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PiggyBac Transfection iPSC

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ABSTRACT

This protocol details the generation of stably transfected iPSC with piggybac constructs. This procedure is used to introduce exogenous NGN2 gene into iPSCs. The established cell lines are used for trans-differentiation into neurons by doxycycline induction.

ATTACHMENTS

dh4cbiqa7.pdf

DOI

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PROTOCOL CITATION

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KEYWORDS

iPSC Transfection, Piggybac constructs, NGN2

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OWNERSHIP HISTORY

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MATERIALS TEXT

Materials:

- 6-well plate
- Cell culture hood
- Pipets (10, 20, 1000p)
- **1.5 mL** autoclaved Eppendorf tubes
- Cell counter

Reagents:

- TransIT®-LT1 Transfection Reagent **Mirus**
- **Bio Catalog #MIR 2304**

  Opti-MEM (Reduced Serum Medium) **Thermo Fisher**
- **Scientific Catalog #31985062**

  Growth Factor Reduced (GFR)
- Matrigel® **Corning Catalog #354230**

  DPBS (Dulbeccos Phosphate Buffered Saline) 1x [-] calcium
- magnesium **Corning Catalog #21-031-CV**

  Accutase - Enzyme Cell Detachment Medium **Thermo**
- **Fisher Catalog #00-4555-56**

  StemFlex Medium **Thermo Fisher**
- **Scientific Catalog #A3349401**

  Y-27632
- Dihydrochloride **peprotech Catalog #1293823**
- Transposase pEf1α-hyPBase
- Piggybac plasmid construct: pEXP-piB-BsD-Tet-NGN2-Puro-IRES-SNAP-PGKtk; Plasmid #1022
- in Khurana's Plasmid logs

Day before transfection: Re-plating the cells 5m

1

Aspirate the medium from one confluent well of 6-well plate, and wash once with PBS.

2 2m

Add **1 mL** accutase and leave in the incubator for **00:02:00** .

3

Add **1 mL** growth medium or hES medium with a 1000p, and pipet up and down to dissociate the cells and break them into single cells.

4 3m

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Transfer the cell suspension to a **15 mL** conical tube. Centrifuge at **800 rpm** for **00:03:00** . Remove the supernatant.

5

Add **1 mL** StemFlex to resuspend and count the cells with cell counter.

6

Take out 6-well plate coated with Matrigel from **37 °C** incubator after at least one-hour incubation.

7

Aspirate the Matrigel carefully without scratching the surface. Wash once with PBS to remove residual Matrigel.

8

Plate 1.5 X 106 cells (when slow growers) in **2 mL** StemFlex supplemented with **10 Micromolar (µM)** ROCK inhibitor in one well of Matrigelcoated 6-well plate.

> You may seed another well with fewer cells at 1.0 X 106 to compare the wells on the day of transfection to choose one. Cells should be about 70% confluent the next day.

Day of transfection 5m

9

Prepare the transfection mix in **1.5 mL** Eppendorf tubes under cell culture hood.

10

Add **200 µl** Opti-MEM medium to the **1.5 mL** tube for each transfection.

11

Add **2 µg** of the piggybac plasmid to the tube.

12

Add **1.5 µg** of the transposase plasmid to the mix.

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13 

Add **10.5 µl** TransIT-LT1 Transfection Reagent and mix well (ratio 1:3 DNA:Reagent).

20m

14 Leave under the hood at **Room temperature** for **00:20:00** .

15 Remove the cell culture plate from day before and choose the well that is about 70-80% confluent in single layer cells.

16 

Remove the medium and feed with **2 mL** StemFlex supplemented with **[M]10 Micromolar (µM)** ROCK inhibitor (Y).

17 

Add the **200 µl** transfection mix evenly and drop-wise on the culture plate.

18 Shake the plate very gently to make sure it is dispersed evenly.

6h

19 

Leave at **37 °C** incubator for **06:00:00** without disturbing it.

20 

Aspirate the medium and feed with **2 mL** StemFlex + Y.

21 Change the medium for every day with StemFlex + Y.

22 

Two days after transfection add **[M]5 µg/ml** blasticidin (BsD) if the backbone of the piggybac plasmid has the selection gene for blasticidin resistance. If cells look unhealthy after the transfection, do **[M]2.5 µg/ml** blasticidin (BsD).

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23 Continue selection for **120:00:00** (max). Changing the medium every day with StemFlex + **10 Micromolar (µM)** Y + **5 µg/ml** BsD. 5d

24 If you see more than 80% of cells die before 5 days, stop selection and feed the cells with StemFlex without BsD until it becomes confluent for expansion.

25 You do not need to supplement StemFlex with ROCK inhibitor after day 7 of transfection, but do use RI at the following passage.

26 Expand the cell line (use RI during the passaging) and freeze a large number of tubes. When passaging the cells for the 1st time, add RI to the media.

Important QC tests on the newly generated polyclonal iPSC lines

27 Depending on the experiment, you may choose to keep the line as polyclonal or monoclonal by subcloning it and select for single clones to use moving forward (2-3 clones for QC and you can pool or keep separate).

27.1 Expand a polyclonal line upfront at the lowest passage possible into a large batch.

27.2 Keep the passage as low as possible. Preferred to not use the line more than 5 passages after the Piggybac transfection.

If the line is stable and has good karyotype, if may be reconsidered to use the line at later passages.

28 Karyotype the newly generated line to determine any abnormal chromosomal events.

28.1 For most purposes karyotyping the polyclonal line on 50 cells to get a sense of the karyotypically unstable population (if any) is fine. Karyotype the lowest passage available.

28.2 For a monoclonal culture of your key cell line you may perform the standard 20 count cell karyotyping.

29

Perform the Splinkerette PCR assay to locate and map the exact location of each transposition event in the genome of the cells that are stably transfected with piggybac construct.

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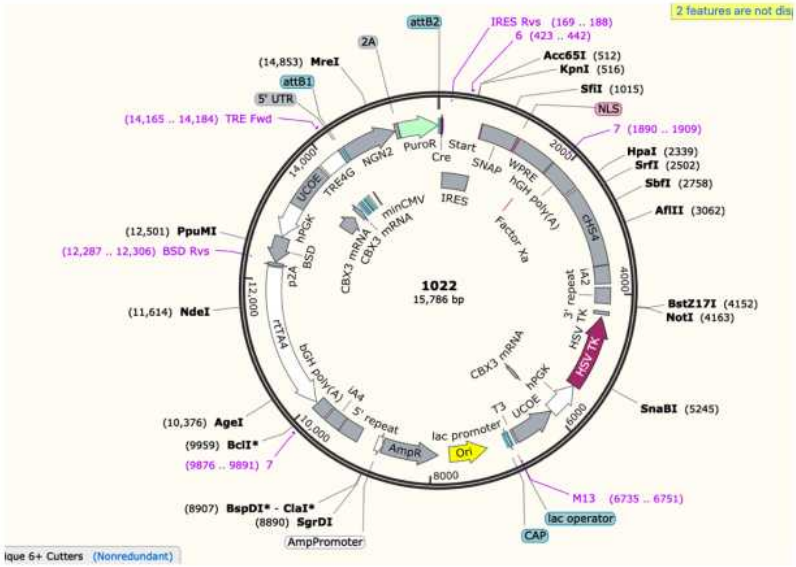
It also shows the copy number for the number of transposon integrations (protocol available at the Khurana Lab/Aazam).

NGN2-Puro-PB Construct Map:



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