genome editing

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Genome editing made easy: **Top 10 tips & tricks**

Designing a knock-in experiment

1 Know the target site	• If interested in expressing a transgene, choose a well-known and characterized safe harbor locus (e.g., *AAVS1* in humans, *ROSA26* in mouse). • If interested in choosing your own target site, whether inserting a gene tag or introducing a simple mutation, good knowledge of your gene and its related pseudogenes is ideal for an efficient experimental strategy.
2 Know the cells	• Establish optimal growth conditions for culture of the cell line of choice—for more information, visit thermofisher.com/cellculture. • Establish optimal transfection conditions for the cell line—for more information, visit thermofisher.com/transfection.
3 Know the copy number of the target site	• Determine the number of copies of the target site. • Decide the number of copies of the transgene you would like to insert into the site.
4 Choose the right editing tool based on your application	• CRISPR-Cas9 system—learn more at thermofisher.com/crispr. • TAL effector nuclease—learn more at thermofisher.com/tals. • The tools need to be specific for the target site and evaluated for off-target effects. If you need help determining which tool to use, check out our selection guide. If you plan on using the CRISPR-Cas9 system, visit our new search and design tool for optimal gRNA designs, **thermofisher.com/crisprdesign.**

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5 Validate the editing tool

- Design at least three CRISPR sequences or three TAL pairs.
- Evaluate the cleavage efficiency of the tool at its target site using the Invitrogen™ GeneArt™ Genomic Cleavage Detection Kit.
- Check the targeted cell population for off-target cleavage.

6 Designing donor DNA

- Choose an appropriate way to make the donor DNA (i.e., Invitrogen™ GeneArt™ gene synthesis or oligo synthesis).
- Choose a sufficient length of homologous sequences to flank the target site—at least 500 bases flanking each side of the mutation site are recommended (this may not be needed if using oligos for single-nucleotide changes).
- Ensure the homologous sequences on the donor DNA are not targeted by the CRISPR or TAL nuclease used to introduce target site cleavage.

7 Codon optimization

- Ensure the transgene to be knocked in is optimized for expression in the target cells; codon optimziation can be performed through the GeneArt gene synthesis service.

8 Knock-in experiment

- Use an optimized transfection protocol to perform the targeting experiment.

9 Setting up clonal isolation

- If possible, enrich for cells likely to have the correct modification using the Invitrogen™ GeneArt™ Genomic Cleavage Selection Kit.
- Colony isolation: If the donor construct is designed to impart antibiotic resistance, the cells should be plated at low density for selection such that the resistant cells can form discrete colonies. These colonies can then be isolated and screened for the correct modification.
- Cloning by limiting dilution or FACS: Limiting dilution or FACS has been used to plate a single cell per well of a 96-well plate for generating clonal populations.

10 Screening clones

- Design an appropriate screening strategy. If using PCR, ensure the junctions between targeted genomic locus and the knocked-in donor are included in the strategy and subsequently sequence-verified. In addition, the integrated donor should be completely sequenced to ensure the integrity of insertion.
- Additional strategies should be employed to understand the ploidy of modification. This may include next-generation sequencing or qPCR-based genotyping approaches.

Find out more at **thermofisher.com/genomeedit**

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