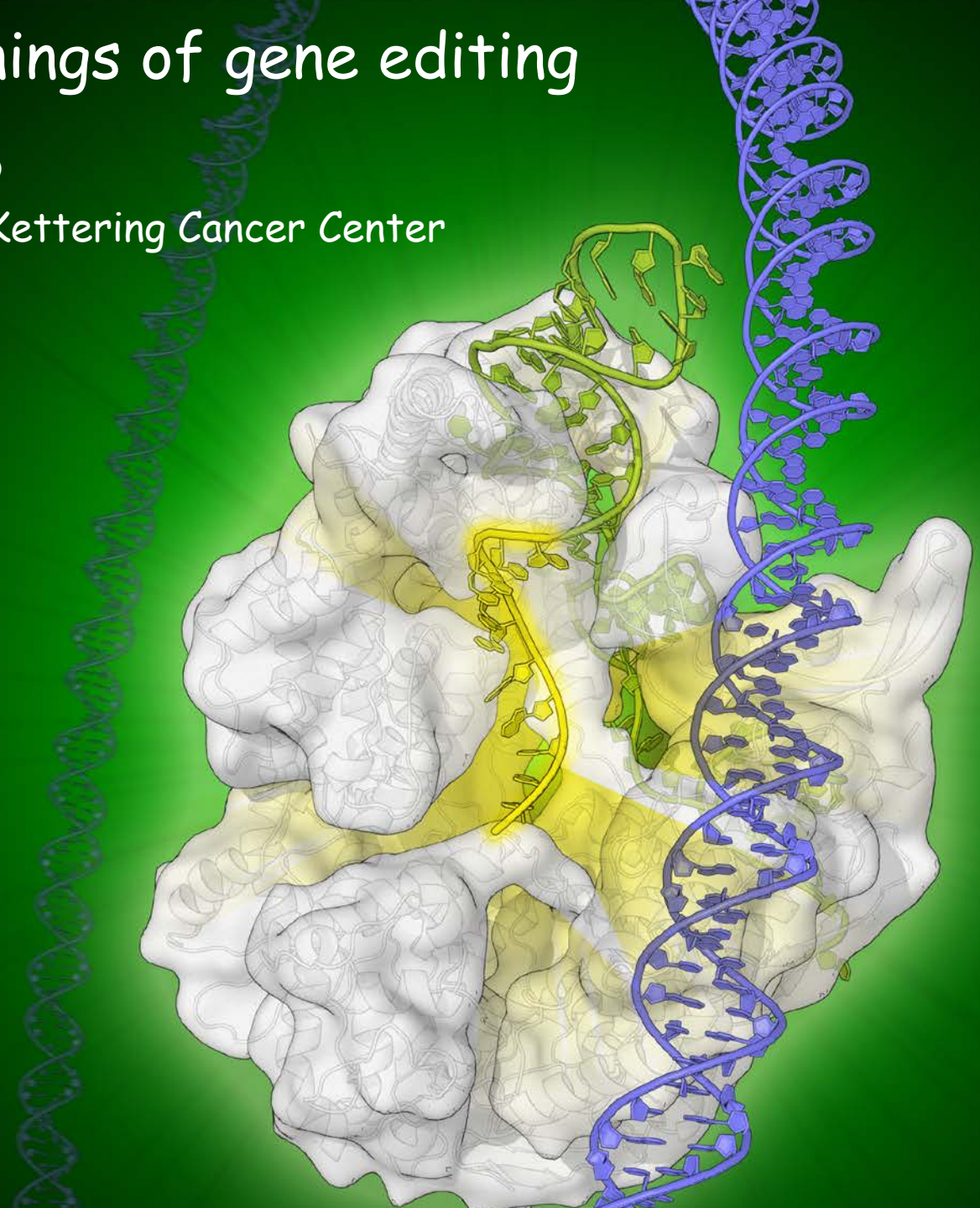
# The beginnings of gene editing

Maria Jasin, PhD

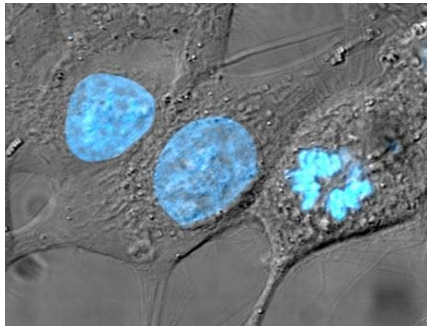
Memorial Sloan Kettering Cancer Center
New York



Courtesy of J Doudna
(H. Adam Steinberg, artist)

# Understanding biological characteristics

Phenotype



Forward genetics

Genotype

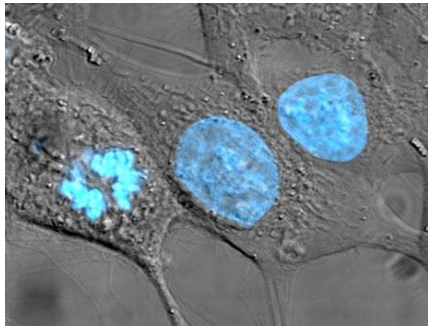
# Why modify the genome?

Scientists: to understanding biological characteristics

Genotype

Forward genetics

Reverse genetics



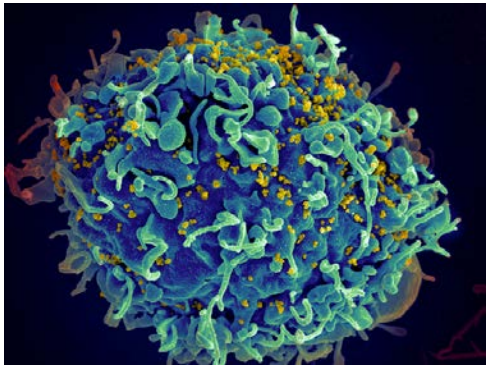
Phenotype

# Why modify the genome?

## Physicians: to ameliorate human disease

Sharon Lees/ Great Ormond Street Hospital

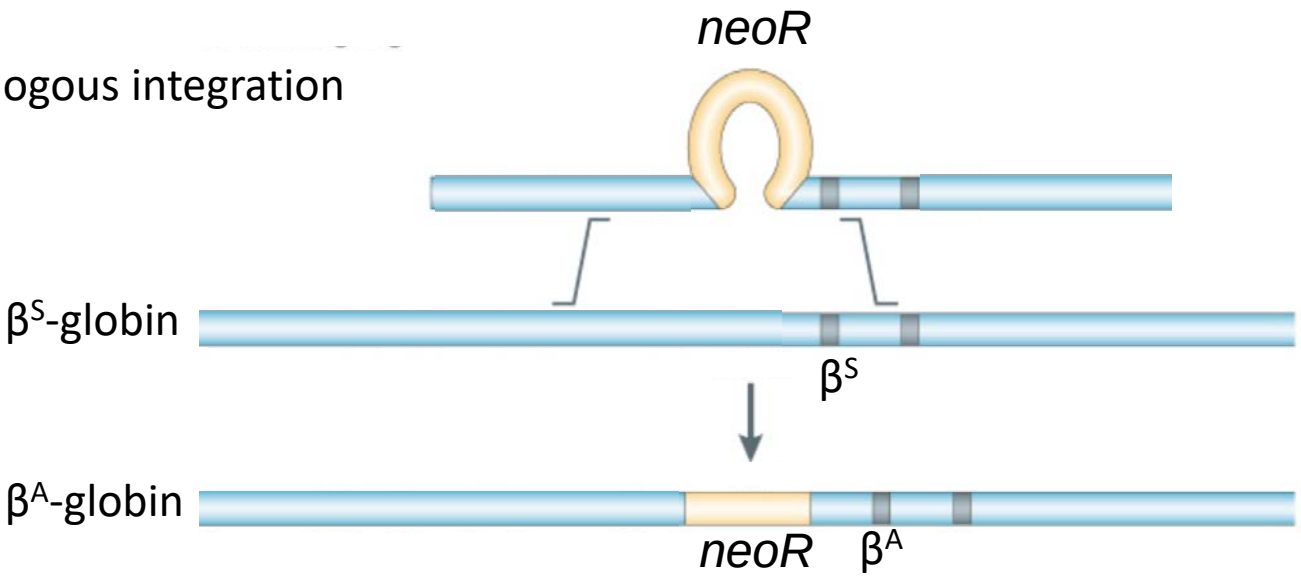
Reverse genetics



NIH.gov/science/hiv

# Targeted genome modification at the end of the 20th century

Homologous integration




1 in 9700 clones

Oliver Smithies and colleagues, 1985, 1991

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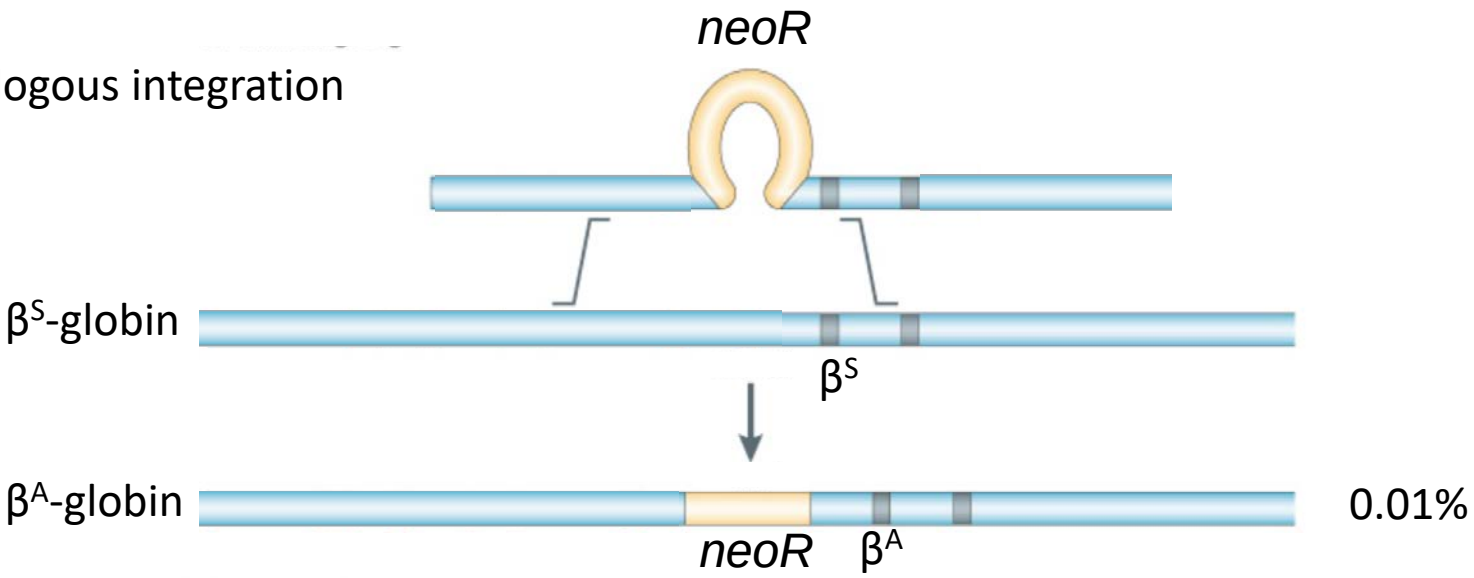
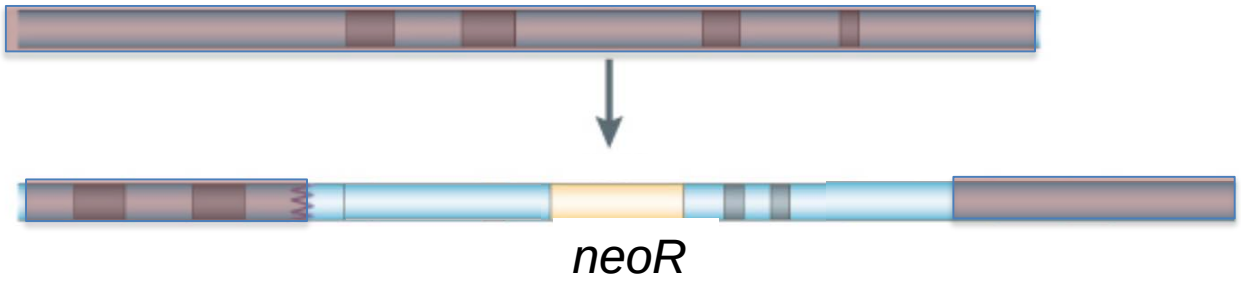



0.01%

Random integration




99.99%

Oliver Smithies and colleagues, 1985, 1991

# Targeted genome modification at the end of the 20th century

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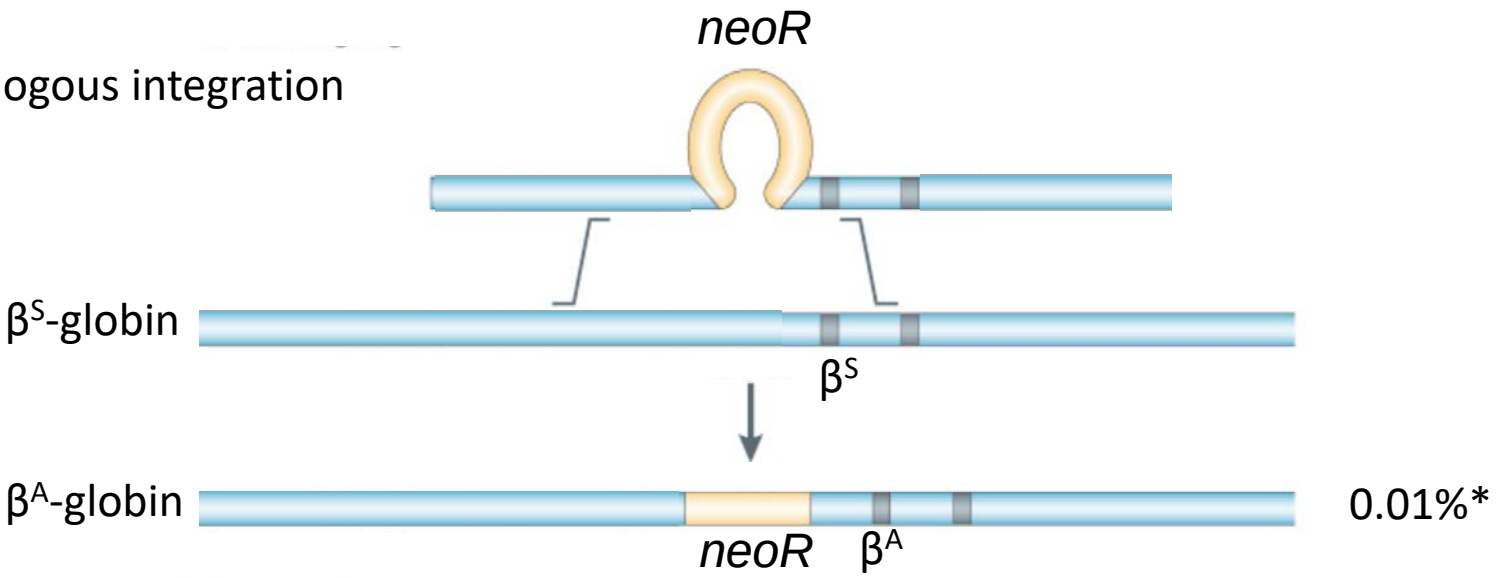
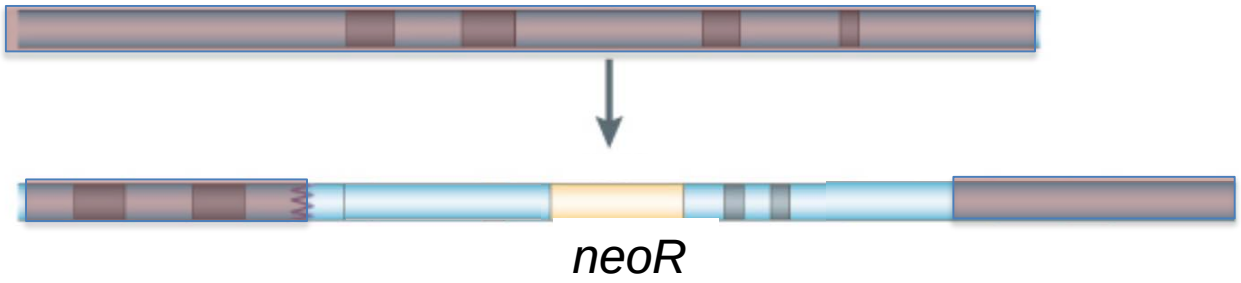



0.01%*

Random integration




99.99%

*~1 in $10^6$ in the total population of total cells

Oliver Smithies and colleagues, 1985, 1991

# Targeted genome modification at the end of the 20th century

- Highly inefficient: ~1 in $10^6$ unselected cells; ~1 in $10^2$-$10^4$ selected cells

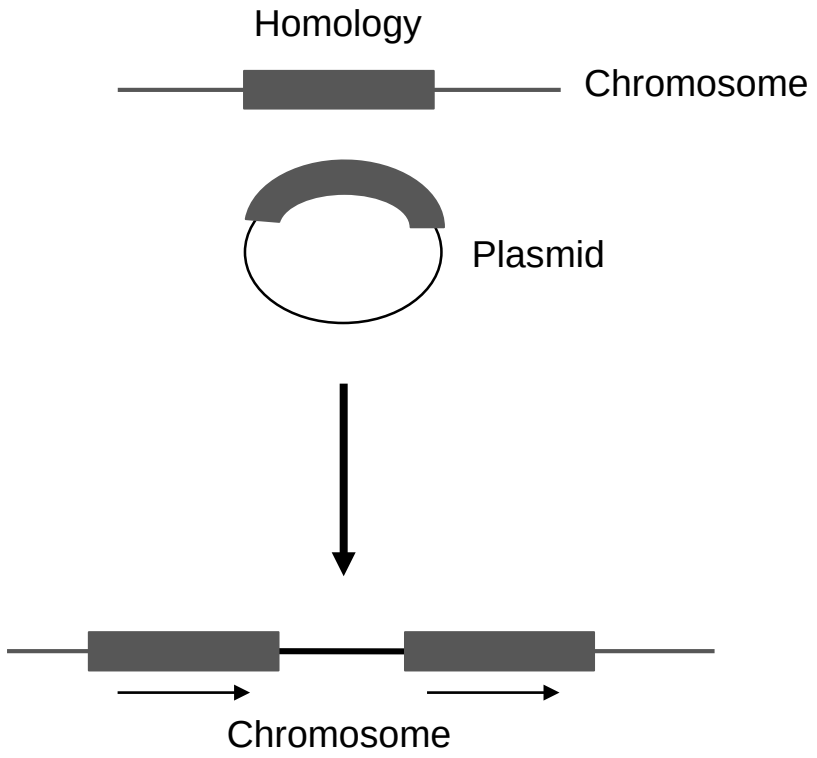
- Possible only in cell lines that can undergo selection, including mouse embryonic stem cells

- Thus, not applicable to most other organisms than the mouse, including humans

- The exception is yeast…

# Yeast: Targeted genome modification occurs in 100% of selected cells

Homologous integration




*Proc. Natl. Acad. Sci. USA*
Vol. 75, No. 4, pp. 1929–1933, April 1978
Genetics

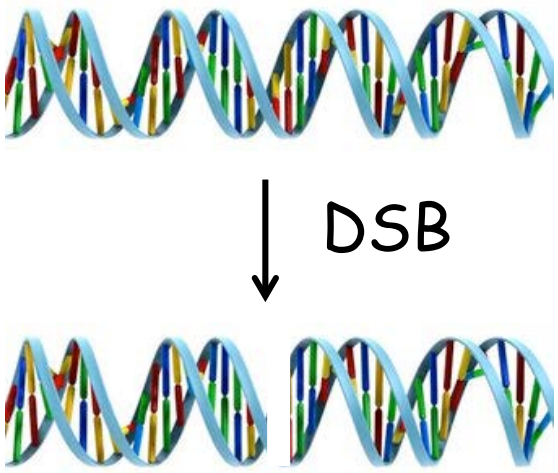
**Transformation of yeast**

(gene exchange/hybrid plasmid/integration)

ALBERT HINNEN, JAMES B. HICKS, AND GERALD R. FINK

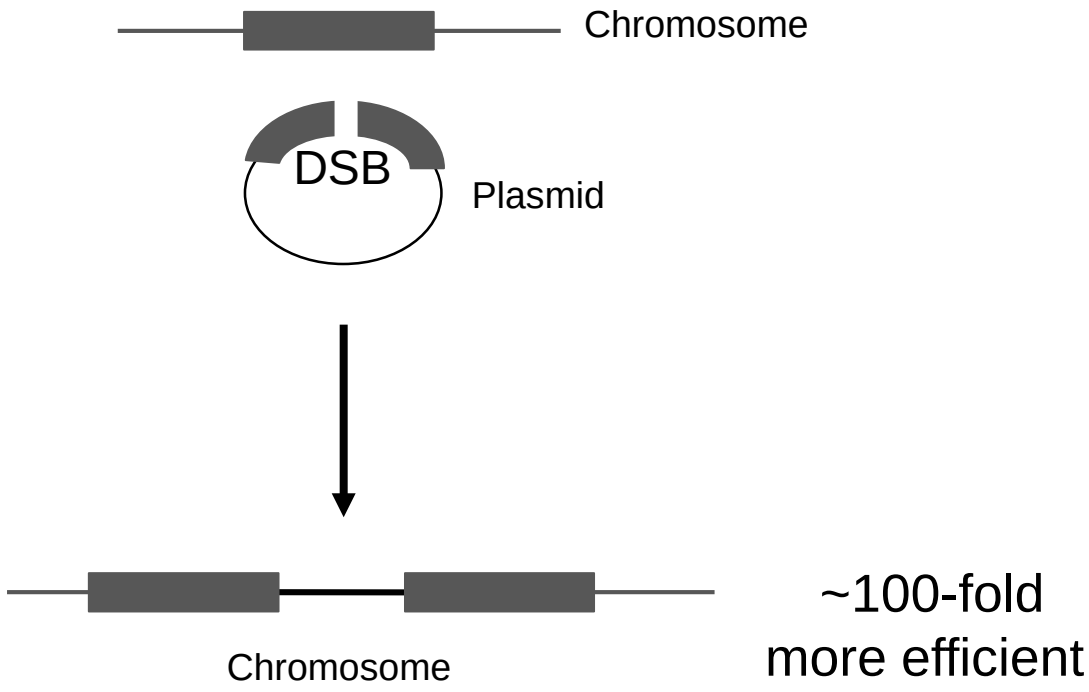
Department of Botany, Genetics and Development, Cornell University, Ithaca, New York 14853

# DNA double-strand break (DSB)



# Yeast: DSB in the plasmid makes an already highly feasible outcome more efficient

Homologous integration




~100-fold more efficient

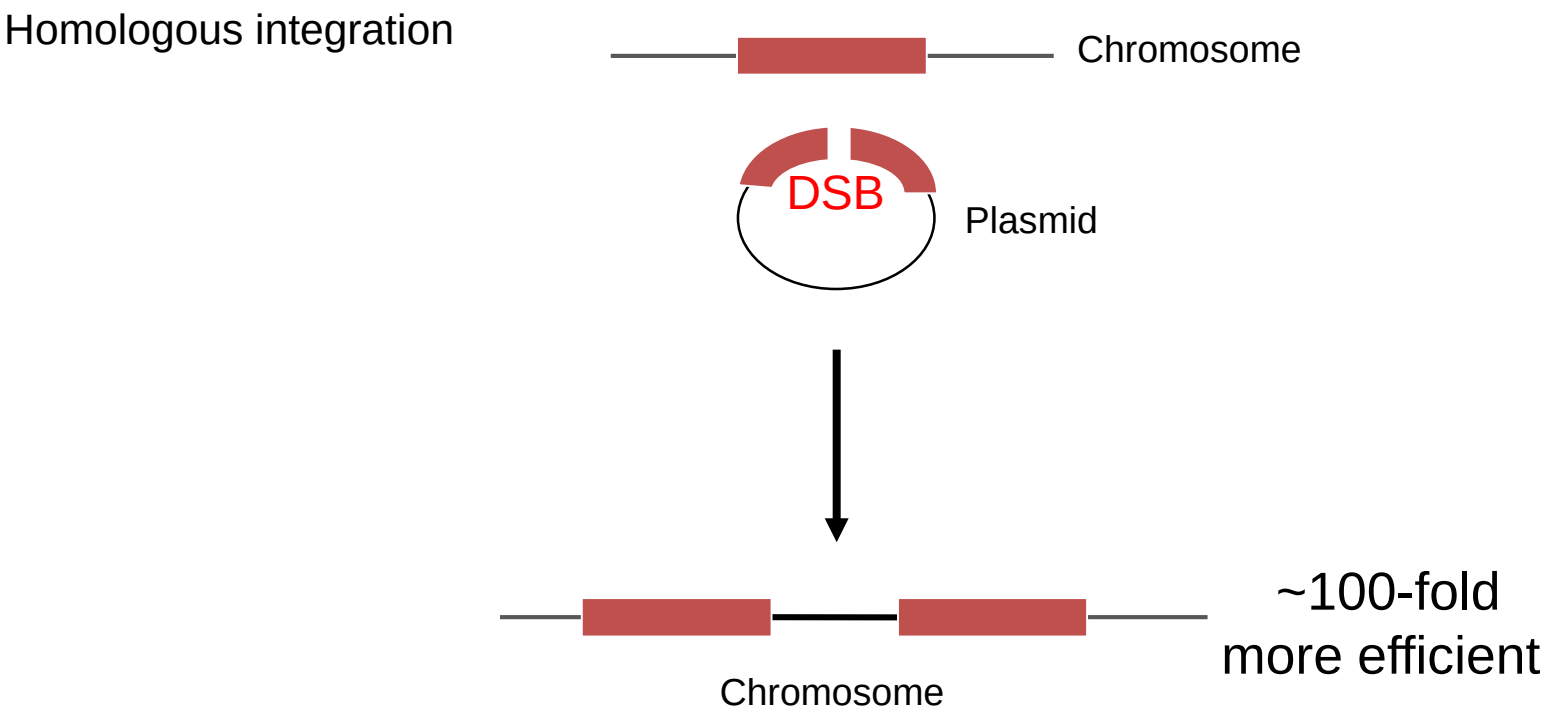
*Proc. Natl. Acad. Sci. USA*
Vol. 78, No. 10, pp. 6354–6358, October 1981
Genetics

**Yeast transformation: A model system for the study of recombination**

(plasmid integration/double-strand break repair/*rad*52-1 mutation/DNA repair synthesis)

TERRY L. ORR-WEAVER*, JACK W. SZOSTAK*†, AND RODNEY J. ROTHSTEIN

# Mammalian cells: DSB in the plasmid also increases homologous integration




**High frequency of homologous recombination in mammalian cells.**
M Jasin, J de Villiers, F Weber, W Schaffner
Cell 1985 Dec;43:695-703.

**Homologous integration in mammalian cells without target gene selection**.
M Jasin, P Berg.
Genes Dev. 1988 Nov;2(11):1353-63

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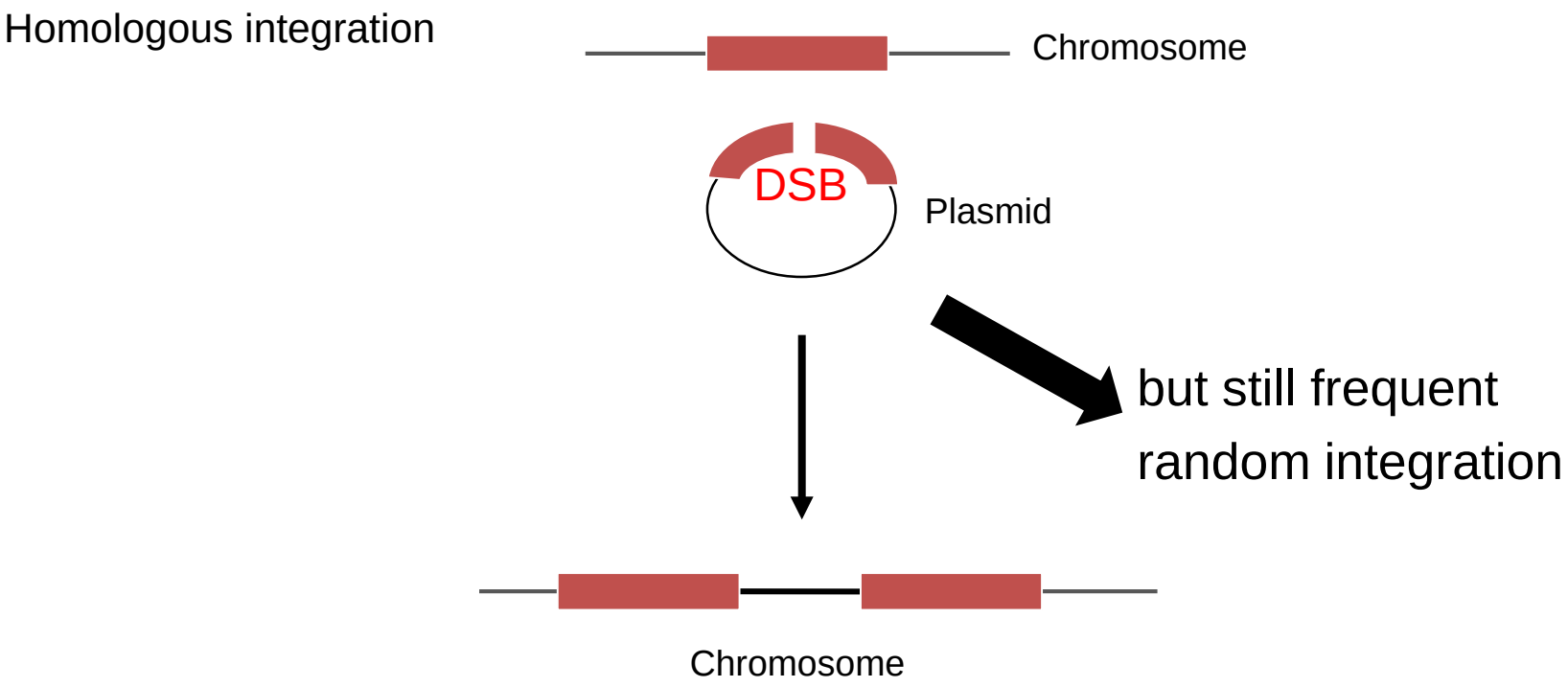
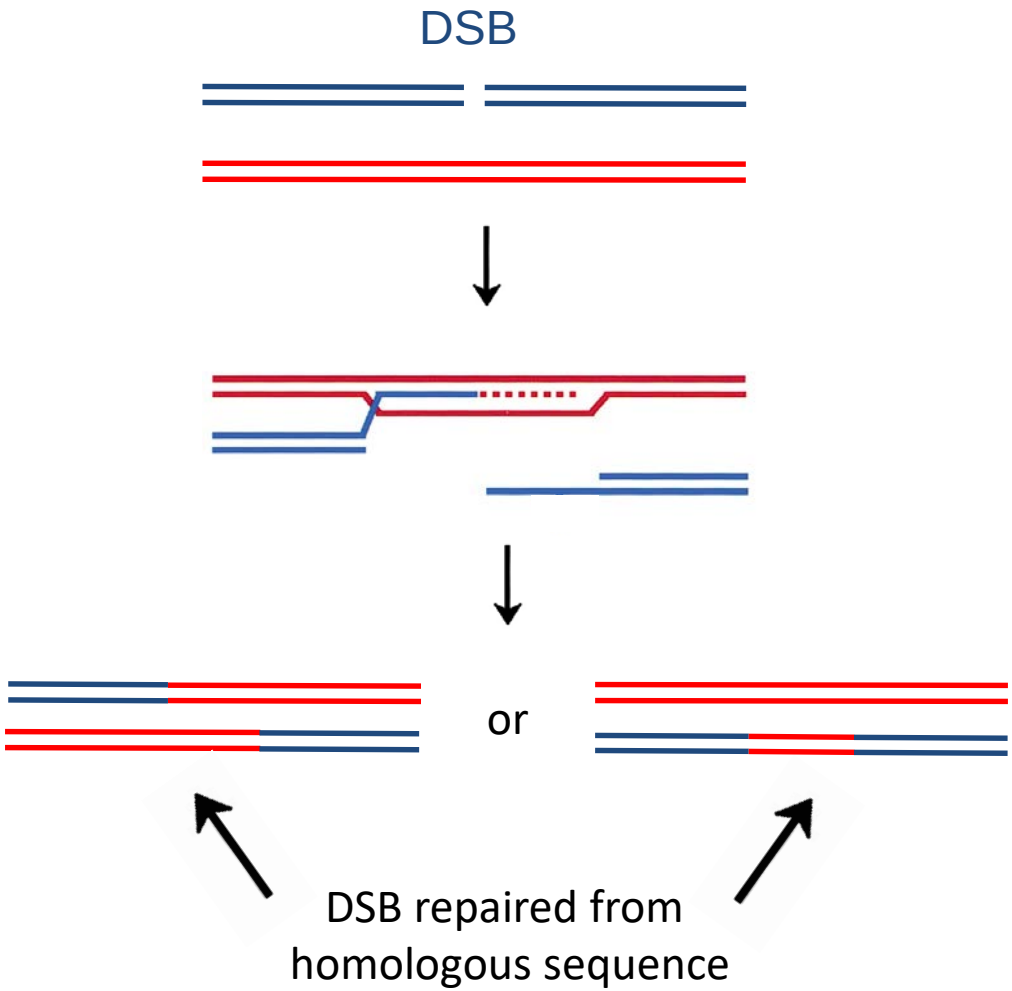



*Some basic principles from yeast hold in other organisms, but others do not*

# Yeast: Double-strand break model for recombination




Jack W. Szostak, Terry L. Orr-Weaver,
Rodney J. Rothstein, and Franklin W. Stahl

Cell, vol 33 25-35 May 1983

In 1994, our basic premise for targeted modification of the genome:

Introduce a DSB into the chromosome, rather than plasmid;
Cellular DNA repair mechanisms will repair the DSB, modifying the chromosome

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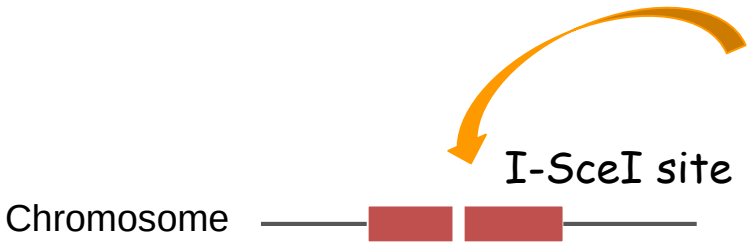
MOLECULAR AND CELLULAR BIOLOGY, Dec. 1994, p. 8096–8106 Vol.
0270-7306/94/$04.00+0


# Introduction of Double-Strand Breaks into the Genome of Mouse Cells by Expression of a Rare-Cutting Endonuclease

PHILIPPE ROUET, FATIMA SMIH, AND MARIA JASIN*

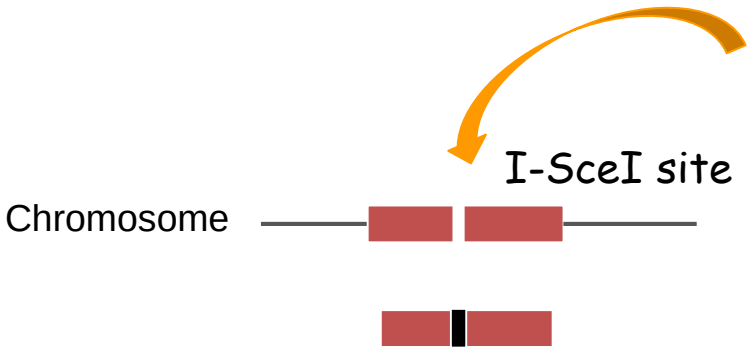
# First gene editing experiment




DSB:
I-SceI endonuclease
(a meganuclease)

Rouet et al, 1994

# First gene editing experiment

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Rouet et al, 1994

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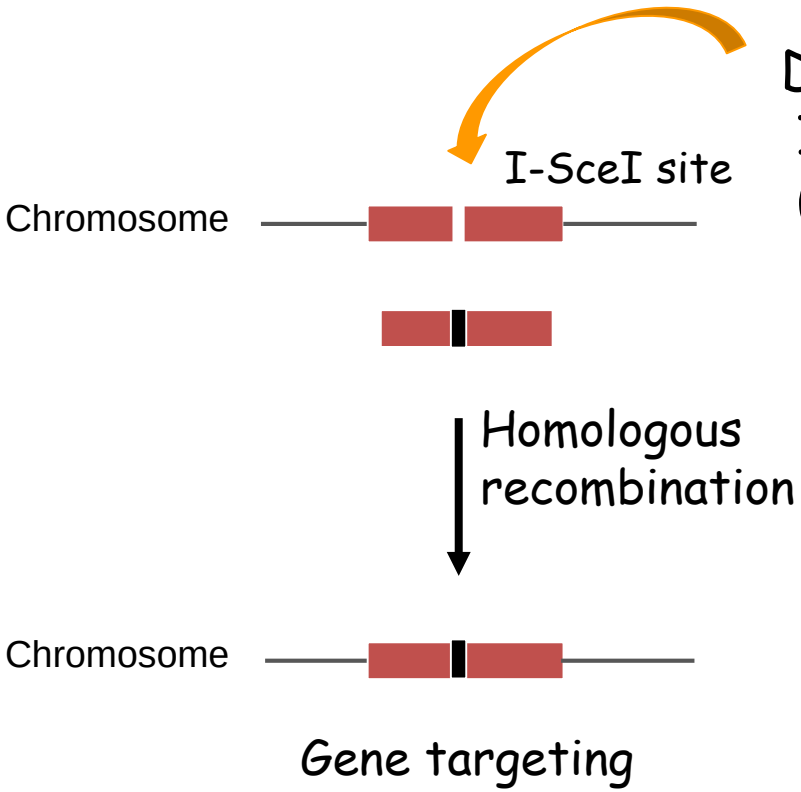
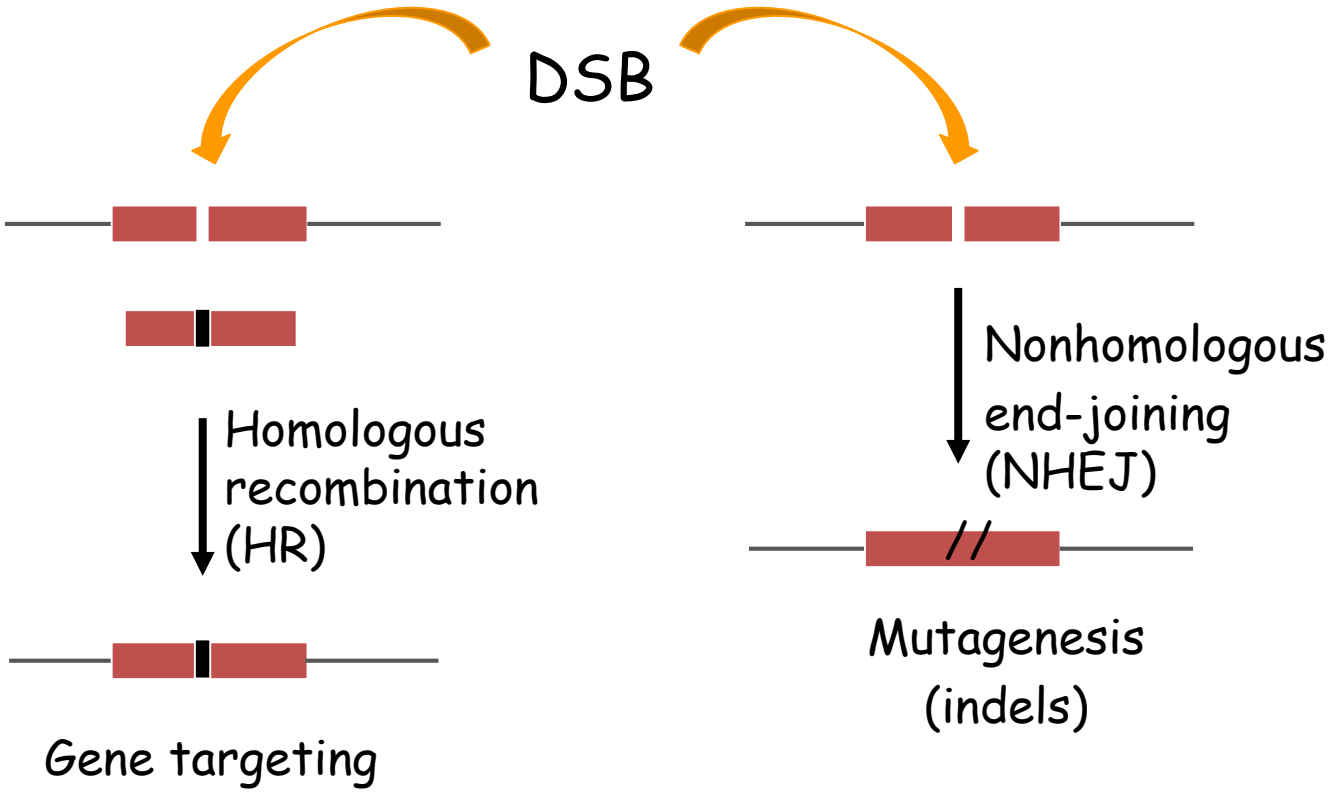



Gene targeting

Rouet et al, 1994

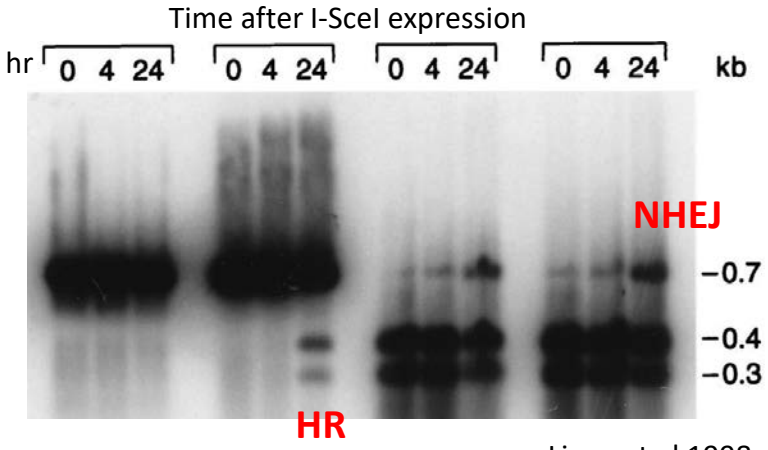
# First gene editing experiment




Rouet et al, 1994

# Gene editing: DSB-induced genome modification

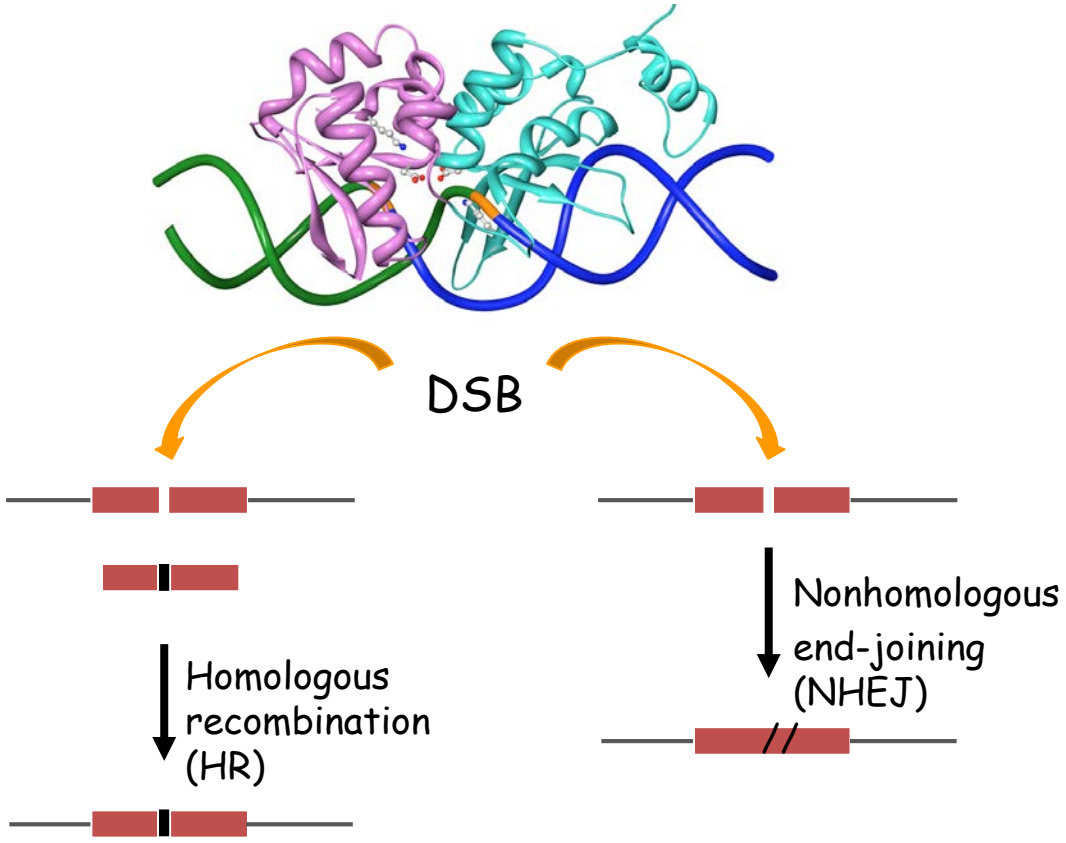
- Two outcomes:
  targeted modification by HR or mutagenesis by NHEJ
- NHEJ known for its use generating diversity in immune system rearrangements
- Highly efficient




Liang et al 1998

- In principle, possible in any cell or organism

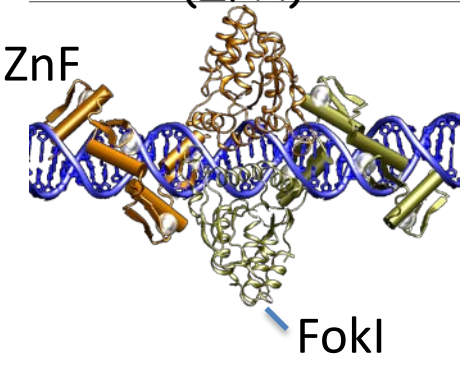
I-SceI
endonuclease/cleavage site



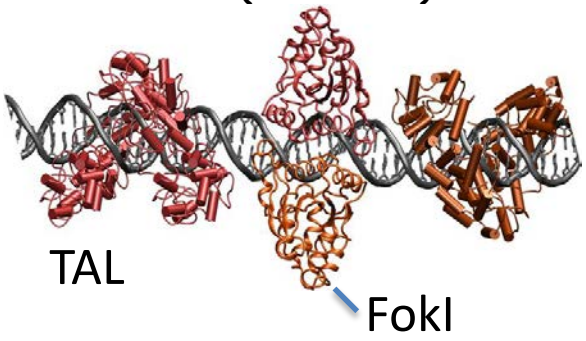

Problem for protein engineering:
Multiple contacts with DNA across the 18bp site

# DSBs at any genomic site using designed endonucleases

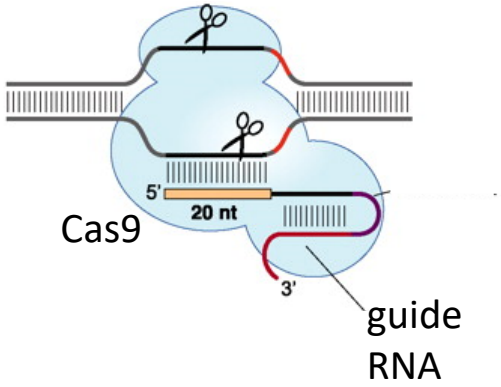
Zn Finger nucleases (ZFN)




TALE nucleases (TALENs)




RNA directed cleavage: Cas9/guide RNA




Code for DNA recognition:

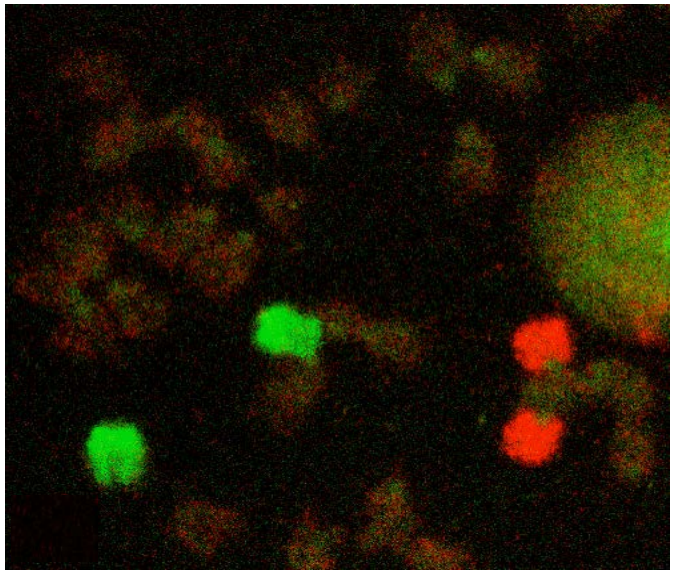
| | | |
|---|---|---|
| ~3 amino acids to 3 bp | ~1 amino acid to 1 bp | Watson-Crick bp |

Gene modification:

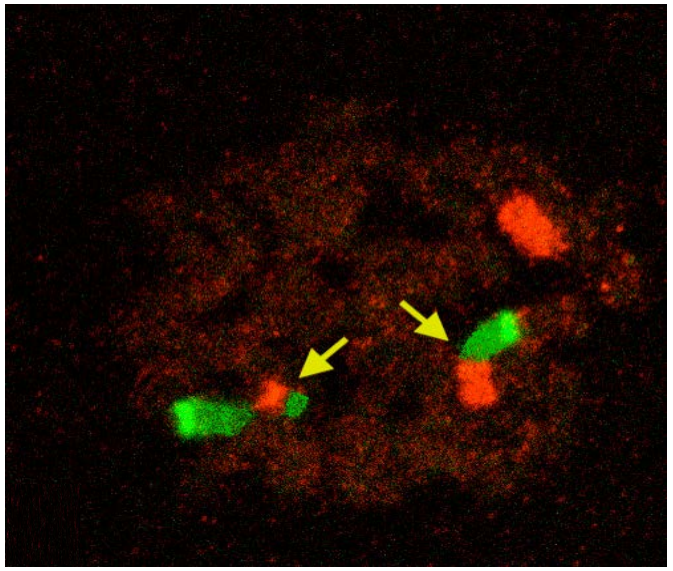
| | | |
|---|---|---|
| Modify some genes (2005) | Modify any gene (2009) | Multiple genes (2012) |

# DSBs induce chromosomal rearrangements in mammalian cells

Parental cell line



Reciprocal translocation



**Richardson and Jasin, Nature 2000**

Also ZFNs, TALENs, Cas9, pnCas9

# Gene editing: DSB-induced genome modification

- Two outcomes:
  targeted modification by HR or mutagenesis by NHEJ
- Highly efficient
- In principle, possible in any cell or organism
- Danger of unintended consequences (e.g., genetic rearrangements)