# Targeted DNA Breakage and Repair

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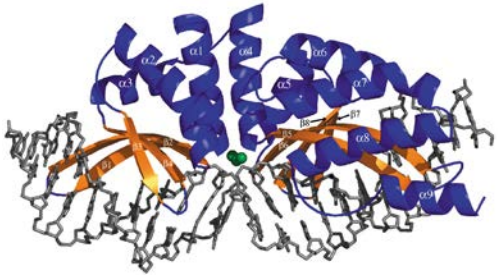
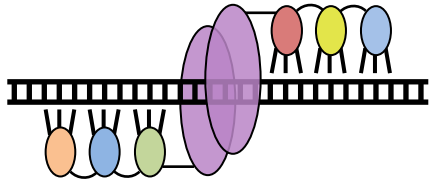
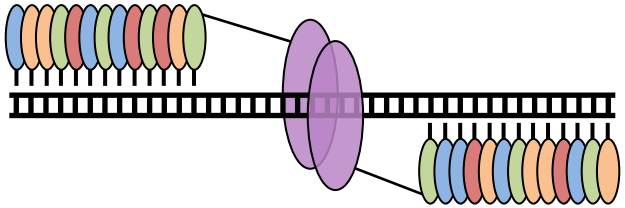
Disclosure: I receive license royalties from Sangamo Biosciences for a patent on targeted chromosomal mutagenesis with zinc-finger nucleases.

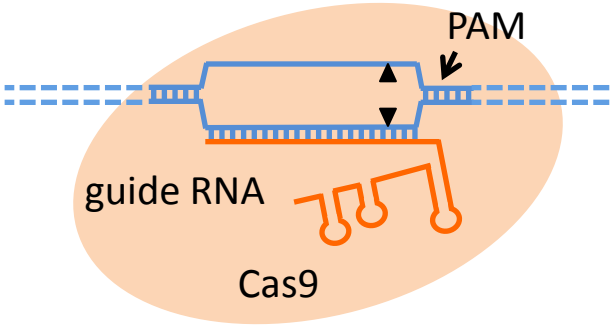
# Targetable Nucleases

| | Recognition |
|---|---|
| HEs (MNs) | Complex |
| ZFNs | 1 module/3 bp |
| TALENs | 1 module/1 bp |
| CRISPR/Cas | 1 base/1 bp |





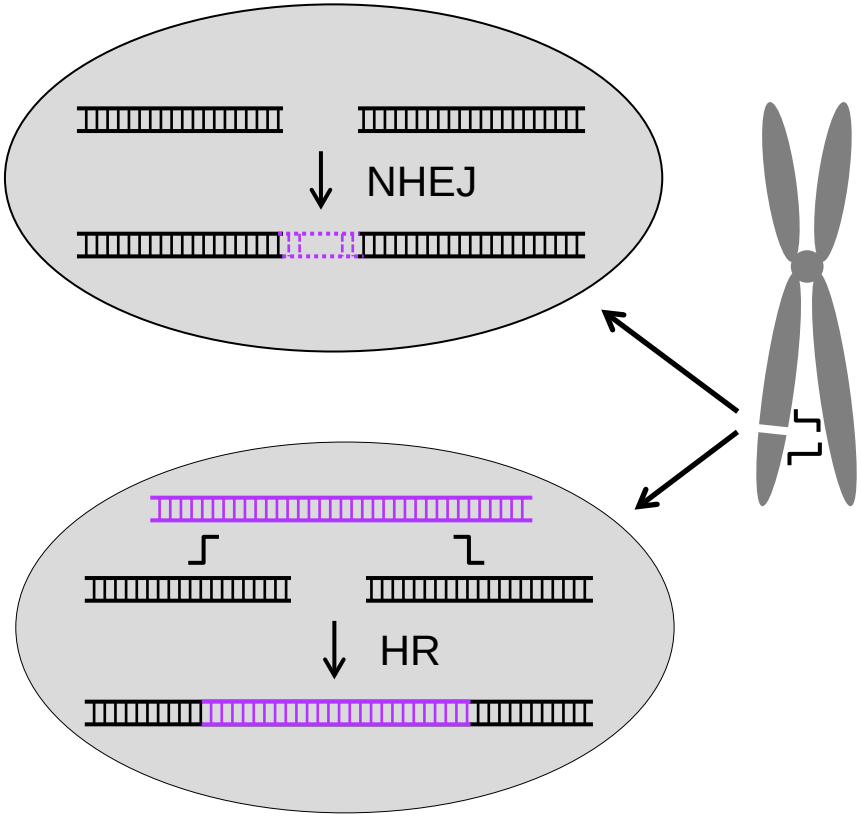




# Expected outcomes from targeted cleavage

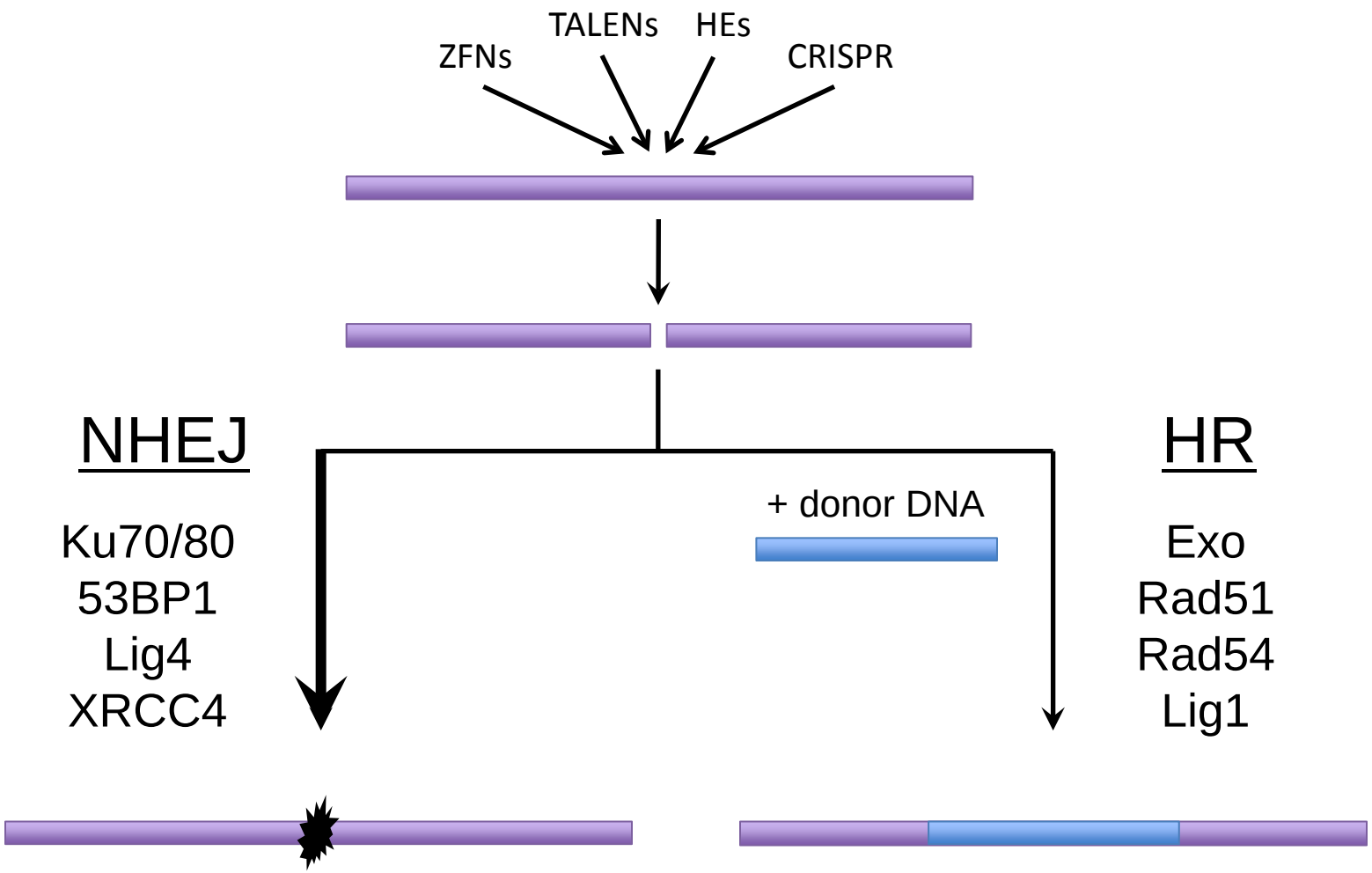
Targeted mutagenesis

Targeted gene replacement

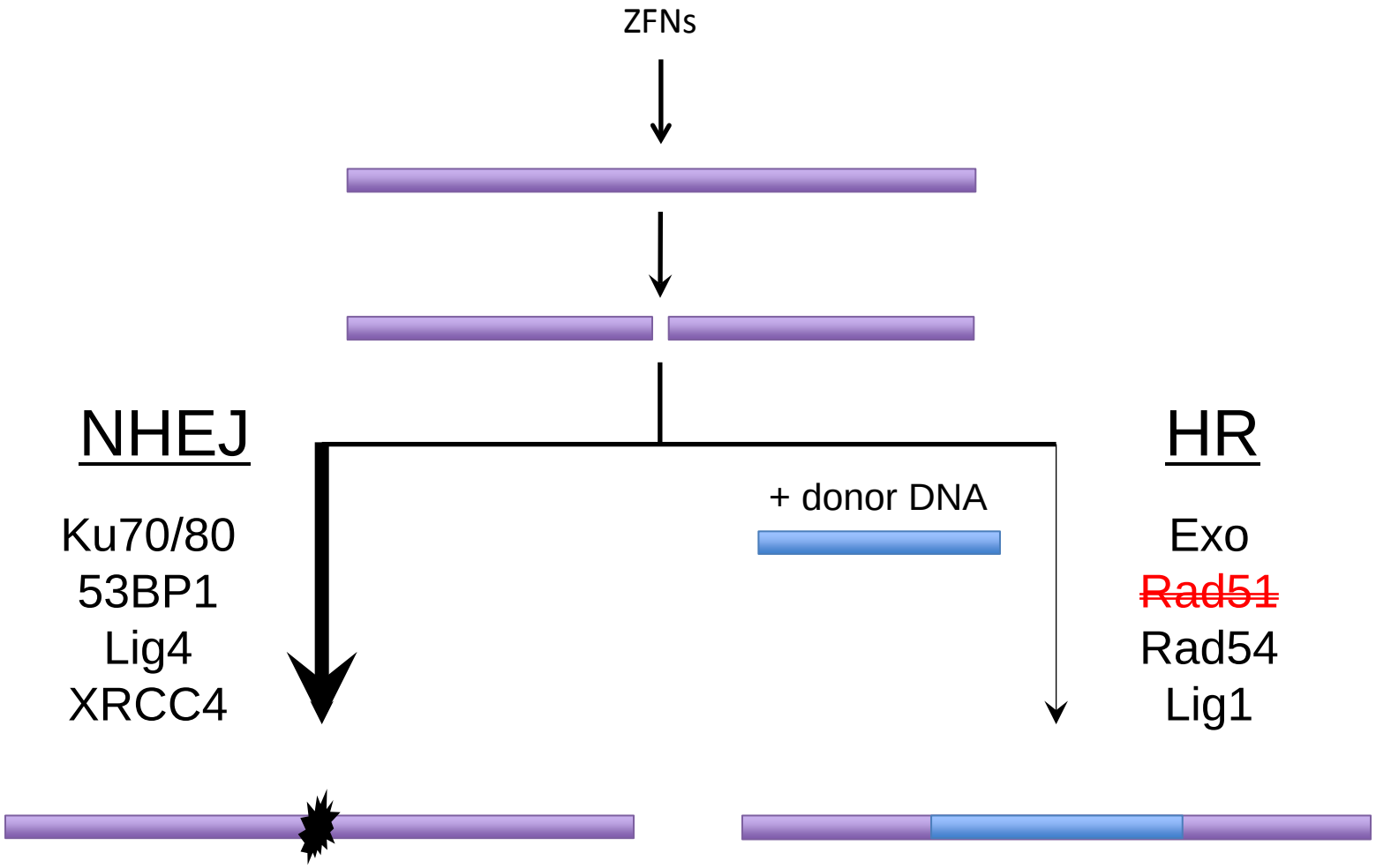



Once the break is made, the outcome depends on cellular DNA repair activities.

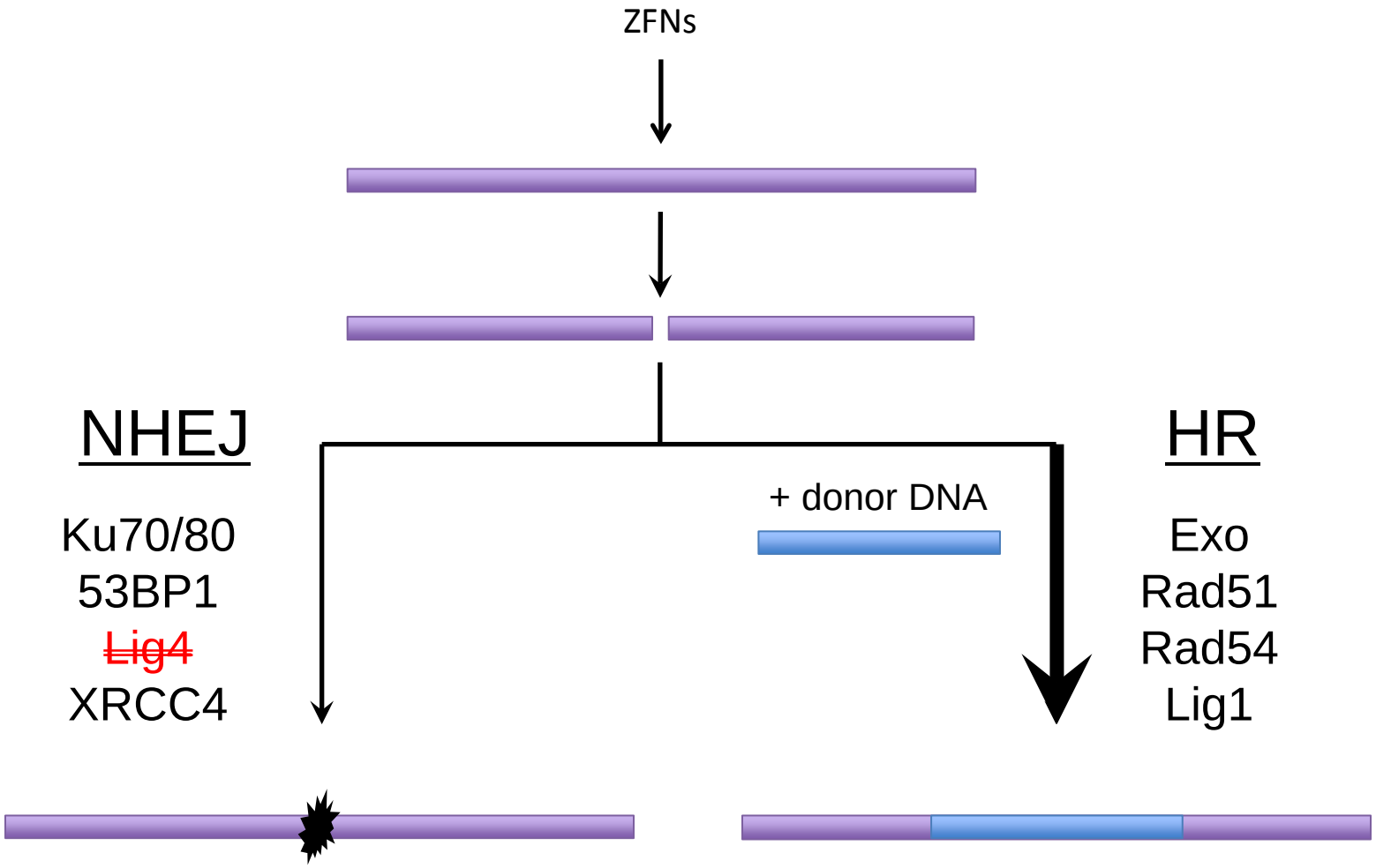
# DSB Repair Pathways



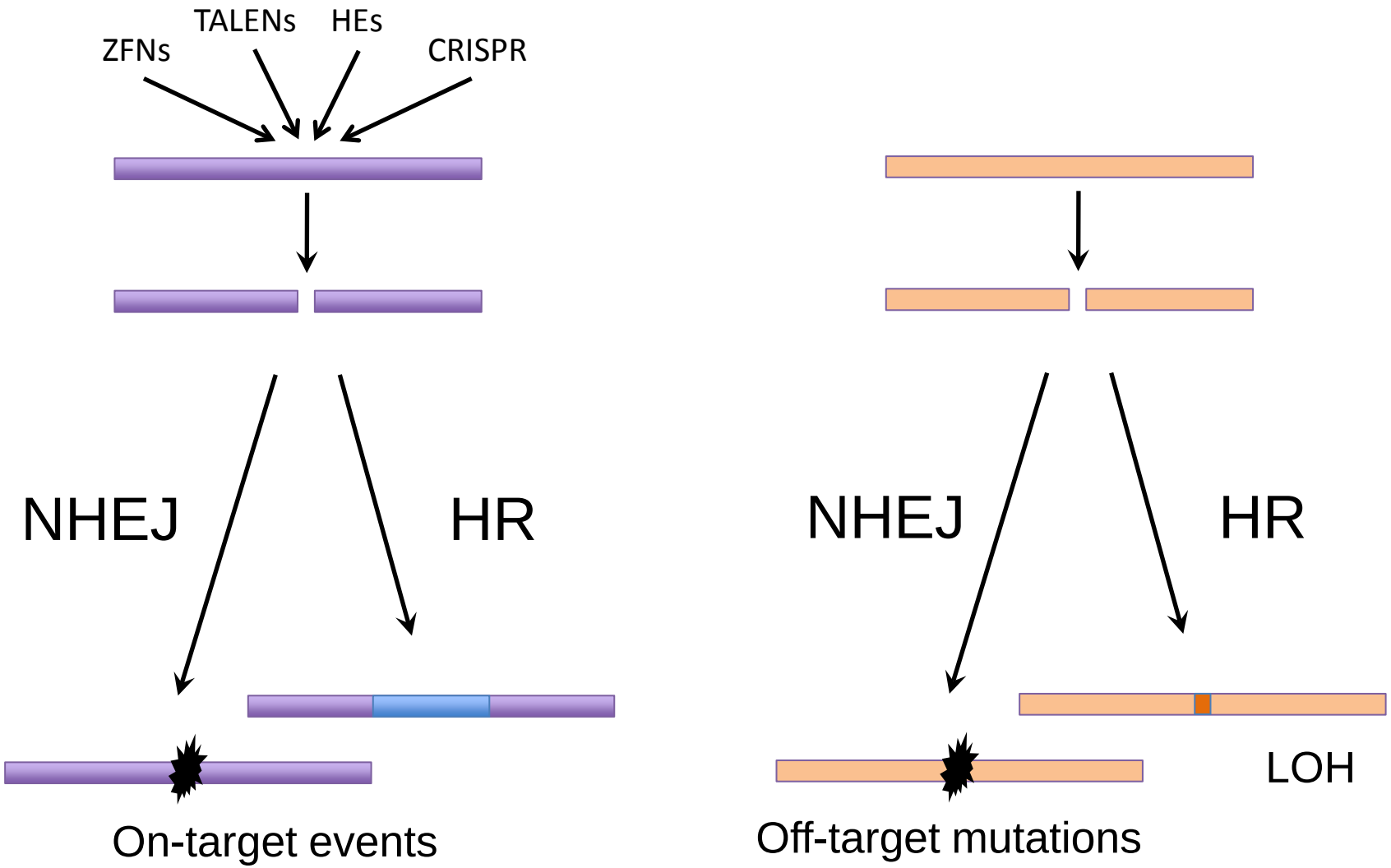
# Shifting the balance toward NHEJ



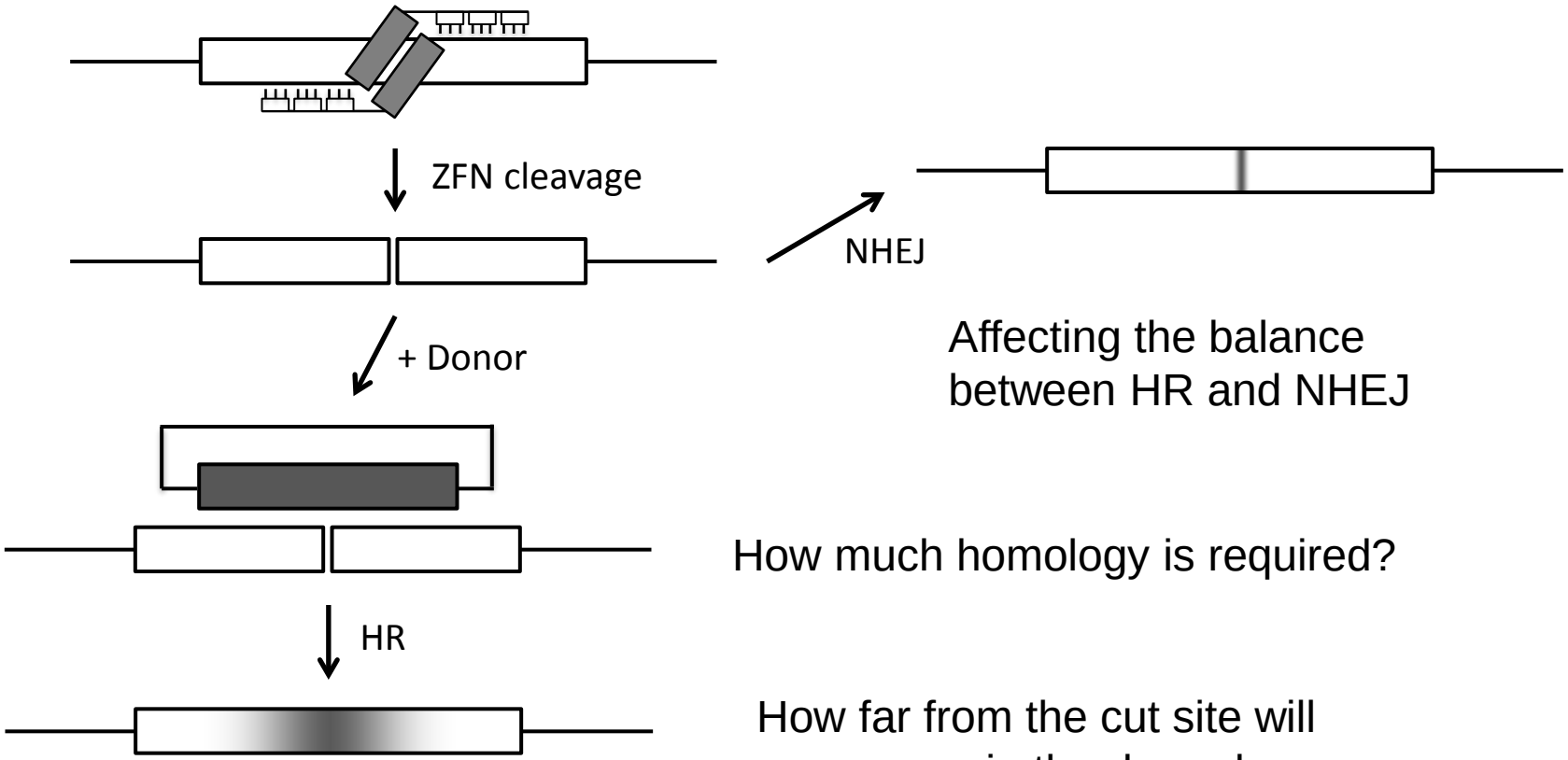
# Shifting the balance toward HR



# Off-target effects



# Donor issues

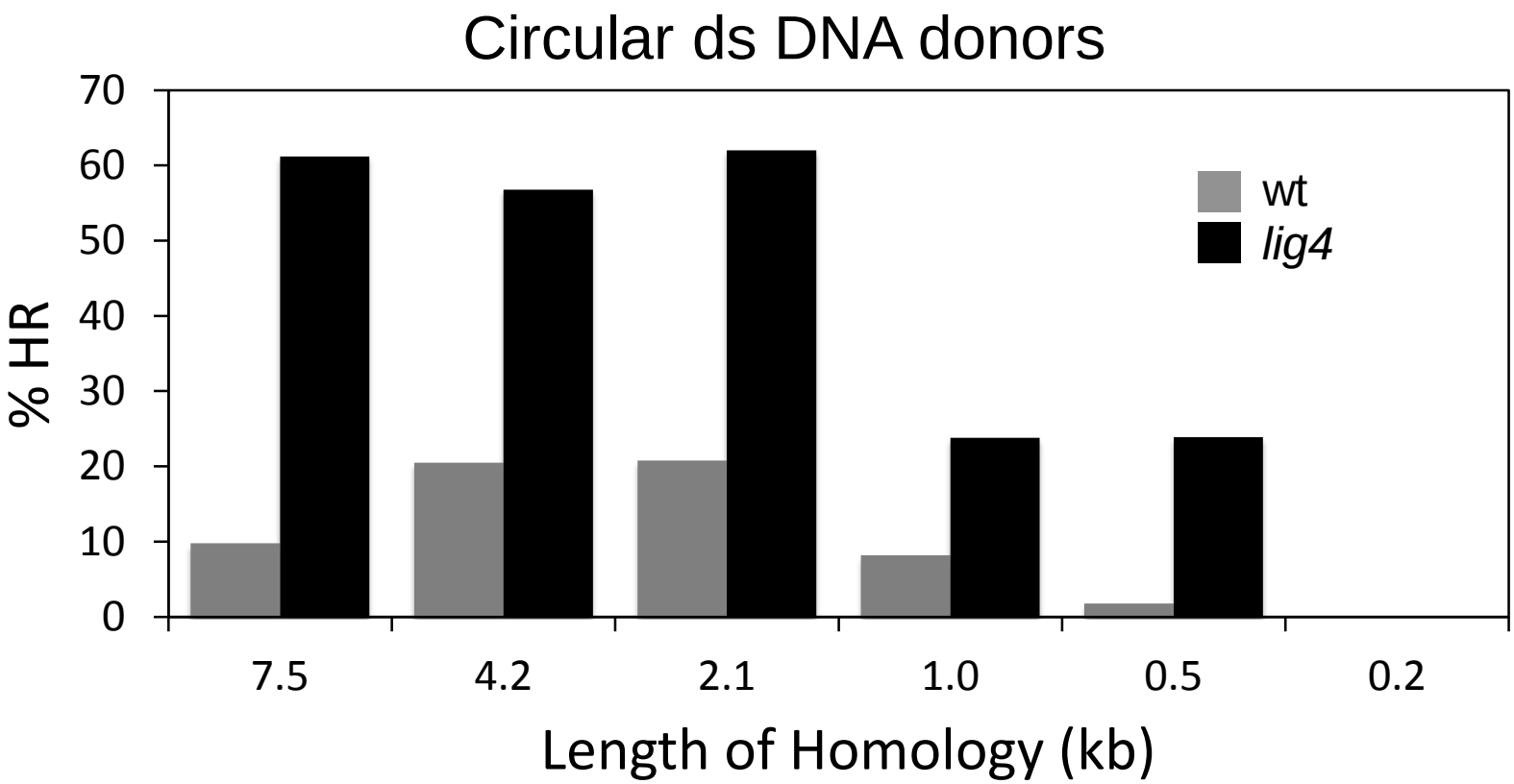



Affecting the balance between HR and NHEJ

How much homology is required?

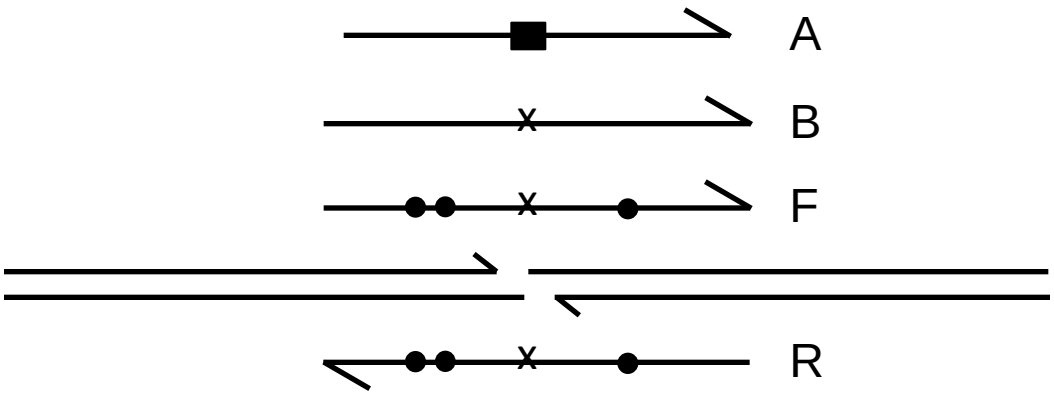
How far from the cut site will sequences in the donor be incorporated at the target?

# How much homology is required?




Beumer et al. (2013) G3 3: 657

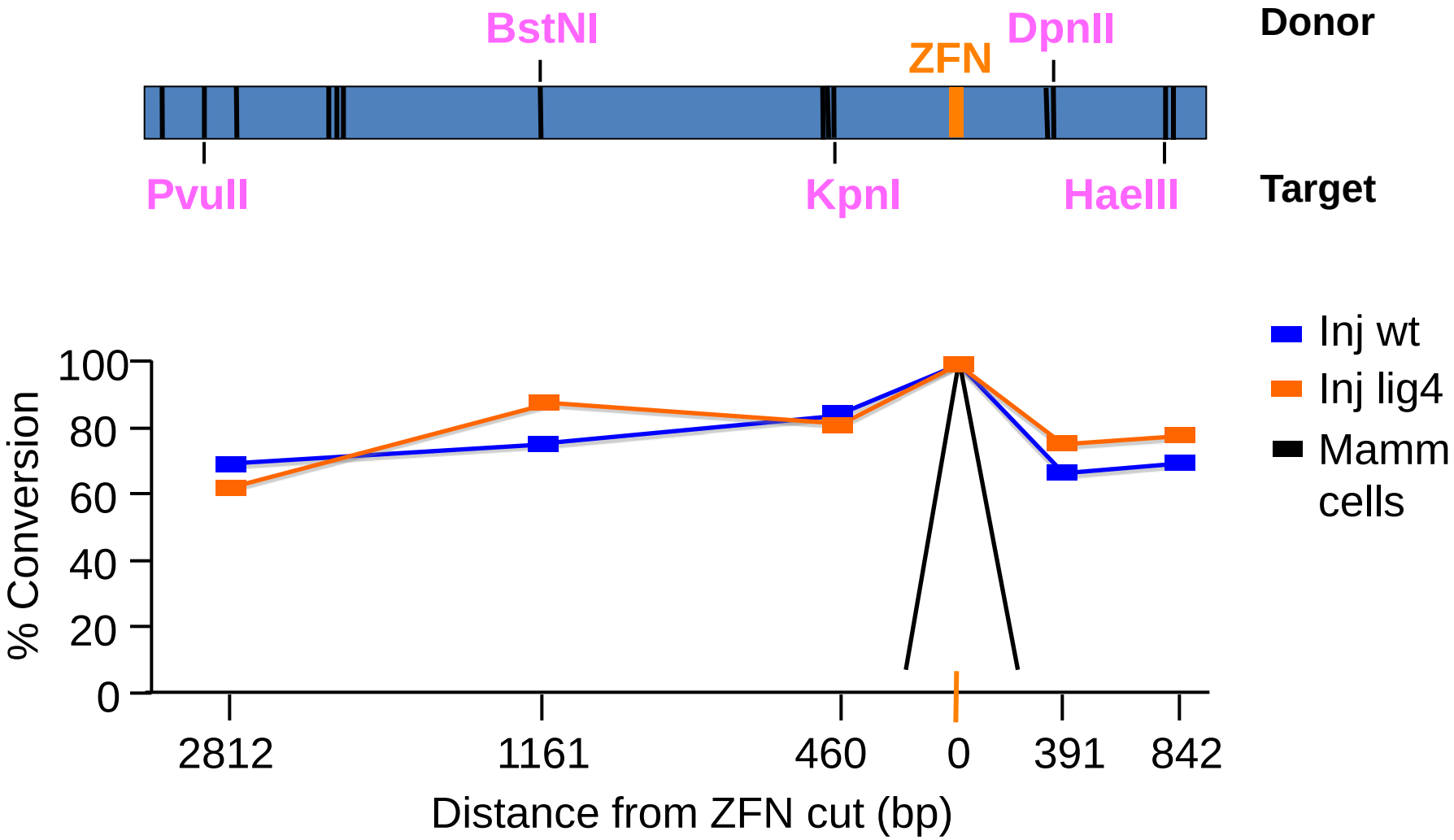
# Oligonucleotide Donors




ss oligo donors:
≥ 30 nt of homology

Beumer et al. (2013) G3 3: 657

# Conversion tracts in Drosophila




Beumer et al. (2013) G3 3: 657

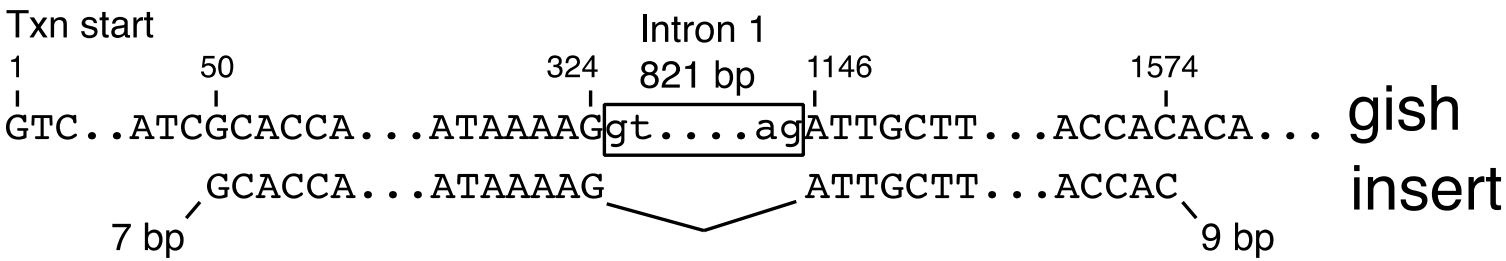
What kinds of NHEJ mutations are produced by the various nucleases?

# Mutation Distribution

| Mutation | ZFNs | TALENs | CRISPR-Cas |
|---|---|---|---|
| Deletion | 51 % | 68 % | 83% |
| Insertion w/ Deletion | 24 % | 30 % | 12% |
| Insertion w/o Deletion | 24 % | 1 % | 5% |
| N | 632 | 148 | 41 |

Deletions somewhat larger with TALENs, CRISPRs

# One very strange insertion




720-bp insertion; 704 bp correspond to *gish* gene

Intron cleanly missing – RNA template for repair?

Junction sequences match neither *ry* nor *gish*

Bozas *et al.* (2009)
Genetics 182: 641.

# Types of events stimulated by targetable nucleases

Local small deletions and insertions - NHEJ

Local small sequence replacements – ss oligo donors

Regional sequence replacements – long ds donors

Large deletions – designed donors, oligo or long

Large deletions – two targets (NHEJ)

Chromosome translocations – two targets

# ZFN/TALEN/CRISPR Success Stories

| Invertebrates | Vertebrates | Plants |
|---|---|---|
| *Drosophila* | Zebrafish | *Arabidopsis* |
| Silkworm | Medaka | Maize |
| *A. aegypti* | Catfish | Tobacco |
| *A. gambii* | Rainbow trout | Soybean |
| Cricket | Tilapia | Rice |
| Butterfly | Frog (*Xenopus*) | Barley |
| *Daphnia* | Newt | Wheat |
| *C. elegans* | Axolotl | Sorghum |
| *C. briggsae* | Amphioxus | Bunch grass |
| *P. pacificus* | Mouse | Cabbage |
| Sea urchin | Rat | Tomato |
| *Platynereis* | Rabbit | Orange |
| *Ciona* | Goat | Petunia |
| *Plasmodium* | Sheep | Liverwort |
| *Toxoplasma* | Pig | *N. benthamiana* |
| Yeast | Cow | *Chlamydomonas* |
| Bacteria | Human | |
| Viruses | Macaque | |
| | Rhesus monkey | |
| | CHO cells | |

As of June 5, 2014

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