

Datasheet for A549/Cas9 AAVS1 Cell Line

Catalog number: SL504 (formerly SCL-76321-G2)

Product: A549 cell line stably expressing CRISPR Cas9 nuclease

Description: This product is a cell line stably expressing the CRISPR Cas9 nuclease. Cas9 is integrated at the human AAVS1 Safe Harbor locus (also known as PPP1R2C). 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

Quantity: 1 vial of 2×10^6 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°C , preferably into the liquid nitrogen vapor phase, until use.

Transgene integration:



Source of parental line:

A549
Organism: *Homo sapiens*, human
Tissue: Lung carcinoma
Cell type: Epithelial

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 °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 F12 Medium. 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 800 µg/mL

Culture temperature:

37 °C with 5% CO₂

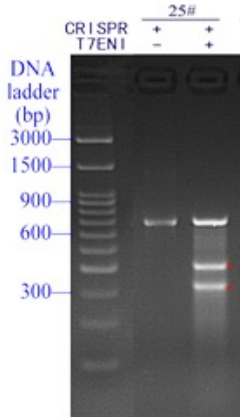
Subculture:

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.

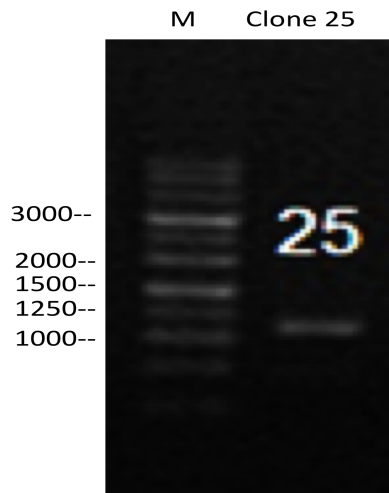
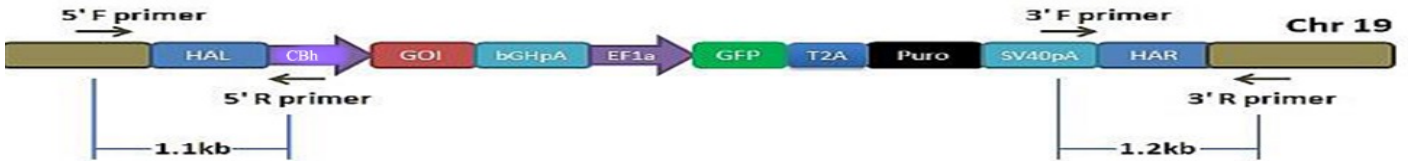
Quality control: >95% viability before freezing. All cells were tested and found to be free of mycoplasma, bacteria, viruses, and other toxins.

1. T7 Endonuclease I (T7E1) Assay



sgRNA targeting to the B2M gene was transduced into A549/CAS9 AAVS1 cell line by lenti-particles LPP-HCP000395-b-LvSG02. B2M was cut by CAS9 expressed within the cells and repaired via NHEJ with a mutation. A 669 bp B2M gene fragment from PCR was then tested by T7E1 Assay. The T7E1 cleavage will results in two additional bands: one ~291bp and the other ~378 bp.

2. Junction PCR (to confirm the Cas9 gene integration into AAVS1 site)

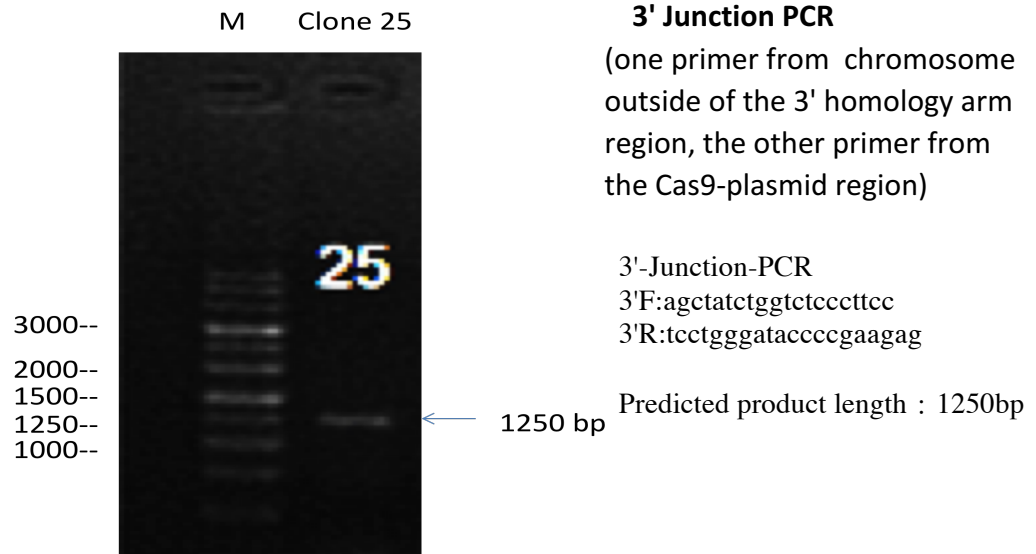


5' Junction PCR

(one primer from chromosome outside of the 5' homology arm region, the other primer from the Cas9-plasmid region)

5' -Junction-PCR
5'F:ccggaactctgccctctaac
5'R:gcgtacttggcatatgat

Predicted product length : 1100bp



Citation of product: If use of this item results in a publication, please use this information:
CRISPR Cas9 stable A549 cell line (SL-504; GeneCopoeia, Inc., Rockville, MD).

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