Datasheet for Neuro2a/LoxP-Cas9-hyg-Rosa26 Cell Line

Catalog number: SL559

Product: Neuro-2a cell line stably expressing CRISPR Cas9 nuclease from ROSA26 locus.

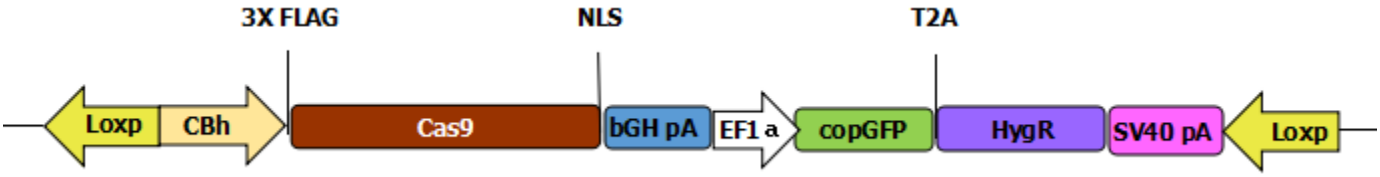
Description: This product is a cell line stably expressing the CRISPR Cas9 nuclease. Cas9 is integrated at the mouse Rosa26 Safe Harbor locus. This cell line also expresses copGFP and the hygromycin resistance gene. In combination with separately transfected or transduced single guide RNAs (sgRNAs), 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 knock-in, mutagenesis, transgene integration, or other genome editing-related applications. The Cas9 and selection cassettes are flanked by LoxP sites and thus can be removed using a transient expression of Cre recombinase or transfection of Cre protein. The TK gene is used in counterselection with ganciclovir to remove clones that have sustained random integration.

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:

Neuro2A
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, bacteria, 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 ℃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 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

Hygromycin to a final concentration of 700 µg/mL

Culture temperature:

37 ℃ with 5% CO_2

Subculture:

Replace culture medium with selection-free medium and incubate for up to 6 hours. 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.

Mycoplasma: Negative
(MycoAllert Mycoplasma Detection Kit from Lonza)

Product QC:

1. Junctional PCR (to confirm the Cas9 gene integration into ROSA26 site)

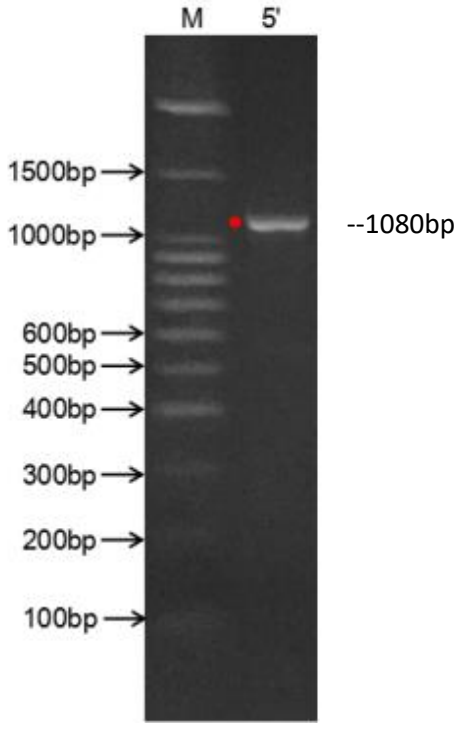
(1) 5' Junctional PCR

One primer recognizes the chromosoel outside of the 5' homology arm region. The other primer recognizes the Cas9-plasmid. This will confirm the ROSA26 site integration of the Cas9 expressing cassette.

Junction-PCR 5' F: GGCGGCCTTAATTAAGCGAATTC

Junction-PCR 5'R: GCGTACTTGGCATATGAT

Predicted product length : 1080 bp



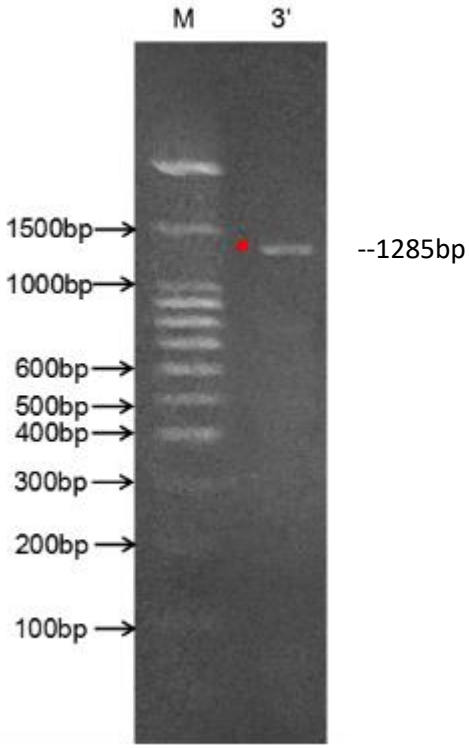
(2) 3' Junctional PCR to confirm 3' integration

one primer from chromosomal outside of the 3' homology arm region, the other primer from One primer recognizes the chromosoel outside of the 5' homology arm region. The other primer recognizes the Cas9-plasmid. This will confirm the ROSA26 site integration of the Cas9 expressing cassette.

Junction-PCR 3' F: GTTAACTTGTTTATTGCAGCTTATAATGG

Junction-PCR 3' R: GAATTGATTTAAATGCTGTCGAC

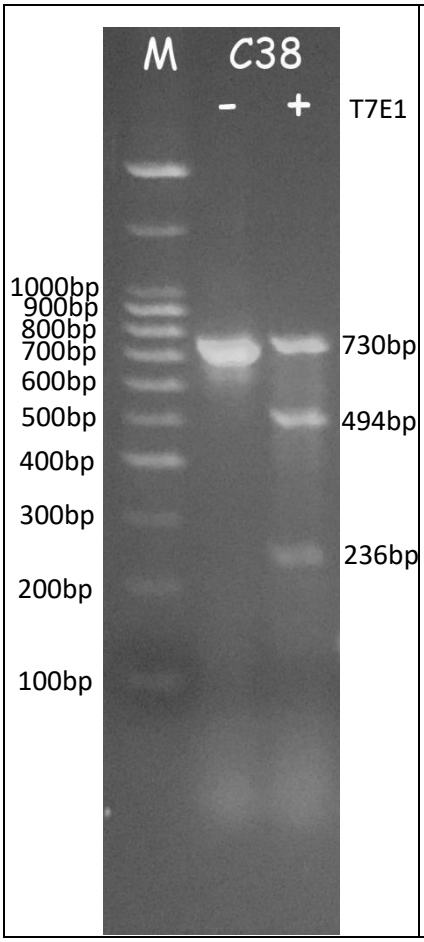
Predicted product length：1285 bp



2. T7 Endonuclease I (T7 E1) Assay

P2X2r Site T7 E1 Assay

One sgRNA targeted to the mouse P2rX2 gene was transfected into the Neuro2a/LoxP-Cas9-hyg-Rosa26 cell line by transient transfection. After transfection, P2rX2 gene was cut by Cas9 expressed inside the cells and repaired through NHEJ with mutation. A 730 bp P2rX2 gene fragment from PCR was then tested by T7 Endonuclease I (T7 E1) Assay. The T7 E1 cleavage will result in two additional bands: one ~494 bp and the other ~236 bp.



sgRNA targeted to the P2rX2 gene was transduced into the Neuro2a/LoxP-Cas9-hyg-Rosa26 cell line. The P2rX2 gene was cut by Cas9 expressed inside the cells and repaired through NHEJ with mutation. The mutations will be recognized and cut by T7 Endonuclease I.

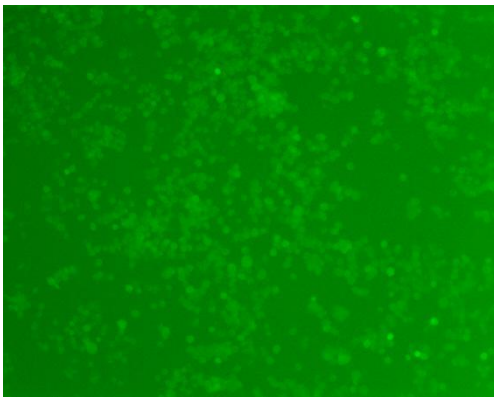
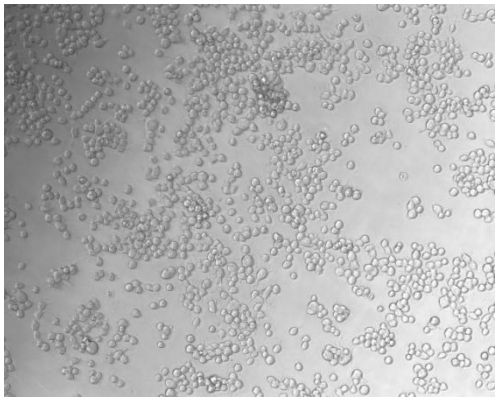
P2rX2 PCR sise 730
Predicted cut size by T7:
494bp and 236bp

3. Cre recombinase inducible conditional gene knockout

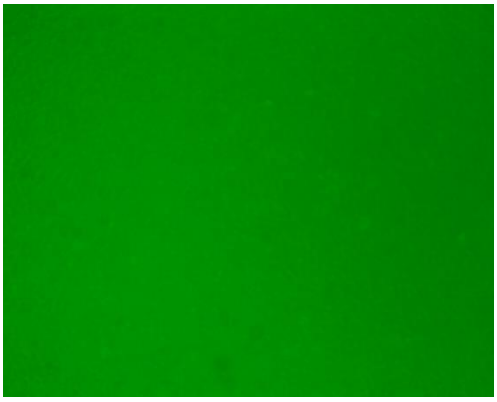
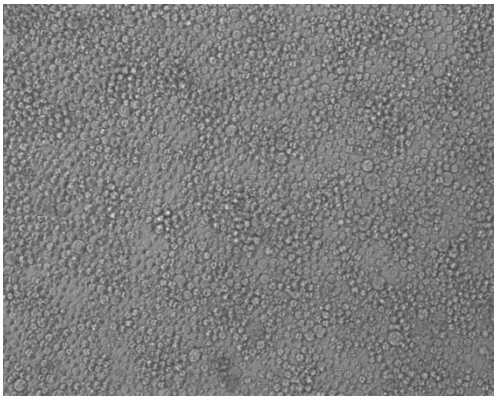
The Cas9 and selection cassettes are flanked by LoxP sites and thus can be removed using transient expression of Cre recombinase or transfection of Cre protein. After transfection, Cre recombinase will recognize the LoxP sites and delete the DNA between the two sites. Therefore, GFP fluorescence will decrease under fluorescence microscopy.

After transient transfection 72hrs:

Exposure time: 1s (fluorescence); 1ms (bright)

Single clone without Cre expression

Single clone transiently transfected with Cre

Citation of product: If use of this item results in a publication, please use this information: CRISPR Cas9 stable Neuro 2a/LoxP-Cas9-hyg-Rosa26 Cell Line (SL559, GeneCopoeia, Inc., Rockville, MD).

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C9SCL-DS-08111704

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