

Datasheet for HCT116/RFP-GFP AAVS1 Cell Line

Catalog number: SL568

Product: HCT116 cell line stably expressing RFP and GFP from AAVS1 Locus

Description: This product is a cell line stably expressing RFP and GFP separately. DNA fragment CMV-RFP-bGH Poly-A-EF1-GFP-T2A-Puro-SV40 Poly-A is integrated at the human AAVS1 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 HCT116 cells in cell based assays, without the adverse effects resulted from the random insertion of RFP-GFP fragment into HCT116 genome.

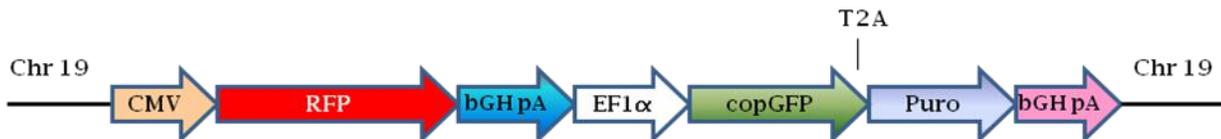
This cell line could also be used as an indicator for genome editing experiments targeting to RFP or GFP gene.

Quantity: 1 vial of 2×10^6 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:

HCT116
Organism: Homo sapiens, human
Tissue: Colon
Cell Type: Epithelial
Disease: Colorectal Carcinoma

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 °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 RPMI1640. 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 °C with 5% CO₂

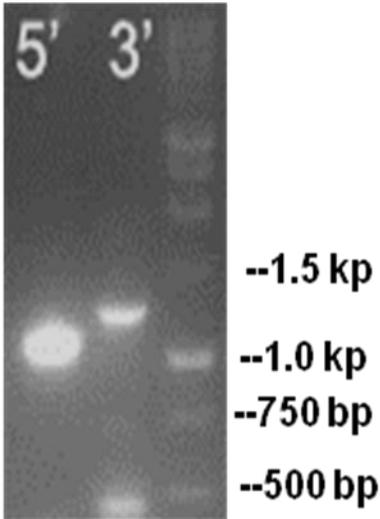
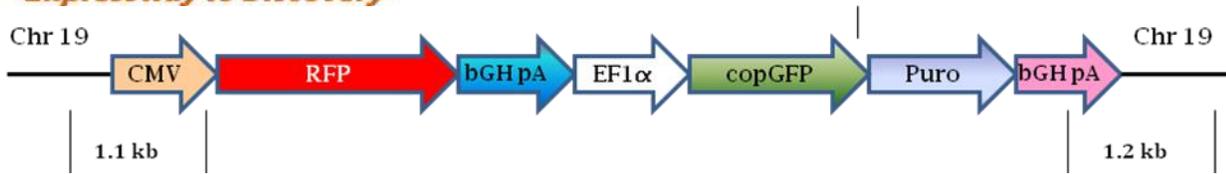
Subculture:

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.

QC Data:

Junctional PCR:



5'-Junctional PCR:

Primers: HQPAVSHR-5' Primer pair-- predicted size 1.1 kb

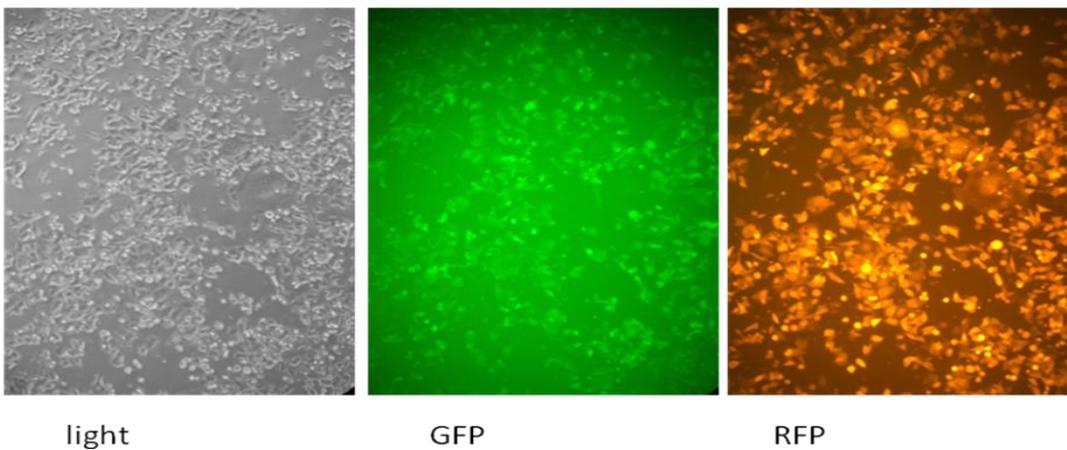
One primer from chromosomal 19 outside of the 5' homology arm region, the other primer from the RFP-GFP plasmid CMV promoter region

3'-Junctional PCR:

Primers: HQPAVSHR-3' Primer pair-- predicted size 1.2 kb

One primer from chromosomal 19 outside of the 3' homology arm region, the other primer from the RFP-GFP plasmid bGH poly A region.

Fluorescent Image of SL568 Cells:





Citation of product: If use of this item results in a publication, please use this information: CRISPR Cas9 stable HCT116 cell line (SL568; GeneCopoeia, Inc., Rockville, MD).

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