Datasheet for Mouse Neuro-2a/Inducible Cas9 Safe Harbor Cell Line

Catalog number: SL508

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

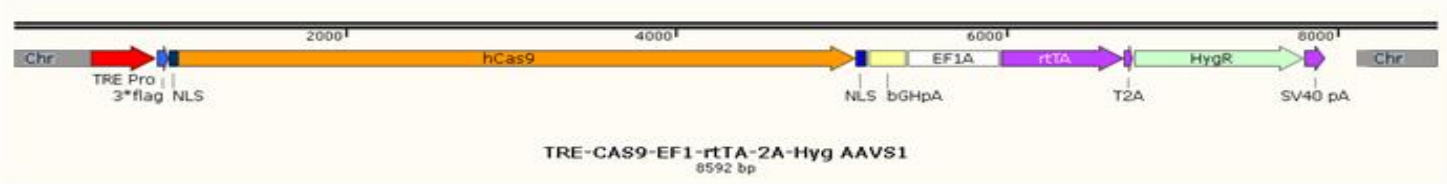
Description: This product is a cell line stably expressing the CRISPR Cas9 nuclease. Cas9 is integrated at the mouse Rosa26 Safe Harbor locus. This cell line also expresses rtTA regulating protein and the hygromycin resistance gene. In combination with separately transfected or transduced single guide RNAs (sgRNAs), this cell line will sustain double-strand DNA breaks (DSBs) at targeted genome sites after Dox induction. Though Tet3G inducible system has very tight control of the leaking expression before Dox induction, we found the basal Cas9 activity before Dox induction is similar to that from Dox induced Cas9 activity by T7 Endonuclease I assay when sgRNA expression at high levels using plasmid transfection. Even this, the controlled Cas9 expression level by Dox induction may reduce the off-target effects of the sgRNA, especially when the cells were transduced with low copies of sgRNA lentivirus. 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, 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 ^{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 DMEM. For optimal growth and maintenance of selection, add the following components to the base medium: dialyzed fetal bovine serum to a final concentration of
10%.

Selection

Hygromycin to a final concentration of 100 µg/mL

Culture temperature:

37 ^{0}C 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)

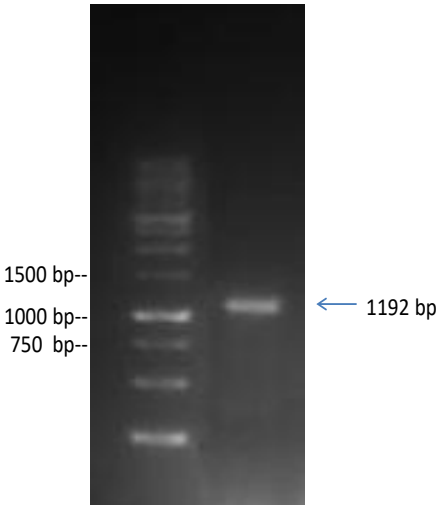
(1) 5' Junctional PCR

one primer from chromosomal outside of the 5' homology arm region, the other primer from the Cas9-plasmid region to confirm the ROSA26 site integration of Cas9 expressing cassette

Junction-PCR 5'F: CTCGTCGCTGATTGGCTTCT

Junction-PCR 5'R: AGGCGATCTGACGGTTCACT

Predict product length：1192 bp



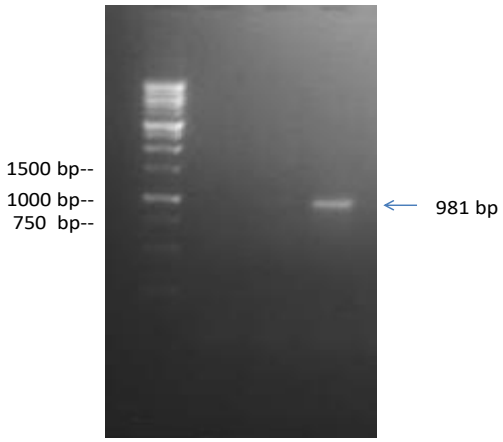
(2) 3' Junctional PCR to confirm 3' integration

one primer from chromosomal outside of the 3' homology arm region, the other primer from the Cas9-plasmid region to confirm the ROSA26 site integration of Cas9 expressing cassette

Junction-PCR 3'F: CTTGCTCTGGTCAACCAGGT

Junction-PCR 3'R: GGAGACATCCACCTGGAAACC

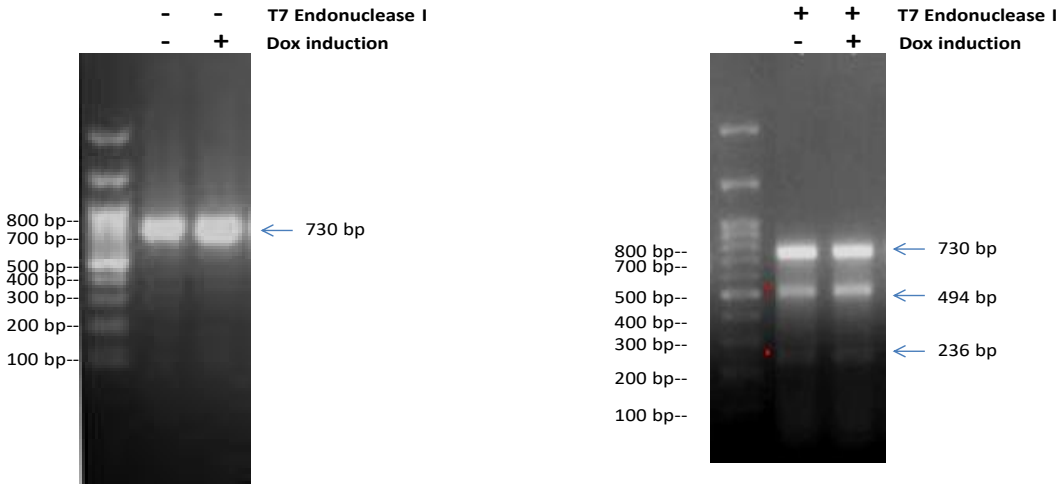
Predict product length：981 bp



2. T7 Endonuclease I (T7 E1) Assay

(1) P2X2r Site T7 E1 Assay

sgRNA targeting to mouse P2X2r gene was transduced into NEURO-2A/Inducible CAS9 ROSA26 cell line by transient transfection. After transfection, Cas9 protein was induced for 72 hours by 1 ug/ml Dox. P2X2r gene was cut by CAS9 expressed inside the cells and repaired through NHEJ with mutation. A 730 bp P2X2R 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.



Left: P2X2r gene PCR without T7 E1, Dox (1 ug/ml)uninduced or induced.

Right: P2X2r gene PCR with T7 E1, Dox (1 ug/ml) uninduced or induced. The uninduced cells still express enough Cas9 for P2X2r sgRNA plasmid to cut the P2X2r gene. It was reported that high copies of sgRNA molecules will only need minimal amount of Cas9 protein in CRISPR assay.

Citation of product: If use of this item results in a publication, please use this information: CRISPR Cas9 stable NEURO-2A/inducible Cas9 ROSA26 cell line (SL508, GeneCopoeia, Inc., Rockville, MD).

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