Applied StemCell, Inc.
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Datasheet

HEK293-LC9 (Cas9 Expressing HEK293 Cells)

Order information

Catalog Number	Quantity
ASE-9313	5.0 x 10^5 cells/ vial

Product Information

Description

This HEK293-derived cell line was engineered to stably express the functional *S. pyogenes* Cas9 (spCas9) nuclease which is driven by CBh promoter and targeted into the ROSA 26 locus. This cell line also contains a puromycin resistant gene.

This Cas9 expressing cell line can be used for gRNA validation and for generating genetically modified isogenic HEK293 cells by transfecting the gRNA into the cell line followed by Surveyor or T7 endonuclease assay.

Shipping

Dry ice

Storage and Stability

Store in liquid nitrogen freezer immediately upon receipt. This product is stable for at least 6 months from the date of receiving when stored as directed.

Quality Control

To ensure the expression of Cas9, the cell line should be periodically cultured in puromycin 2 µg/ml.

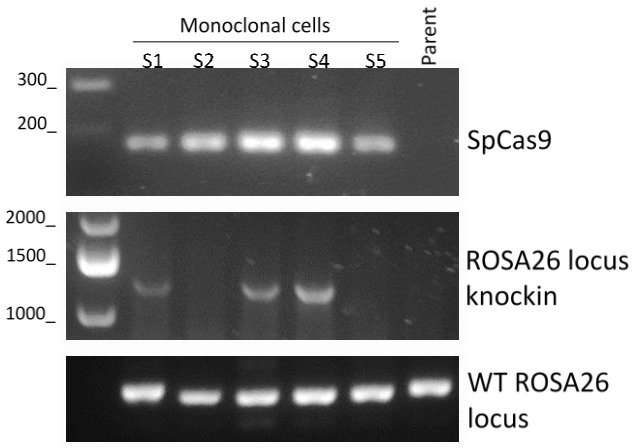

Figure 1. PCR analyses of SpCas9 expressing HEK293 cell lines. Five monoclonal cell lines, designated as S1-S5, were developed from parent HEK293 cells with SpCas9 expression (upper panel). Junction PCR products amplified across both ROSA26 locus and SpCas9 sequence indicated that the cell line S1, S3 and S4 have correct SpCas9 targeting into the ROSA26 locus (middle panel). ASE-9313 was derived from monoclonal cell line S4, which is a heterozygous cell line containing one wildtype allele of ROSA26 locus (bottom panel).

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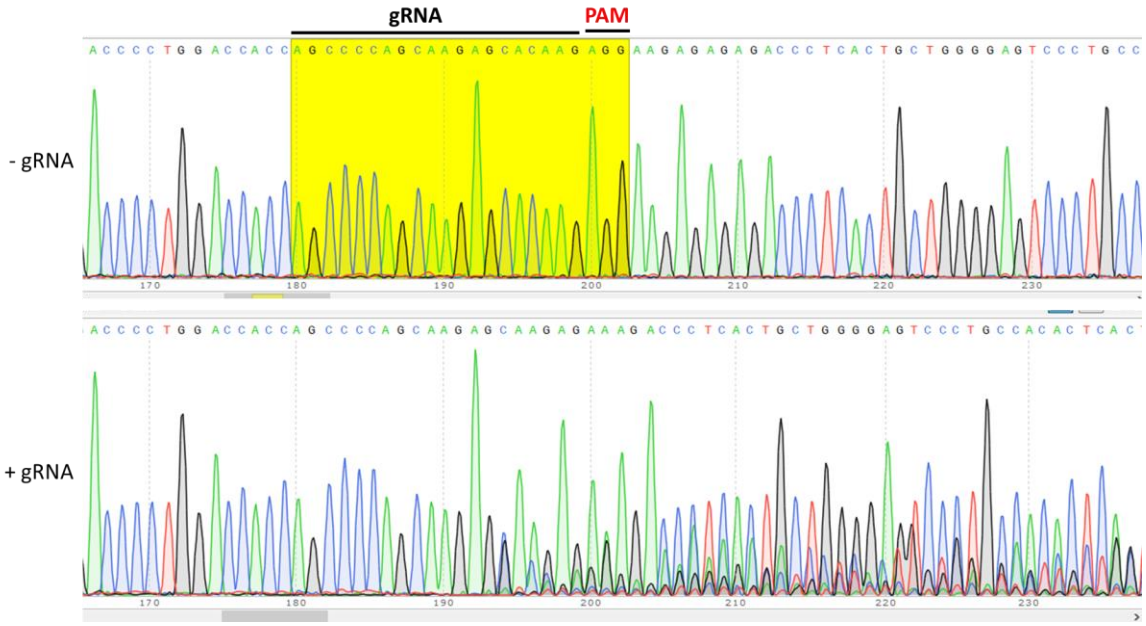

Figure 2. Genome sequence analyses of *GAPDH* locus in ASE-9313 cells without or with transduction of gRNA/AAV virus which targets *GAPDH* gene. Indel was seen in the cells only after gRNA treatment.

Safety Precaution

PLEASE READ BEFORE HANDLING ANY FROZEN VIALS. Please wear the appropriate Personal Protection Equipment (lab coat, thermal gloves, safety goggles and a face shield) when handling the cells. Handle the frozen vials with due caution. Please be aware that the following scenario can occur: Liquid nitrogen can leak into the vials when the vials are submerged in liquid nitrogen. Upon thawing, the liquid nitrogen returns to the gas phase, resulting in a dangerous build-up of pressure within the vial. This can result in the vial exploding and expelling not only the vial contents but also the vial cap and plastic fragments of the vial.

Restricted Use

This product is for research use only and not intended for human or animal diagnostic or therapeutic uses.

Protocol

1. Cell culture protocol

Media Components	Final Conc.
Eagle's Minimum Essential Medium	
Fetal bovine serum (FBS)	10%
Streptomycin	100 µg/mL
Penicillin	100 U/mL

1.1 Seeding density: 1-3 x 10^5 cells/ mL

1.2 Passage interval: Cells should be passaged before reaching a density of 3 x 10^6 cells/mL

2. Guide RNA preparation

Important note: Please handle all reagents with care in an RNase-free environment

For gRNA preparation, choose either method 2.1 or 2.2 described below:

2.1 Oligo-synthesis: Design and order synthetic guide RNA(s) based on genomic sequence to be targeted and the predicted "on-target" and/or "off-target" activity.

2.2 *In vitro* transcription (IVT): Use HiScribe T7 High Yield RNA Synthesis Kit (NEB, Cat. #E2040S) to transcribe the gRNA(s).

2.2.1 Clean the lab bench and pipettors by wiping with RNase*Zap*® RNase Decontamination Solution (ThermoFisher, Cat. #AM9780).

2.2.2 Keep the T7 RNA Polymerase Mix on ice.

2.2.3 Thaw the T7 10X Reaction Buffer, four ribonucleotide solutions, and ddH_2O at room temperature.

- Briefly vortex the T7 10X Reaction Buffer and ribonucleotide solutions and centrifuge before opening the tubes.

2.2.4 Prepare the template:

- HiScribe T7 High Yield RNA Synthesis Kit suggests using 1 µg template DNA for short RNA transcription (0.3 kb).
- Ambion MEGAshortscript Kit (ThermoFisher, Cat. #AM1354) suggests using 260 ng DNA template in 20 µL reaction to yield best amount of RNA (~90 µg) from 100 bp-length DNA.

2.2.5 Assemble the reaction mix in an RNase-free microfuge tube at room temperature, as shown below.

2.2.6 The dNTPs, 10X Buffer and Enzyme Mix can be premixed and added to the template DNA.

- For short RNA transcripts (0.3 kb)

Reaction mixture	Volume (µL)
T7 10X Reaction Buffer	1.5
dNTPs mix (75 mM)	1.5 X 4
T7 Enzyme Mix	1.5
Template DNAs (100 ng - 1 µg)	11

2.2.7 Mix the contents thoroughly by gently flicking the tube.

2.2.8 Incubate the reaction at 37°C for at least 4 hours.

3. Transfection:

Transfection using Lipofectamine®

3.1 Plate ASE-9313 cells at a density of 2-3 x 10^5 cells/mL with fresh medium in one well of a 12-well plate, the day before transfection.

3.2 gRNAs can be purchased directly or prepared by IVT: choose appropriate method for transfection.

3.2.1 Add 2.5 µL of crRNA (20 µM) and 2.5 µL of trRNA (20 µM) to 50 µL OPTI-MEM; add 3 µL Lipofectamin 3000 and mix together for 5 minutes; or

3.2.2 Add 2 µg IVT gRNA to 50 µL OPTI-MEM and add 3 µL Lipofectamin 3000 to 50 µL OPTI-MEM. Mix together for 5 min.

3.3 Add 100 µL of the transfection solution to cells.

3.4 Collect a portion of the transfected cells 72 hours after transfection for a T7 endonuclease assay (mismatch assay). Refresh the medium if necessary during culturing.

Electroporation using Neon® Transfection System

3.1 In one well of a 6-well plate, seed 5 X 10^5 ASE-9313 cells in 2 mL of EMEM growth medium (containing 10% FBS).

3.2 The next day, count the cells and harvest 1 x 10^6 cells and spin down at 300g for 3 minutes.

3.3 Wash the cells with 4 mL PBS.

3.4 Re-suspend the cells with 200 µL buffer R (provided in Neon® transfection kit).

3.5 gRNAs can be purchased directly or prepared by IVT: choose appropriate method for transfection.

3.5.1 Mix 2.5 µL of crRNA (20 µM) and 2.5 µL of trRNA (20 µM); OR

3.5.2 Prepare 5 µg of IVT gRNA.

3.6 Add 110 µL of cells (in buffer R) to the gRNA solution.

3.7 Perform the electroporation using a 100 µL tip (provided in Neon® transfection kit) with the following parameters:

1350 v (pulse voltage) / 20 ms (pulse width) / 1 (pulse number)

3.8 Transfer the electroporated cells into one well of 6-wells plate with 2.5 mL fresh medium.

3.9 Collect a portion of the transfected cells 72 hours after transfection for a T7 endonuclease assay (mismatch assay) or PCR/genome sequencing analyses. Refresh the medium if necessary during culturing.

4. T7 Endonuclease Assay

4.1 Harvest 10-20% of total cells in a culture well and extract genomic DNA.

4.2 Perform PCR with 100 ng of genomic DNA using 2x Phusion PCR Master Mix (ThermoFisher, Cat. #F548L).

4.3 Run 2 µL of PCR sample on a 2% agarose gel with Low DNA Mass ladder (Invitrogen, Cat. #10068-013) to estimate DNA concentration.

4.4 Adjust concentration of genomic DNA to 400 ng in 17 µL volume using diH2O.

4.5 Hybridize PCR sample to form heteroduplexes in a thermocycler using the program given below:

Temperature	Time	Temperature ramp
95°C	10 min	
95°C to 85°C		(-2.0°C/s)
85°C	1 min	
85°C to 75°C		(-0.3°C/s)
75°C	1 min	
75°C to 65°C		(-0.3°C/s)
65°C	1 min	
65°C to 55°C		(-0.3°C/s)
55°C	1 min	
55°C to 45°C		(-0.3°C/s)
45°C	1 min	
45°C to 35°C		(-0.3°C/s)
35°C	1 min	
35°C to 25°C		(-0.3°C/s)
25°C	1 min	
4°C	Hold ∞	

4.6 Add 2 µL of 10X NEBuffer 2 and 1 µL of T7 Endonuclease I (NEB, cat. #M0302S).

4.7 Incubate at 37°C for 15 minutes. Add 2 µL of 0.5M EDTA to stop the reaction.

4.8 Run 16 µL of sample on an acrylamide gel.

5. Cryopreservation

Resuspend the cells in the culture medium containing 10% DMSO and place vials into an isopropanol freezing container and place the container at -80°C overnight. Transfer to a liquid nitrogen tank on the following day.