1. Identify target and find genomic sequence

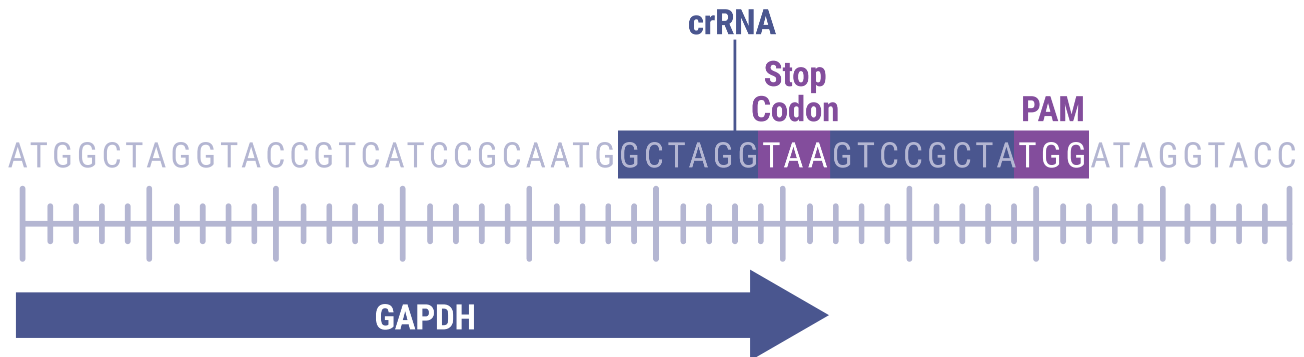
Download full genomic sequence.
NCBI: https://ncbi.nlm.nih.gov/gene
Ensembl: http://useast.ensembl.org

Ensure you have the complete genomic sequence that includes 5' and 3' UTR. In NCBI these RefSeq numbers start with NG or NC.

Find region for HiBiT insertion.
Create C-terminal fusions before stop codon
Generate N-terminal fusions after start codon

2. Design crRNA and order guide RNA (crRNA + tracrRNA)

Select a region to search for guide RNA.
Start with 40 nucleotides upstream (for N-terminal fusions) and downstream (for C-terminal fusions) of insert site.



Use an online program to identify guide RNA.
We suggest using one of the following sites:
http://chopchop.cbu.uib.no/
http://portals.broadinstitute.org/gpp/public/analysis-tools/sgrna-design/

Order a duplex guide (crRNA and tracrRNA). For example, the IDT Alt-R CRISPR-Cas9 system.

We recommend choosing guides based on PAM sites within 30nt of the start or stop codon.

Order 3–5 different guide RNAs.
We recommend Protospacer Adjacent Motif (PAM) sites downstream of stop codon for C-terminal fusions and upstream of start codon for N-terminal fusions.

Order the crRNA without including the PAM sequence. Remember, Cas9 will cut 3nt upstream of the PAM sequence.

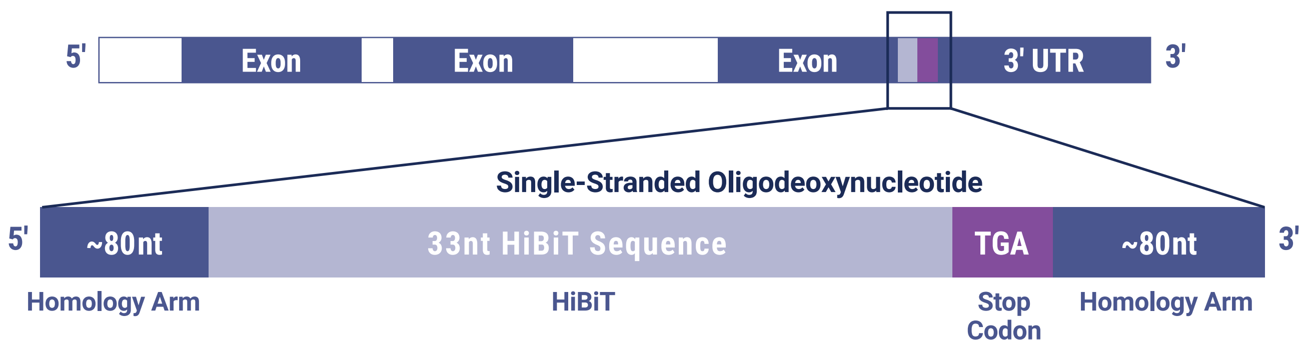
3. Design and order HiBiT Donor DNA template

Find regions of identity for donor DNA.
Use 50–80 nucleotides upstream and downstream of insert site.

Position the HiBiT sequence symmetrically between homology arms. This facilitates the CRISPR homologuous recombination.

Order donor DNA template.
Request a single-stranded oligodeoxynucleotide (ssODN). For example, IDT Ultramer DNA Oligonucleotide. Obtain synthesis rights and access HiBiT sequence: www.promega.com/HiBiT-synthesis

Introduce a silent mutation in the PAM sequence of the donor DNA template to avoid recutting.



4. Deliver guide RNA, donor DNA and Cas9

Prepare ribonucleoprotein (RNP) complex.
Anneal the crRNA to tracrRNA (IDT) to form guide RNA. Incubate purified Cas9 with guide RNA to form RNP complex.

1. Prepare 24µM tracrRNA:crRNA duplex.

a. Combine the following in a PCR tube:

COMPONENT	VOLUME
100µM tracrRNA	12µl
100µM crRNA	12µl
Nuclease-Free Duplex Buffer	26µl
Total	50µl

b. Heat at 95°C for 5 minutes and cool to room temperature on bench top.

2. Prepare RNP complexes (sufficient for 200,000 cells).

a. Combine 100pmol Cas9 and 120pmol tracrRNA:crRNA:

COMPONENT	VOLUME
20µM Cas9	5µl
24µM trRNA:crRNA	5µl
Total	10µl

Note: To avoid precipitation, add the Cas9 very slowly to the tracrRNA:crRNA and swirl with pipette tip.

b. Allow RNP to form for 10–20 minutes at room temperature.

4. Deliver guide RNA, donor DNA and Cas9 (continued)

Deliver RNP and donor DNA to cell by electroporation.
We have successfully electroporated commonly used cell lines (e.g., HeLa, U2OS, HepG2, HEK 293) suspended in Nucleofector Reagent using the cell line-specific program on the 4D-Nucleofector.

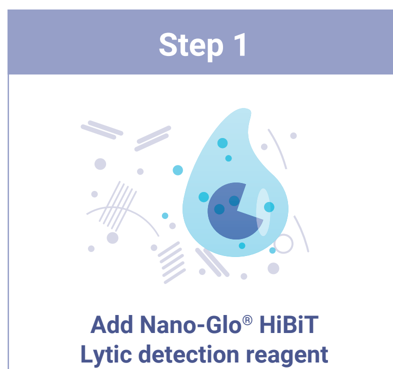
We typically use 1µl of 100µM donor DNA template with 10µl of RNP for 20µl of cell suspension.

We obtain highest efficiency using electroporation, however, lipid-based reagents designed for RNP transfection may be an alternative.

5. Validate editing event in cells (2 options)

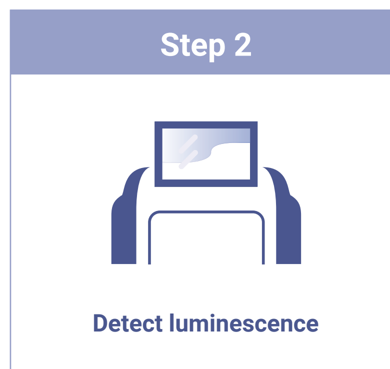
Option 1: Use Lytic plate-based assay to confirm edited gene expression level.
Take a sample of the edited cells 24–48 hours post-electroporation. Use Nano-Glo® HiBiT Lytic Detection System to assess luminescence for each guide RNA tested. Use unedited cells as negative control for background.

Step 1



Add Nano-Glo® HiBiT Lytic detection reagent

Step 2



Detect luminescence

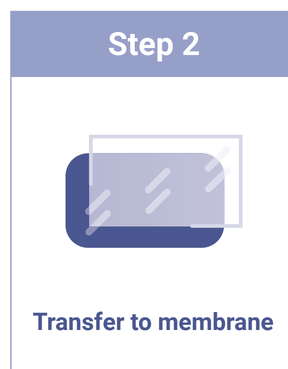
We recommend trypsinizing cells, resuspending to 200,000 cells/ml in growth medium and transferring 100µl (20,000 cells) per well of a 96-well plate. Transfer unused cells to a six-well plate to scale up for future experiments.

Option 2: Use HiBiT Blotting to confirm expression of full-length protein.
Take a sample of the edited cells and lyse with preferred buffer. Run samples on a gel, transfer proteins to membrane and detect using the Nano-Glo® HiBiT Blotting System. Use unedited cells as negative control for background.

Step 1

Run lysates on gel

Step 2

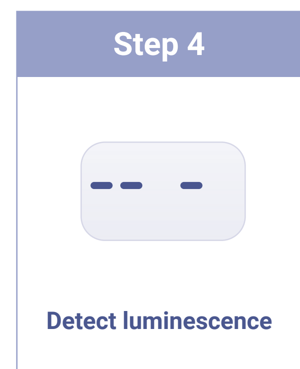


Transfer to membrane

Step 3

Add Nano-Glo® HiBiT Blotting System reagents

Step 4



Detect luminescence

Additional Resources

DeWitt, M., Corn, J.E. and Carroll, D. (2017) Genome editing via delivery of Cas9 ribonucleoprotein. *Methods* Epub ahead of print. doi: http://dx.doi.org/10.1016/j.ymeth.2017.04.003

Detailed protocols from the Innovative Genomics Initiative at University of California–Berkeley at: https:// https://www.protocols.io/groups/innovative-genomics-institute

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