Datasheet for HEK293/EGFP-AAVS1-Puro Stable Cell Line

Catalog number: SL573

Product: HEK293 cell line stably expressing EGFP from AAVS1 locus.

Description: This product is a cell line stably expressing the EGFP protein. EGFP gene is integrated at the human AAVS1 Safe Harbor locus without the possibility of disrupting any endogenous gene in HEK293 cells.

This product could be used as an EGFP labeled HEK293 cell line for many applications. One of the applications is as the Genome Editing Learning Tool. When an sgRNA lentivirus targeting to the EGFP was transduced into this cells, the majority of eGFP fluorescence will disappear if the EGFP gene knockout is successful. A following T7 Endonuclease 1 assay should also show positive result using the eGFP knockout cells.

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 ºC, preferably into the liquid nitrogen vapor phase, until use.

Transgene integration:

Source of parental line:

HEK293
Organism: Homo sapiens, human
Tissue: embryonic kidney
Cell type: epithelial

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 ºC 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
Puromycin to a final concentration of 0.5 µg/mL

Culture temperature
37 ºC with 5% CO_2

Subculture
Replace culture medium with selection-free medium and incubate for up to 6 hours. Rinse the cells with PBS, digest cells with 0.25% (w/v) Trypsin-EDTA (0.53 mM) solution and split at 1:6 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)

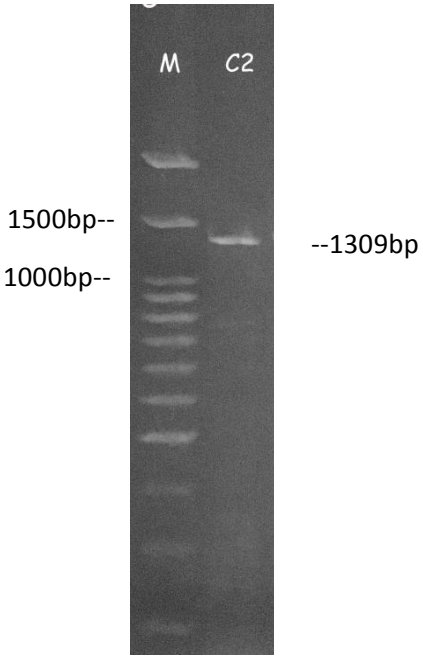
QC Data:

1. **Junctional PCR**
   1) **5' Junctional PCR**
   one primer from chromosomal outside of the 5' homology arm region, the other primer from the Ex-NEG-Lv201 plasmid region to confirm the AAVS1 site integration of EGFP expressing cassette

   `Junction-PCR 5'F: TGCTTTCTCTGACCAGCATTC`

   `Junction-PCR 5'R: CCACAACTAGAATGCAGTG`

   Predict product length : 1309 bp

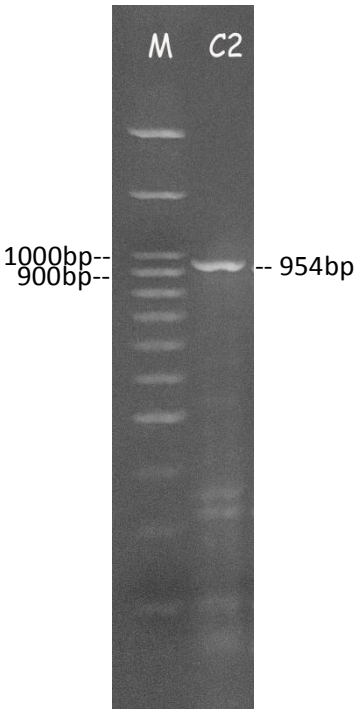


   2) **3' Junctional PCR to confirm 3' integration**
   one primer from chromosomal outside of the 3' homology arm region, the other primer from the Ex-NEG-Lv201 plasmid region to confirm the AAVS1 site integration of EGFP expressing cassette

   `Junction-PCR 3'F: CCAATGCTTAATCAGTGAGGC`

   `Junction-PCR 3'R: GGAAGTGTAAGGAAGCTGCA`

Predict product length : 954 bp

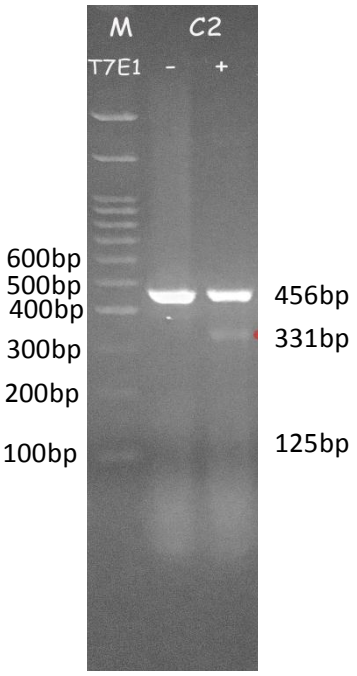


2. EGFP gene knockdown and T7 Endonuclease I (T7 E1) Assay

EGFP Site T7 E1 Assay

Primer	eGFP-F	eGFP-R	PCR length	Tm
eGFP	CCGGGAGCTTGTATATCCAT	CTCCTTGAAGTCGATGCCCT	456	55

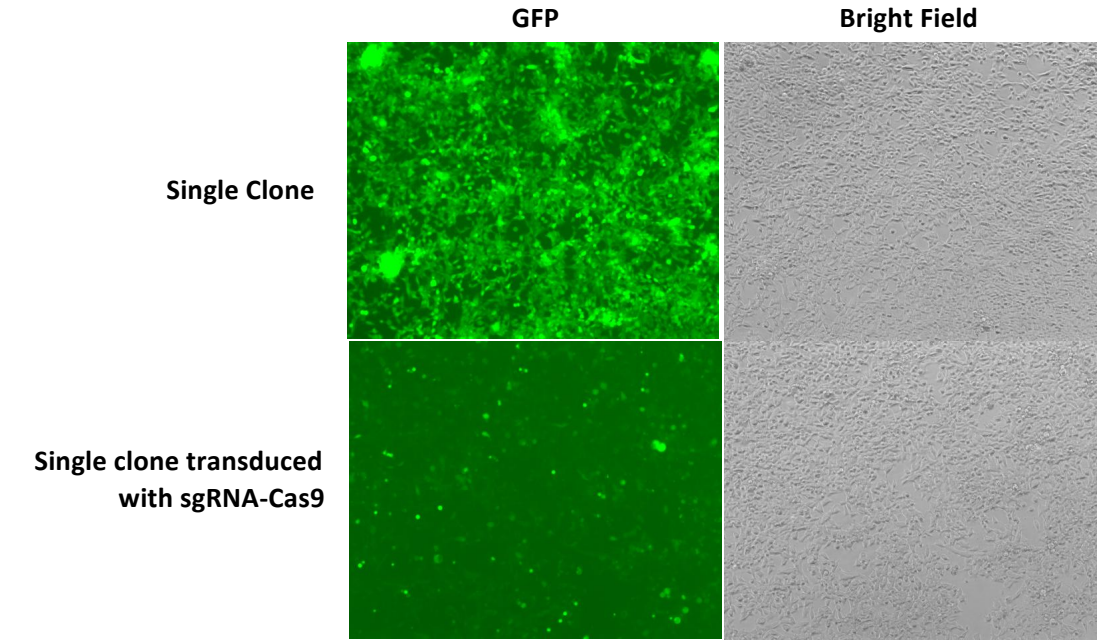
Lentivirus sgRNA-Cas9 targeting to EGFP gene (LPP-CS-OCP001145-LvSG06-3-10-a-050) was transduced into HEK293/EGFP-AAVS1 Stable cell line. After transduction, EGFP gene was cut by Cas9 and repaired through NHEJ with mutations. A 456bp EGFP gene fragment from PCR was then tested by T7 Endonuclease I (T7 E1) Assay. The T7 E1 cleavage will result in two additional bands: one ~125bp and the other ~331bp.



sgRNA-Cas9 targeting to EGFP gene was transduced into HEK293/EGFP-AAVS1 Stable cell line. EGFP gene was cut by Cas9 and repaired through NHEJ with mutation. The mutations will be recognized and cut by T7 Endonuclease I.

EGFP PCR size: 456bp
Predicted cut size by T7: 331bp and 125bp

Drug selection 48hrs after transduction:



Citation of product: If use of this item results in a publication, please use this information: HEK293/EGFP-AAVS1 Stable cell line (SL573, GeneCopoeia, Inc., Rockville, MD)

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