Bioedit

Modify any genome, in any way,
with zinc finger nuclease technology

CompoZr® Knockout ZFNs

Permanently Knockout Any Human, Mouse or Rat Gene

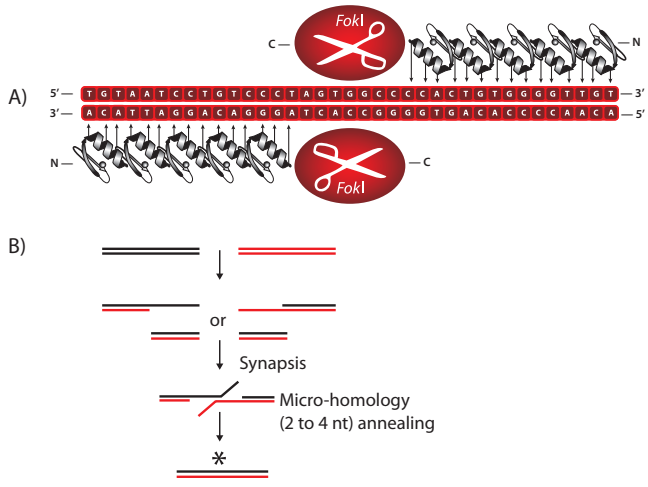
Complete Genomic Coverage

Knockout any gene in the entire human, mouse or rat genomes with the power of CompoZr Knockout Zinc Finger Nuclease (ZFN) technology from Sigma® Life Science. Access to the proven and award-winning ZFN technology has never been easier. With unprecedented access to all three species, CompoZr Knockout ZFNs are the ideal technology to generate knockout cell lines or research models.

- Zinc finger target sequence is pre-designed in the first available open reading frame site (ORF)
- All Knockout ZFNs are functionally validated prior to shipment
- Immediately transfect cell lines upon receipt of Knockout ZFNs
- Gene editing occurs in 1–20% of clonal population

To learn more, visit
sigma.com/knockout

Zinc Finger Mediated Gene Knockout



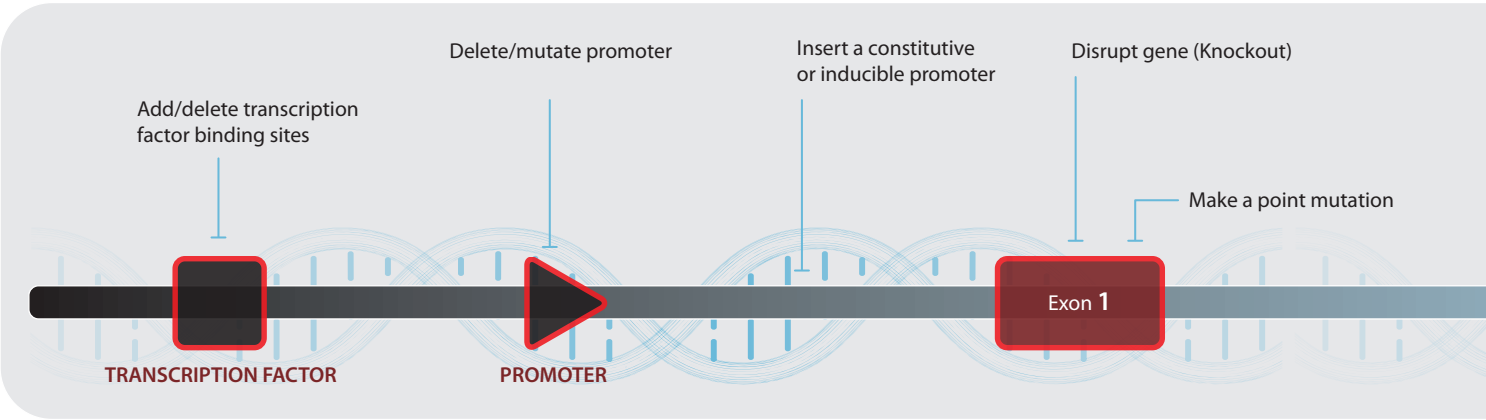
*Mutation due to addition/deletion of bases

Fig 1. ZFN-mediated Targeted Genome Editing (48-72 Hours)

A. The ZFNs bind a specific DNA sequence within the target gene, inducing a double stranded break. **B.** This break is repaired by non-homologous end joining (NHEJ). As an imperfect repair mechanism, a percentage of the double stranded breaks result in nucleotide addition or deletion. These disruptions in DNA sequence result in the loss of functional protein resulting in gene knockout.

The Ability of CompoZr Zinc Finger Nucleases

The specificity of CompoZr ZFNs enables researchers to engineer any genome they research in the following ways:



CompoZr® Custom ZFN Service

Genome Editing in Any Organism or Cell Line

Sophisticated Genome Editing

With the CompoZr Custom ZFN Service any type of genome engineering is possible. Genes may be knocked out or modified (i.e., introduction of SNPs) and transgenes may be integrated — all at a user specified target sequence. Researchers have this ability thanks to the capability of CompoZr ZFNs to target within 50-200 base pairs of genomic DNA.

- Researchers select the target sequence and approve ZFN design prior to manufacture
- All Custom ZFNs are functionally validated in the appropriate model prior to shipment
- Immediately transfect ZFNs upon receipt
- Gene editing occurs in 1–20% of clonal population
- Receive technical support directly from Sigma® ZFN scientific staff

To learn more visit
sigma.com/customzfn

Organisms Modified with Custom ZFNs

The Custom ZFN Service has modified the genome of several different organisms and cell lines:

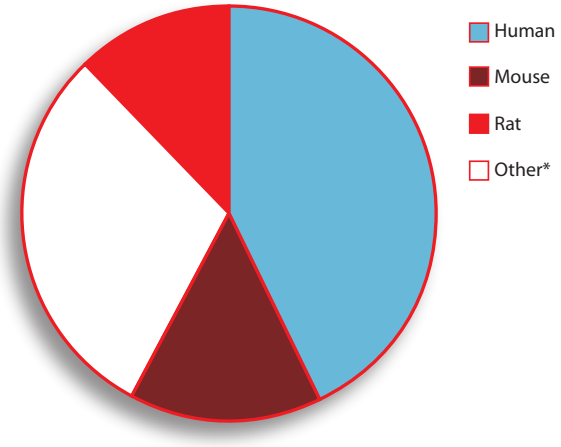
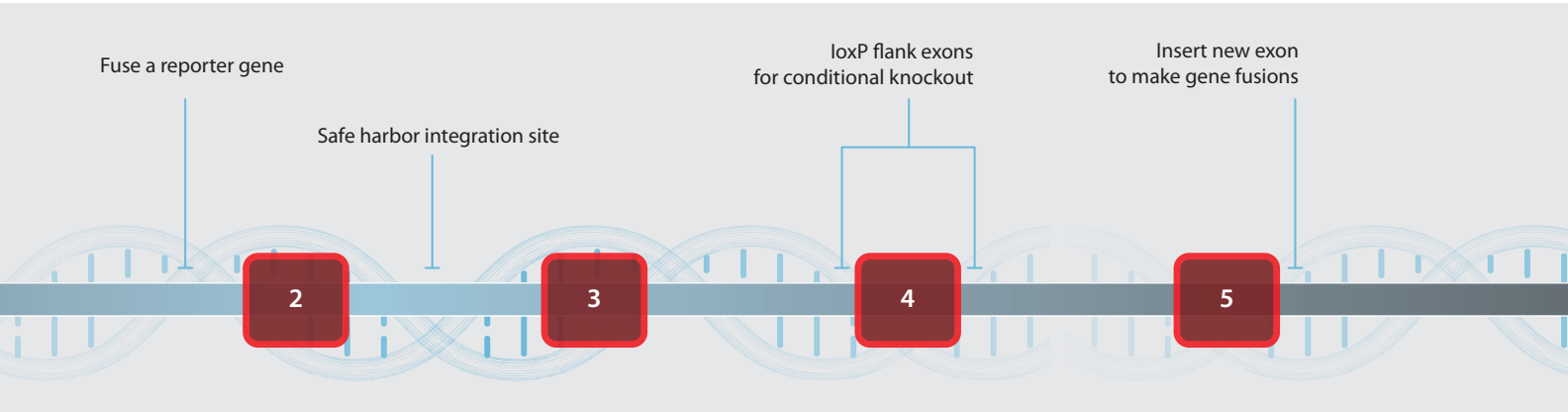


Fig 2. Organisms Modified with Custom ZFNs

* Includes the following organisms: *Drosophila*, zebrafish, worm, snail, frog, rabbit, goat, sheep, pig, cattle and rhesus monkey.

To view zinc finger nuclease references, visit
sigma.com/zfnreferences



CompoZr® Targeted Integration Kits

Rapid Gene Insertion into the Human, Mouse, or Rat Cell Line of Your Choice

Predictable Integration of a Gene of Interest

The CompoZr line of Target Integration Kits is designed for rapid integration of a user-specified gene of interest in specific loci. Zinc finger mediated targeted integration is ideal since a transgene is inserted in a constitutively expressed safe harbor locus that doesn't interfere with normal cellular processes.

- Highly efficient ZFNs delivered in each kit
- Biallelic insertions through a single transfection
- No need to engineer a transgene landing pad
- Any promoter system may drive transgene expression
- Antibiotic selection is not required

To learn more visit
sigma.com/targetedkits

Ordering Information	
Cat No.	Product Description
CTI1-1KT	CompoZr Targeted Integration Kit– AAVS1
CTIM-1KT	CompoZr Targeted Integration Kit – mRosa26
CTIR-1KT	CompoZr Targeted Integration Kit – rRosa26

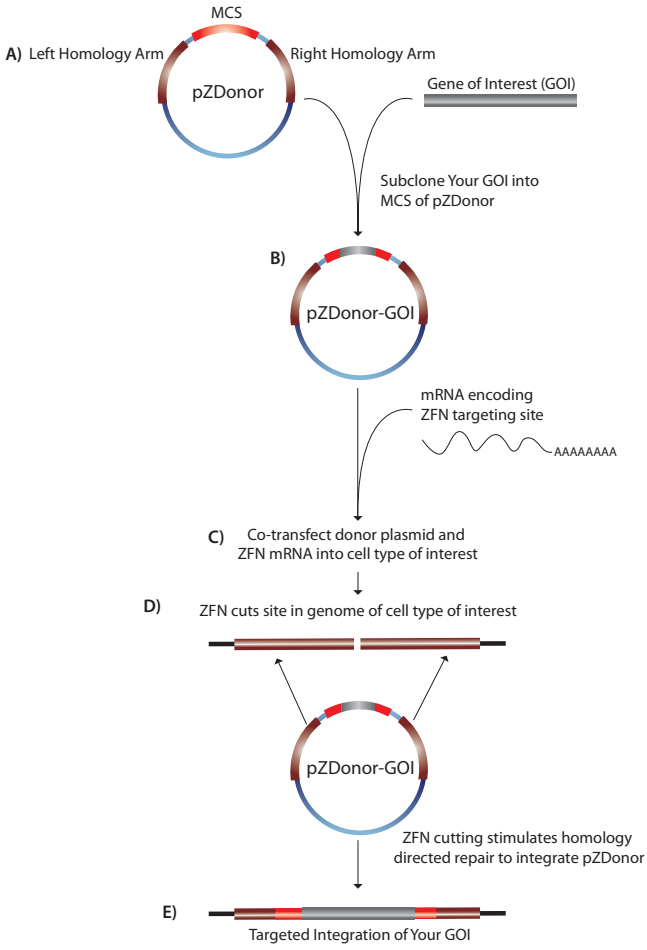
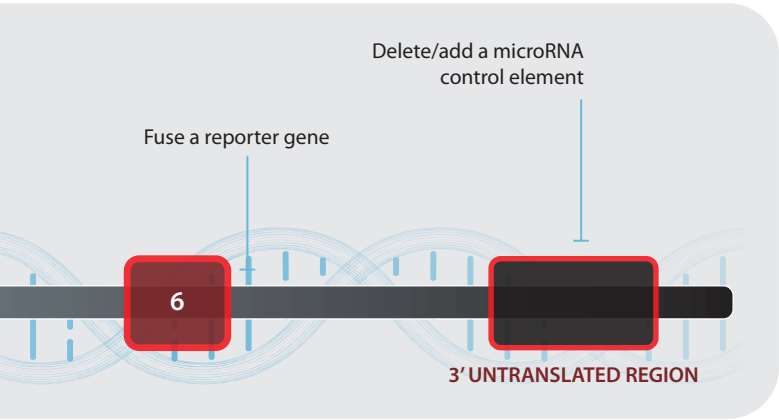


Fig 3. Schematic of CompoZr Targeted Integration Kits

A. Your Gene of Interest (GOI) is subcloned into the supplied pZDonor plasmid at the multiple cloning site (MCS). The MCS is flanked by left and right homology arms to the genomic integration site. **B.** The modified pZDonor now contains your GOI between the homology arms. **C.** The modified pZDonor and supplied mRNA, encoding ZFNs that target the genomic integration site, are co-transfected into the cell line of your choice. **D.** The ZFNs bind and cut the genomic site in the nucleus, creating a double strand break. **E.** The cell uses the modified pZDonor plasmid as a repair template. ZFN stimulated homology-directed repair leads to targeted integration of your gene into your cell line of choice.



ZFN Method of Action

Zinc finger nucleases are engineered proteins comprised of a zinc finger DNA-binding domain fused to the cleavage domain of the *Fok*I restriction endonuclease. When bound as a heterodimer, ZFNs create a double-stranded break at a user-specified DNA sequence. This results in the stimulation of one of two cellular repair mechanisms, non-homologous end joining or homologous recombination.

Delivery of ZFNs may be accomplished with standard methods of transfection including: lipid-based, electroporation and nucleofection.

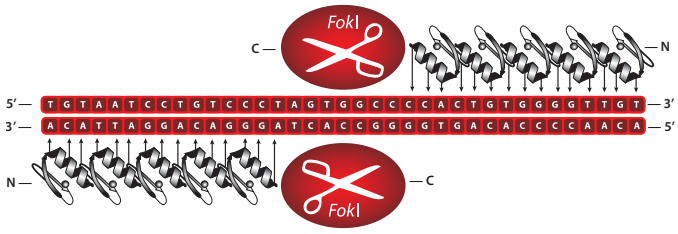


Fig 4. ZFNs are comprised of two functional domains 1) the *Fok*I cleavage domain 2) the user-specified DNA binding domain

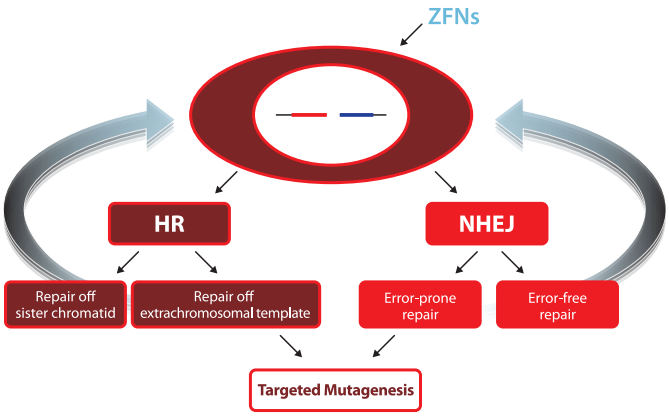


Fig 5. Targeted Mutagenesis

ZFN induced double-strand breaks are repaired by NHEJ or HR. NHEJ is used for gene knockouts, HR is used for gene integration.

Potential CompoZr® ZFN Applications

Gene Knockout

ZFNs specifically target the first available site in the open reading frame (ORF) for cleavage. During the repair process, nucleotides may be added or deleted at the cleavage site due to the imperfect nature of NHEJ. This either prevents transcription or causes nonsense-mediated decay of truncated protein leading to the loss of functional protein.

Gene Integration

In addition to the ZFNs, a donor plasmid with the Gene of Interest (GOI) and homology arms to the ZFN cut site is transfected. The ZFNs then bind and cleave at the user-specified cut site. Through homologous recombination the GOI is then integrated at the ZFN cut site.

Gene Modification

In addition to the ZFNs, a donor plasmid with the desired genetic modification and homology arms to the ZFN cut site is transfected. The ZFNs then bind and cleave at the user-specified cut site. Through homologous recombination the gene modification is then integrated at the ZFN cut site.

Application Data

ZFN-Mediated Gene Knockout

Objective

Inactivate DHFR, a gene used as a selection marker for many bioproduction applications.

Results

Using ZFN-mediated gene deletion, new genetically distinct DHFR-/- cell lines were generated. Each new cell line exhibited growth and functional properties consistent with the specific knockout of the DHFR gene. Biallelic gene disruption of DHFR was observed at a frequency of >1% without the need for selection markers.

Fig 6. Genotype of ZFN-induced DHFR-/- Clones

Each pair of sequences represents the two alleles of the DHFR gene in the designated cell line. For each mutant allele, inserted bases are boxed and deleted bases are represented by dots.

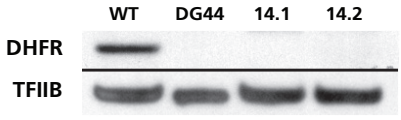


Fig 7. Western blot for DHFR protein in wild type CHO cells (WT) and the mutant cell lines 14.1 and 14.2 as well as the commercially available DHFR-null CHO cell line DG44

Input is normalized against TFIIB expression levels as indicated.

ZFN-Mediated Gene Knockin

Objective

Rapidly insert a 7.8 kb sequence carrying three distinct promoter-transcription units, two of which encode the heavy and light chains of a human IgG molecule, into the endogenous IL2Rγ locus.

Results

These data indicate that designed ZFNs can be used to drive the targeted integration of inserts up to 7.8 kb in length at a frequency of >5%, without the need for selection markers.

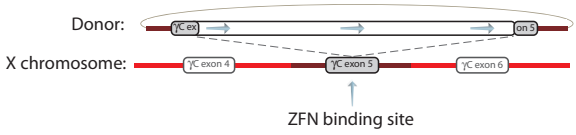


Fig 8. A schematic representation indicating the design of the donor DNA plasmid containing the 750-bp homologous flanking sequence of the IL2Rγ exon5 region and the three promoter transcription units (line arrows).

The site of cleavage of the ZFNs for IL2Rγ is indicated.

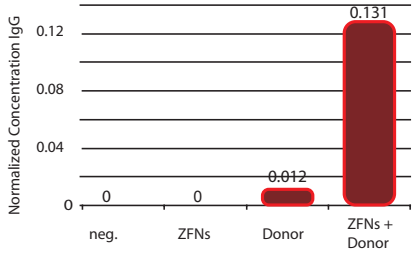


Fig 9. ELISA for IgG in Culture Medium

Medium was collected from K562 cells after the indicated treatments, and levels of secreted IgG were measured by performing an ELISA with an antibody for the heavy and light chains of IgG. IgG concentration is expressed in nanograms per milliliter per cell.

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