Highly efficient endogenous human gene correction using designed zinc-finger nucleases

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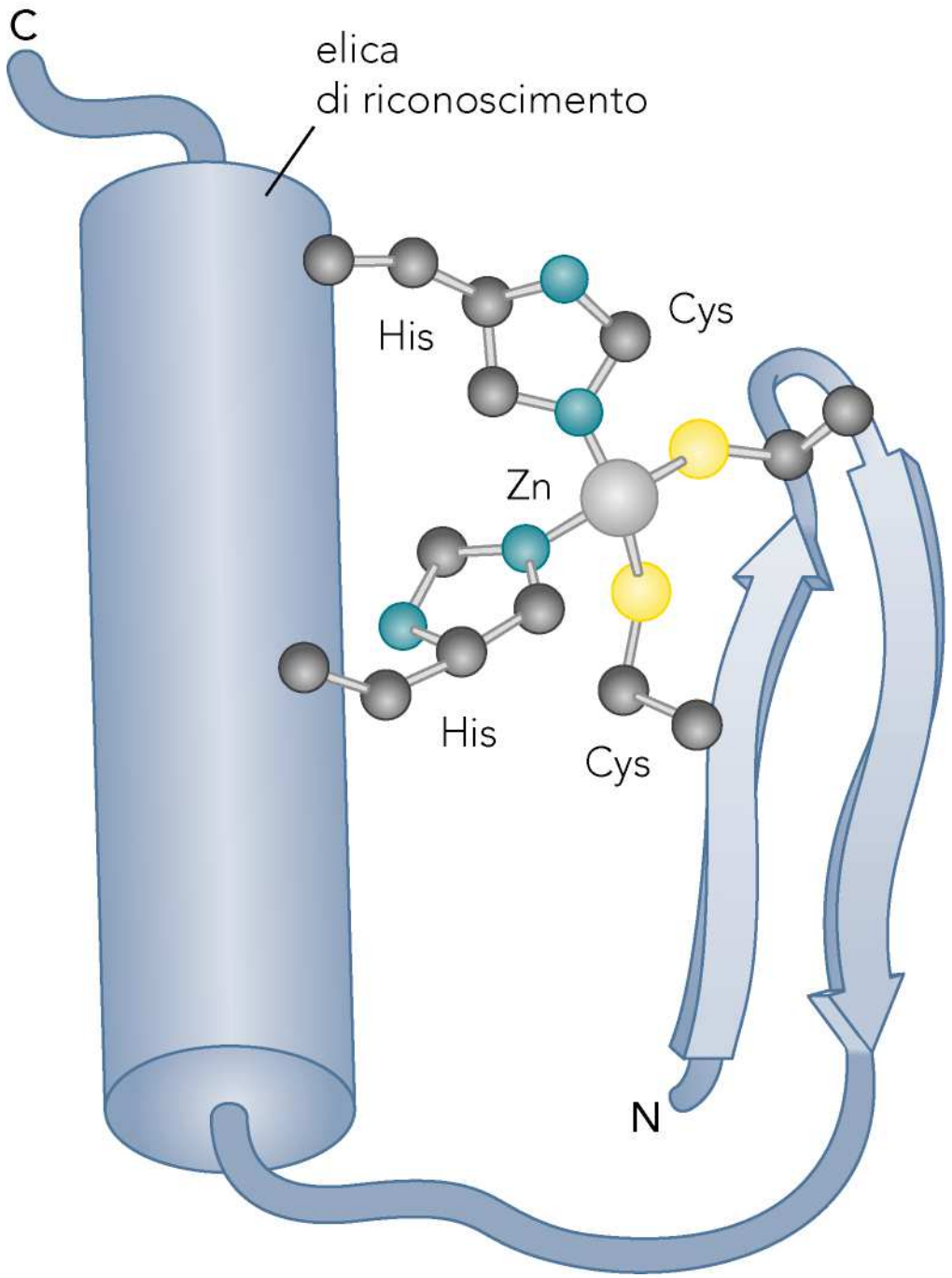
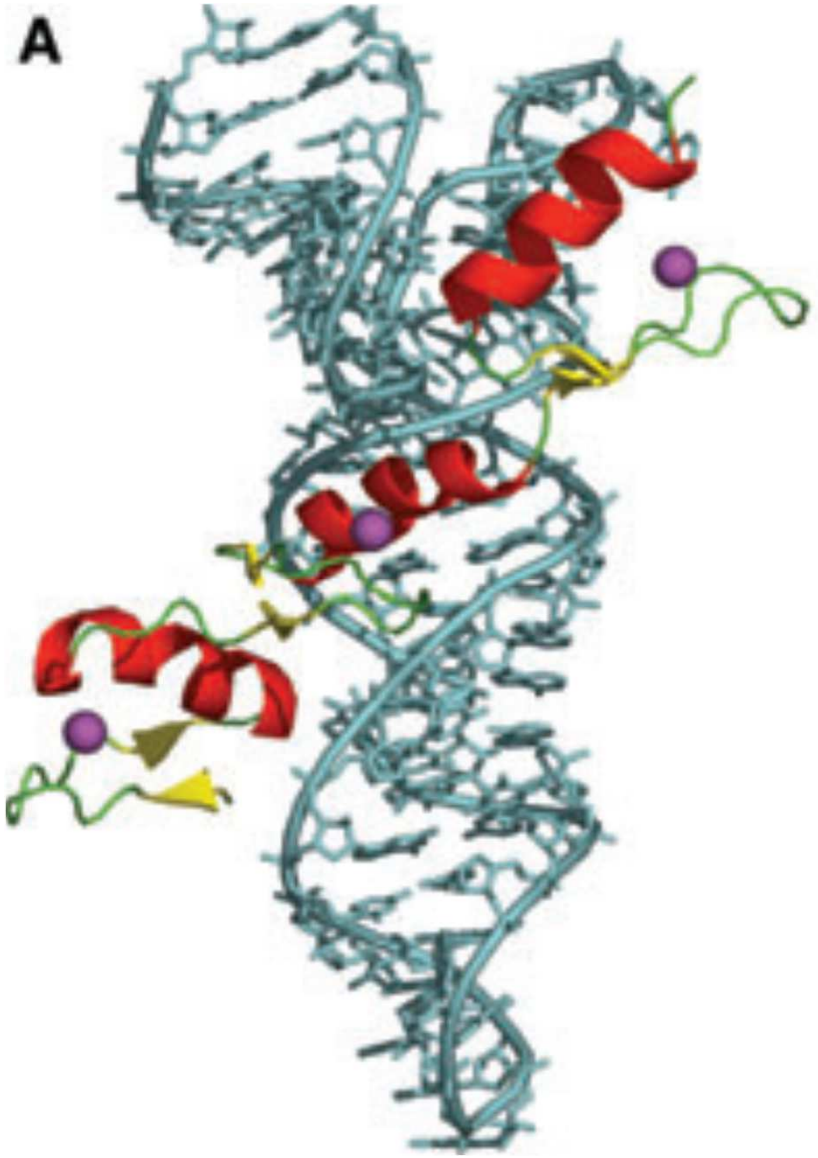
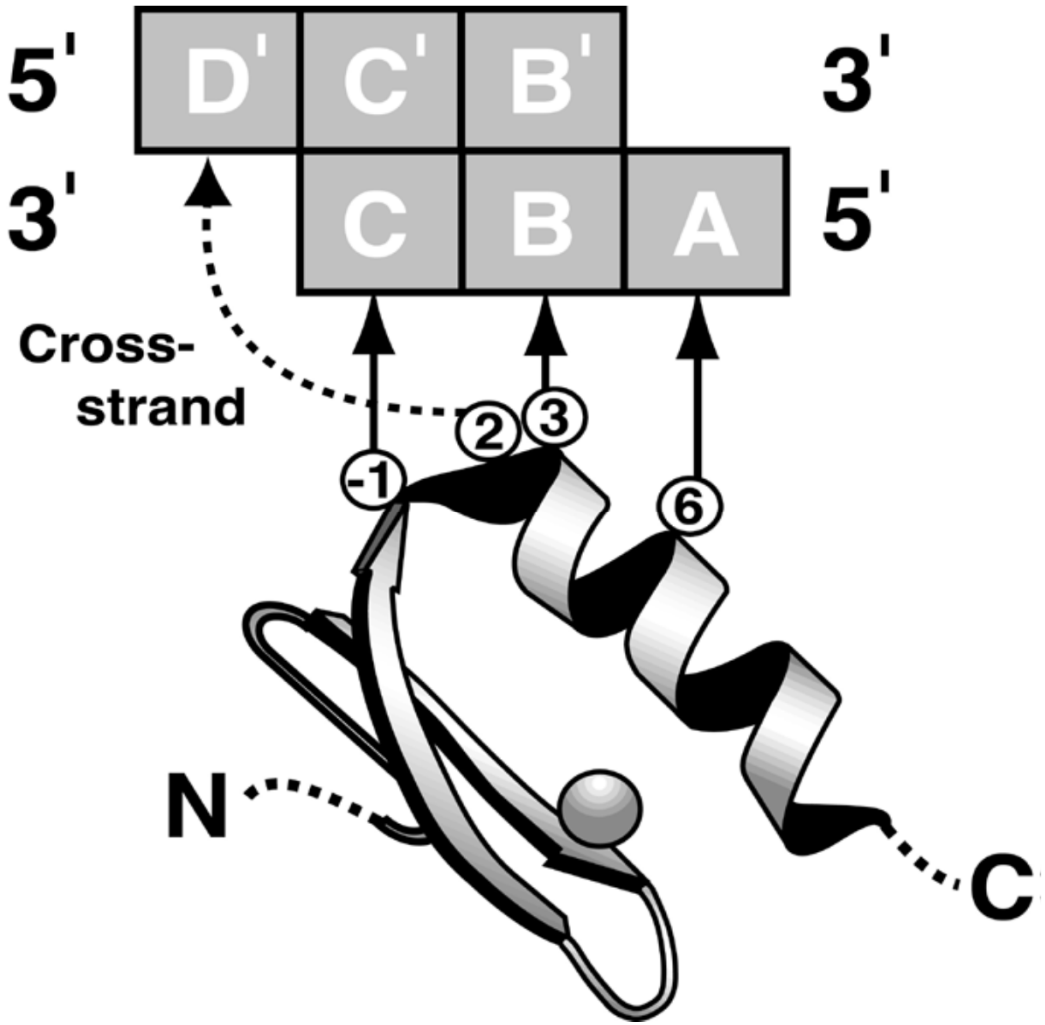
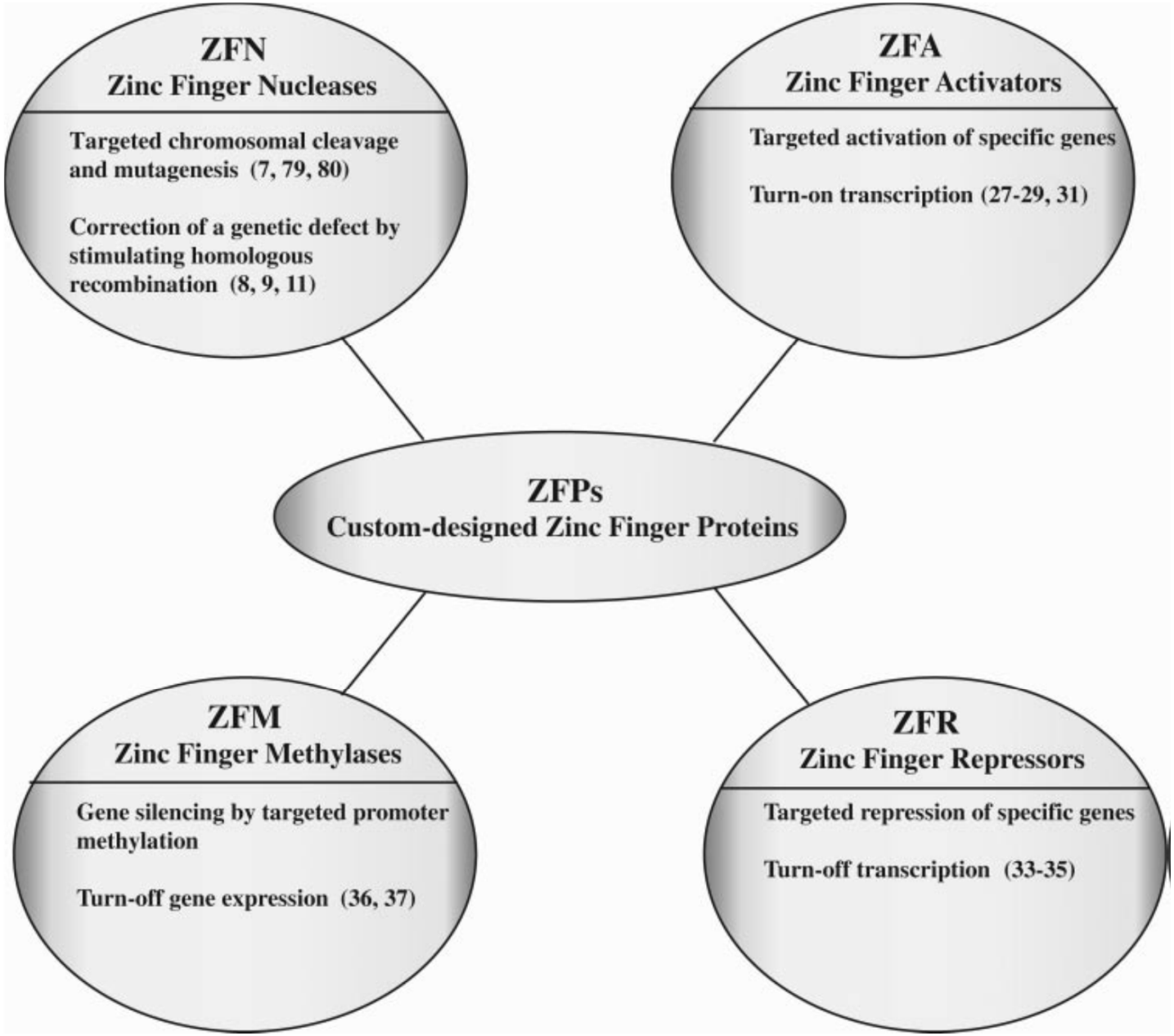
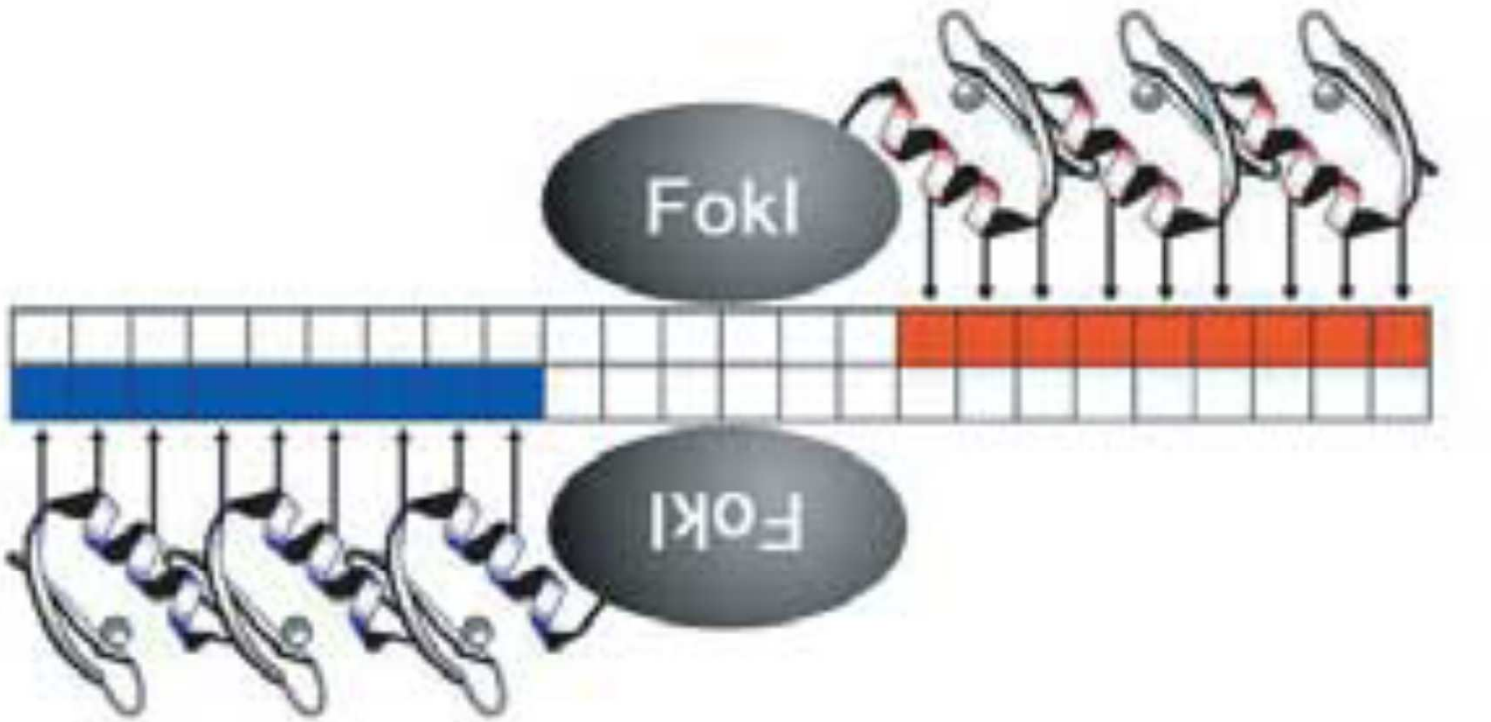

C
elica
di riconoscimento
His
Cys
Zn
His
Cys
N


A


5'
D'
C'
B'
3'
3'
C
B
A
5'
Cross-
strand
2
3
-1
6
N
C

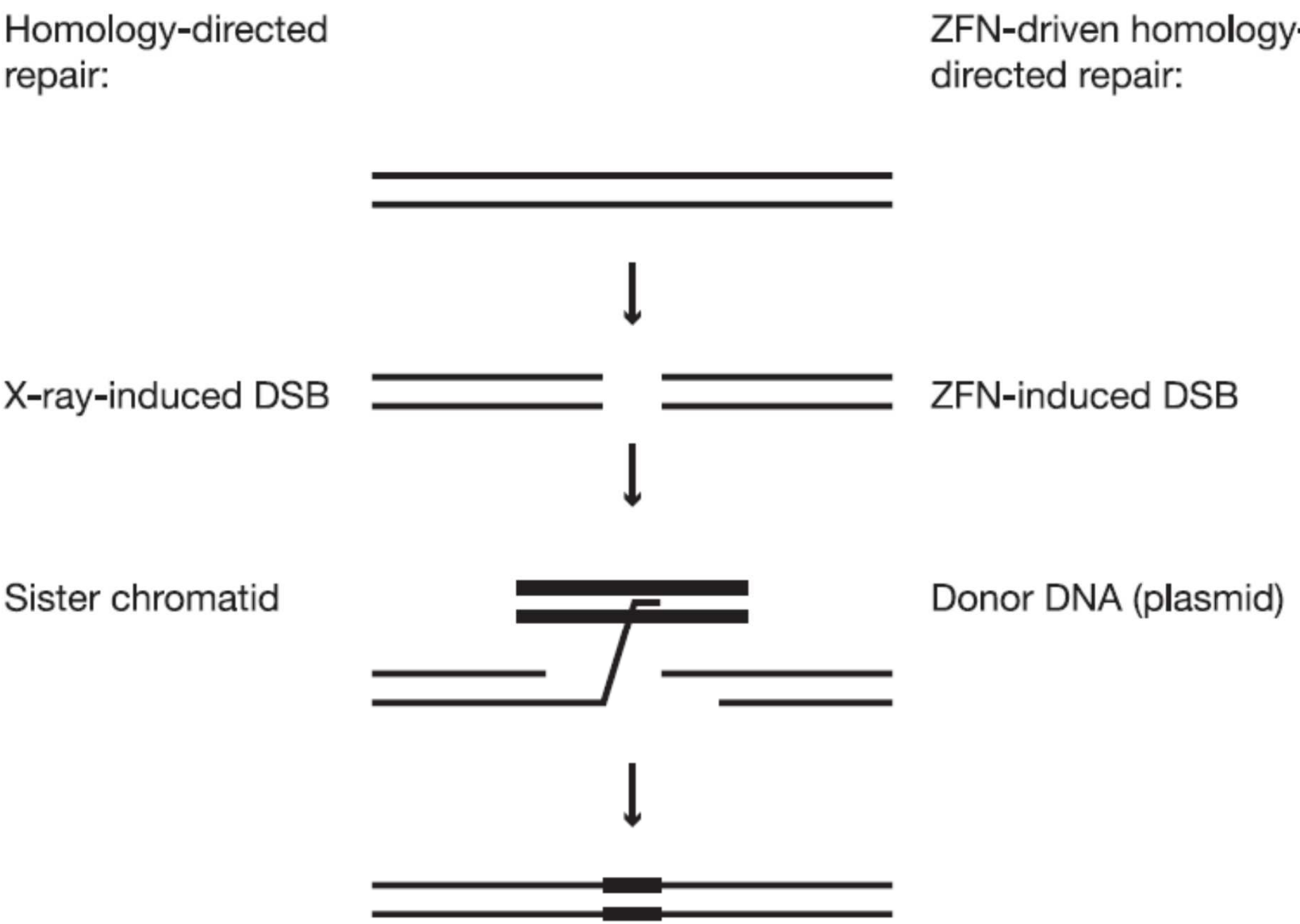

ZFN
Zinc Finger Nucleases
Targeted chromosomal cleavage and mutagenesis (7, 79, 80)
Correction of a genetic defect by stimulating homologous recombination (8, 9, 11)
ZFA
Zinc Finger Activators
Targeted activation of specific genes
Turn-on transcription (27-29, 31)
ZFPs
Custom-designed Zinc Finger Proteins
ZFM
Zinc Finger Methylases
Gene silencing by targeted promoter methylation
Turn-off gene expression (36, 37)
ZFR
Zinc Finger Repressors
Targeted repression of specific genes
Turn-off transcription (33-35)

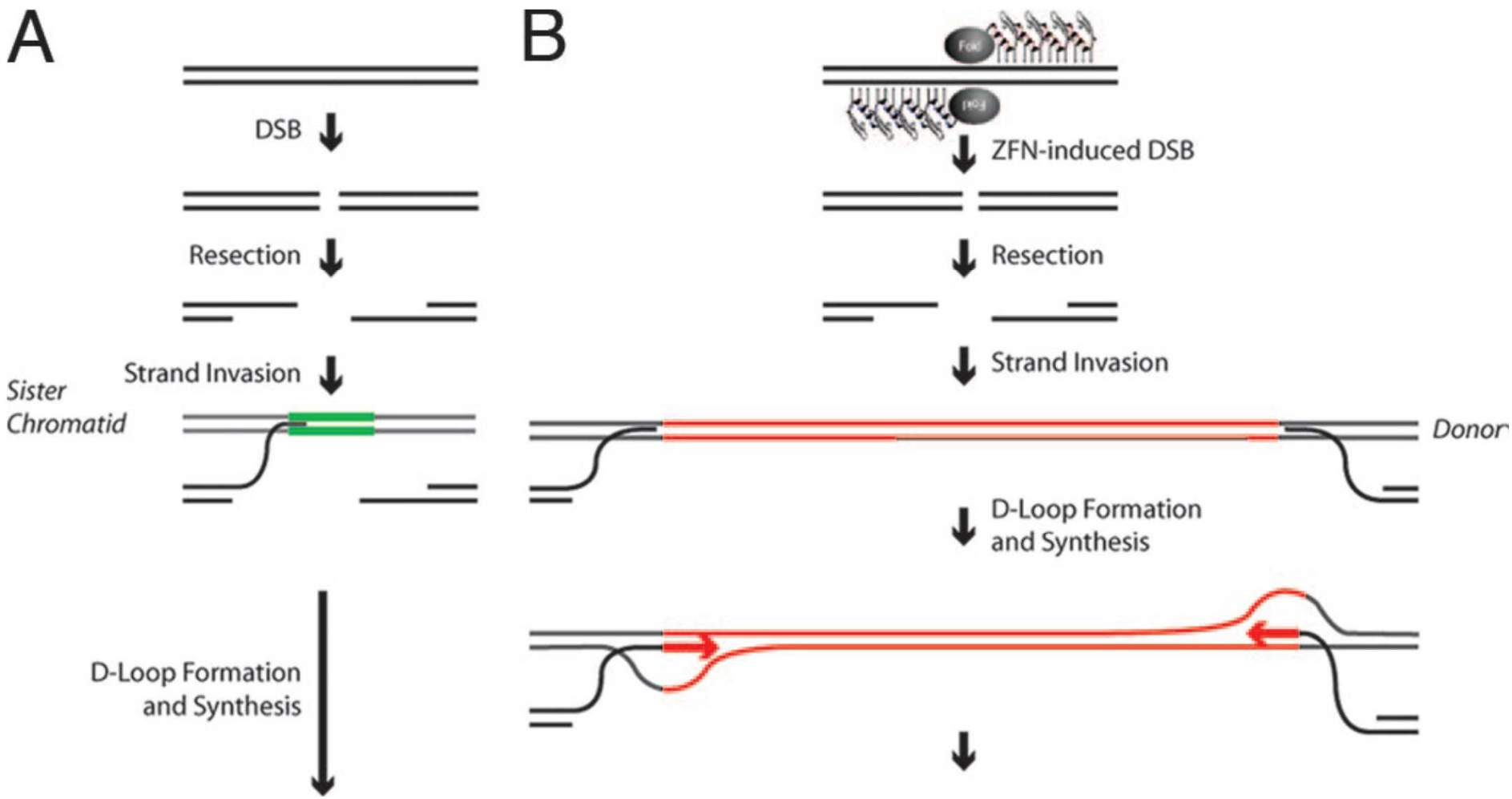
- Two approaches were originally used for zinc finger protein (ZFP) engineering in order to expand the DNA recognition code and to create zinc fingers that bind desired base triplets
- a combinatorial approach using libraries of zinc fingers displayed on the surface of filamentous phage that were selected against target DNA sequences
- a rational design approach that used databases to predict rules for amino acid–base interactions.

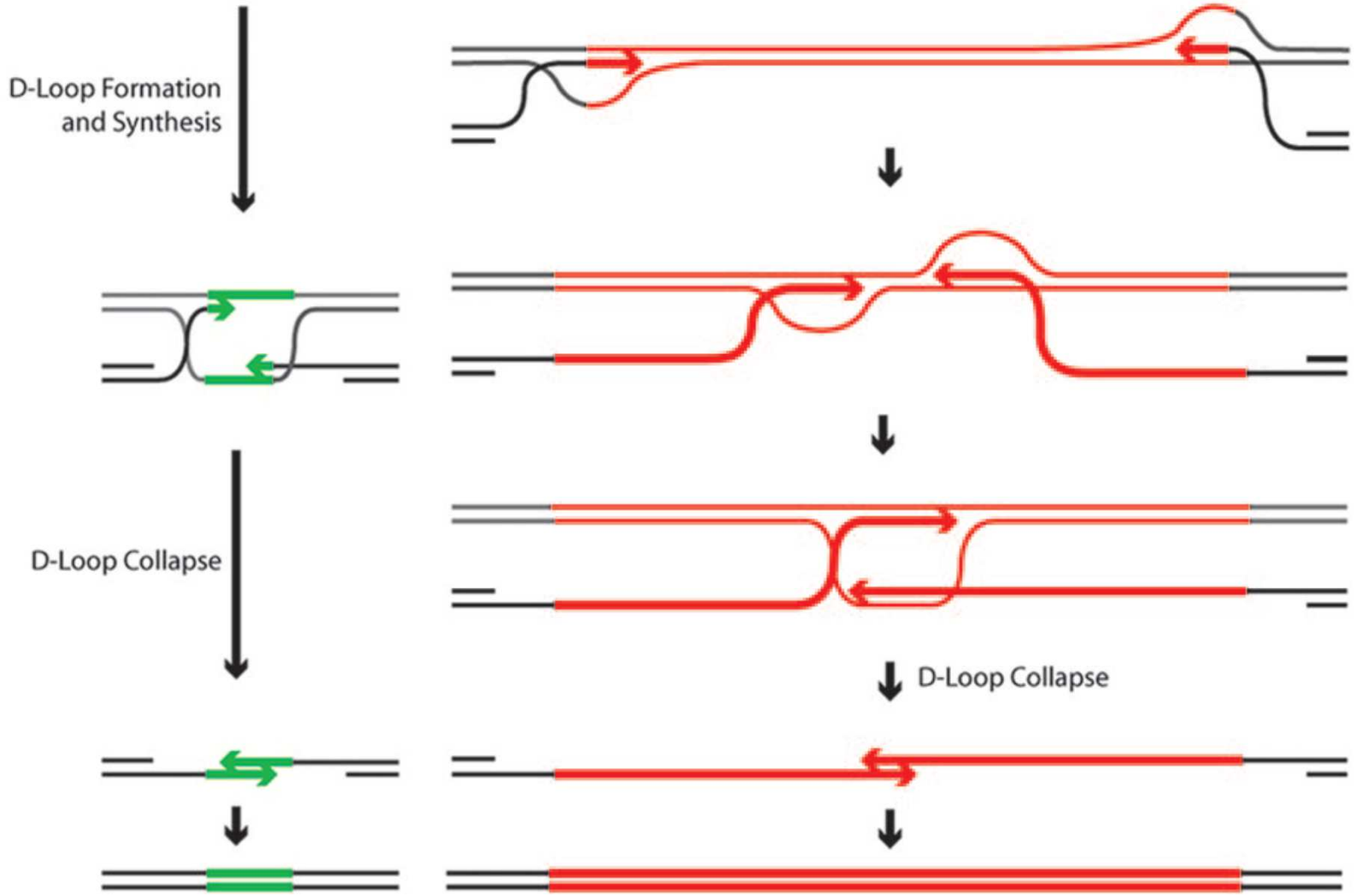


Dimerization of FokI domains is required for its DNA binding-dependent endonuclease activity

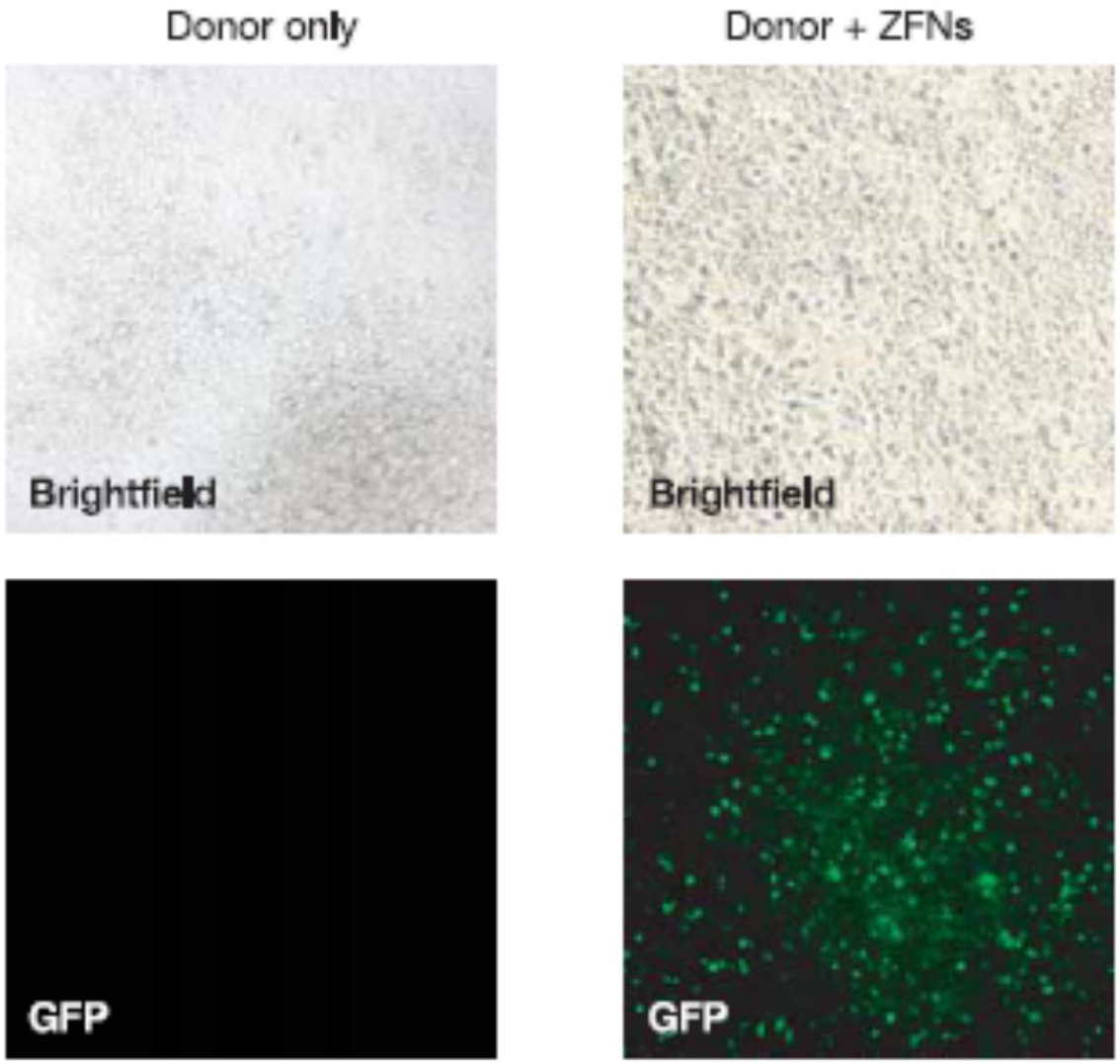
Targeted gene knockout in mammalian cells by using engineered zinc-finger nucleases

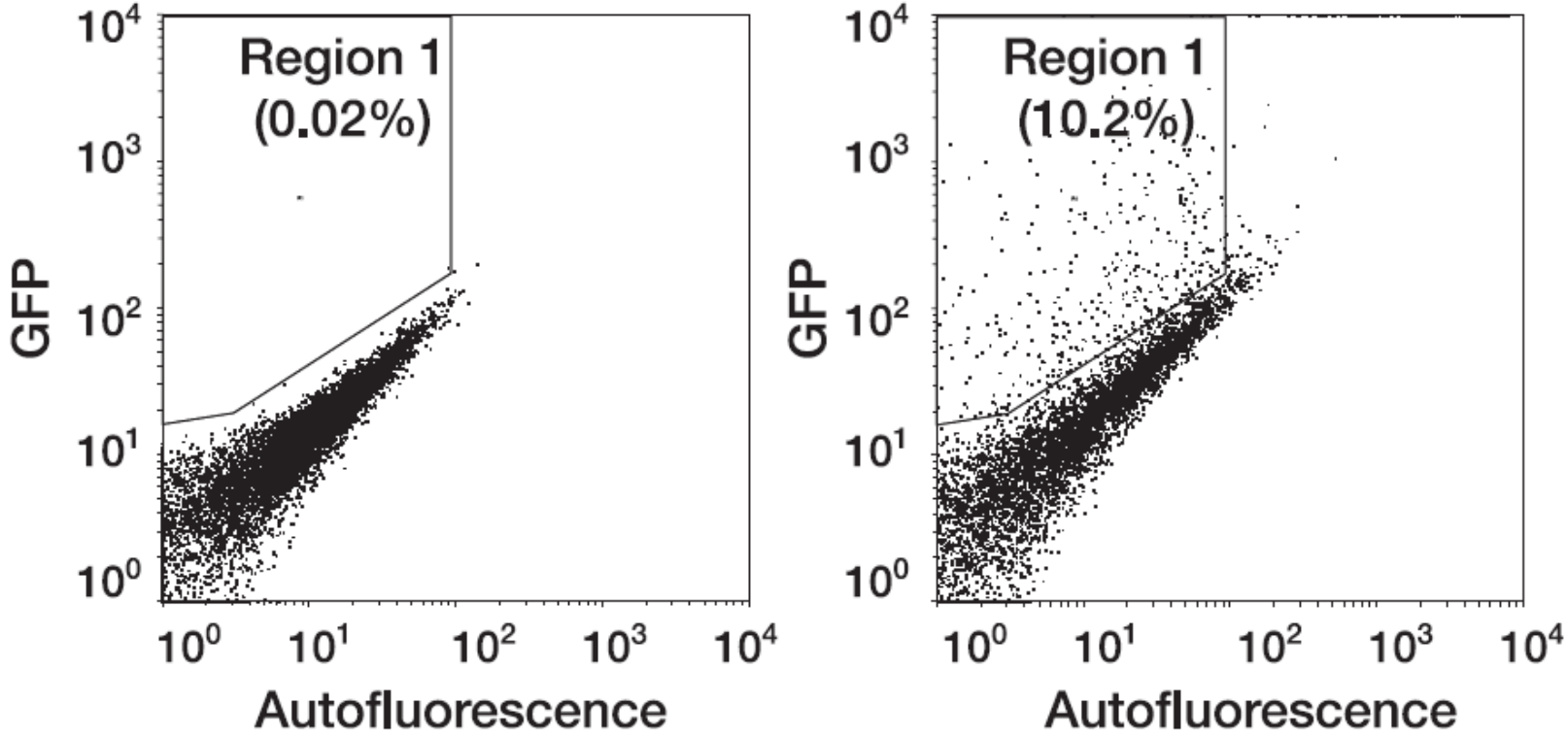

Homology-directed repair:
ZFN-driven homology-directed repair:
X-ray-induced DSB
ZFN-induced DSB
Sister chromatid
Donor DNA (plasmid)


A
DSB
Resection
Strand Invasion
Sister
Chromatid
D-Loop Formation
and Synthesis
B
ZFN-induced DSB
Resection
Strand Invasion
Donor
D-Loop Formation
and Synthesis

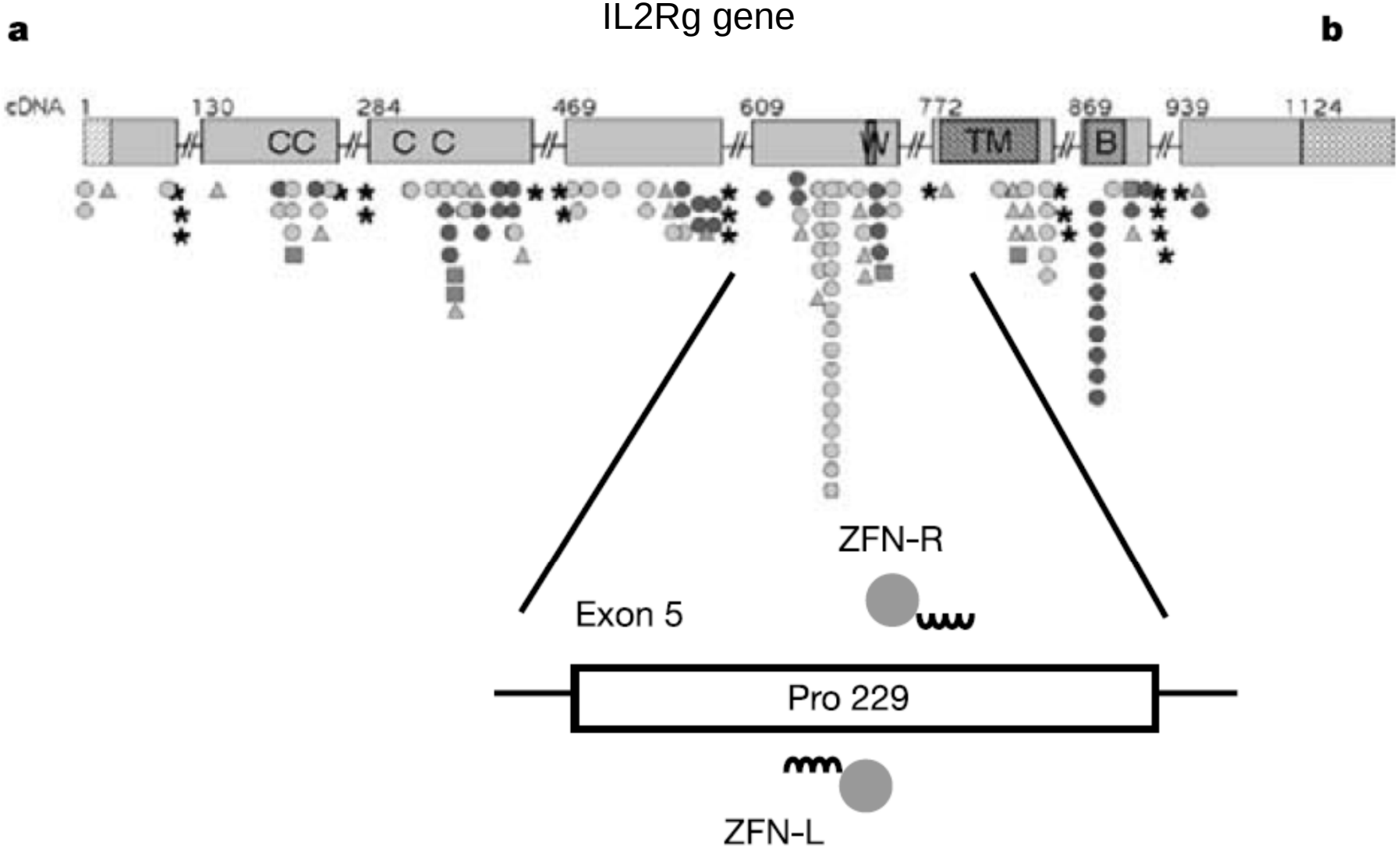

D-Loop Formation
and Synthesis
D-Loop Collapse
D-Loop Collapse

Cells carrying a mutated GFP reporter were transiently transfected with a donor plasmid carrying a fragment of wild-type GFP (left column), orthe donor plasmid and the ZFNs (right column).



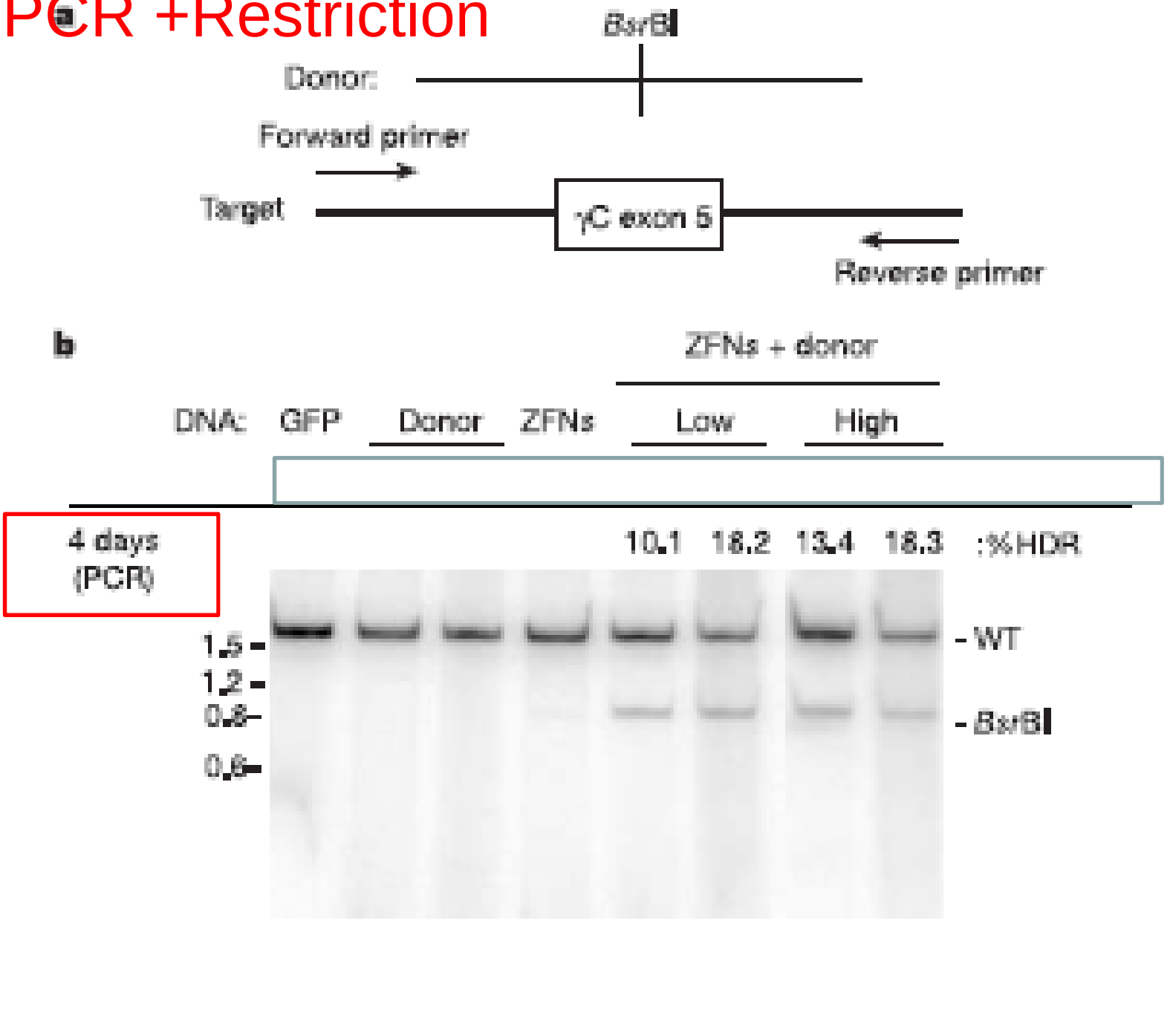

Region 1
(0.02%)
Region 1
(10.2%)
GFP
Autofluorescence
10^0
10^1
10^2
10^3
10^4

IL2Rg gene

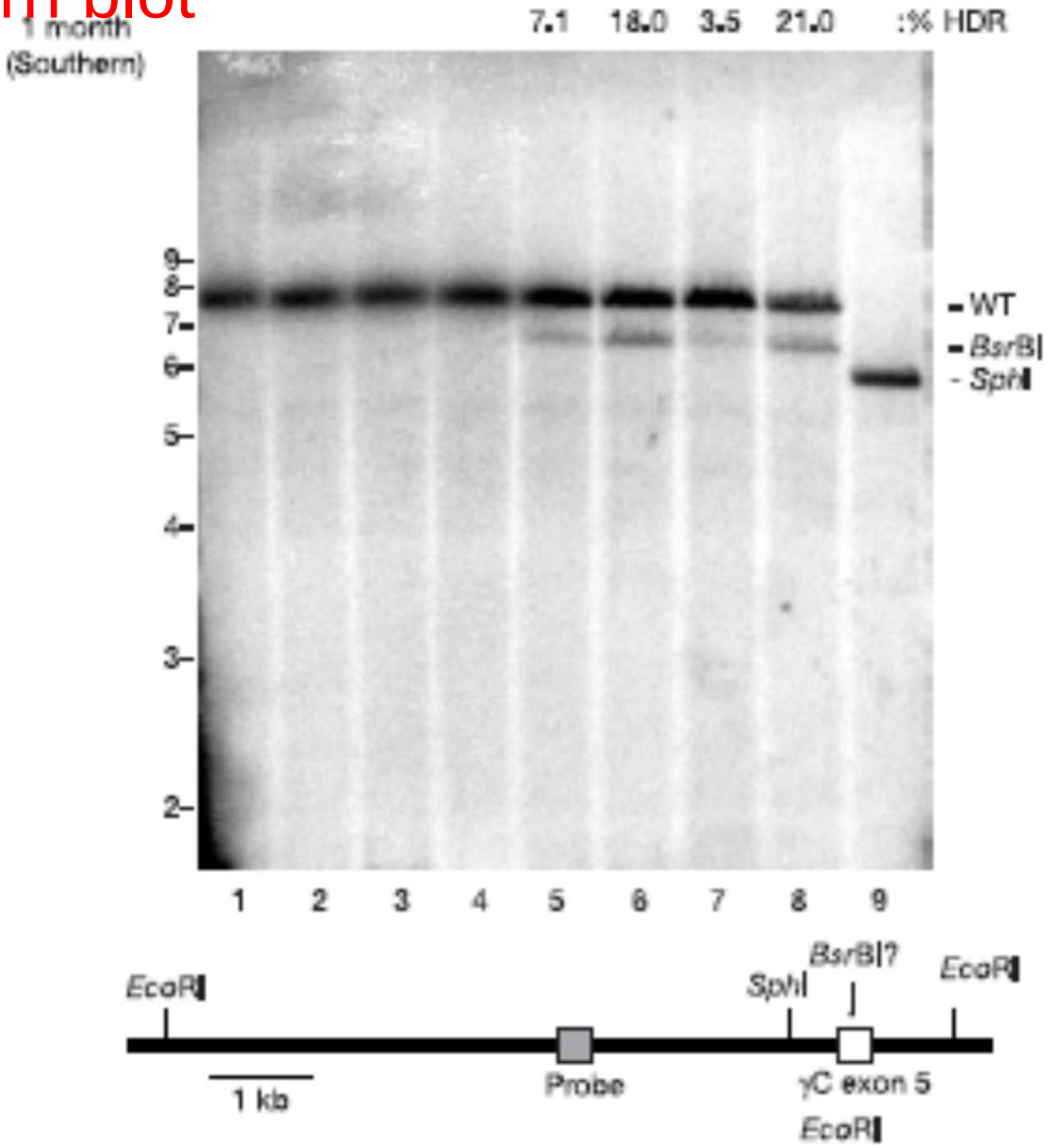


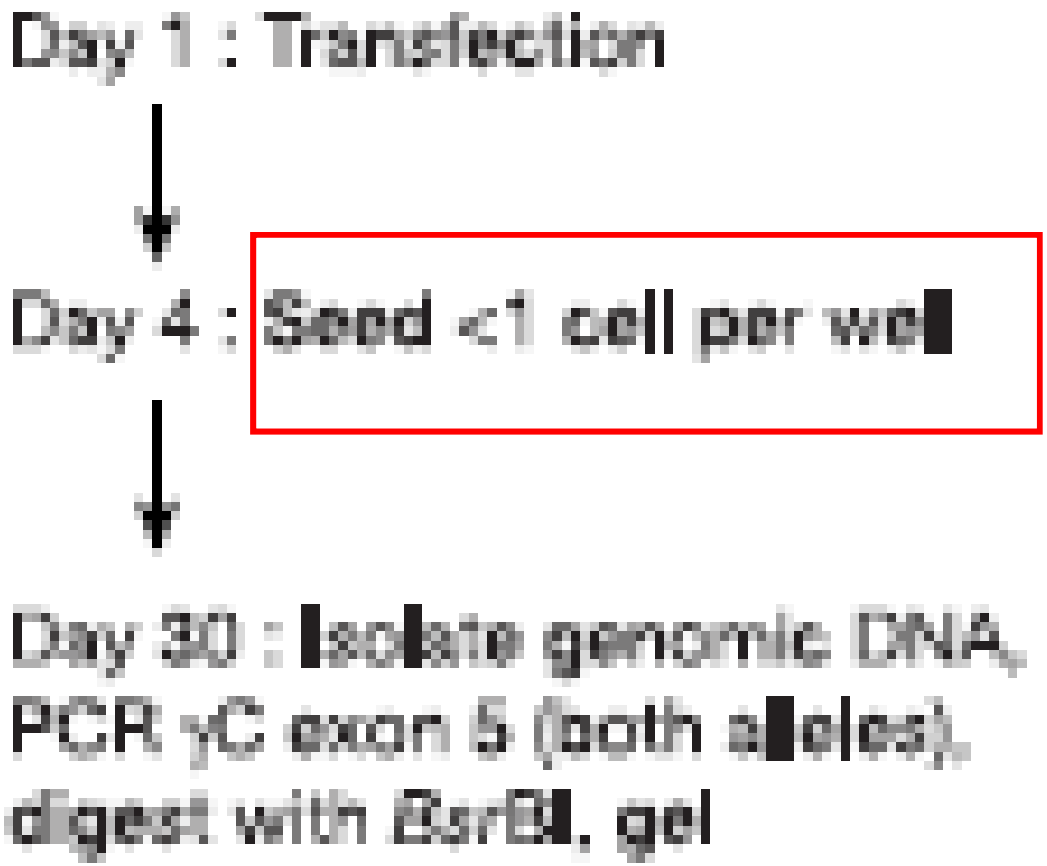
X-linked severe combined immune deficiency (SCID)

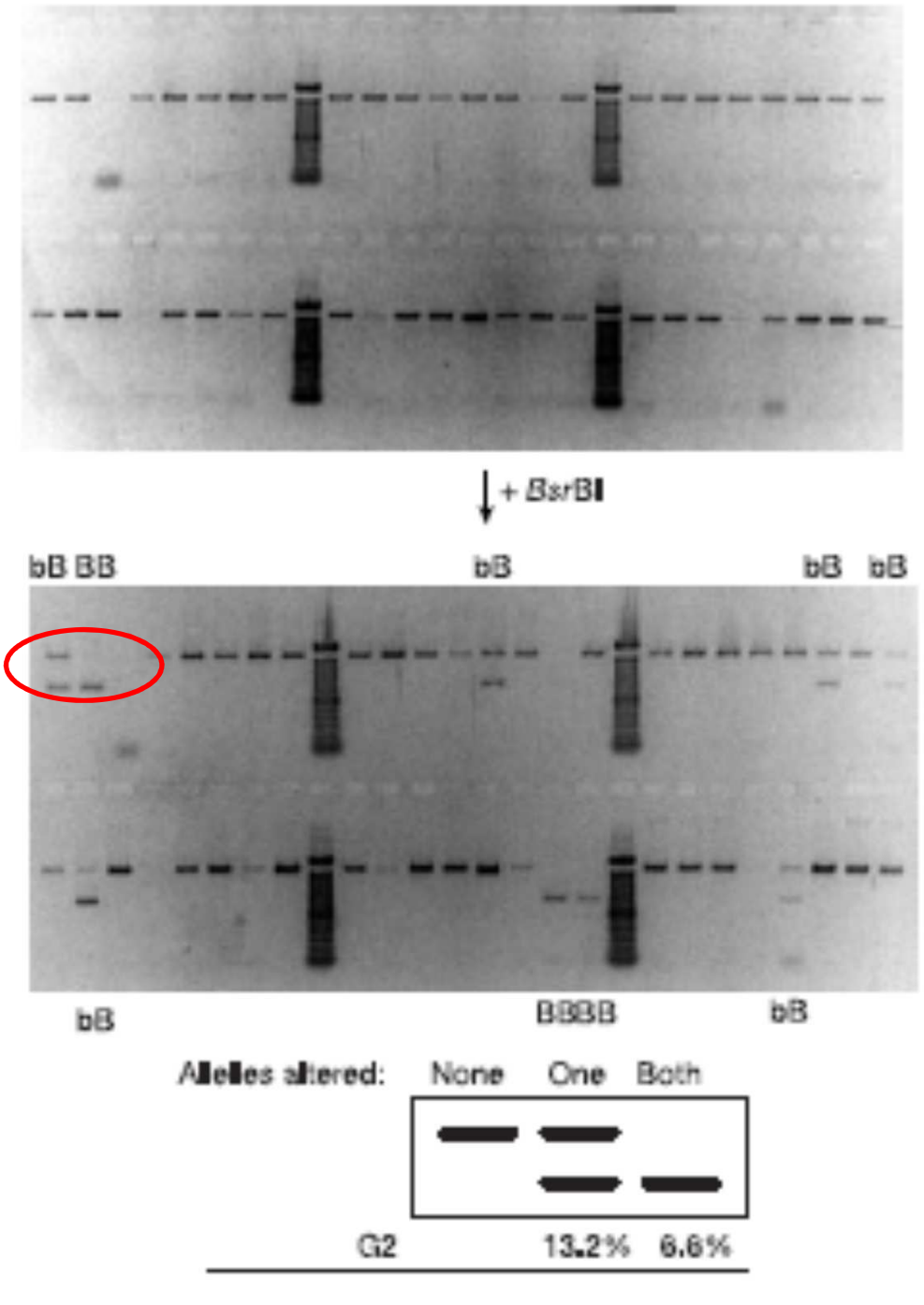
PCR +Restriction



Southern blot



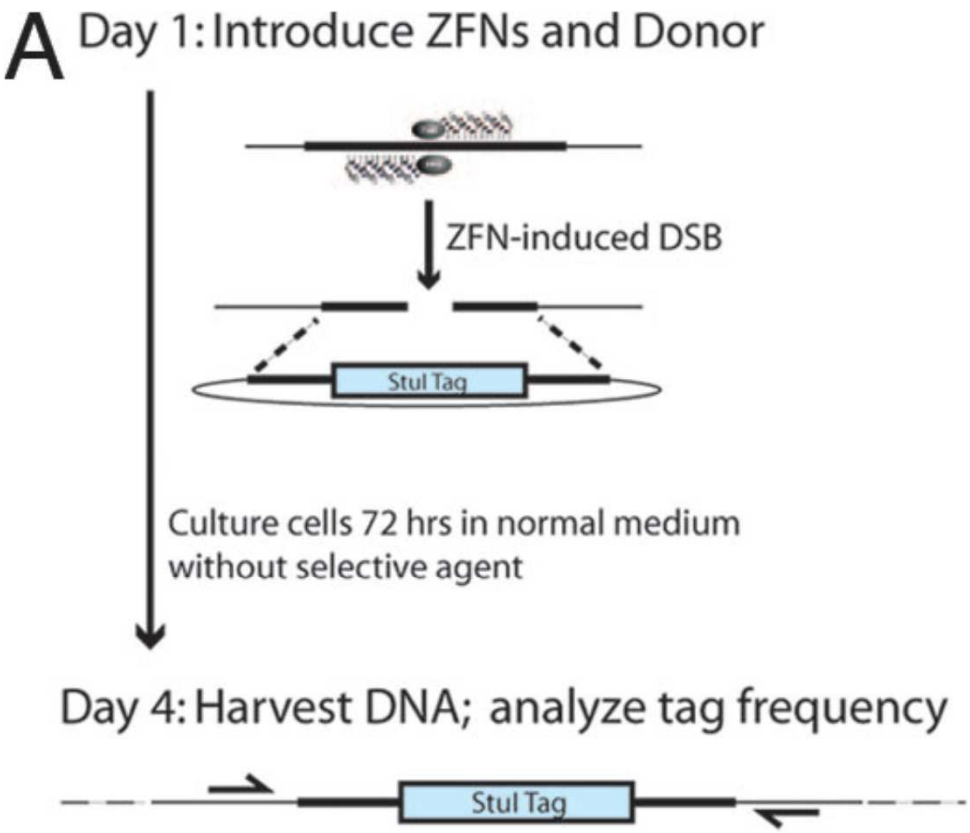

Day 1 : Transfection
Day 4 : Seed <1 cell per well
Day 30 : Isolate genomic DNA, PCR γC exon 5 (both alleles), digest with BsrBI, gel


+ BsrBI
bB BB
bB
bB bB
bB
BBBB
bB
Alleles altered:
None
One
Both
G2
13.2%
6.6%

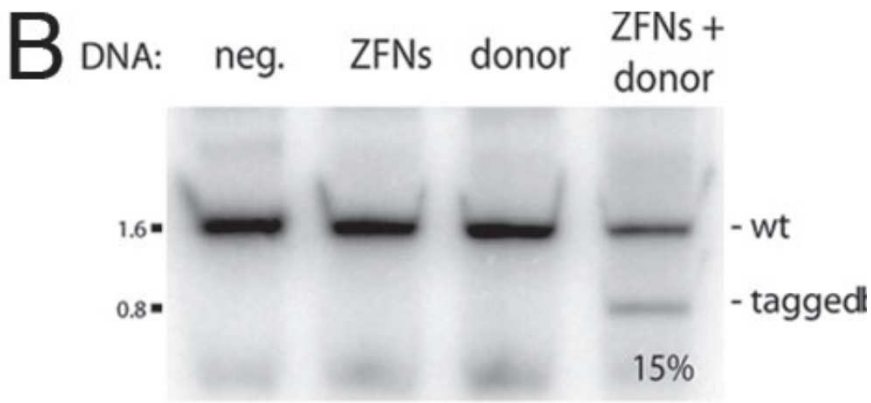
Targeted gene addition into a specified location in the human genome using designed zinc finger nucleases Moehle PNAS 2007

- A precisely placed double-strand break induced by engineered zinc finger nucleases (ZFNs) can stimulate integration of long DNA stretches into a predetermined genomic location, resulting in high-efficiency site-specific gene addition.
- Using an extrachromosomal DNA donor carrying a 12-bp tag, a 900-bp ORF, or a 1.5-kb promoter-transcription unit flanked by locus-specific homology arms, we find targeted integration frequencies of 15%, 6%, and 5%, respectively, within 72 h of treatment, and with no selection for the desired event.
- The integration event occurs in a homology-directed manner and leads to the accurate reconstruction of the donor specified genotype at the endogenous chromosomal locus, and hence presumably results from synthesis-dependent strand annealing repair of the break using the donor DNA as a template.
- This site-specific gene addition occurs with no measurable increase in the rate of random integration. Remarkably, we also find that ZFNs can drive the addition of an 8-kb sequence into an endogenous locus at a frequency of 6%, also in the absence of any selection.
- Surprising versatility of the specialized polymerase machinery involved in double-strand break repair
- Powerful approach to mammalian cell engineering
- Possibility of ZFN-driven gene addition therapy for human genetic disease.

Experimental outline and a schematic of the process whereby a ZFN-induced DSB is repaired by using an extrachromosomal donor as a template



PCR-based measurements of ZFN-driven tag integration frequency into the IL2R locus



PCR products were digested with StuI