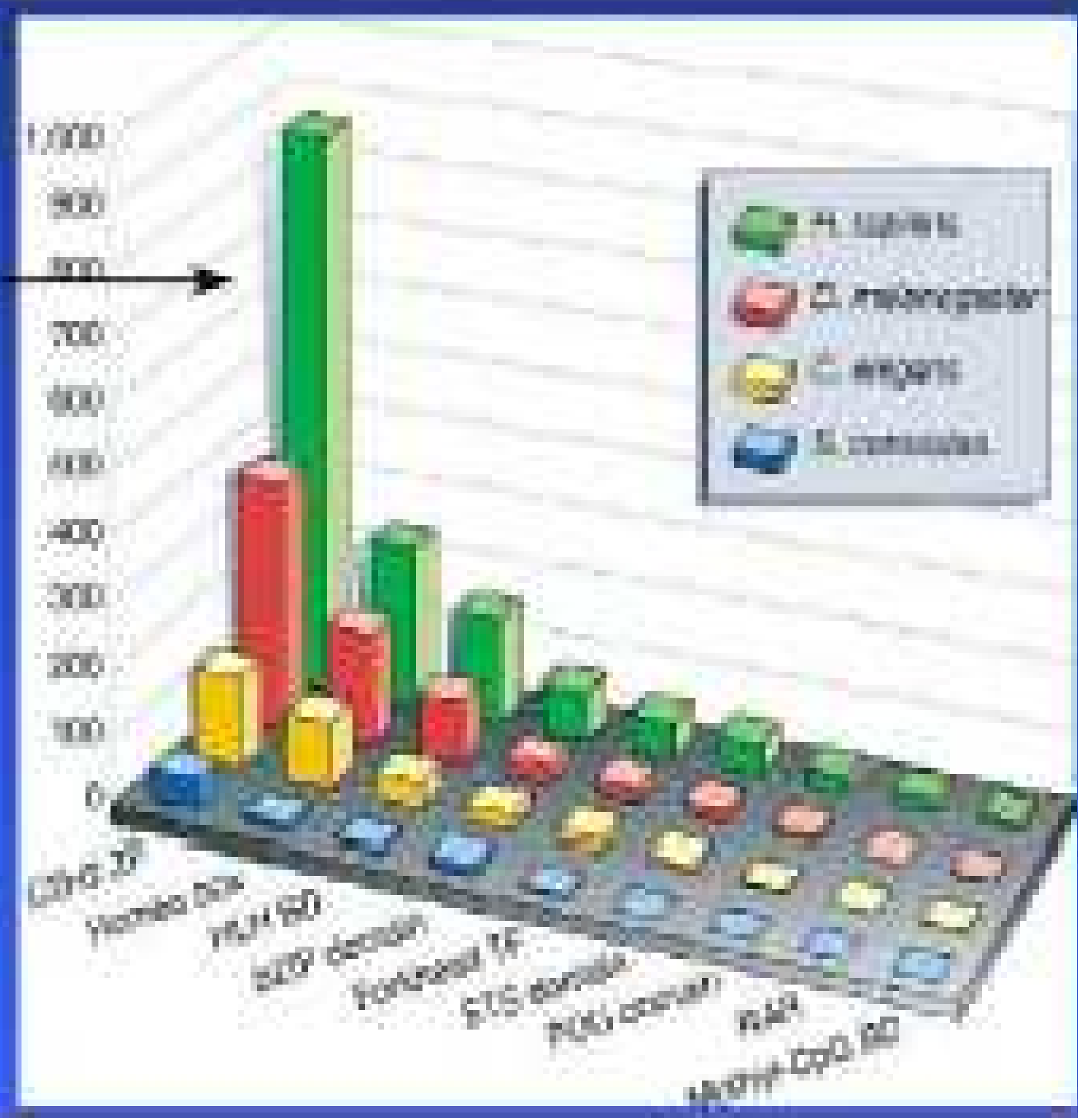
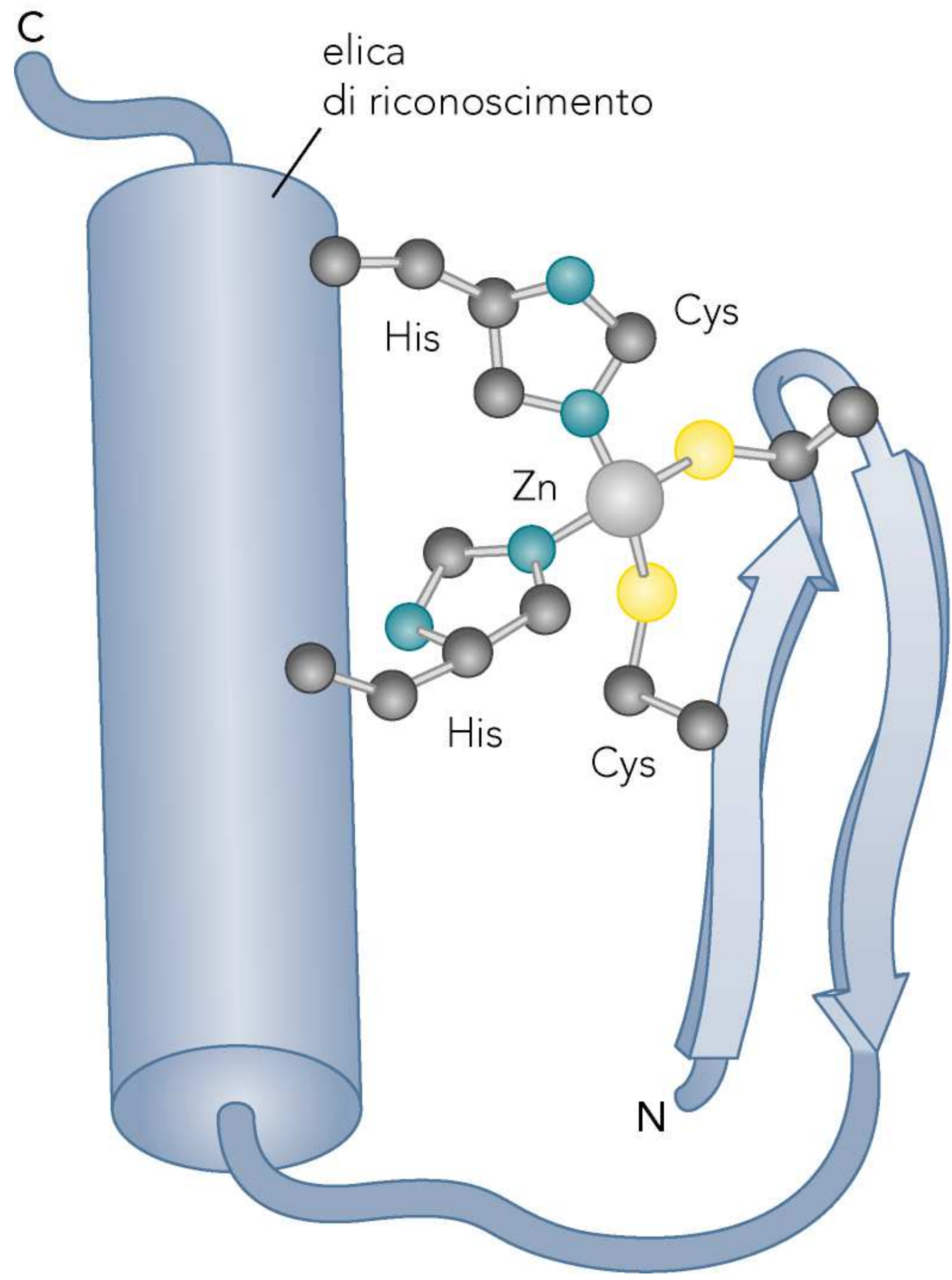
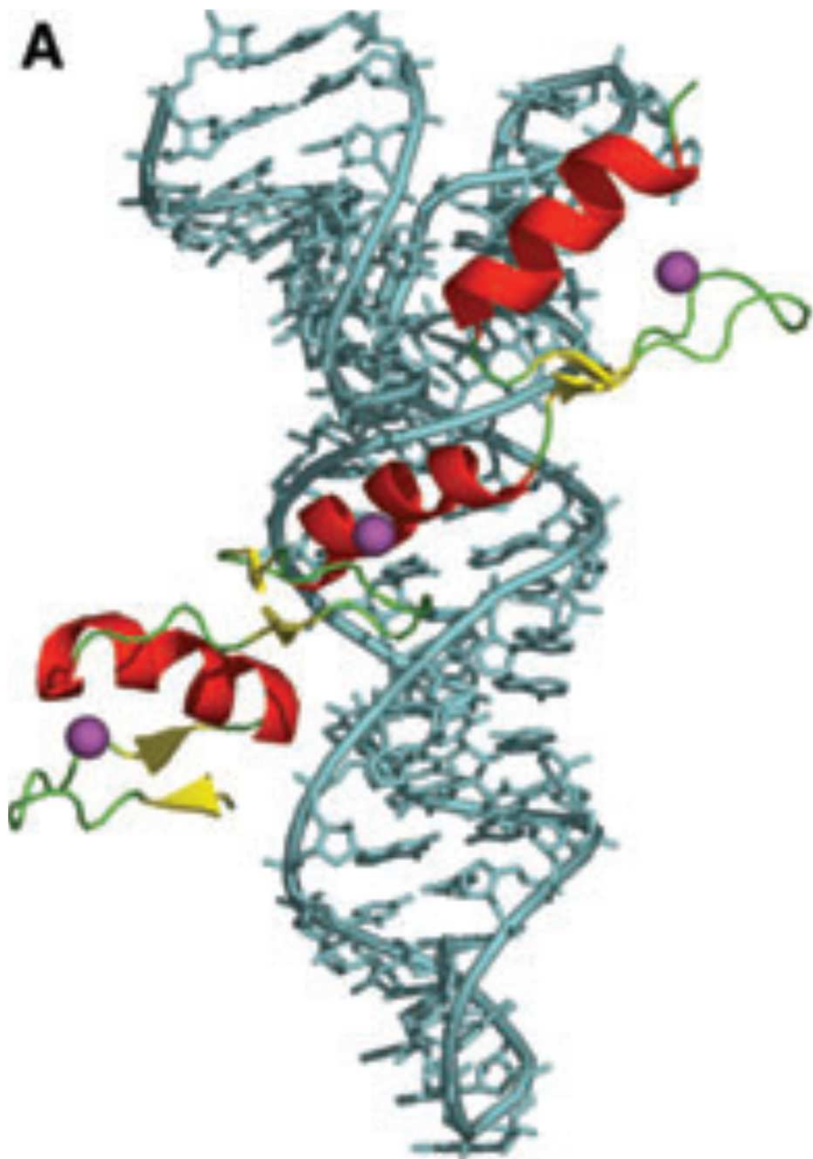
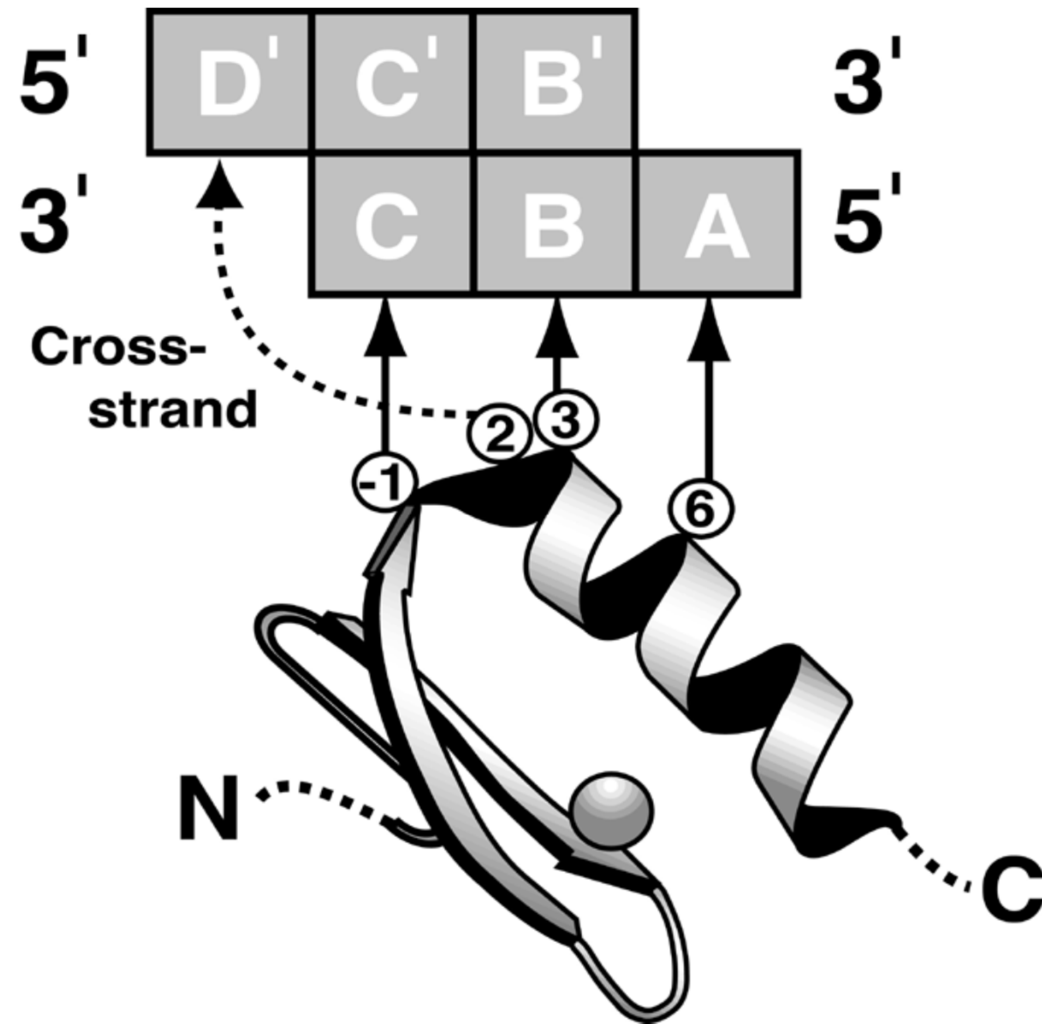
Zinc finger proteins



DNA binding protein families


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

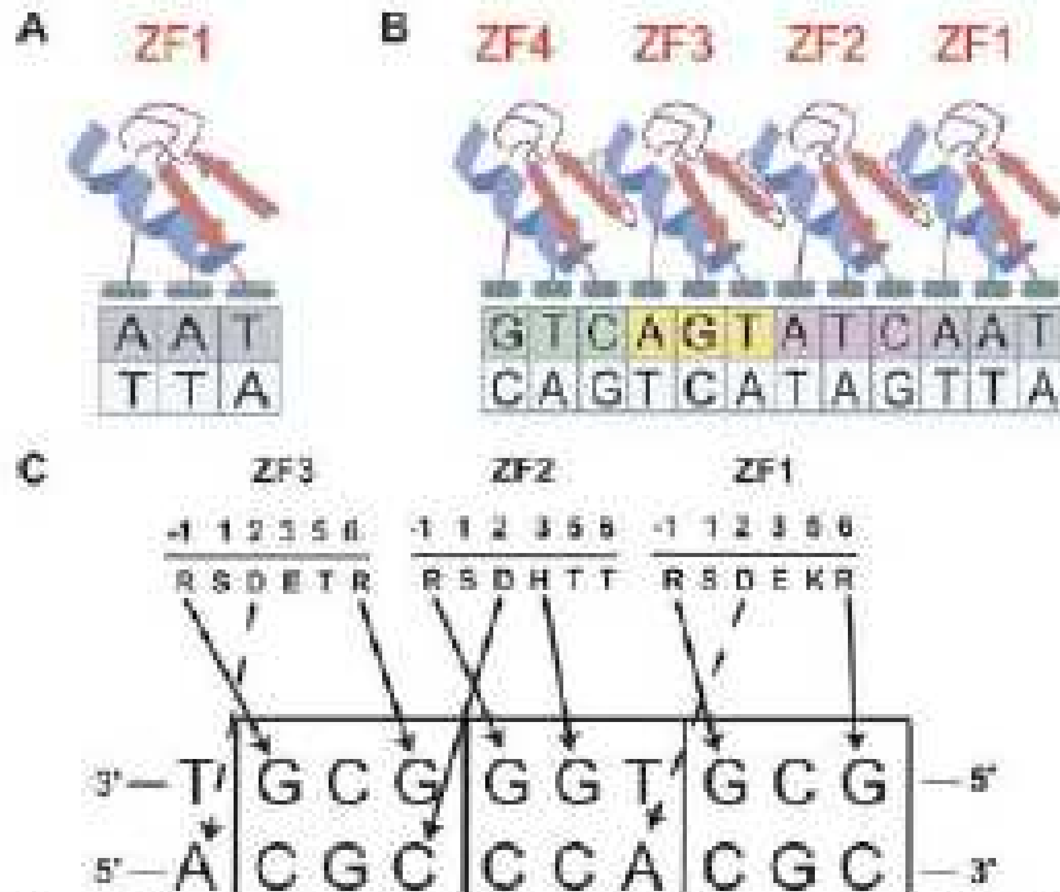


Figure 1. Structures of an individual ZF motif (A) and a four-finger ZFP, which is formed by linking four such individual ZF motifs (B). Zif268 bound to its cognate DNA site (C) and potential binding of Zif268 to degenerate sites (D). The key base contacts were deduced from the crystal structure of Zif268-DNA complex (44). Each finger makes contact with its target 3 bp site. In addition, Asp^2 at position 2 in each finger makes contact with a base outside the 3 bp site (C). Fingers 1 and 3 of Zif268 make specific contacts only with two bases of their cognate DNA triplets, while specific base contacts are seen with all the three bases of finger 2. Zif268 could potentially bind to other secondary or degenerate sites (D) as indicated, where N = G, A, T or C.

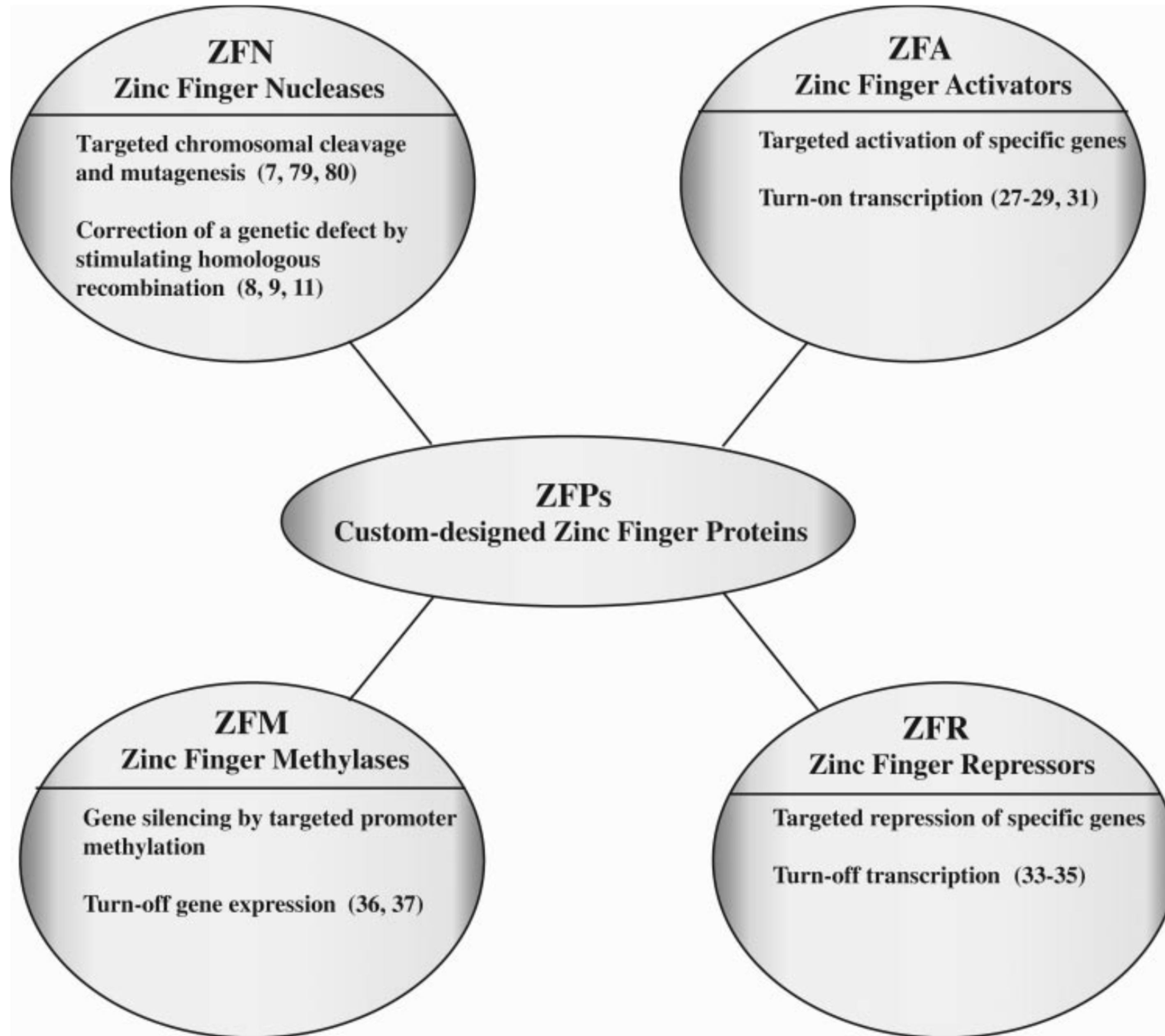
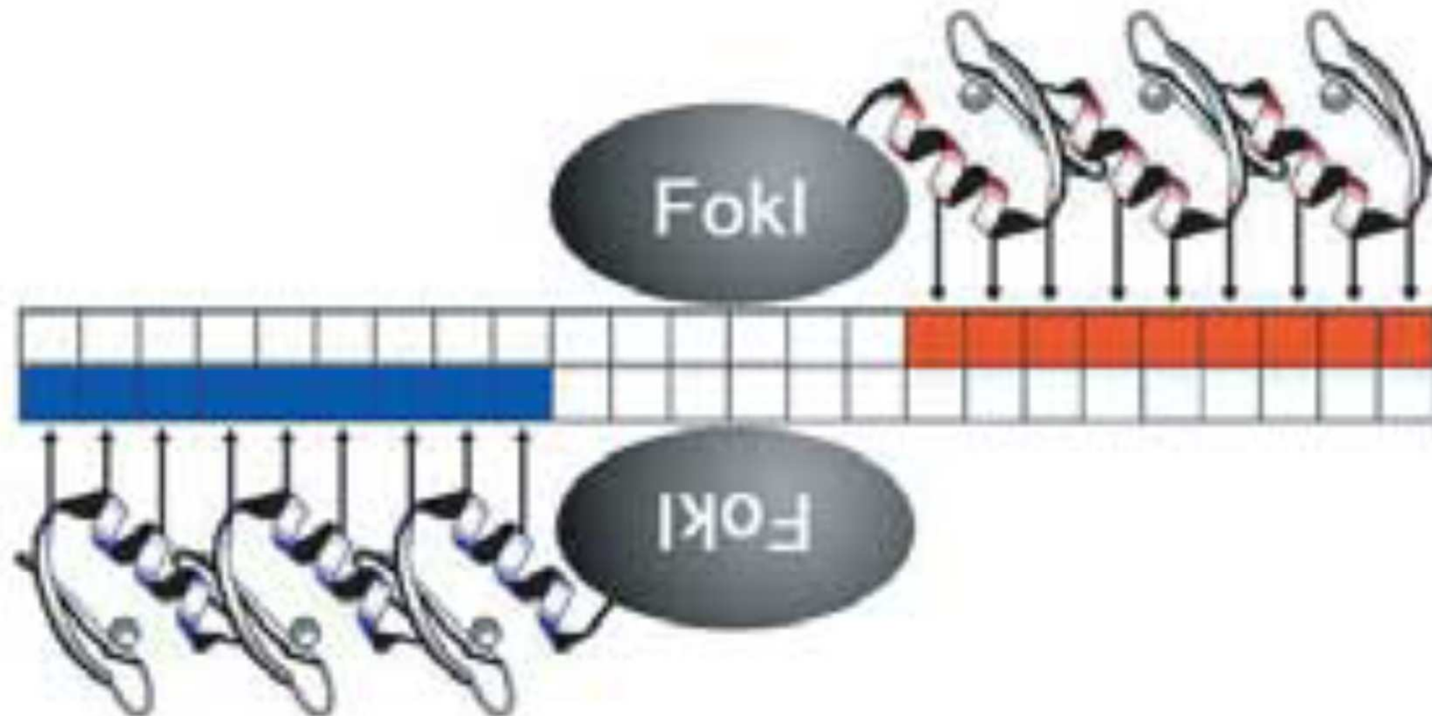

ZFN
Zinc Finger Nucleases
Targeted chromosomal cleavage and mutagenesis (7, 79, 80)
Correction of a genetic defect by stimulating homologous recombination (8, 9, 11)
ZFA
Zinc Finger Activators
Targeted activation of specific genes
Turn-on transcription (27-29, 31)
ZFPs
Custom-designed Zinc Finger Proteins
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)

Table 1 Potential applications of zinc finger nucleases

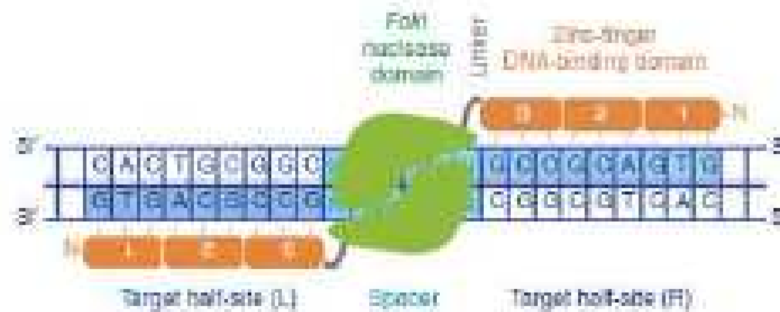
Experimental uses	Drug development
Create knockout genes (cell lines, primary cells, transgenic animals)	Create humanized cell lines
Create point mutations or small deletions in permanent or primary cell lines	Create cell lines for drug target validation
Improve efficiency of gene targeting in ES cells	Create cell lines for high-throughput screening for novel compounds
Create targeted transgenics with insertions into precise genomic locations	
Genome manipulation in model organisms currently without gene targeting mechanism (worms, zebrafish)	

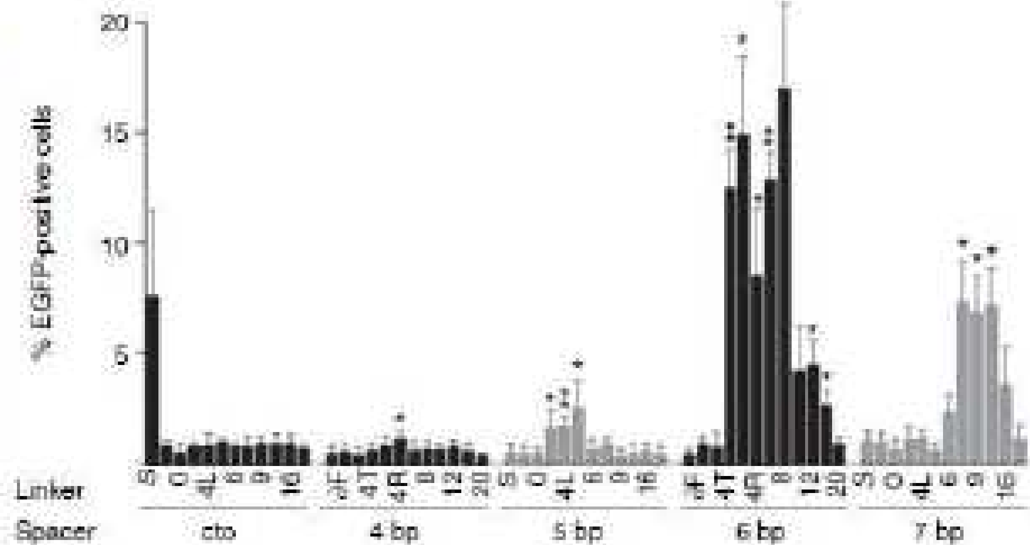
Therapeutics
Correction of genes in monogenic diseases (e.g., Huntington disease)
Inserting genes into precise (safe and permissive) locations for correcting complex mutations (hemophilia A) and introducing RNAi, for example
Altering alleles; for example, the CCR5 gene to create resistance to HIV.
Designer immunotherapeutics
Modification of stem cells

- 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



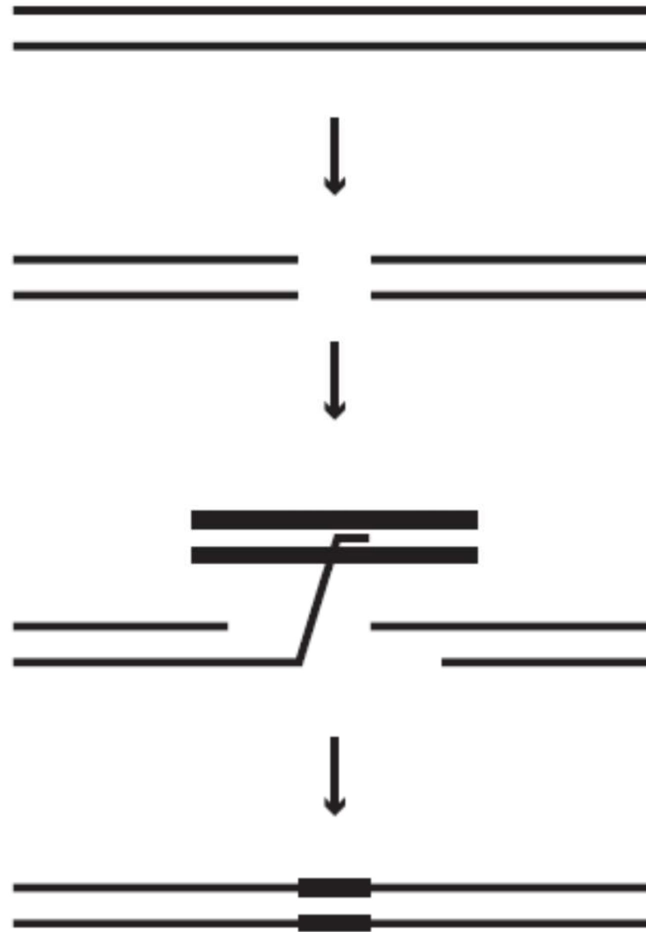


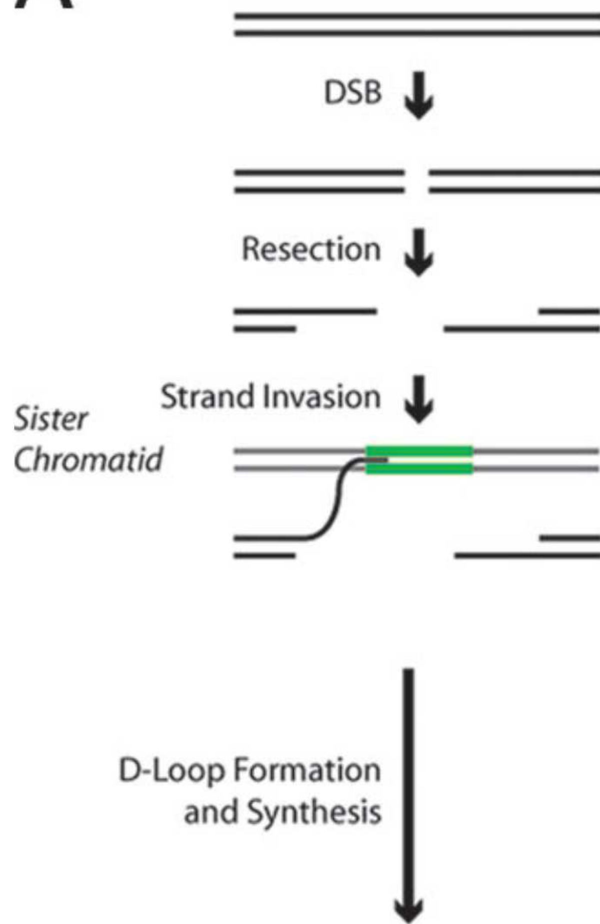
Expanding or Restricting the Target Site Repertoire of Zinc-finger Nucleases: The Inter-domain Linker as a Major Determinant of Target Site Selectivity

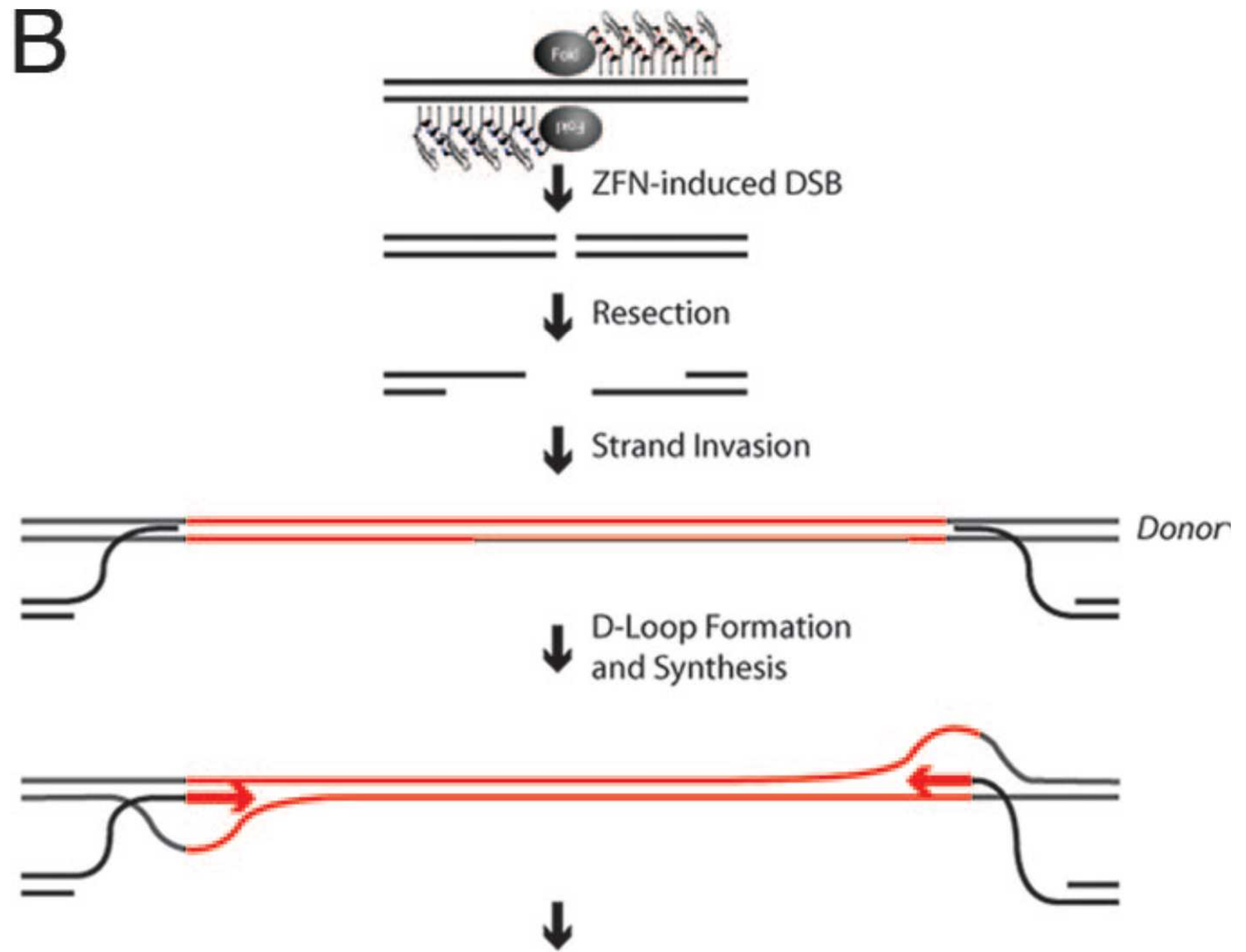
Highly efficient endogenous human gene correction using designed zinc-finger nucleases

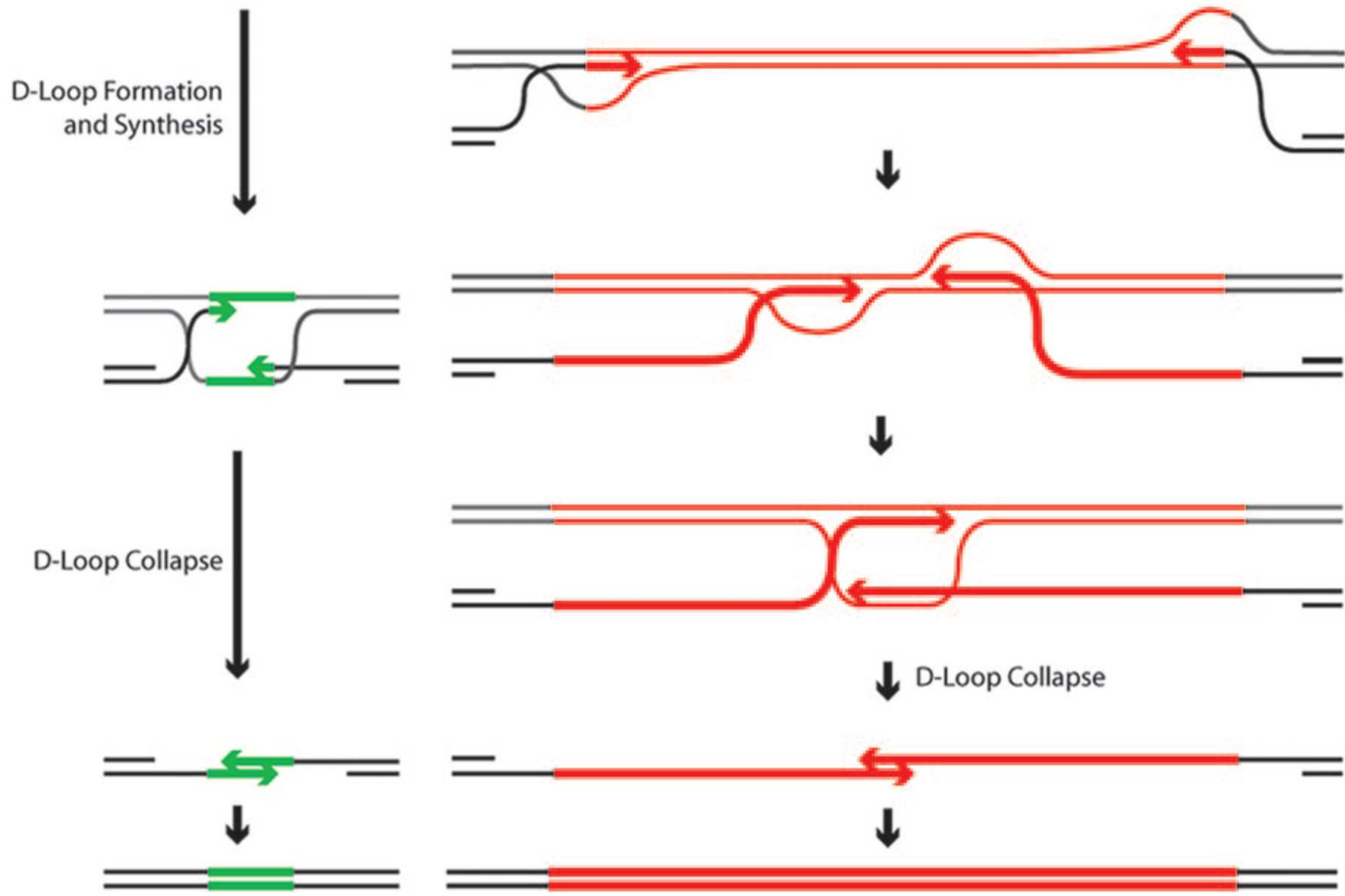
Fyodor D. Urnov[1], Jeffrey C. Miller[1], Ya-Li Lee[1], Christian M. Beausejour[1], Jeremy M. Rock[1], Sheldon Augustus[1], Andrew C. Jamieson[1], Matthew H. Porteus[2], Philip D. Gregory[1] & Michael C. Holmes[1]

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

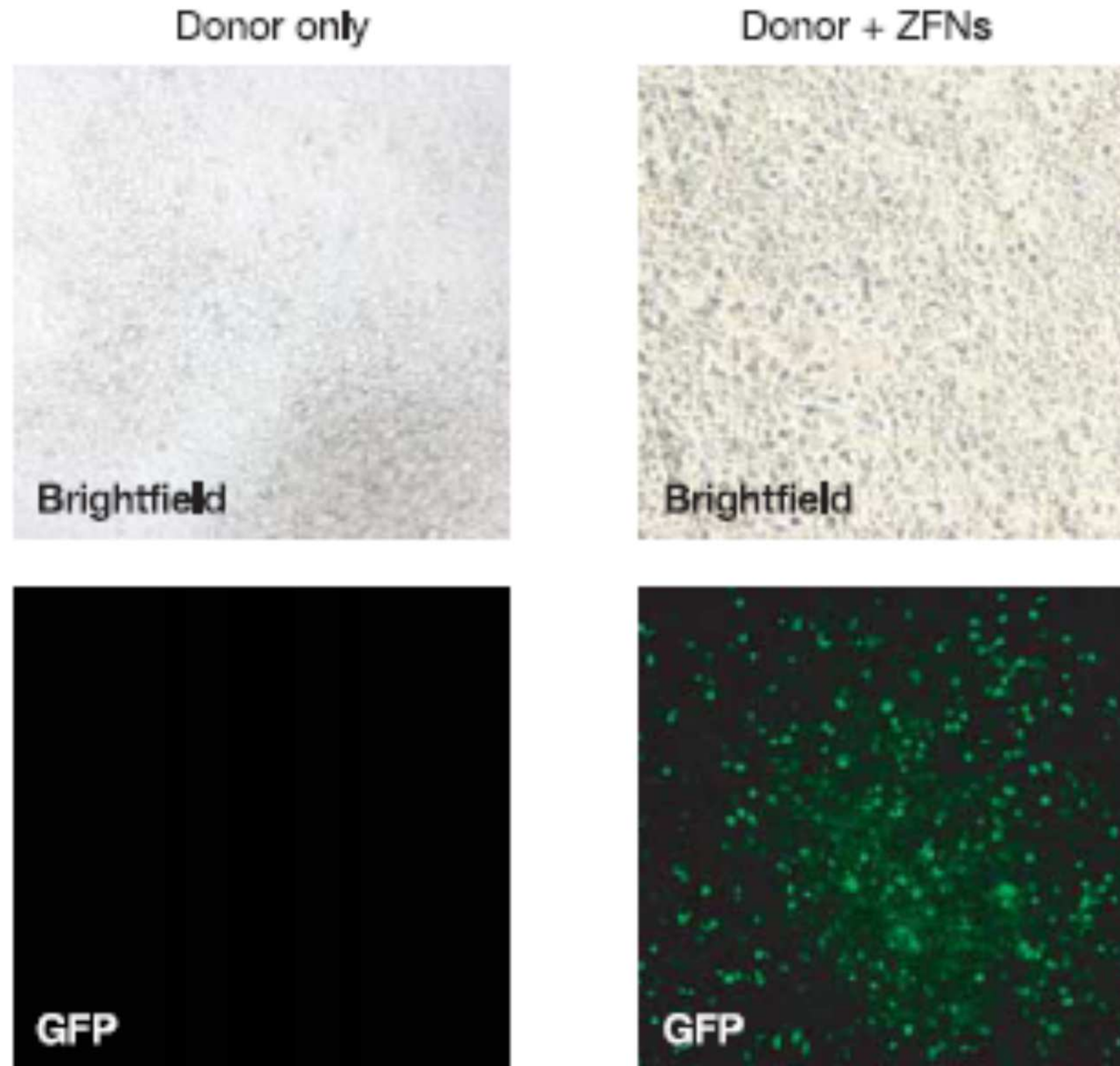

Homology-directed repair:
ZFN-driven homology-directed repair:
X-ray-induced DSB
ZFN-induced DSB
Sister chromatid
Donor DNA (plasmid)

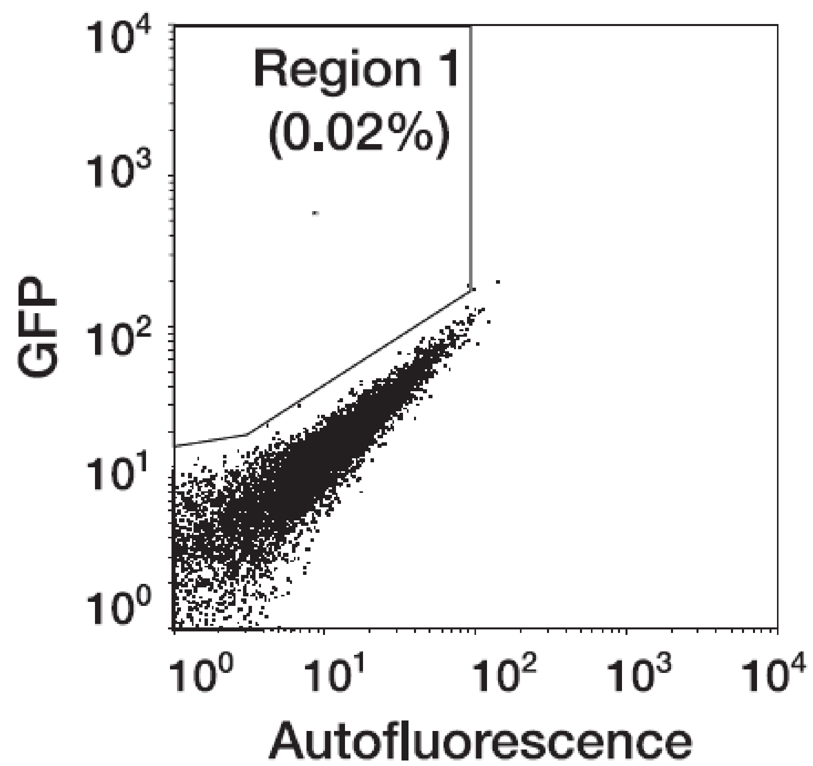

A
DSB
Resection
Strand Invasion
Sister
Chromatid
D-Loop Formation
and Synthesis

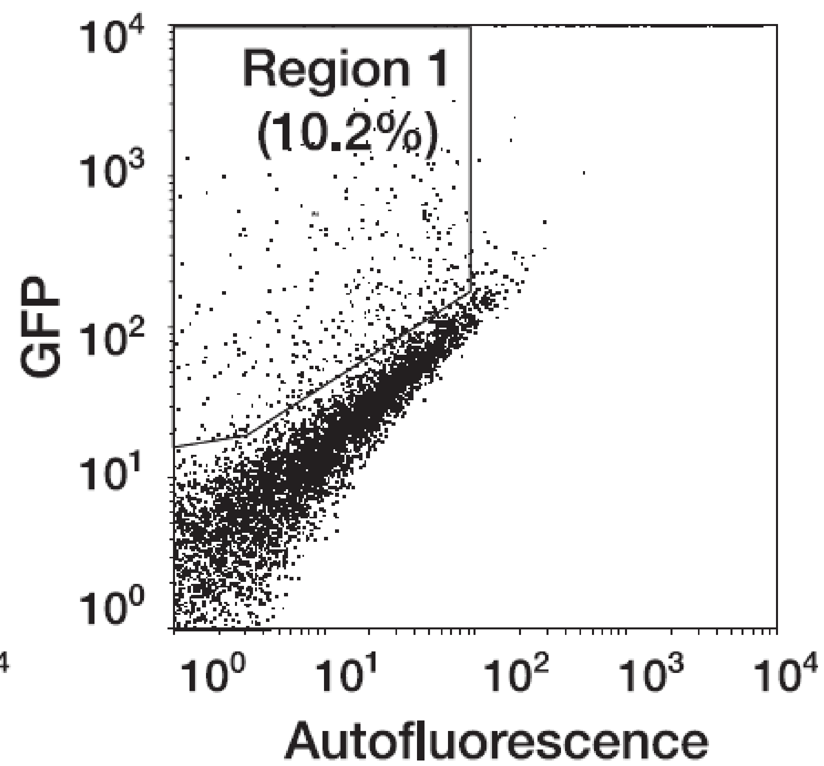

B
ZFN-induced DSB
Resection
Strand Invasion
Donor
D-Loop Formation
and Synthesis

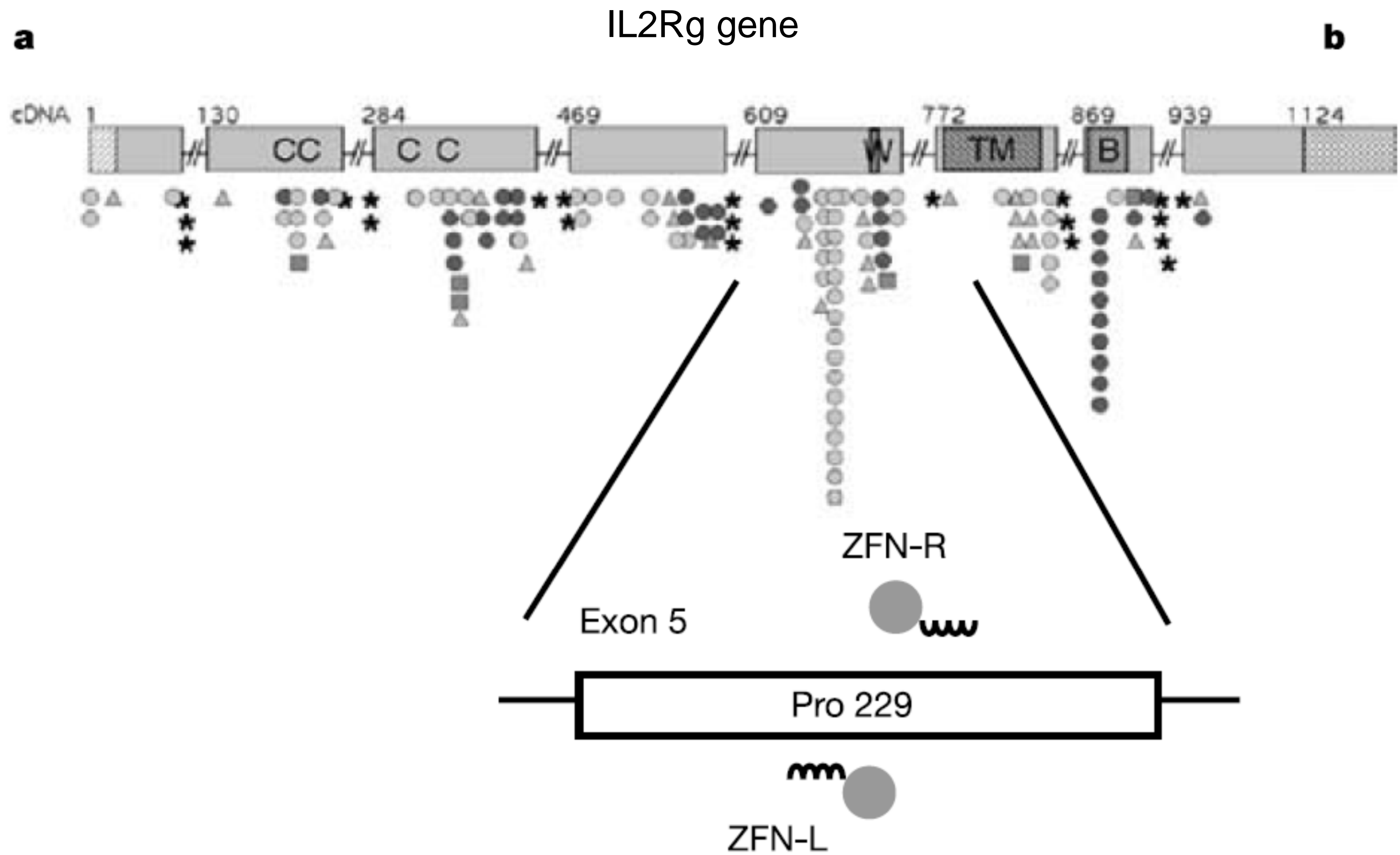

D-Loop Formation
and Synthesis
D-Loop Collapse
D-Loop Collapse

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).



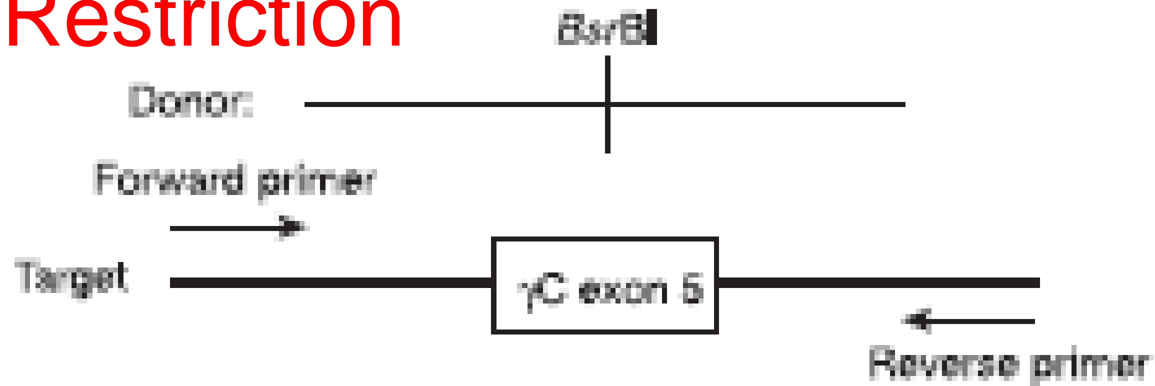

Region 1
(0.02%)
GFP
Autofluorescence
10^4
10^3
10^2
10^1
10^0

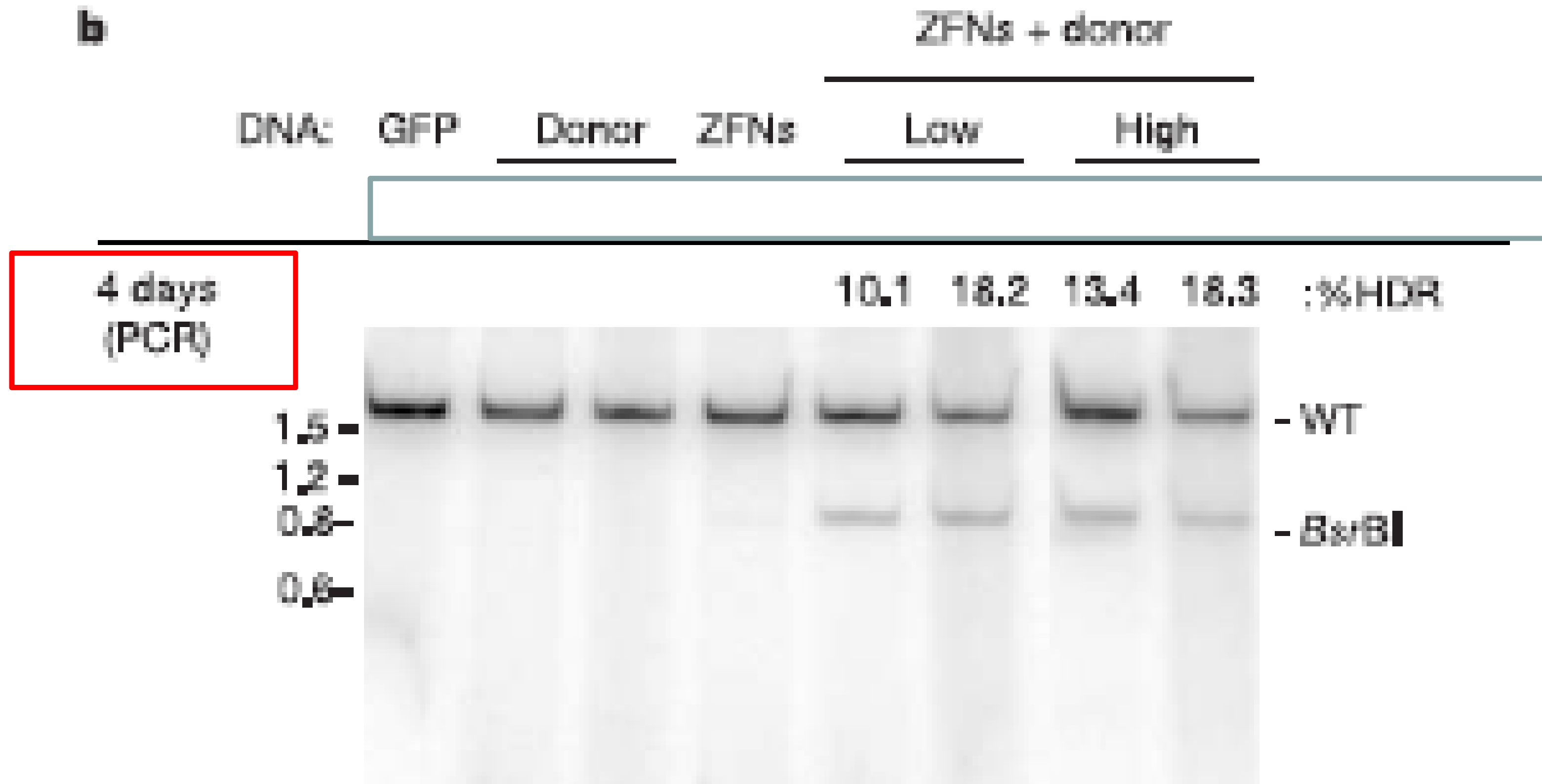

Region 1
(10.2%)
GFP
Autofluorescence
10^4
10^3
10^2
10^1
10^0



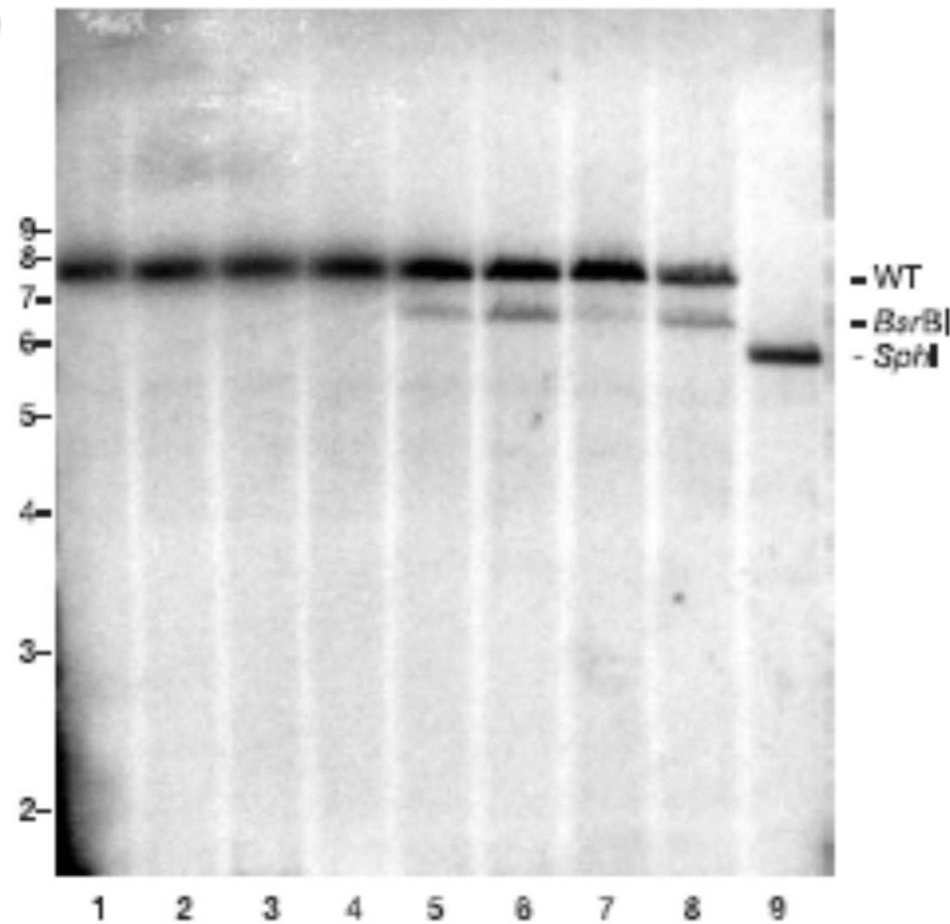
X-linked severe combined immune deficiency (SCID)

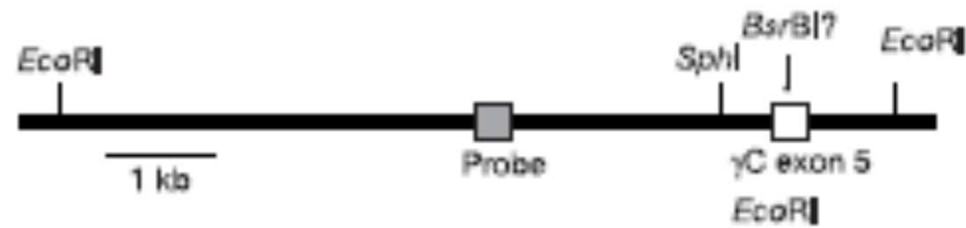
PCR +Restriction





Southern blot

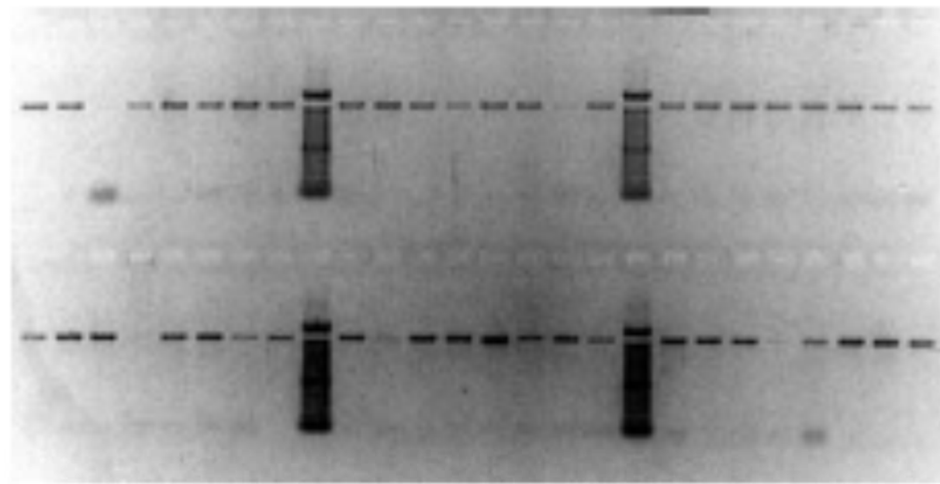




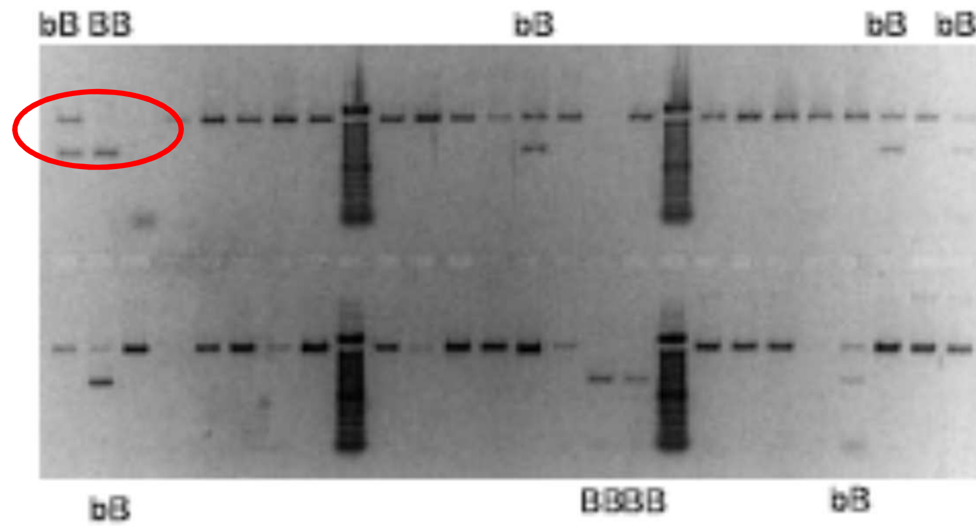
Day 1 : Transfection

Day 4 : Seed <1 cell per well

Day 30 : Isolate genomic DNA,
PCR γC exon 5 (both alleles),
digest with *BsrBI*, gel



↓ + *BsrBI*



Alleles altered: None One Both

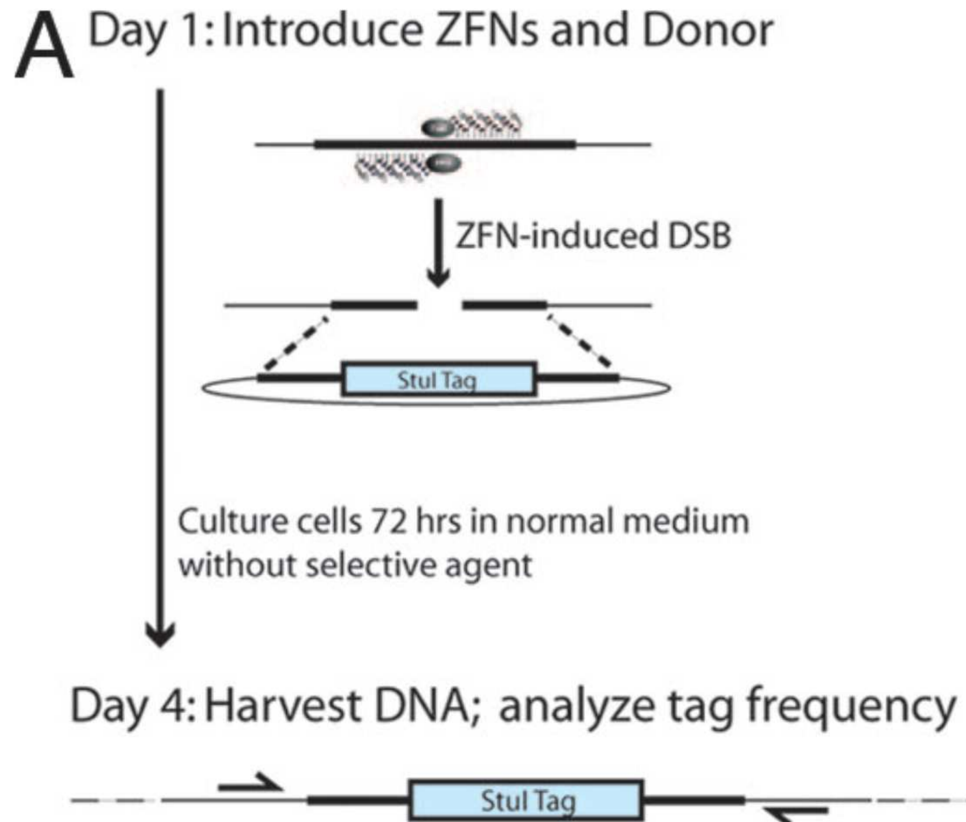
G2 13.2% 6.6%

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