Hanna LAB Reprogramming Protocol

Robust and Rapid Reprogramming of Adult Primary Human Fibroblasts

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Last Updated: 30/3/2014

We have recently described that combined action of 1) adequate and robust **OKSM** induction 2) **Mbd3 depletion** and 3) **Naïve pluripotency promoting conditions** (e.g. for mouse cells – 5% O2, 2i/LIF and KSR that contains Vitamin C and Albumax), dramatically promotes **mouse** naive iPSC induction.

We also showed that **in vitro differentiated secondary human fibroblast-like cells** can reprogram up to 100% efficiency by 1) **STEMCCA-OKSM induction** 2) **Human Naïve WIS-NHSM conditions (including ingredients like 2i/LIF, ROCKi, 5% O2 and KSR)** and 3) **MBD3 genetic depletion**. When applied on human BJ primary fibroblasts, their reprogramming was not deterministic, but we were able to increase efficiency and achieve iPSCs within 7 days indicating the MBD3 depletion promotes rapid human iPSC reprogramming.

Below we describe modifications that render primary naïve human iPSC from other primary fibroblasts cells (not only BJ) and at increased efficiency. We achieve this by addition of **ERAS** reprogramming factor (Takahashi et al. Nature 2003), which is induced during mouse reprogramming, however is not expressed in human cells ((1) **STEMCCA-OKSM + ERAS induction** 2) **Human Naïve WIS-NHSM conditions (including WIS-NHSM, 5% O2 and Vitamin** C) and 3) **MBD3 depletion**) (Manuscript in Preparation).

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Media needed:

A) Human conventional ES medium (hES medium)

Used for Reprogramming (First 48 hours of reprogramming)

- 450mL DMEM F-12 (**no HEPES**) (BI 06-1170-50-1A) (throw away 50 ml)
- 50ml KSR. NEVER HEAT-INACTIVATE.
- 50ml Heat inactivated FBS (Human primed ES compatible)
- 5mL Pen-strep (Penicillin + Streptomycin)
- 5mL L-Glutamine
- 5mL Non-essential Amino-Acid
- 50µl β-Mercaptoethanol
- FGF2 (Peprotech 100-18c) 8ng/ml final concentration
- Human LIF 20ng/ml final concentration
- L-ascorbic acid 2-phosphate (Sigma-A8960) (50 µg/ml final concentration)
- When DOX inducible vector are used: include Doxycycline 2µg/ml final concentration

B) Human Naïve ES Medium (WIS-NHSM; Version 3, 2 or 1)

Used for Reprogramming (starting from 48 after OKSM induction until completion)

-Protocols available on our website. Must include ROCKi.

C) MEF medium

To be used for human fibroblast expansion and collecting of viral supernatants.

- **500mL** DMEM (Invitrogen 41965-039)
- **100mL** FBS (MEF type). **MAKE SURE HEAT-INACTIVATED**.
- **5mL** Pen-strep (Penicillin + Streptomycin)
- **5mL** L-Glutamine
- **5mL** Non-essential Amino-Acid
- **5mL** Sodium-Pyruvate

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DNA Plasmids Needed:

1) **FUW-Ubi-M2rtTA(LoxP). (Jaenisch lab – Addgene 20342)**
2) **STEMCCA-TetO-OKSM (LoxP) (Kindly provided – Gustavo Mostuslavsky)**
3) **FUW-TetO-ERAS or FUW-CAGGS-ERAS (Hanna Lab- Addgene)**
4) **TRIPZ Human MBD3 shRNA Clone ID: V3THS_392206 (#1) (Thermo Scientific).**
5) **TRIPZ Human MBD3 shRNA Clone ID V3THS_392210 (#3) (Thermo Scientific).**

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Lentiviral Production and iPSC Generation:

1) **Lentiviral production of plasmids 1-5 indicated above separately.**
2) **Viral supernatants are mixed at 1:2:2:1:1 ratio.**
3) **Human Fibroblasts expanded in 20% O2 and in MEF medium (Medium C) are subjected to 2-4 rounds of lentiviral transduction (at 12 hours intervals).**
4) **At day -1 - Human fibroblasts are plated on 10c"m plates pre-coated with 0.2% gelatin / 1μg/ml Vitronectin (1 hour in 37C). 200,000 or 400,000 cells are plated per 10 c"m dish.**
5) **At day 0 – transfer fibroblasts to 5% O2 and initiate reprogramming in Medium A (hES medium).**
6) **At day 2 – switch to Reprogramming medium B (naïve WIS-NHSM).**
7) **iPSC colonies are evident starting from day 6. Colonies can be picked days 6-10 and expanded in WIS-NHSM conditions independent of DOX (preferably retain naïve hiPSCs in in 5% O2, but maintenance can also be conducted in 20% O2 although less optimal).**

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