genome editing

invitrogen

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

TAL effector–mediated genome editing

1	**Design and test at least 2–3 pairs of transcription activator–like (TAL) effectors per gene**	• Testing multiple constructs increases the chance of obtaining a highly specific and active TAL effector pair.
2	**Design the TAL pair to cleave the DNA as close as possible to the desired position**	• Cleavage efficiency may vary based on the accessibility of the target loci. • Target accessibility could be affected by chromatin structure and DNA methylation. • GeneArt™ PerfectMatch TALs can be designed to target any desired locus, since there is no 5′ base restriction (5′ base at target site can be A, T, G, or C).
3	**Design TAL repeats to target 18 or 24 bp of DNA sequence, and design TAL pair targets with 16 bp of spacing in between; then add one N (A, T, G, or C) to the 5′ end of each target sequence**	• GeneArt PerfectMatch TALs are available with 18 or 24 repeats to target 18- or 24-bp DNA-binding sequences, respectively. To order PerfectMatch TALs, a 5′ N has to be included in the target sequences. N could be A, T, G, or C. • The optimized spacing between forward TAL target and reverse TAL target is 16 bp.
4	**GC content should be distributed throughout the target site when possible**	• Avoid long runs of As and Ts, especially at the 5′ end of the binding site.

ThermoFisher SCIENTIFIC

5 **Select the TAL vector of interest**

- Choose the N-TAL Fok1 CMV expression vector to drive high-level expression of the TAL in mammalian systems. It can be used directly without extra subcloning.
- Choose the N-TAL Fok1 entry vector (Gateway™-adapted vector) for easy transfer of your target-specific TAL to a destination vector designed for expression in your specific cell type.

6 **Validate TALs with the GeneArt™ Genomic Cleavage Selection Kit and GeneArt™ Genomic Cleavage Detection Kit**

- TAL function can be validated using the GeneArt Genomic Cleavage Selection Kit and the GeneArt Genomic Cleavage Detection Kit prior to performing full-scale genome engineering experiments.

7 **Optimize transfection conditions**

- High transfection efficiency is important for efficient genome modification with TALs.
- Use our transfection reagent selection guide to find the best transfection reagent for your cell line and optimize transfection conditions according to cell type.
- Optimize the amount of TAL pair for your cell type and culture condition. A good starting point is 750 ng of DNA or 500 ng of mRNA for each TAL pair, per well, in 12-well format.

8 **Use mRNA instead of DNA**

- To circumvent random integration and cell type–specific promoter requirements, use TAL mRNA instead of TAL expression plasmid.
- Order ready-to-use TAL mRNA from our Custom Services team or make your own.
- GeneArt PerfectMatch TAL vectors (pDEST40 or N-TAL CMV) have a T7 promoter upstream of the TAL Fok1 sequence, which can be used for *in vitro* transcription of TAL mRNA using the mMESSAGE mMACHINE™ T7 ULTRA Transcription Kit. We recommend Lipofectamine™ MessengerMAX™ reagent for mRNA transfection.

9 **Test modification efficiency with the GeneArt Genomic Cleavage Detection Kit**

- Refer to the GeneArt Genomic Cleavage Detection Kit manual for analyzing genome modification efficiency after transfection.

10 **Enrich modified cells with the GeneArt Genomic Cleavage Selection Kit**

- In case of low transfection efficiency, modified cells can be enriched using the GeneArt Genomic Cleavage Selection Kit.

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

ThermoFisher SCIENTIFIC