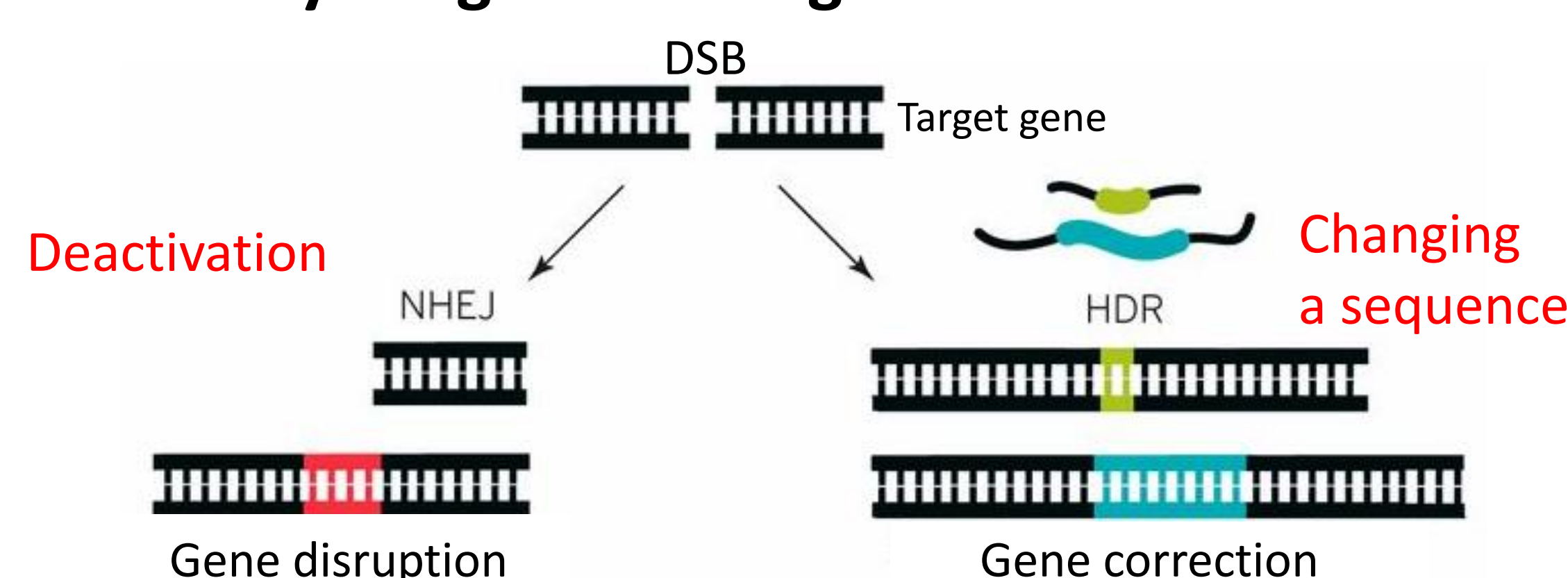
Project Purpose

To Reduce off-target effects in genome-editing

- Confirm efficiency of on-target cleavage
- Identify potential off-target cleavage sites

Introduction

Two ways of gene-editing



Application of Gene-editing^[1]

- Gene therapy
- Transgenic animals
- Gene labeling
- Targeted transgenic addition
- Study gene function with stem cells

CRISPR/Cas9^[1]

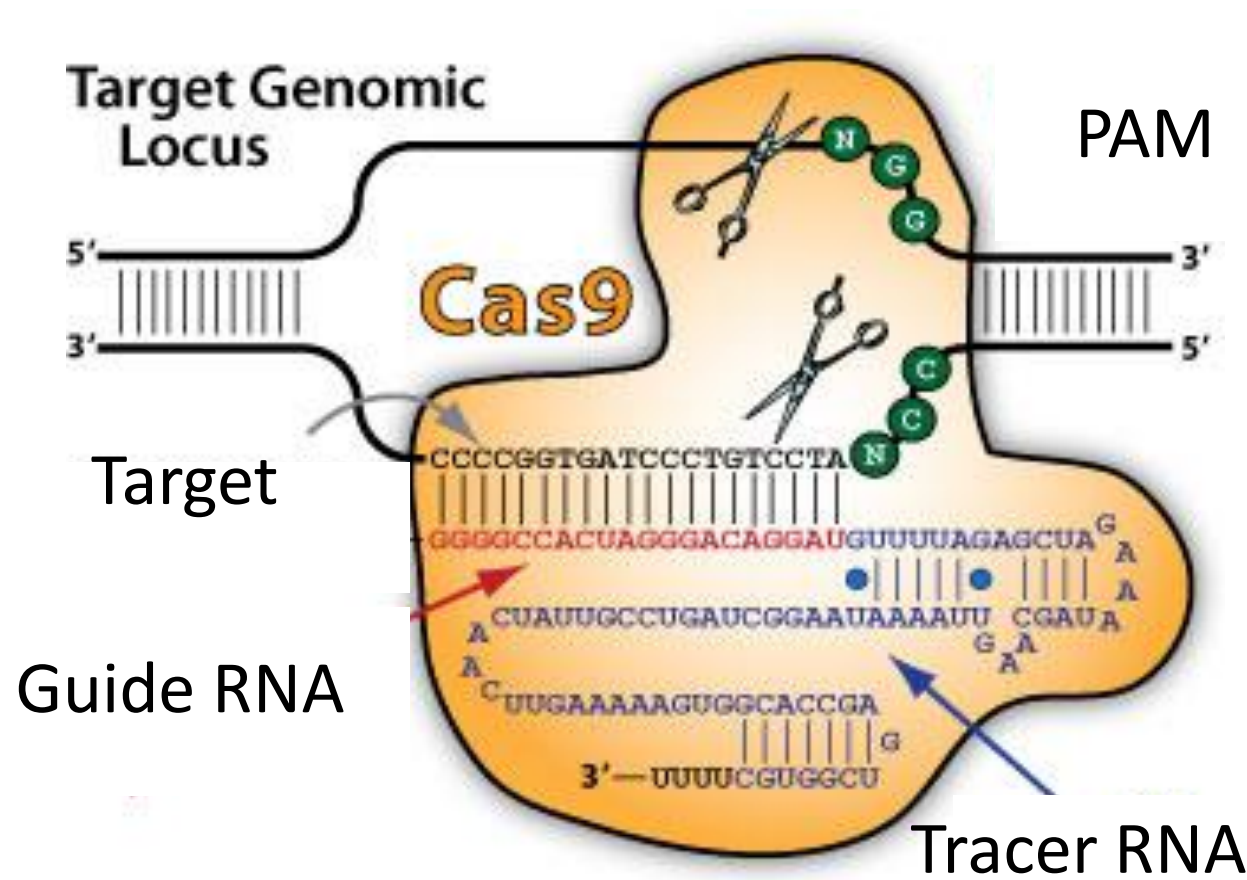
Pros

Simplicity
Efficiency
Multiplexing

Cons

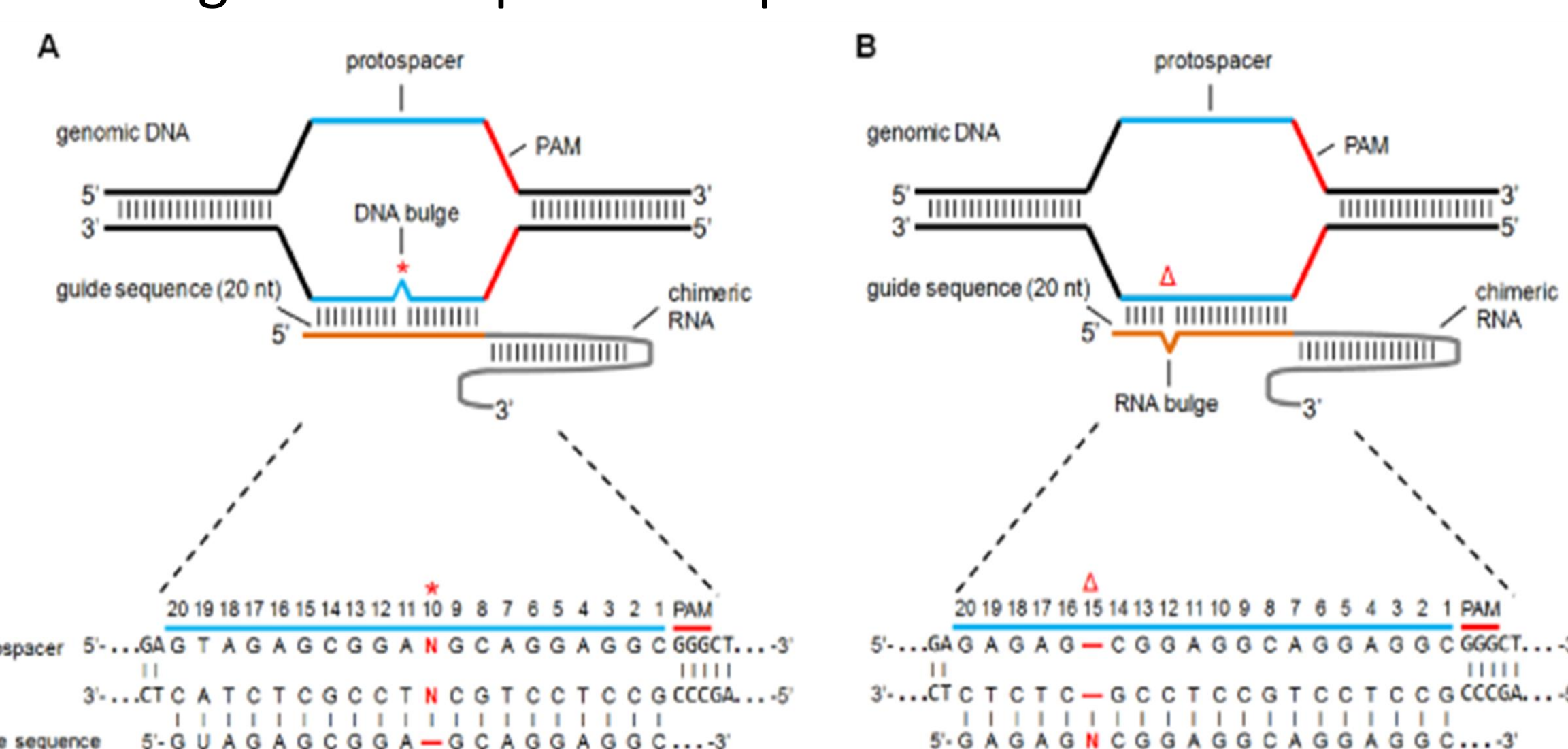
Off-target effect

Mechanism of CRISPR/Cas9



CRISPR Cas9 Off-target^[2]

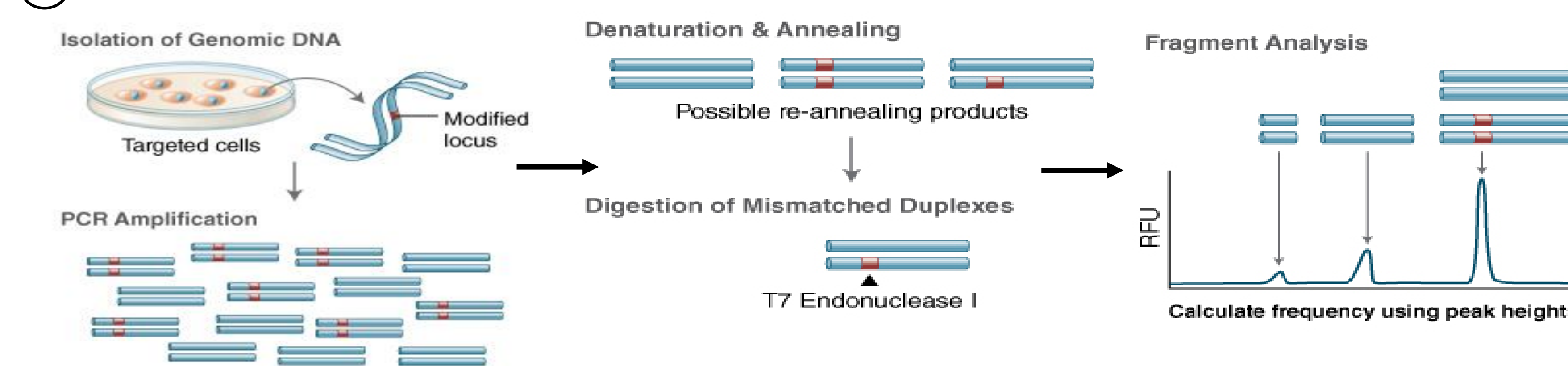
Cleavage of non-specific sequences



- Off-target mutation in the human cells
- Gross chromosomal deletions between two cut sites

Experimental steps: Determining Gene Targeting Efficiency

① T7 Endonuclease I^[3]

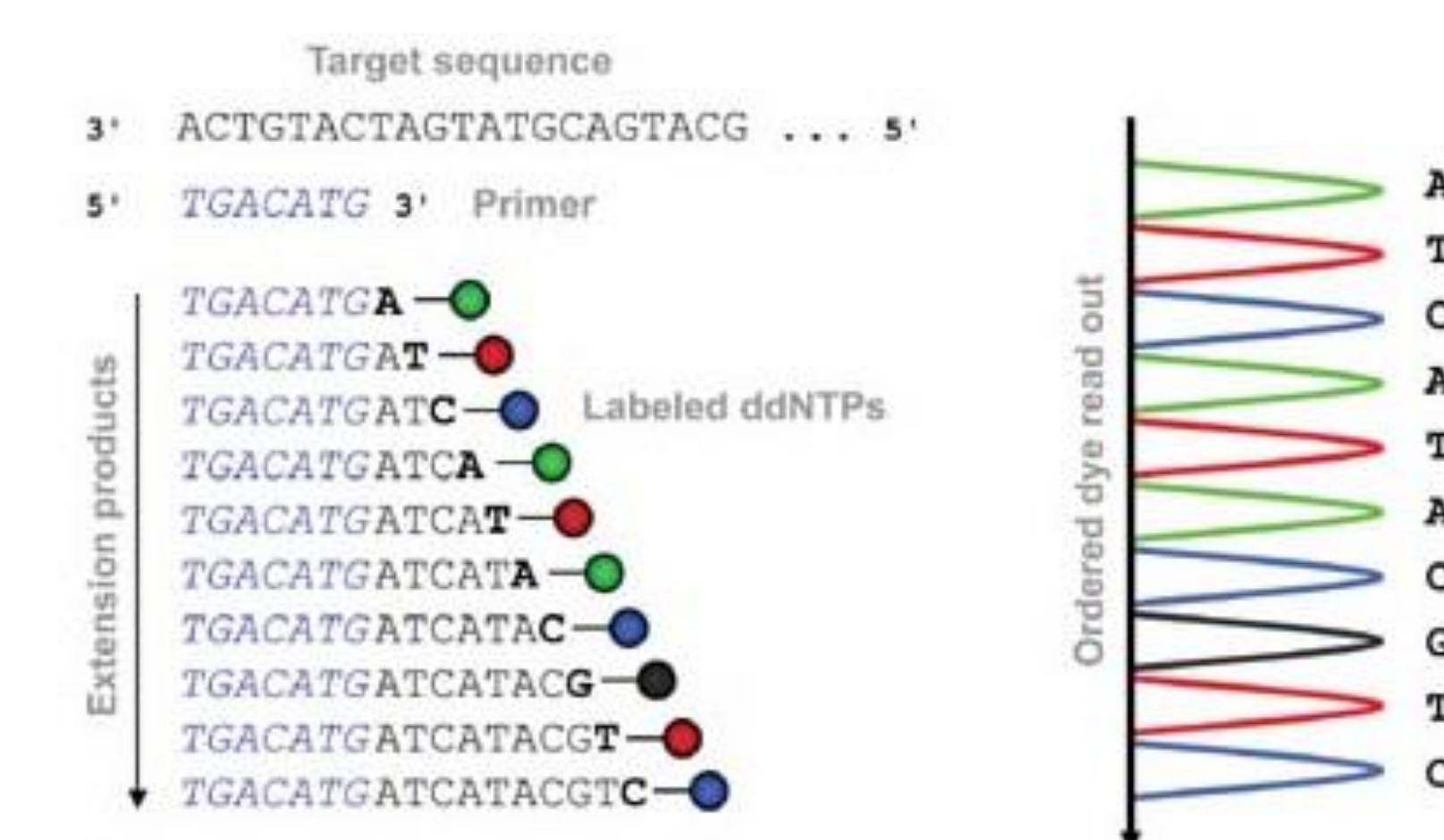
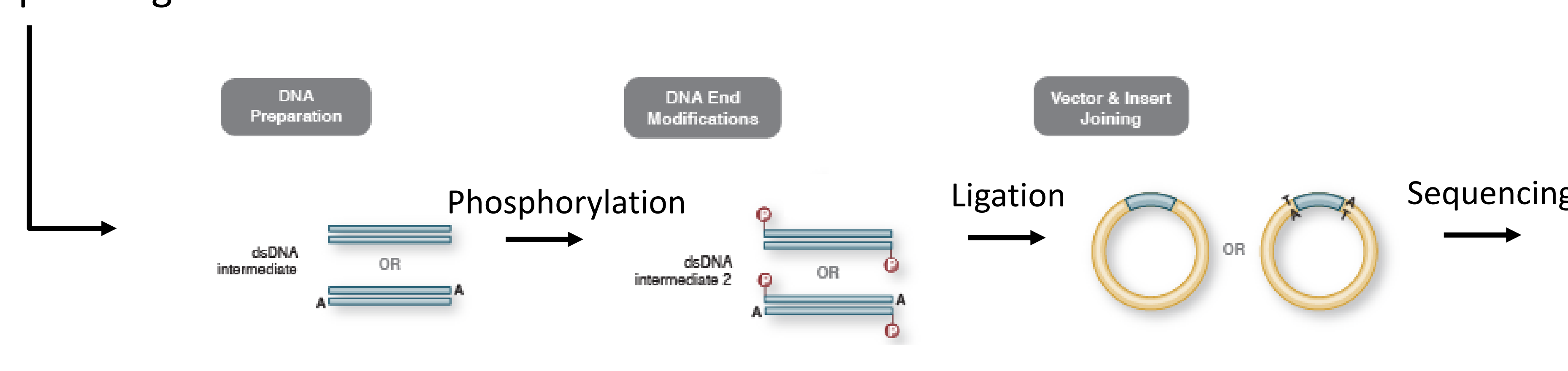


Calculate percentage of gene targeting

$$f = \frac{cut}{cut + uncut}$$

$$\% \text{indel} = (1 - \sqrt{1 - f}) \times 100$$

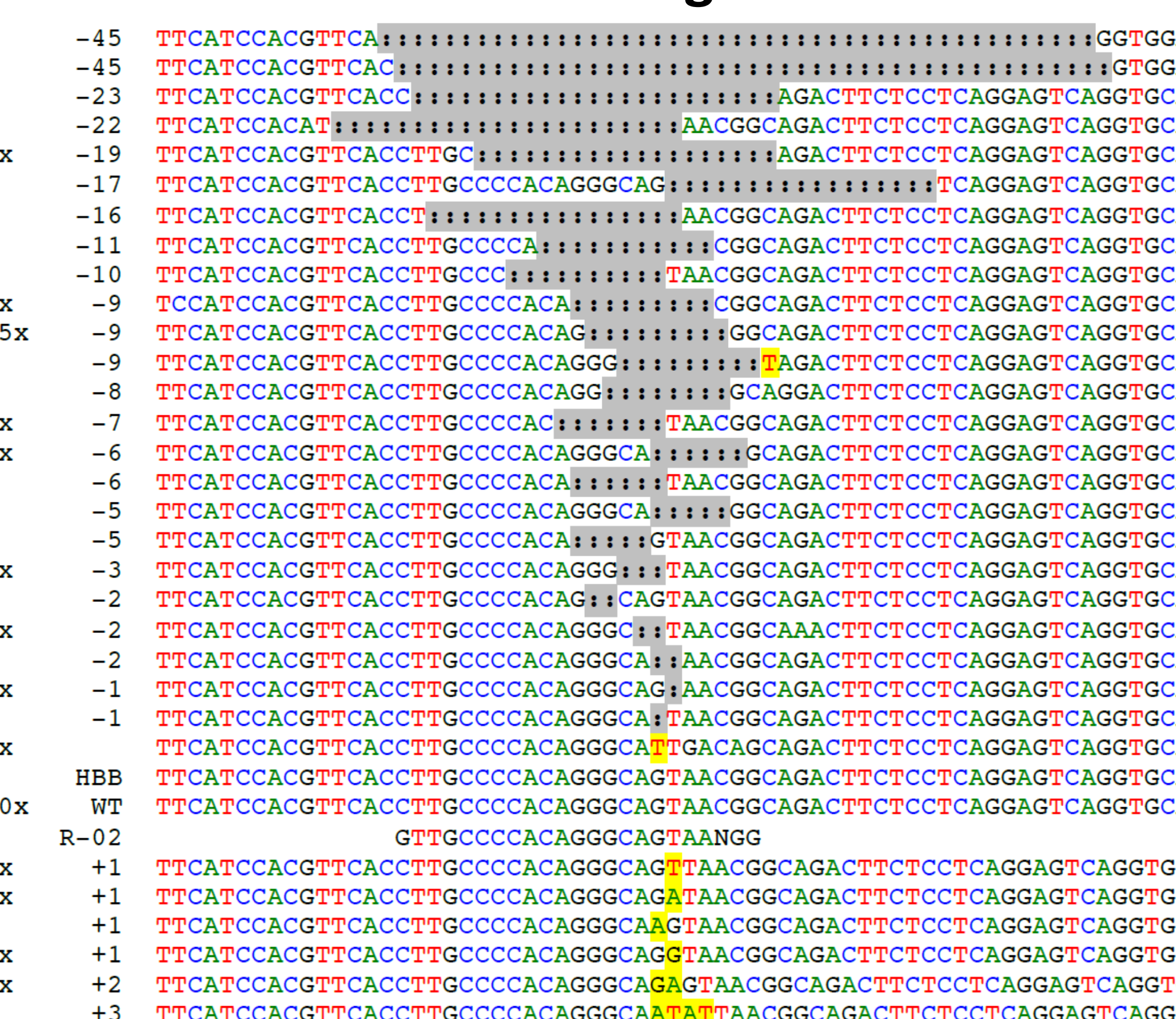
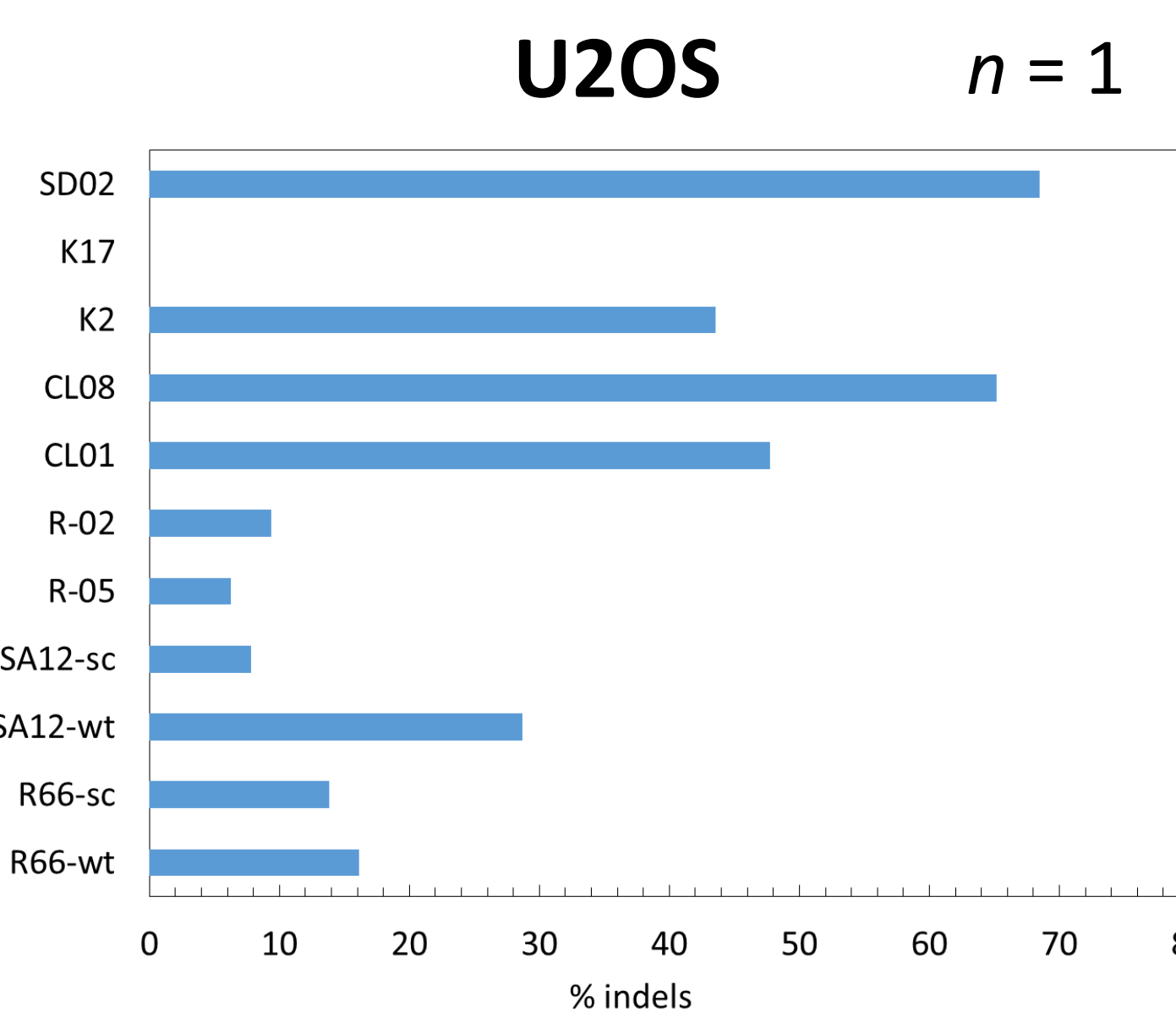
② DNA sequencing



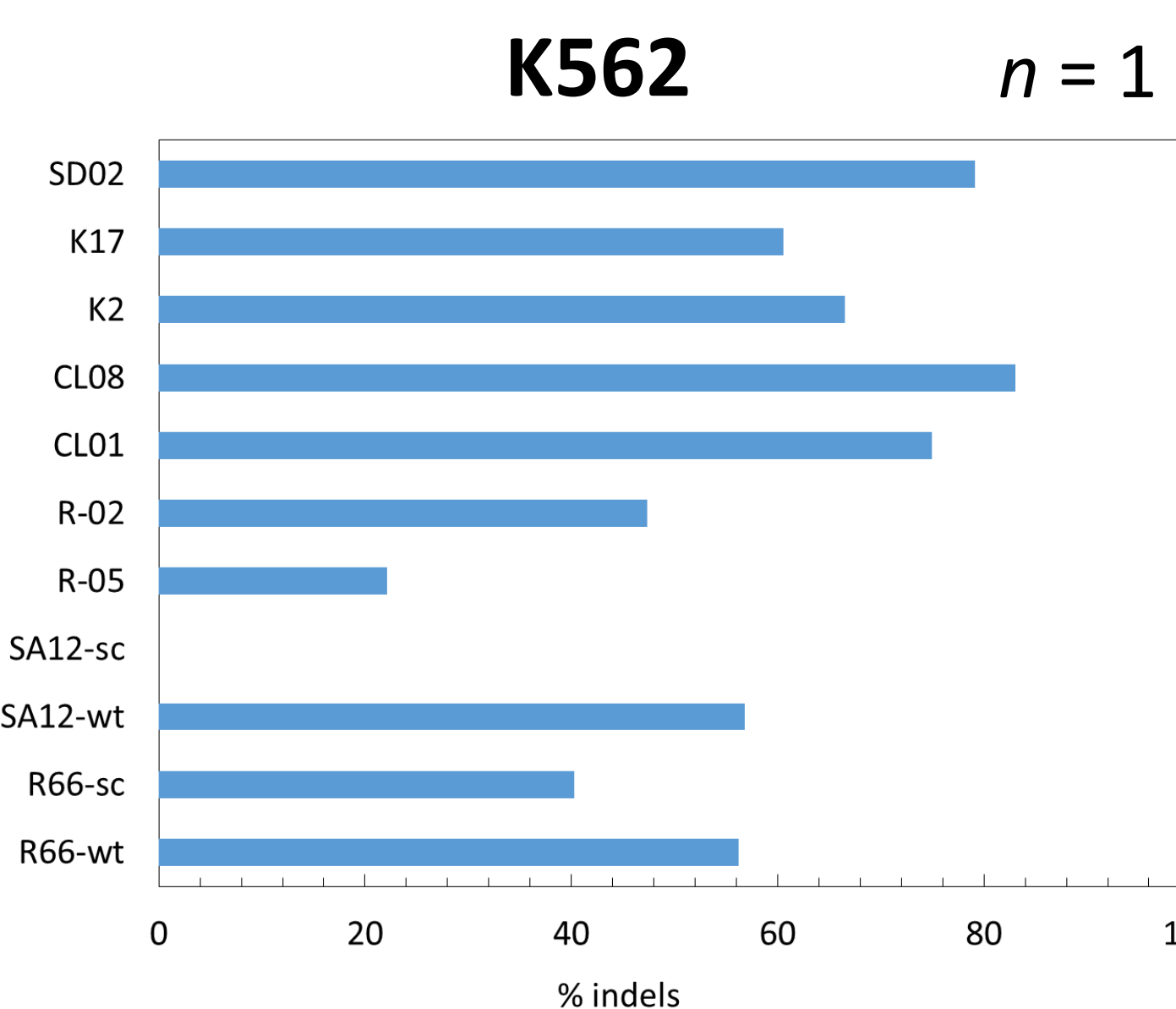
Results

- Efficiency of on-target cleavage
- Gene modification with R-02 at on-target and off-target sites^[5]

On-target site



Off-target site



- Left number : no. of reads
- Center number : the size of deletion (-) or insertion (+)

Conclusion

- Achieved the high level of on-target modification
- Determined the on-target and off-target gene modification

Future work

- Examine further off-target cleavage
- Investigate the effect of cell types
- Redesign guide RNAs to reduce off-target cleavage

References

- Jennifer A. Doudna. et.al. (2014) PMID : 25430774
- Yanni Lin. et.al. (2014) PMID : 24838573
- Kim HJ et.al. (2009) PMID : 19470664
- Matin Kircher. et.al. (2010) PMID : 20486139
- Gang Bao. et.al. (2013) PMID : 23939622

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