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Culturing 3T3 Cells for Validating GEARBOCS 2.0 Constructs V.1

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Abstract

This protocol is for culturing Cas9 NIH/3T3 cells for the validation of GEARBOCS 2.0 and similar constructs for use in in vivo assays.

Materials

- **NIH3T3/Cas9 Cell Line**, Calibre Scientific, CBIO-AKR-5104
- DMEM, GIBCO, Cat# 11960
- Fetal Bovine Serum, Thermo Fisher Cat# 10-437-028
- 100 U/mL Pen/Strep, GIBCO, Cat# 15140
- 2 mM L-Glutamine, GIBCO, Cat# 25030-081
- 1 mM Sodium Pyruvate, GIBCO, Cat# 11360-070
- Opti-MEM, GIBCO, Cat# 31985070
- X-tremeGENE HP DNA Transfection Reagent, Sigma-Aldrich, 6366244001
- DNeasy Blood & Tissue Kit, Qiagen, Cat# 69504

1 **Validation by indel sequencing in immortalized murine cell lines:**

2 Prepare HEK293 cell media

3 1. DMEM 450ml
2. Fetal Bovine Serum 50ml
3. L-Glutamine, 2 mM, 5ml
4. Sodium Pyruvate, 1 mM, 5ml
5. Pen-Strep, 100 U/mL, 5ml

4 Seed **Cas9 NIH/3T3** cells at 250k per well of a 6-well plate (if starting from frozen cells, you. will need to passage at least once prior to transfection. So thaw to 10cm dish, then passage to 6-well)

5 24 hours after seeding, transfect Cas9 NIH/3T3 cells with GBOCS plasmid:

6 For each gene of interest, combine the following components in a 1.5 mL tube:

6.1 1. 200 uL optimum
2. 1 uL of GBOCS2.0 (at 1ug/uL)

7 Mix by vortexing

8 Add 6 uL of Xtreme Gene

9 Pipette up and down rapidly. Do not touch pipette tip to the wall of the tube (Xtreme gene is sticky and extremely finicky. You want to minimize contact between XG and plastic surfaces)

10 Mix tube contents by gently flicking

11 Incubate 30 min at room temperature.

12 Add 200 uL of optimem/XG/DNA mixture per well in drop-wise swirlfashion.

13 Return cells to incubator for 48 hours

14 48 hours after transfection, collect cells and isolate genomic DNA for Sanger sequencing:

15 Place 6-well plate on ice

16 Aspirate media and wash once with 1 mL of ice-cold PBS per well

17 Aspirate PBS wash and add fresh 1 mL cold PBS for collection

18 Use rubber scraper to collect cells into 1.5 mL tube and keep on ice

19 Use Qiagen DNeasy kit to isolate genomic DNA