Background

Cas9 (*Streptococcus pyogenes* CRISPR associated protein 9) is an endonuclease enzyme that, when recruited to a specific DNA sequence by the sgRNA (single guide RNA), introduces a double stranded break into the DNA. This double stranded break is repaired in mammalian cells either through Non-Homologous End Joining or Homologous Recombination. Non-Homologous End Joining often results in the deletion or insertion of several base pairs at the cut site, which, when resulting in a frameshift, causes the functional inactivation of the targeted gene. Cas9-expressing MCF7 cells can be transduced or electroporated with sgRNA targeting a gene of interest to quickly generate knock-out cell pools or cell lines.

Application

1. Quickly generating knock-out cell pools or cell lines in MCF7 cells.
2. Implementing sgRNA screens in Cas9-expressing MCF7 cells.

Materials Provided

Components	Format
2 vials of frozen cell pool	Each vial contains 2 x 10^6 cells in 1 ml of 10% DMSO

Host Cell

MCF7 human breast mammary gland cell line. Adherent epithelial cells.

Mycoplasma Testing

This cell pool has been screened using the MycoAlert™ Mycoplasma Detection Kit (Lonza, #LT07-118) to confirm the absence of Mycoplasma contamination. MycoAlert Assay Control Set (Lonza, #LT07-518) was used as a positive control.

Materials Required but Not Supplied

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

Materials Required for Cell Pool Culture

Name	Ordering Information
Thaw Medium 1	BPS Bioscience #60187
Growth Medium 1R	BPS Bioscience #78180
Insulin Solution from Bovine Pancreas	Sigma-Aldrich #I0516

Storage Conditions

Cells will arrive upon 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.

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Troubleshooting Guide

For all questions, please email support@bpsbioscience.com.

Media Formulations

For best results, it is *highly recommended* to use these validated and optimized media from BPS Bioscience. To formulate a comparable but not BPS validated media, formulation components can be found below.

Note: Thaw Medium 1 does *not* contain selective antibiotics. However, Growth Medium 1R *does* contain selective antibiotics, which are used for maintaining cell pools over many passages. Cells should be grown at 37°C with 5% CO_2 using Growth Medium 1R + 10 µg/ml Insulin.

Media Required for Cell Culture

Thaw Medium: Thaw Medium 1 (BPS Bioscience, #60187) **+ 10 µg/ml Insulin** (Sigma-Aldrich #I0516):
MEM medium (Hyclone, #SH30024.01) supplemented with 10% FBS (Life technologies, #26140-079), 1% Penicillin/Streptomycin (Hyclone, #SV30010.01), 1% Non-Essential amino acids (Hyclone, #SH30238.01), and 1 mM Na pyruvate (Hyclone, #SH30239.01).

Note: the final concentration of 10 µg/ml Insulin (Sigma-Aldrich #I0516) will need to be added to Thaw Medium 1R for cell culture

Growth Medium: Growth Medium 1R (BPS Bioscience, #78180) **+ 10 µg/ml Insulin** (Sigma-Aldrich #I0516):
Thaw Medium 1 (BPS Bioscience, #60187) plus 0.25 µg/mL Puromycin (Invivogen, #ant-pr-1) to ensure recombinant expression.

Note: the final concentration of 10 µg/ml Insulin (Sigma-Aldrich #I0516) will need to be added to Growth Medium 1R for cell culture

Cells should be grown at 37°C with 5% CO_2 using Growth Medium 1R plus 10 µg/ml Insulin (Sigma-Aldrich #I0516) to ensure recombinant expression is maintained.

Recommended Culture Conditions

Frozen Cell Pool:

1. Prepare T-75 culture flask with 20 ml of pre-warmed Thaw Medium (**no Puromycin**).
2. Quickly thaw cells in a 37°C water bath with constant and slow agitation.
3. After cleaning the outside of the vial with 70% ethanol, immediately transfer the entire content to Thaw Medium (**no Puromycin**). Avoid pipetting up and down, and gently rock the flask to distribute the cells.
4. Incubate the cells in a humidified 37°C incubator with 5% CO_2.
5. 24-48 hours after incubation, change to fresh Growth Medium (**contains Puromycin**), without disturbing the attached cells.
6. Continue to change medium every 2-3 days until cells reach desired confluency.

Subculture:

1. When cells reach 90% confluency, remove Growth Medium and wash twice with PBS (without Magnesium or Calcium).
2. Treat cells with 2-3 ml of 0.05% Trypsin/EDTA and incubate for 2-5 minutes at 37°C.
3. After confirming cell detachment by light microscopy, add 10 mL pre-warmed Growth Medium and gently pipette up and down to dissociate cell clumps.
4. Transfer cells to a 15 ml conical tube and centrifuge at 200 x g for 5 minutes.
5. Remove the medium and resuspend cells in 10 ml pre-warmed Growth Medium.
6. Dispense 1 mL of the cell suspension into a new T75 flask containing pre-warmed 9 ml Growth Medium (a subcultivation ratio of 1:2 to 1:10 is recommended).
7. Incubate cells in a humidified 37°C incubator with 5% CO_2.

Cryopreservation:

1. When cells have reached 90% confluency, use trypsin to remove cells from plate as above, spin cells and remove medium from the pellet.
2. Resuspend the cells in freezing medium (BPS Bioscience #79796).
3. Freeze cells using a reduced rate freezing box (-0.5°C to -1°C per minute) down to -80°C, then move cells to liquid nitrogen for long term storage.

Cells have been demonstrated to be stable for at least 15 passages; BPS recommends preparing frozen stocks early so cells are not used beyond passage 20.

Validation Data

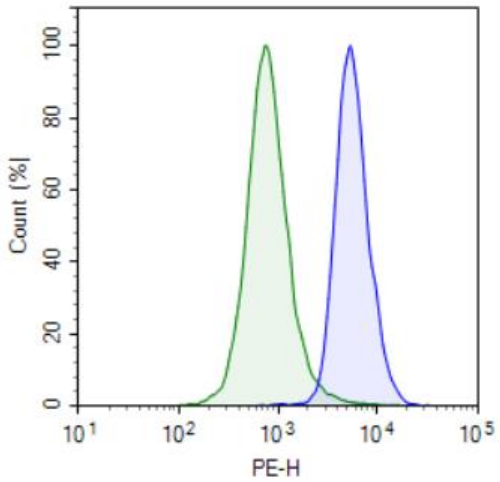

Figure 1. Flow cytometry analysis of intracellular expression of Cas9 in MCF7 cell pool.
Cells were stained with PE-labeled anti-FLAG antibody (BioLegend, #637309) and analyzed by flow cytometry. The parental MCF7 cells are shown in green, and the Cas9-expressing MCF7 Cell Pool (BPS Bioscience, #78179) is shown in blue.

Vector and Sequence

Streptococcus pyogenes Cas9, including a C-terminal FLAG tag, was transduced via lentivirus (BPS Bioscience, #78066).

MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSN
EMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNP
DNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKL
QLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFF
DQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKI
EKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKV
KYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDIL
EDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLT
FKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGI
KELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEE
VVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKS
KLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFK
TEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGF
DSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKG
NELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHL
FTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDKRPAATKKAGQAKKKKDYKDDDD

Related Products

Products	*Catalog #*	*Size*
Cas9 Lentivirus (puromycin selection)	78066	500 µl x 2
Cas9, His-tag (*S. pyogenes*)	100206-1	50 µg
Thaw Medium 1	60187	100 ml
Growth Medium 1R	78180	500 ml
Cell Freezing Medium	79796	50 ml or 100 ml
Cas9 Expressing MDA-MB-231 Cell Pool	78069	2 vials

Notes

The CRISPR/CAS9 technology is covered under numerous patents, including U.S. Patent Nos. 8,697,359 and 8,771,945, as well as corresponding foreign patents applications, and patent rights.