Datasheet for Neuro 2a/HF-Cas9- ROSA26 Cell Line

Catalog number: SL571

Product: Neuro 2a cell line stably expressing CRISPR SpCas9-HF nuclease

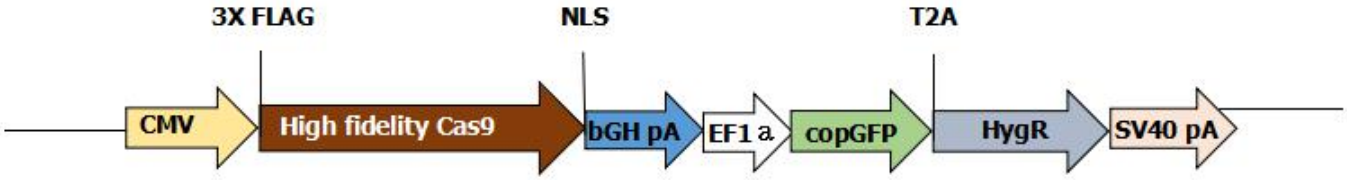
Description: This product is a cell line stably expressing the CRISPR SpCas9-HF Cas9 nuclease. High-fidelity CRISPR-Cas9 nucleases with no detectable genome-wide off-target effects. SpCas9-HF Cas9 is integrated at the mouse ROSA26 Safe Harbor locus. This cell line also expresses copGFP and the hygromycin resistance gene. In combination with separately transfected or transduced single guide RNAs (sgRNAs), this cell line will sustain double-strand DNA breaks (DSBs) at targeted genome sites. This cell line can be used *in vitro* for gene knockout, transgene knockin, mutagenesis, transgene integration, or other genome editing-related applications

Quantity: 1 vial of 2 x 10^6 cells; frozen

Shipping conditions: Dry ice

Storage conditions: Liquid nitrogen vapor phase. Remove the item from the dry ice packaging and check all items for damage and leakage. Place immediately into storage at or below -140 ^{0}C, preferably into the liquid nitrogen vapor phase, until use.

Transgene integration:



Source of parental line:

Neuro 2a
Organism: *Mus musculus*, mouse
Tissue: Brain
Cell type: Neuroblast

Quality control: >95% viability before freezing. All cells were tested and found to be free of mycoplasma, bacterial, viruses, and other toxins.

Safety instructions: To ensure safety, protective gloves, clothing, and a face mask should be worn when handling frozen vials. Some leakage may occur into the vial during storage. The liquid nitrogen will be converted to gas upon thawing. Due to the nature of nitrogen gas, pressure may build within the vial and possibly result in the vial exploding or losing its cap. This may cause flying debris.

Thawing procedure: The vial of cells should be thawed in a 37 ^{0}C water bath with gentle agitation. For optimal performance, the vial should be thawed in under two minutes. Ensure that the cap of the vial did not loosen upon thawing, and re-tighten as needed. Spray the vial with 70% EtOH and wipe off. Repeat. Using aseptic technique, add the contents of the vial to 9 ml of complete growth medium (without selection). Centrifuge for 5 min. at 125 x g. Aspirate the medium, being careful not to disturb the pellet. Resuspend in 10 mL of complete growth medium, and place into a culture vessel of your choice. Only add selection to the medium after 24 hours in culture.

Culture conditions:

Complete Growth Medium

The base medium for this cell line is Dulbecco's Modified Eagle's Medium (DMEM). For optimal growth and maintenance of selection, add the following components to the base medium: fetal bovine serum to a final concentration of 10%.

Selection

Hgromycin to a final concentration of 600 μg/mL

Culture temperature:

37 ^{0}C with 5% CO_2

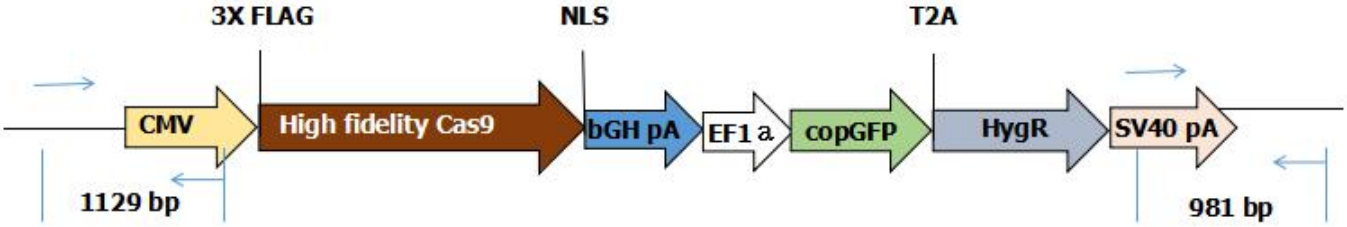
Subculture:

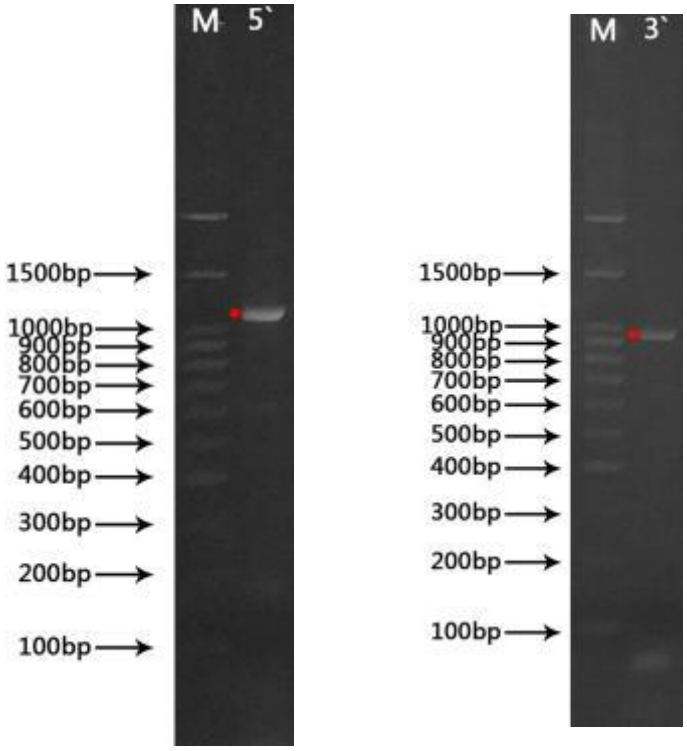
Rinse the cells with PBS without cations, digest cells with 0.25% (w/v) Trypsin-EDTA (0.53 mM) solution and split at 1:3 to 1:10 ratio.

Cryopreservation: Freeze slowly in complete growth medium supplemented with 5% (v/v) DMSO.

QC Data:

1. Cas9 gene integration at ROSA26 site in Neuro 2a/HF-Cas9 cell line by Junctional PCR from genomic DNA



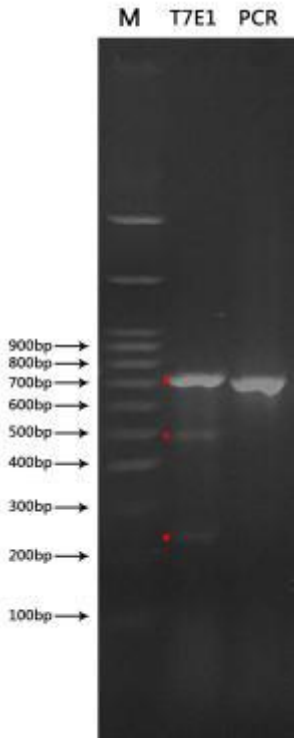


5'-Junctional PCR size was 1129 bp. One primer from chromosomal outside of the 5' homology arm region, the other primer from the Cas9-plasmid region.

3'-Junctional PCR size was 981 bp. One primer from chromosomal outside of the 3' homology arm region, the other primer from the Cas9-plasmid region.

2. Neuro 2a/Cas9 Activity by T7 Endonuclease I assay (T7E1)

sgRNA targeting to P2X2 gene were transduced into Neuro 2a/HF-CAS9 ROSA26 cell line by lenti-particals. P2X2 gene was cut by SpCas9-HF CAS9 expressed inside the cells and repaired through NHEJ with mutation. The mutations will be recognized and cut by T7 Endonuclease I.



For P2X2r sgRNA transduced cells, a 730 bp P2X2r gene fragment from PCR was tested by T7E1 Assay. The T7E1 cleavage will results in two additional bands: 494 bp and 236 bp.

Citation of product: If use of this item results in a publication, please use this information: CRISPR SpCas9-HF Cas9 stable Neuro 2a cell line (SL571; GeneCopoeia, Inc., Rockville, MD).

Limited Use License

A limited use license is granted to the Buyer of the Product. The Product shall be used by the Buyer for internal research purposes only and shall not be transferred (in any way) to any third party. The Product is expressly not designed, intended, or warranted for use in humans or for therapeutic or diagnostic use. The Product must not be resold, repackaged or modified for resale, or used to manufacture commercial products without prior written consent from GeneCopoeia. This Product should be used in accordance with NIH guidelines developed for recombinant DNA and genetic research. Use of any part of the Product constitutes acceptance of the above terms.

C9SL-DS-090518

GeneCopoeia, Inc.
9620 Medical Center Drive, #101
Rockville, MD 20850 USA
Tel: 301-762-0888; Fax: 301-762-3888
Email: support@genecopoeia.com
Web: www.genecopoeia.com