Description

Recombinant stable HEK293 cell line constitutively expressing a FLAG-tagged Cas9 nuclease and CopGFP (*Pontellina plumata* green fluorescent protein), which have been stably integrated into the AAVS1 safe harbor locus on chromosome 19. When transfected or transduced with single guide RNAs (sgRNAs), this cell line will sustain double-strand DNA breaks (DSBs) at targeted genome sites.

Expression of Cas9 is driven by a CBh promoter, whereas CopGFP is driven by an EF1A promoter. The combined DNA fragment (CBh-Cas9-bGH Poly A-EF1A-CopGFP-T2A-Puro-SV40 Poly A), shown in Figure 1, was integrated at the AAVS1 safe harbor locus using CRISPR/Cas9 technology. The construct also contains a puromycin resistance gene.

Cells were cloned by limiting dilution to obtain a monoclonal population. Without the adverse effects resulting from random integrations of Cas9/GFP into the HEK293 genome, this cell line behaves like parental HEK293 cells.

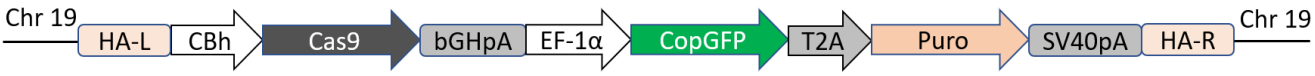

Figure 1: Transgene integration at the AAVS1 locus.

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. GFP (green fluorescent protein) presents green fluorescence, and it was first identified in *Aequorea Victoria*. *Pontellina plumata* GFP (CopGFP) was later identified and is a superbright and fast maturation rate. The presence of fluorescent proteins allows for cell identification and quantification by flow cytometry or fluorescence microscopy, and localization studies *in vivo*, providing easy assay readouts.

Application

Use for gene knockout, transgene knock-in, mutagenesis, transgene integration, or other genome editing-related applications.

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains >1 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, the use of validated and optimized media from BPS Bioscience is *highly recommended*. Other preparations or formulations of media may result in suboptimal performance.

Note: Cells should be grown at 37°C with 5% CO_2. BPS Bioscience's cell lines are stable for at least 10 passages when grown under proper conditions.

Media Required for Cell Culture

Thaw Medium 6 (BPS Bioscience #60183)
DMEM medium supplemented with 10% Fetal Bovine Serum (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) without Ca^{2+}/Mg^{2+}, 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.

3. Spin down cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in Thaw Medium 6.

4. Seed into new culture vessels at the recommended sub-cultivation ratio of 1:6 to 1:8 once or twice per week.

Cell Freezing

1. Aspirate the medium, wash the cells with PBS without Ca^{2+}/Mg^{2+},, 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 Cell Freezing Medium (BPS Bioscience #79796) 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.

B. Validation Data

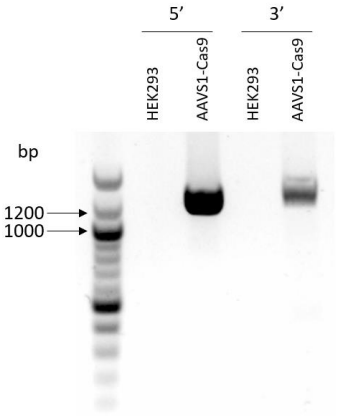

Figure 2: Stable integration into the AAVS1 safe harbor locus by PCR (polymerase chain reaction). On the 5' end of the integration, the region spanning the AAVS1 locus in chromosome 19 and the beginning of the CBh Cas9 integration was amplified by PCR, with a predicted size of 1.1 kb. On the 3' end of the integration, the region spanning the EF1α GFP-Puro integration and the AAVS1 locus in chromosome 19 was amplified by PCR, with a predicted size of 1.2 kb.

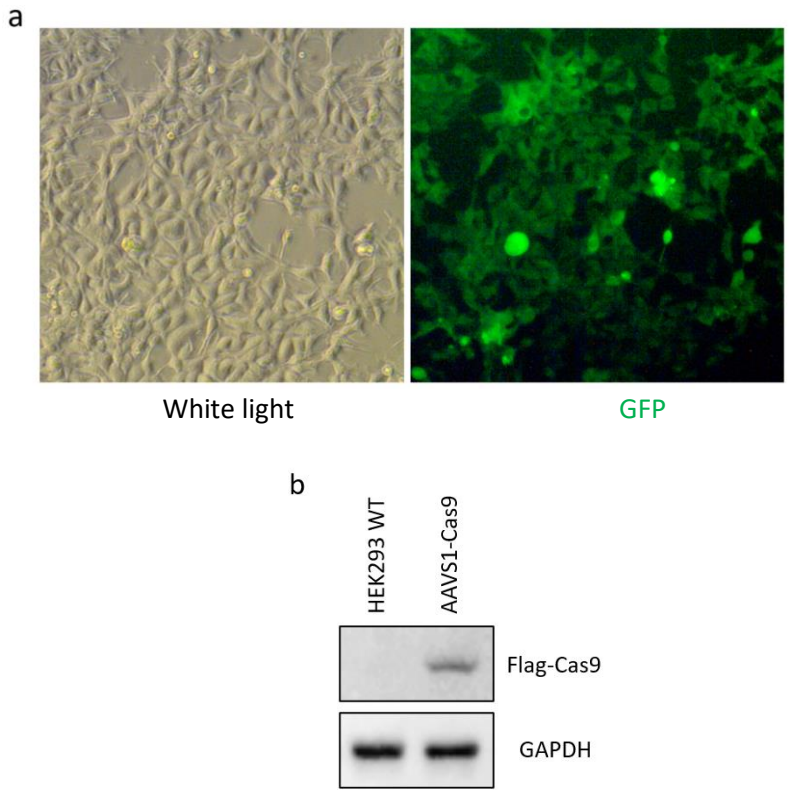

Figure 3: Expression of Cas9/GFP in the Safe Harbor HEK293 Cell Line.
a. The Cas9/GFP Safe Harbor HEK293 cells were observed under a fluorescence microscope using a 10x objective. b. Expression of Cas9 in control HEK293 cells and Cas9/GFP Safe Harbor HEK293 cells was analyzed by Western Blot using an ANTI-FLAG® M2 antibody, Mouse monoclonal (Sigma Aldrich #F1804). GAPDH was used as a protein loading control.

Data are representative. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com.

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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*
RFP/GFP Safe-Harbor HEK293 Cell Line	78581	2 vials
ZsGreen/Luciferase Safe-Harbor Hek293 Cell Line	82091	2 vials
Cas9 Lentivirus (Puromycin Selection)	78066	500 µl x 2

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