Description

Recombinant stable HEK293 cell line constitutively expressing RFP (Red Fluorescent Protein) and GFP (Green Fluorescent Protein), which have been stably integrated into the AAVS1 safe harbor locus on chromosome 19.

Expression of RFP is driven by a CMV promoter, whereas GFP is driven by an EF1A promoter. The combined DNA fragment (CMV-RFP-bGH Poly A-EF1A-GFP-T2A-Puro-SV40 Poly A) (Figure 1) was integrated at the AAVS1 safe harbor locus using CRISPR/Cas9 technology. Cells were cloned by limiting dilution to obtain a monoclonal population. Without the adverse effects resulting from random integrations of RFP-GFP into the HEK293 genome, this cell line behaves like parental HEK293 cells.

Background

AAVS1 (also known as the PPP1R12C locus) on human chromosome 19 is a well-validated "safe harbor" site for hosting DNA transgenes. AAVS1 has an open chromatin structure and is transcription competent. Most importantly, disrupting the AAVS1 locus by inserting DNA transgenes has no known adverse effects on the cells. Specifically targeting the AAVS1 locus is a major advantage compared to the random integration obtained using other approaches such as lentivirus infection or cell transfection, which may cause insertional mutagenesis or disrupt important genes or cellular processes.

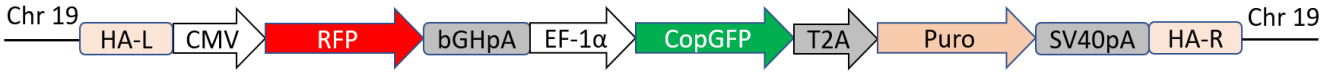


Figure 1: Transgene integration at the AAVS1 locus.

Application

Use as a control in cell-based assays.

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains 2 x 10^6 cells in 1 ml of cell freezing medium (BPS Bioscience #79796)

Parental Cell Line

HEK293, Human Embryonic Kidney, epithelial-like cells, adherent

Mycoplasma Testing

The cell line has been screened to confirm the absence of Mycoplasma species.

Materials Required but Not Supplied

These materials are not supplied with the cell line but are necessary for cell culture and cellular assays. BPS Bioscience's reagents are validated and optimized for use with this cell line and are highly recommended for best results. Media components are provided in the Media Formulations section below.

Media Required for Cell Culture

Name	Ordering Information
Thaw Medium 6	BPS Bioscience #60183

Storage Conditions

Cells are shipped in dry ice and should immediately be thawed or stored in liquid nitrogen upon receipt. Do not use a -80°C freezer for long term storage. Contact technical support at support@bpsbioscience.com if the cells are not frozen in dry ice upon arrival.

Media Formulations

For best results, it is *highly recommended* to use these validated and optimized media from BPS Bioscience. Other preparations or formulations of media may result in suboptimal performance.

Note: Thaw Media do *not* contain selective antibiotics. However, Growth Media *do* contain selective antibiotics, which are used for maintaining the presence of the transfected gene(s) over passages. Cells should be grown at 37°C with 5% CO_2. BPS Bioscience's cell lines are stable for at least 15 passages when grown under proper conditions.

Media Required for Cell Culture

Thaw Medium 6 (BPS Bioscience #60183):
DMEM medium supplemented with 10% FBS and 1% Penicillin/Streptomycin.

Cell Culture Protocol

Cell Thawing

1. Swirl the vial of frozen cells for approximately 60 seconds in a 37°C water bath. As soon as the cells are thawed (it may be slightly faster or slower than 60 seconds), quickly transfer the entire contents of the vial to a tube containing 10 ml of pre-warmed Thaw Medium 6.
   Leaving the cells in the water bath at 37°C for too long will result in rapid loss of viability.

2. Immediately spin down the cells at 300 x g for 5 minutes, remove the medium and resuspend the cells in 5 ml of pre-warmed Thaw Medium 6.

3. Transfer the resuspended cells to a T25 flask or T75 flask and incubate at 37°C in a 5% CO_2 incubator.

4. After 24 hours of culture, check for cell attachment and viability. Change medium to fresh Thaw Medium 6, and continue growing in a 5% CO_2 incubator at 37°C until the cells are ready to passage.

5. Cells should be passaged before they are fully confluent.

Cell Passage

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS), and detach the cells from the culture vessel with 0.05% Trypsin/EDTA.

2. Once the cells have detached, add Thaw Medium 6 and transfer to a tube. Spin down cells at 300 x g for 5 minutes, remove the medium and resuspend the cells in Thaw Medium 6. Seed into new culture vessels at the desired sub-cultivation ratio of [1:6 to 1:8 weekly or twice per week].

Cell Freezing

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS), and detach the cells from the culture vessel with 0.05% Trypsin/EDTA.

2. Once the cells have detached, add Thaw Medium 6 and count the cells.

3. Spin down the cells at 300 x g for 5 minutes, remove the medium and resuspend the cells in 4°C Freezing Medium (BPS Bioscience #79796, or 10% DMSO + 90% FBS) at ~2 x 10^6 cells/ml.

4. Dispense 1 ml of cell aliquots into cryogenic vials. Place the vials in an insulated container for slow cooling and store at -80°C overnight.

5. Transfer the vials to liquid nitrogen the next day for storage.

Note: It is recommended to expand the cells and freeze at least 10 vials at an early passage for future use.

Validation Data

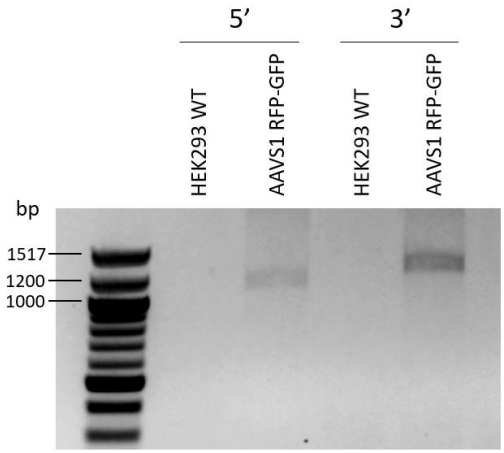
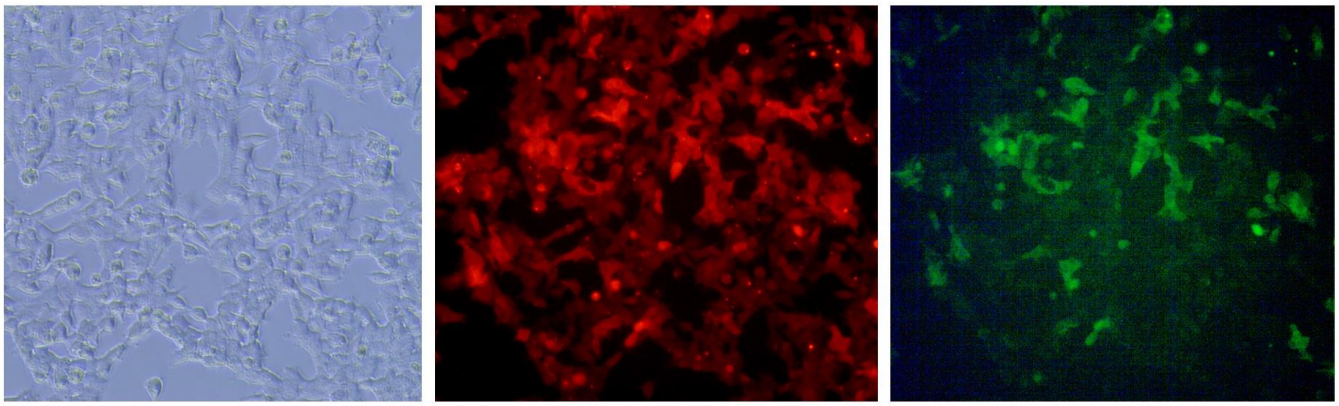


Figure 2: PCR confirmation of stable integration into the AAVS1 safe harbor locus.
On the 5' end of the integration, the region spanning the chromosome 19 AAVS1 locus and the beginning of the CMV RFP integration was amplified by PCR, with a predicted size of 1.1 kb. On the 3' end of the integration, the region spanning the EF1α GFP-Puro integration and the chromosome 19 AAVS1 locus was amplified by PCR, with a predicted size of 1.2 kb.



White light RFP GFP

Figure 3: Expression of RFP and GFP in the RFP/GFP AAVS1 Safe Harbor HEK293 Cell Line.
The RFP/GFP AAVS1 Safe Harbor HEK293 cells were observed under a 10x fluorescence microscope.

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Troubleshooting Guide

Visit bpsbioscience.com/cell-line-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com.

Related Products

Products	*Catalog #*	*Size*
eGFP NK-92 Cell Line	78399	2 vials
AP-1 Reporter – HEK293 Recombinant Cell Line (JNK signaling pathway)	60405	2 vials
PD-1 / NFAT Reporter - Jurkat Recombinant Cell Line	60535	2 vials
RFP Lentivirus	78347	500 µl x 2
Enhanced GFP Lentivirus (G418, Hygromycin and Puromycin)	78639	500 µl x 2