Datasheet for Neuro-2a/RFP-GFP Rosa26 Cell Line

Catalog number: SL506

Product: Neuro-2a cell line stably expressing RFP and GFP from ROSA26 Locus

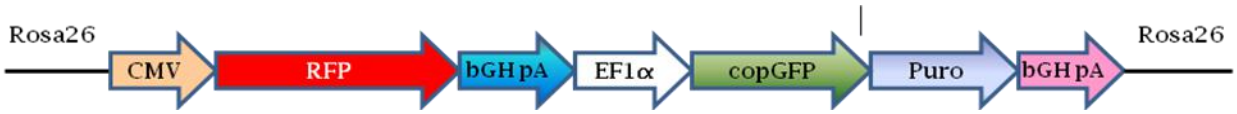
Description: This product is a cell line stably expressing RFP and GFP separately. DNA fragment CMV-RFP-bGH Poly-A-EF-1-GFP-T2A-Puro-SV40 Poly-A is integrated at the human ROSA26 Safe Harbor locus (also known as PPP1R2C) using CRISPR/Cas9 technology. This cell line can be used to replace the random integrated RFP-GFP labeled NEURO-2A cells in cell based assays, without the adverse effects resulted from the random insertion of RFP-GFP fragment into Neuro-2A genome.
This cell line could also be used as an indicator for genome editing experiments targeting to RFP or GFP gene.

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
Disease: Neuroblastoma

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

Puromycin to a final concentration of 1 µg/mL

Culture temperature:

37 ^{0}C with 5% CO_2

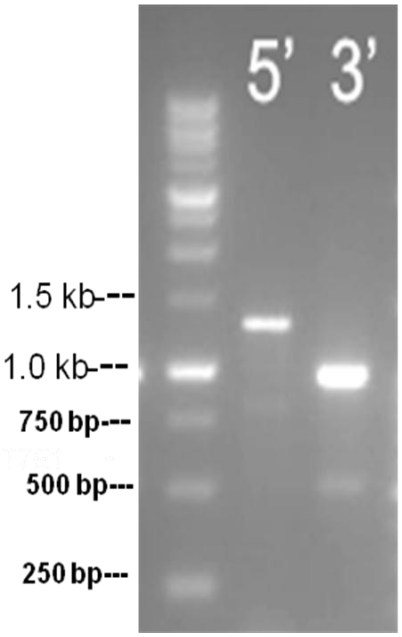
Subculture:

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.

QC Data:

Junctional PCR:



5'-Junctional PCR: 1192 bp:

Primers

Fowrard: 5'-ctcgtcgctgattggcttct-3'

Reverse: 5'-aggcgatctgacggttcact-3'

One primer from chromosomal 6 Rosa26 locus outside of the 5' homology arm region, the other primer from the RFP-GFP plasmid CMV promoter region

3'-Junctional PCR: 981 bp:

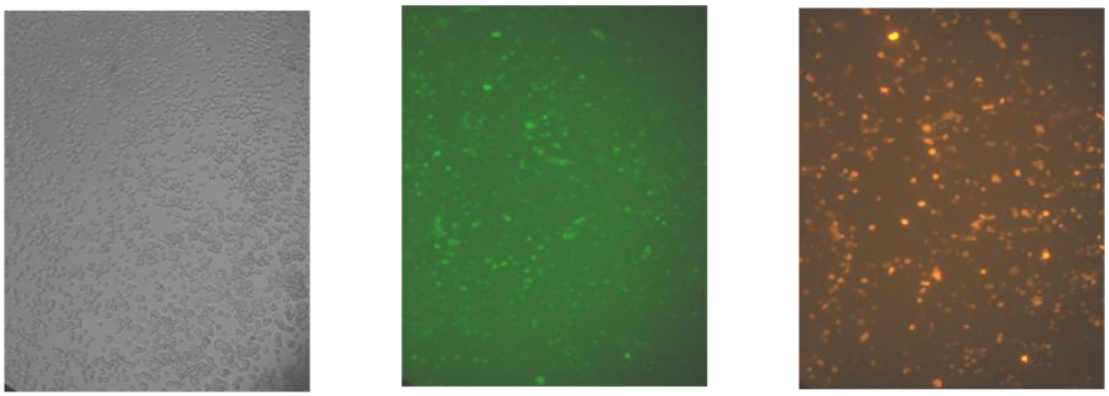
Primers:

Forward: 5'-cttgctctggtcaaccaggt-3'

Reverse: 5'-ggagacatccacctggaaacc-3'

One primer from chromosomal 6 Rosa 26 locus outside of the 3' homology arm region, the other primer from the RFP-GFP plasmid after bGH poly A and before homology arm region.

Fluorescent Image of SL506 Cells:



Light GFP RFP

Citation of product: If use of this item results in a publication, please use this information: CRISPR Cas9 stable Neuro-2a cell line (SL506; GeneCopoeia, Inc., Rockville, MD).

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