genome editing

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

Improve your genomic cleavage detection (GCD) assay

1	**Optimize cell culture conditions**	• Pay attention to passage number, seeding density, and media. • Find the best culture reagents for your cell line at thermofisher.com/cellculture.
2	**Optimize transfection conditions**	• High transfection efficiency and minimal cellular toxicity will maximize efficient genome modification and improve genomic cleavage detection analysis. • Find the best transfection reagent for your cell line and optimize transfection conditions with our transfection reagent selection guide.
3	**Optimize the amount of nuclease**	• Find the best quantity of nuclease for your cell type and culture conditions for TALs and for CRISPRs in the corresponding product user manuals.
4	**Include negative controls**	• Plan to include a mock transfection with an irrelevant nuclease as a negative control to distinguish background from cleavage banding in the gel analysis portion of the GeneArt™ genomic cleavage detection assay.

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5	**Prepare cell lysate**	• Ensure that the cell lysate contains at least 50,000 and no more than 2 x 10^6 cells in a 50 µL cell lysis reaction—lysate from less than 50,000 cells will be insufficient for PCR, and lysate from more than 2 x 10^6 cells will inhibit PCR.
6	**Generate a strong PCR product**	• Generate a reaction that produces a single PCR product that migrates on a gel as a crisp band with minimal background. • We recommend designing a PCR fragment approximately 500 bp in length to allow for analysis of cleaved products. • Include a PCR sample from the mock transfection with the irrelevant nuclease (see Tip 4).
7	**Verify PCR product before proceeding**	• A single band is crucial for obtaining accurate cleavage detection. If a single band of correct size is present with intensity similar to 50 ng of 400 bp band in the mass ladder, proceed to the denaturing and reannealing step.
8	**Denature and reanneal PCR fragment**	• It is also good practice to include a negative control sample that will not be treated with the GCD enzyme, to distinguish background from true cleavage banding.
9	**Optimize enzyme digestion**	• To prevent over-digestion of the sample, be sure not to exceed the incubation time with the GCD enzyme, and continue immediately to gel loading.
10	**Optimize gel analysis**	• Optimize the gel imaging conditions to achieve the best signal/background ratio.

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

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