Nov 20, 2025

Genome Edited (KO) Cell Line Generation – PCR Validation

Forked from Genome Edited (KO) Cell Line Generation –

DOI
https://dx.doi.org/10.17504/protocols.io.5jyl88wj6l2w/v1

Amanda Bentley-DeSousa[1,2,3,4,5], Devin Clegg[1,2,3,4,5], Shawn Ferguson[1,2,3,4,5,6]

[1]Department of Cell Biology, Yale University School of Medicine, New Haven, Connecticut 06510, USA;
[2]Neuroscience, Yale University School of Medicine, New Haven, Connecticut 06510, USA;
[3]Program in Cellular Neuroscience, Neurodegeneration and Repair;
[4]Wu Tsai Institute Yale University School of Medicine, New Haven, Connecticut 06510, USA;
[5]Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD, 20815, USA;
[6]Kavli Institute for Neuroscience, Yale University School of Medicine, New Haven, Connecticut 06510, USA

Devin Clegg
Yale University

DOI: https://dx.doi.org/10.17504/protocols.io.5jyl88wj6l2w/v1

Protocol Citation: Amanda Bentley-DeSousa, Devin Clegg, Shawn Ferguson 2025. Genome Edited (KO) Cell Line Generation – PCR Validation. **protocols.io** **https://dx.doi.org/10.17504/protocols.io.5jyl88wj6l2w/v1**

Manuscript citation:
https://doi.org/10.1101/2023.10.31.564602

Protocol status: Working
We use this protocol and it's working

Created: October 01, 2025

Last Modified: November 20, 2025

Protocol Integer ID: 228804

Keywords: RAW 264.7, KO, Synthego, ko cell lines in raw, ko cell line, cell line generation, cell lines in raw, genome edited, genome, cell, line, ko, raw, pcr validate ko cell line, pcr validate ko cell lines in raw, pcr validation, pcr validation this protocol details step

Funders Acknowledgements:
Aligning Science Across Parkinson's Disease
Grant ID: ASAP-025173

Abstract

This protocol details steps taken to create and PCR validate KO cell lines in RAW 264.7 cells.

Materials

DMEM (Thermo Fisher Scientific, 11965-092)
FBS (Thermo Fisher Scientific, 16140-071)
Penicillin/Streptomycin (Thermo Fisher Scientific, 15140122)
CellStripper (Corning, 356230)
Synthego CRISPR Gene Knockout V2 Mouse Kits
Lipofectamine CRISPRiMAX (ThermoFisher Scientific, CMAX00003)
Cas9 (Synthego CRISPR Gene Knockout V2)
Opti-Mem (Thermo Fisher Scientific, 31985062)

Day 0

1 Plate 2.5 × 10^5 RAW 264.7 cells per well in a 6-well dish.

Day 1

2 Transfect cells using Lipofectamine CRISPRiMAX, gene specific sgRNAs and recombinant Cas9 (Synthego CRISPR Gene Knockout V2).

2.1 **CRISPRiMAX protocol**
In Tube 1 combine 62.5 µL Opti-MEM, 3.25 µL sgRNA (from 3 uM stock), 2.5 µL Cas9 (from 3 uM stock), and 2.5 µL Cas9+ then incubate for 00:05:00 . In Tube 2 combine 62.5 µL Opti-MEM and 3.75 µL CRISPRMAX then incubate for 00:05:00 . Combine tube 1 and tube 2. Incubate for 00:20:00 . Add drop-wise to 1 well containing 1.8 mL of media.

Day 3

3 Change the media.

Daty 4

4 Plate single cells into 96-well dishes to obtain clonal cell lines. After subculturing, confirm cell lines via immunoblotting

4.1 Expand clonal cell lines to 24-well dish
(Design primers flanking Cas9 cut sites)

Harvesting DNA using Quick Extract 8m 20s

5 Dissociate cells from 24-well dish

5.1 Centrifuge Cells 1000 rpm, 00:03:00

5.2 Discard supernatant

6 Add 200 µL of Quick Extract to cells

7 Vortex sample for 00:00:10 10s

8 Incubate sample at 65 °C for 00:06:00 6m

9 Vortex sample for 00:00:10 10s

10 Incubate sample at 98 °C for 00:02:00 2m

PCR Validation 33m

11 Run PCR using designed primers

11.1 PCR Reaction Setup
GoTaq® Green Master Mix, 2X 12.5 µL
upstream primer, 10µM 1 µL
downstream primer, 10µM 1 µL
DNA template 3 µL
Nuclease-free water 7.5 µL
Total 25 µL

12 Thermocycler: 3m
Initial denaturation 98 °C 00:02:00
denaturation 98 °C 00:00:30
Annealing 00:00:30 Temp. is determined by primers

Extension 72 °C 1 minute per kilobase

30 Cycles

Hold 4 °C

13 Run PCR reactions on 1% agarose gel

100v for 00:30:00

30m