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Protocol to generate stable cell line using HEK293 Flp-In TREx cells

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Julia Heiby[1]

[1]FLI Leibniz Institute on Aging

Julia C. Heiby
FLI Leibniz Institute on Aging

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

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Disclaimer

Abstract

Transfection of HEK293 FlpIn TREx cells, to generate stable cell lines.

Materials

- Centifuge 5810R (#5811000015, Eppendorf)
- Safety cabinet (Thermo, #Safe S2020 1.2)
- Automated cell counter (Biorad, #TC20)
- Trypan blue solution for cell counting (Sigma, #T8154)
- Counting Slides for cells (Biorad, #145-0011)
- Incubator (Thermo, #BBD 6220, CO2 Incubator), settings: 5% CO2, 95%rH, 37°C
- Milli-Q water system (Merck, Advantage A10)

- Flp-In T-REx 293 Cell Line (RRID: CVCL_U427)
- XtremeGENE9 A Transfection Reagent (Roche, #6365779001)
- 6-well plate (Falcon,# 353046)
- 15ml and 50ml Falcon (Corning, #352096 and #352070, respectively)
- 1.5ml tubes (Eppendorf, #0030 120.086)
- Serologicla Pipettes (Greiner, cellstar)
- Opti-MEM (Gibco, #31985-047)
- pOG44 Flp-Recombinase Expression Vector (Invitrogen, #V600520)
- Blasticidin (Gibco, #R210-01)
- HygromycinB (Invitrogen, #10687010)
- Trypsin-EDTA (0,05%, Gibco, #25300-062)
- DMEM high glucose (4,5g/L) (Sigma, #D6429)
- FBS, fetal bovine serum (Sigma,#F7524)
- DMSO, dimethyl sulfoxide (Sigma, #D2438-5)
- Penicillin/Streptomycin (Gibco, #15140-122)

Day 1

1 Seed 200 000 on a 6-well (at this point use medium with no antibiotics at all)
Do not forget to seed a well for no-DNA control transfection.

Day 2

2 Transfect cells on a 6-well plate with XtremeGENE9 from Roche (XTG9-RO), following instructions of the manufacturer. *Pre-Mix under the hood:* Use 1:9 of plasmid : pOG44

- 100µL Opti-MEM (or DMEM with no FBS, no antibiotics)

+ 3µL X-tremeGENE9 Transfection medium
+ 1µl of Plasmid DNA (100ng; containing target sequence to transfect)
+ 4.3µl pOG44 (900ng; pOG44 Flp-Recombinase Expression Vector from Invitrogen)
Incubate 15 min at RT (change to medium with no antibiotics if required)
Add dropwise to wells
Distribute evenly by gently moving flask

Day 3

3 Trypsinize and transfer cells onto a p150 plate (25ml medium)

Day 4

4 Add the corresponding antibiotics for selection (usually, Blasticidin + HygromycinB. For 20 mL of medium: 30 µL Blasticidine + 40 µL HygromycinB (from stocks 10 mg/mL Blasticidine and 50 mg/mL HygromycinB).

Following days / weeks

5 - Replace medium with fresh antibiotics twice a week until colonies are seen in your transfection but not in the negative control (no-DNA).
- When colonies start being too big, trypsinize them onto the same dish to separate the cells and allow them to grow better. The day after trypsinizing them, add fresh antibiotics.

Final antibiotics concentrations:
Blasticidin: 15 µg/mL
HygromycinB: 100 µg/mL

This usually takes 2-3 weeks for HEK FIpIn TREx cells.

Advice

6 For more information see info given by Invitrogen or Roche

Preparing cryo-cultures

7 Buffers and reagents

growth medium – pre-warmed to 37°C: (stored in the fridge)
DMEM high glucose (4,5g/L) -500ml
10% FBS (heat inactivated for 30min at 56°C) -50ml

+ antibiotics depending of the cell type

freezing medium (4°C):
FBS (heat inactivated) -45ml
10% DMSO -5ml

PBS – pre-warmed to 37°C (without calcium and magnesium/ stored in the fridge)

0,05% Trypsin-EDTA (1x)-pre-warmed to RT (contains 0,05% phenol red as pH indicator, keep it only short time outside the fridge because of autodigestion- stored in the fridge)

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1. grow cells to a density of 80% confluence in growth media with antibiotics (1 day)
2. wash the cells one time with 5ml PBS
3. add 0,05% Trypsin-EDTA and incubate at 37°C
4. stop trypsinization by adding of growth media, transfer cell suspension to a conical tube
5. pellet cells at 500xg for 5min at 20°C
6. aspirate the supernatant and resuspend the pellets in 4°C freezing medium (at about 1 to 4×10^6 cells/ml)
7. dispense 0.5ml up to 1ml aliquots into labeled vials and place them in a cell freezing container

8. place freezing container into -80°C freezer o/n
9. Transfer vials in liquid nitrogen for long term storage

Protocol references

Following the Manufacturers instruction for the X-tremeGENE™ 9 DNA Transfection Kit from Roche.