Genome Editing: Challenges and Opportunities

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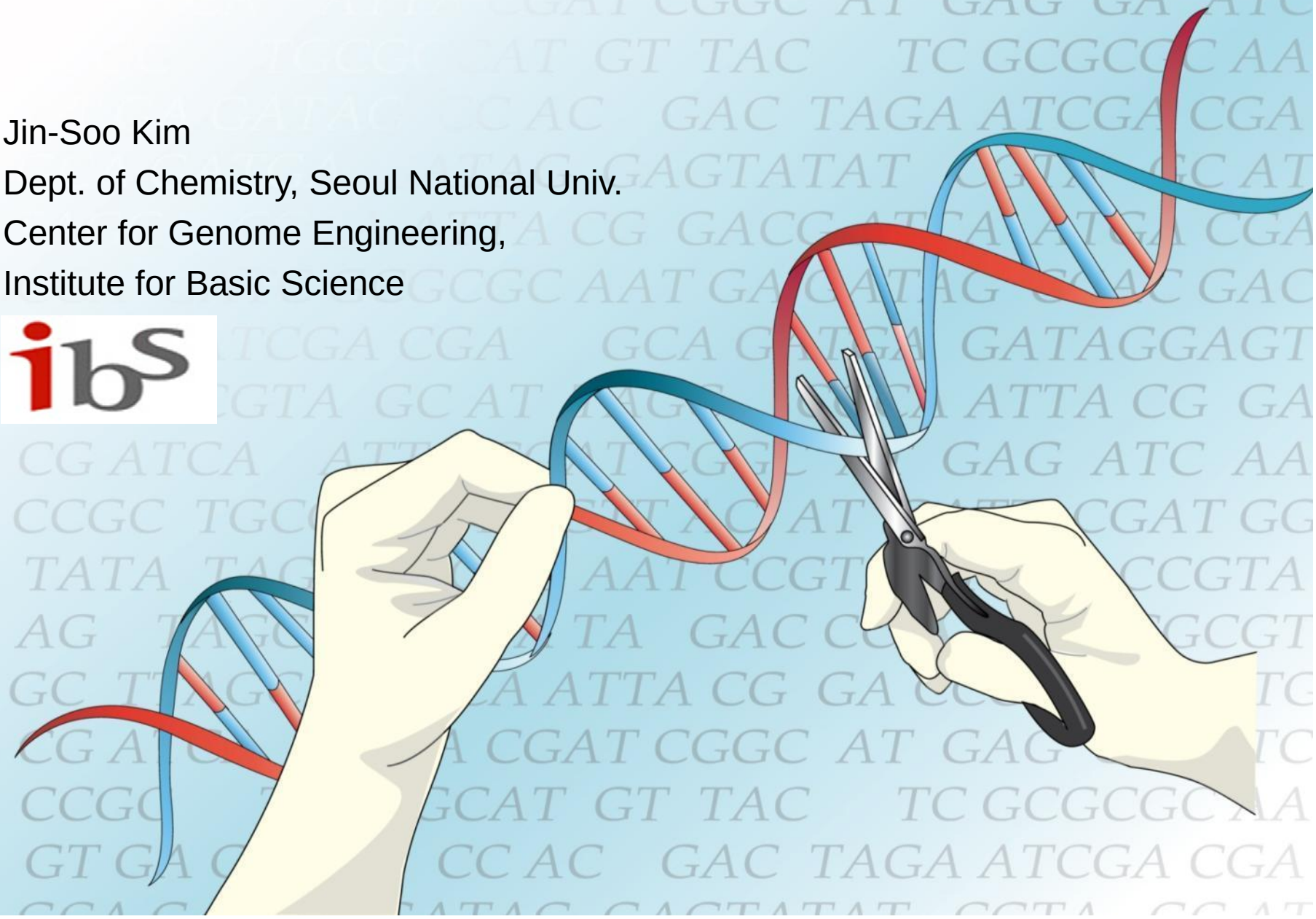
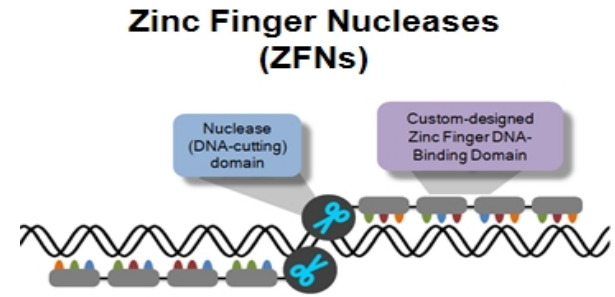
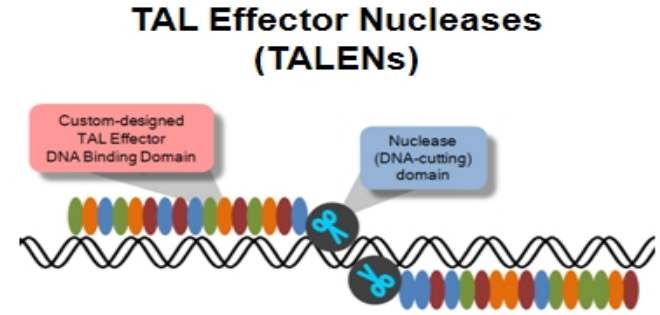
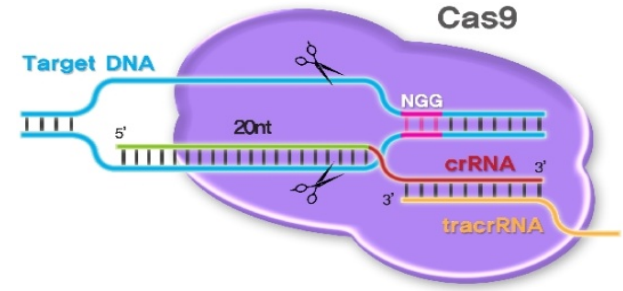


Challenges in Genome Editing

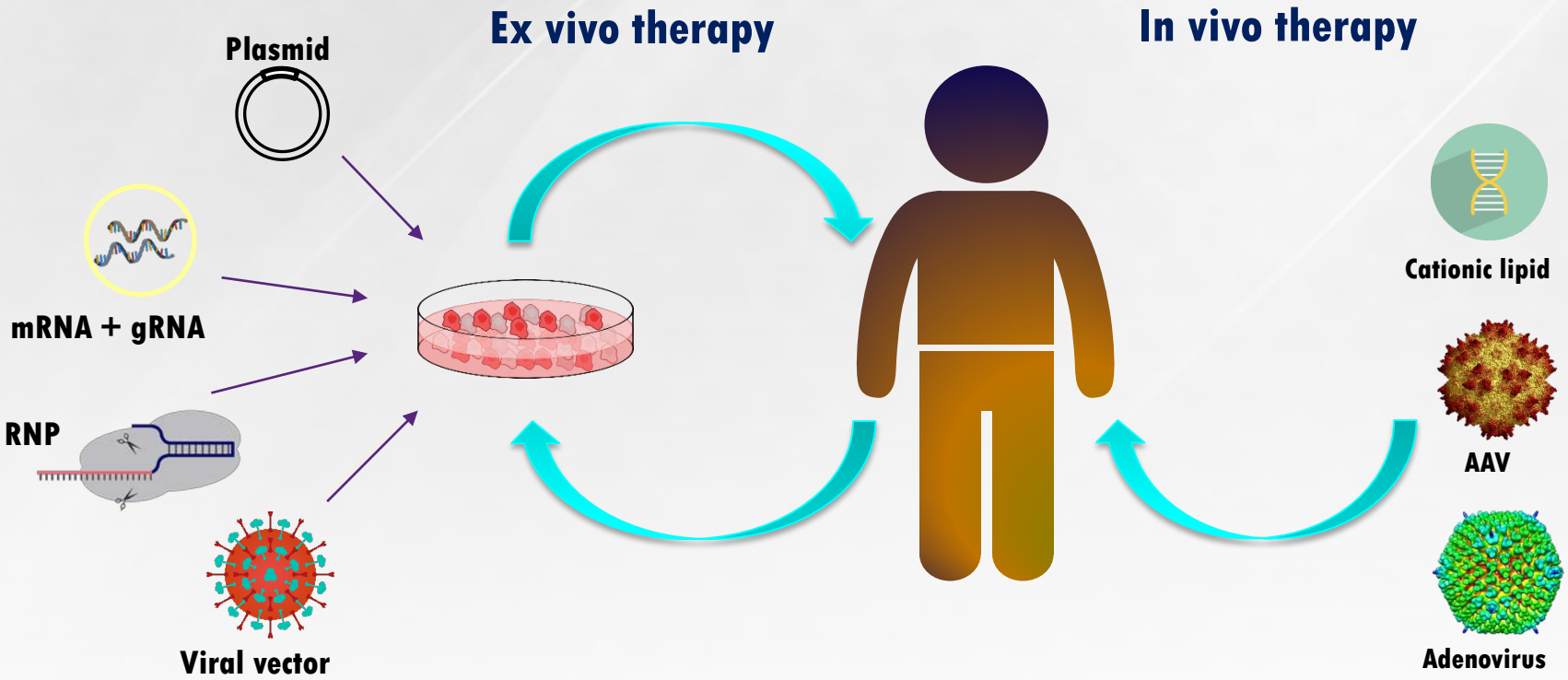
- Delivery
- Immunogenicity
- Mosaicism
- HDR vs. NHEJ
- Specificity: Off-target mutations
- Regulatory guidelines



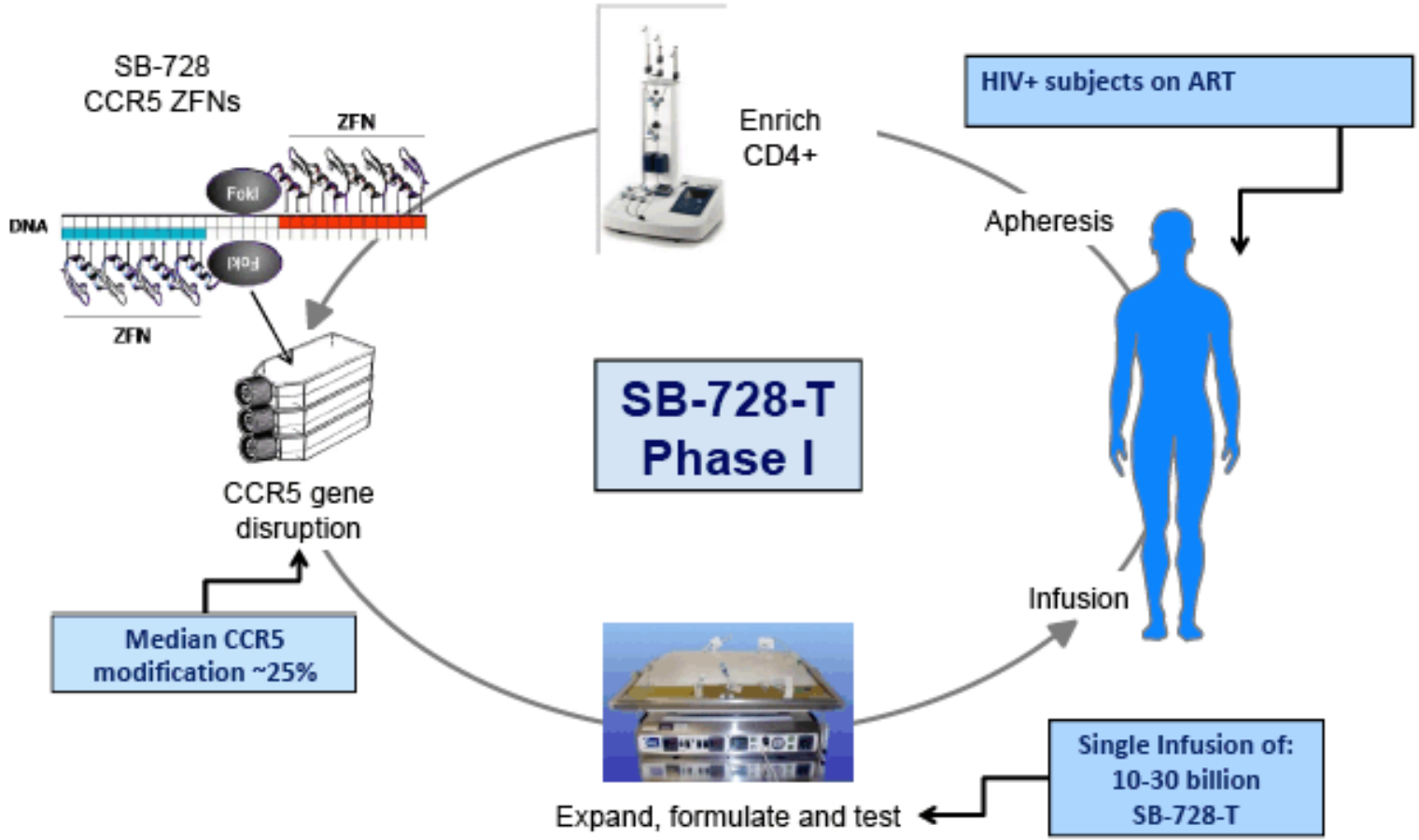




Genome Surgery

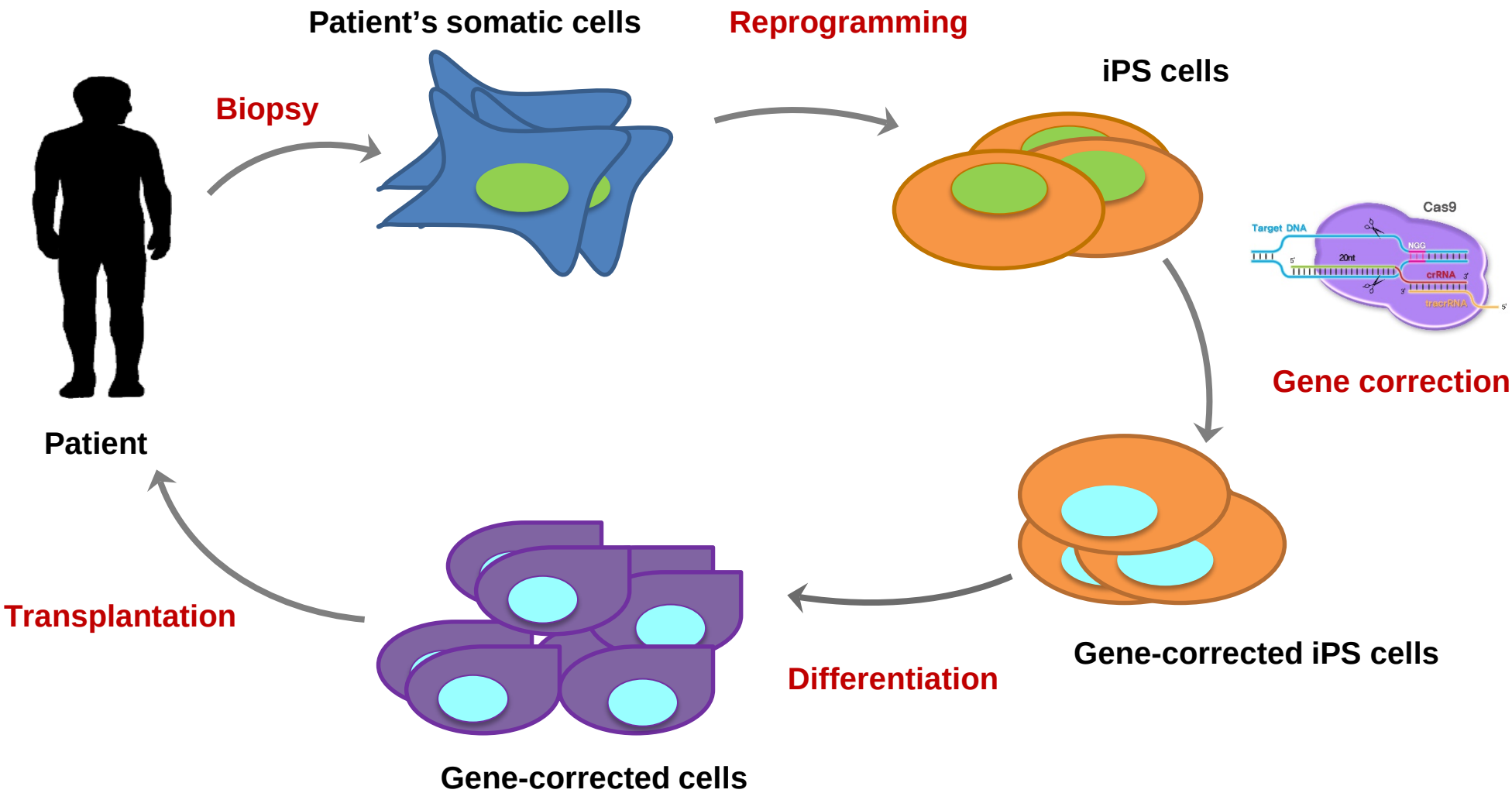


SB-728-T: Zinc Finger Nuclease Driven CCR5 Modified Autologous CD4+ T-cells



- T cells from HIV+ patients are treated with a programmable nuclease.
- CCR5-inactive T cells are delivered back to patients

Stem Cell Therapy: Gene Correction in iPS Cells

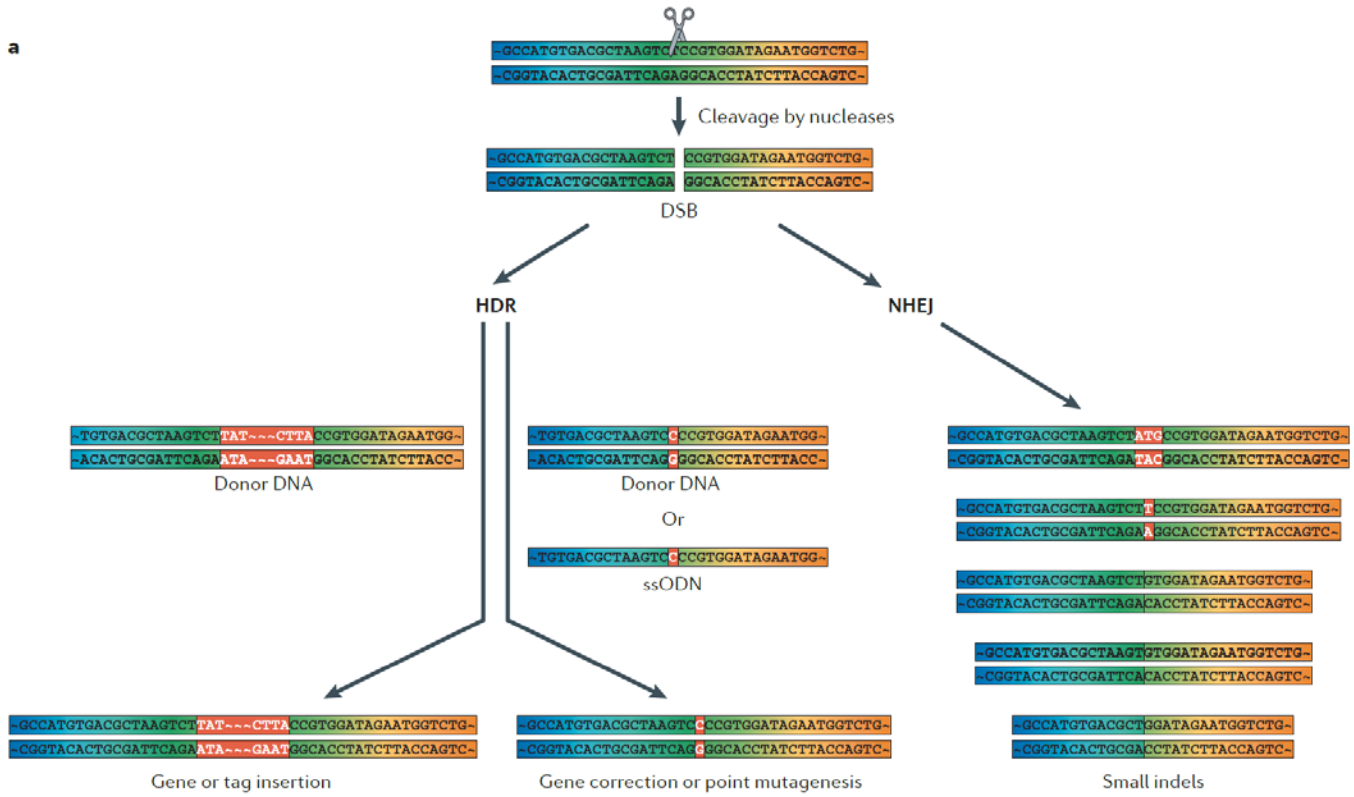


Park et al. Cell Stem Cell (2015)

Nuclease Immunogenicity

- Programmable nucleases are potential immunogens
- In vivo delivery and long-term expression can elicit immune responses
- Transient delivery of Cas9 RNPs or ex vivo delivery can be a solution

Double-Strand Break Repair

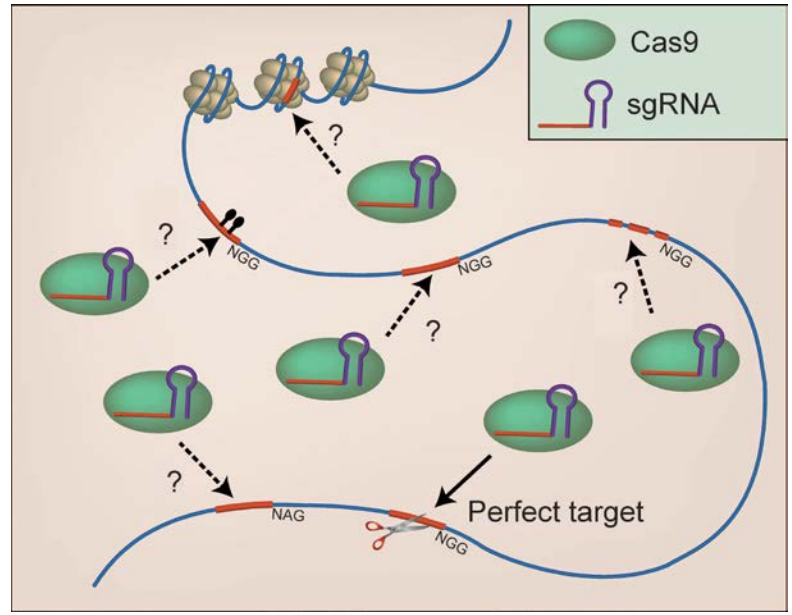


Kim H & Kim JS, Nat. Rev. Genet. (2014)

- Error-prone NHEJ is useful for targeted gene knockout
- Homology-directed repair is needed to correct genetic defects
- How to suppress NHEJ and enhance HDR?

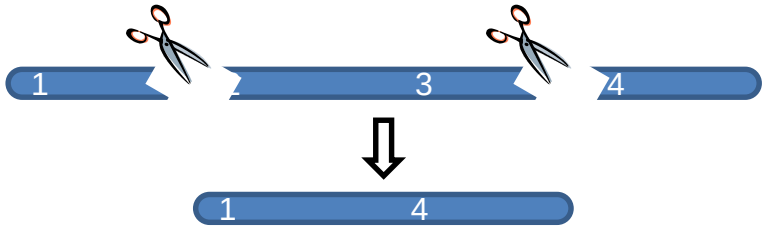
Nuclease Off-target Effects

- ZFNs, TALENs, and CRISPR-Cas9 can cleave off-target sites
- Off-target mutations can
  - Inactivate essential genes
  - Activate oncogenes
  - Cause chromosomal rearrangements

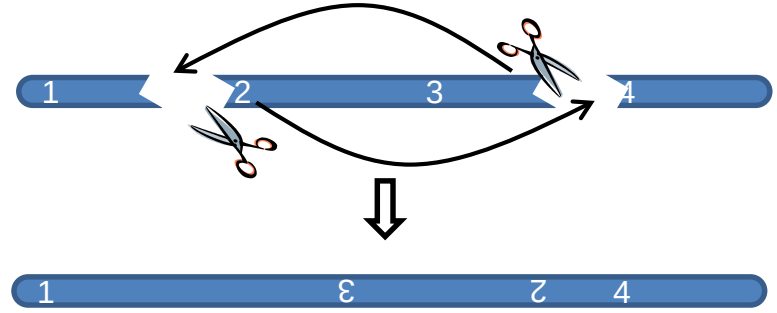


Wu et al. Quant. Biol. (2014)

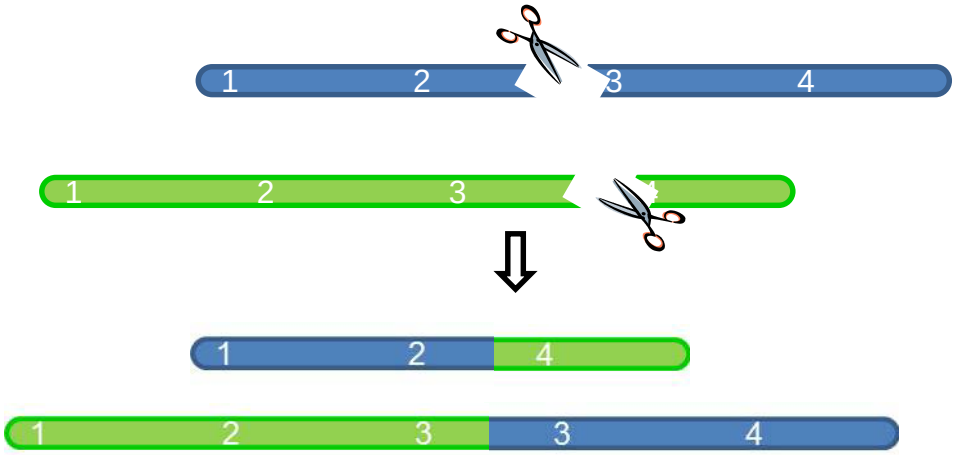
Off-target Chromosomal Rearrangements



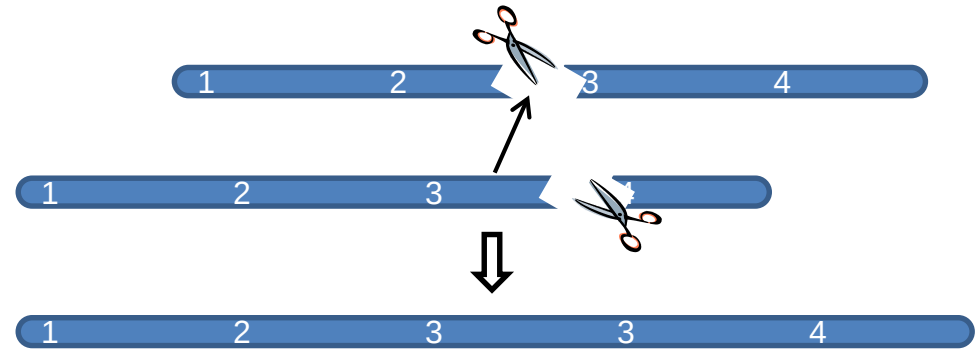
Deletion



Inversion



Translocation

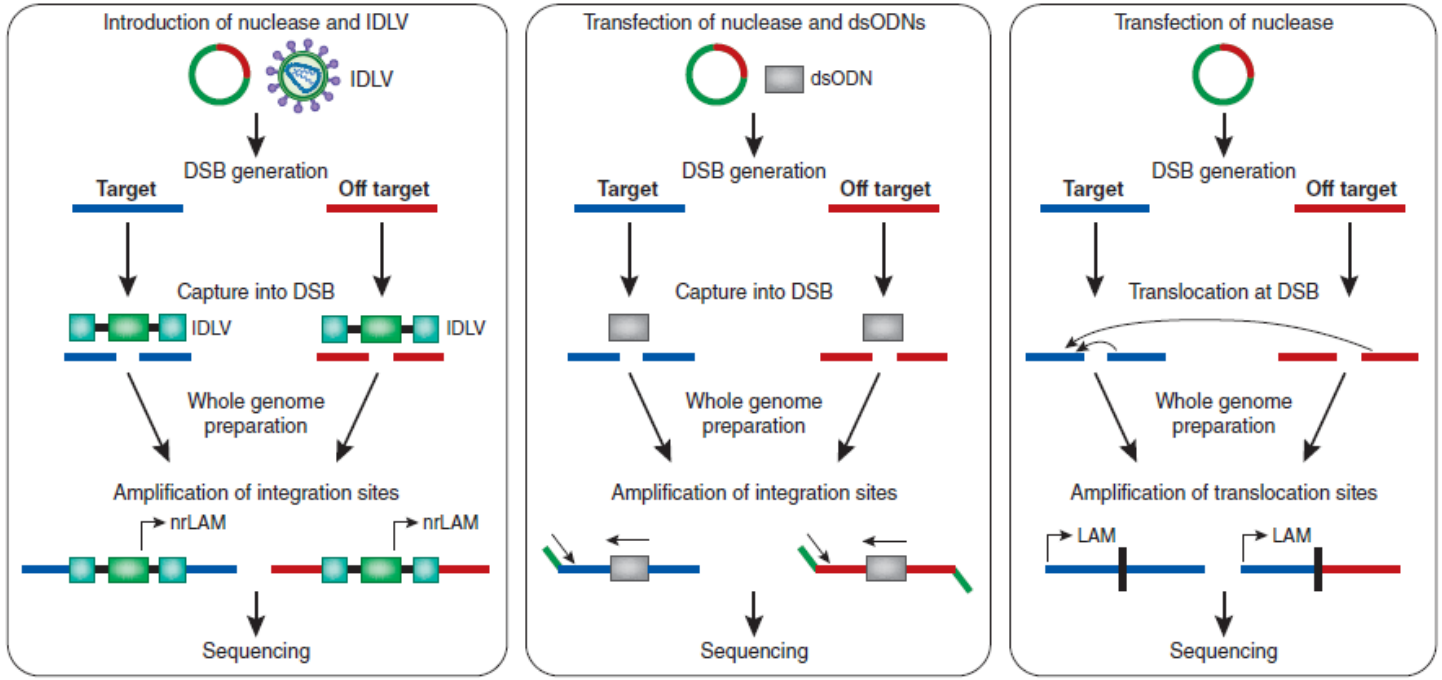


Duplication

How to Assess Genome-wide Off-target Effects

- Whole genome sequencing: Limited by sequencing depth
- Digenome-seq: Nuclease-digested whole genome seq.
- Cell-based methods: GUIDE-seq, Translocation seq., BLESS

Kim et al. Nat. Methods (2015)



Gabriel et al. Nat. Biotechnol. (2015)

How to Avoid Off-target Effects

- Choose a unique target site
- Use purified Cas9 protein rather than Cas9 plasmid
- Use modified guide RNAs
- Use modified Cas9: paired Cas9 nickases, Cas9-ZFP, dCas9-FokI

Koo et al. Molecules and Cells (2015)

Do Off-target Effects Matter?

- No drugs are free from off-target effects, often leading to repositioning
- Etoposide, an anti-cancer drug, cleaves DNA randomly, inducing mutations
- Biological consequences rather than mutations per se are more relevant
- CCR5-targeted ZFN has been proven safe in a clinical test (thus far)