Genome editing made easy: Top 10 tips & tricks

Genome cleavage selection (GCS)–based screening

1 Choose a genome editing tool

- Choose the appropriate genome editing tool for the desired application and evaluate the tool for target site specificity and potential off-target effects.

2 Design 2–4 CRISPR target sequences or TAL pairs and corresponding GCS vectors per gene

- Using the design tool, choose 2–4 of the top-scoring CRISPRs or TALs.
- Design and clone individual GCS vectors for each CRISPR or TAL.
- If you plan on using the CRISPR-Cas9 system, visit our new search and design tool for optimal gRNA designs at thermofisher.com/crisprdesign.

3 Establish optimal growth conditions for the cell lines being used

- Optimize the cell culture growth conditions for the cell line being used, such as the culture medium and optimal passage number prior to transfection.

4 Transfect the GCS vector and editing tool using optimal transfection conditions and reagents

- Use an optimized transfection protocol for the reagent and format being used. We recommend titering the GCS vector to determine the optimal quantity for delivery and screening. The optimal reagents and dose will be dependent on the cell line, the tool (CRISPR or TAL), and format (DNA or mRNA) being used. We recommend:
  - DNA only: Invitrogen™ Lipofectamine™ 3000 reagent
  - DNA and RNA cotransfection: Invitrogen™ Lipofectamine 2000™ reagent or Lipofectamine™ MessengerMAX™ reagent
  - To check if the transfection reagent used is suitable for the cell line of interest, refer to our Transfection Reagent Selection Guide at **thermofisher.com/transfection**

5 Use appropriate controls to establish cleavage efficiency

- For each sample transfected with a GCS vector and genome editing tool, also transfect with a genome editing tool that does not target the DNA sequence in the GCS vector. This provides the control for background fluorescence needed for analysis by flow cytometry, as well as for downstream applications such as quantification of genomic DNA cleavage using the Invitrogen™ GeneArt™ Genomic Cleavage Detection Kit.
- Included in the GeneArt Genomic Cleavage Detection Kit are positive control oligonucleotides that contain target sites for the TAL pair specific for the *AAVS1* safe harbor locus (also known as the *PPP1R12C* locus on human chromosome 19). If you want to include a positive control in your transfection, you can obtain our off-the-shelf validated TAL pair or CRISPR gRNA expression plasmid by contacting Technical Support at techsupport@thermofisher.com.

6 Screen for cells expressing Orange Fluorescent Protein (OFP) using fluorescence microscopy or flow cytometry

- As early as 24 hours posttransfection, observe genome editing tool cleavage efficiency on the GCS vector using an Invitrogen™ EVOS™ Imaging System or other fluorescence microscope.
- Cleavage efficiency can be further quantified using flow cytometry. Excite transfected cells using the 488 nm laser on the Invitrogen™ Attune™ or Attune™ NxT Acoustic Focusing Cytometer, and gate on the negative control to determine cleavage efficiency.

7 Correlate OFP expression with genome editing efficiency on the endogenous loci

- Once nuclease functionality has been confirmed by OFP expression, confirm and quantify genomic cleavage efficiency on the endogenous loci using the GeneArt Genomic Cleavage Detection Kit. Cleavage efficiency is expected to deviate from the proportion of OFP expression observed, as endogenous cleavage will be affected by factors such as chromatin structure.

8 Confirm GCS vector functionality in a workhorse cell line

- Nuclease expression can vary tremendously between cell lines and can also be heavily influenced by cell culture conditions and general cell health. Prior testing of the GCS vector in a workhorse cell line such as HEK 293T will help confirm nuclease functionality.

9 Enrich for nuclease-modified cells

- Cells transfected with the GCS vector can be subsequently enriched based on expression of OFP or the CD4 membrane protein. Enrichment of OFP-positive cells requires the use of a cell sorter, while enrichment based on CD4 expression only requires the use of our Invitrogen™ Dynabeads™ CD4 Positive Isolation Kit.

10 Analyze CD4- or OFP-positive cells

- After enriching for and isolating CD4- or OFP-positive cells, proceed with the downstream method of choice such as sequencing or qPCR to verify nuclease efficiency in producing the desired mutation.

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

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