Dec 03, 2024

Stable Cell Line Generation – RAW 264.7

The Journal of Cell Biology

DOI
https://dx.doi.org/10.17504/protocols.io.yxmvm9nr5l3p/v1

Amanda Bentley-DeSousa[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

Amanda Bentley-DeSousa
Yale University

DOI: https://dx.doi.org/10.17504/protocols.io.yxmvm9nr5l3p/v1

Protocol Citation: Amanda Bentley-DeSousa, Shawn Ferguson 2024. Stable Cell Line Generation – RAW 264.7. **protocols.io** https://dx.doi.org/10.17504/protocols.io.yxmvm9nr5l3p/v1

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

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

Created: December 02, 2024

Last Modified: December 03, 2024

Protocol Integer ID: 113412

Keywords: RAW 264.7, piggybac, stable expression, stable cell line generation, cell line generation, cell lines in raw, expressing cell line, cell, line, raw, protocol details step, generation

Funders Acknowledgements:
Aligning Science Across Parkinson's Disease
Grant ID: ASAP-000580

Abstract

This protocol details steps taken to create stably expressing 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)
Lipofectamine 2000 (Invitrogen, 11668019)
FuGene HD (Promega, E2311)
Opti-Mem (Thermo Fisher Scientific, 31985062)
Puromycin (Gibco A11138-03)
Blasticidin (Invivogen ant-bl)

Day 0

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

Day 1

30m

2 Transfect cells using Lipofectamine 2000 or FuGene HD. Ensure to use a 1:2 ratio of Piggybac transposon plasmid:gene-of-interest plasmid for a total of 1 uG DNA.

2.1 **For Lipofectamine 2000 (HALO-LRRK2):** 30m
In Tube 1 combine 150 µL Opti-MEM and 8 µL Lipofectamine 2000 then incubate for 00:05:00 . In Tube 2 combine 150 µL Opti-MEM and 1 uG total DNA (see above) then incubate for 00:05:00 . Combine tube 1 and tube 2. Incubate for 00:20:00 . Add drop-wise to 1 well containing 1.7 mL of media.

Day 3

3 Change the media.

Day 4

4 Add media containing selection-specific antibiotics.

4.1 Use Puromycin (3.5 uG/mL) or Blasticidin (10 uG/mL).

Day 6-7

5 Change the media.

Day 8+

6 After cells have recovered from antibiotic selection, plate single cells into 96-well dishes to obtain clonal cell lines. After subculturing, confirm cell lines via immunoblotting.