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| Title | CD34+ cell reprogramming using episomal vectors |
| Date Submitted | May 5, 2012 |
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| Adapted from - | |
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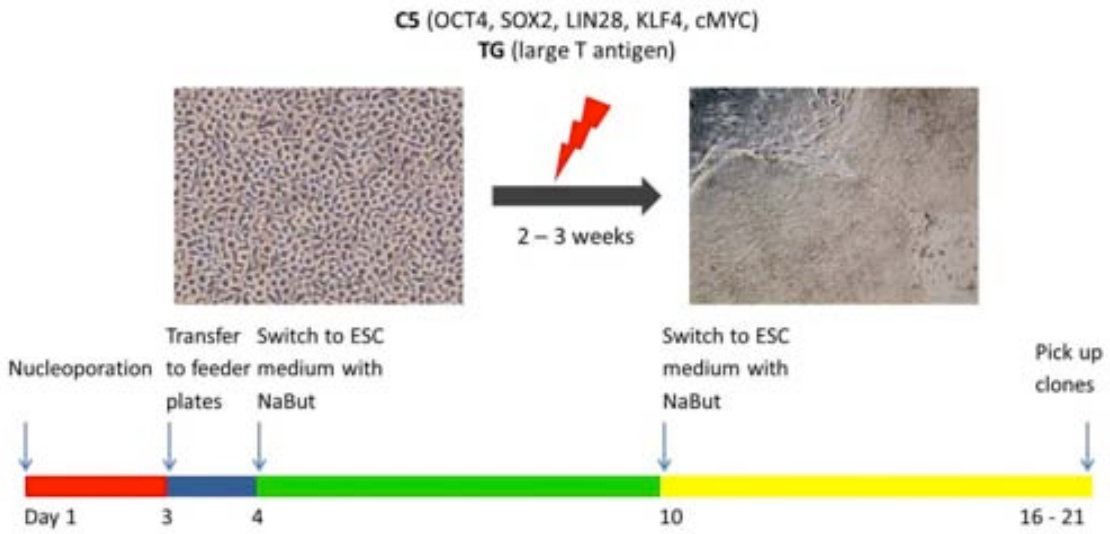
## ❖ Introduction:




iPSC colony derived from CD34+ cord blood cells by reprogramming with non-integrating plasmids.

## ❖ Protocol:

1. Prime CD34+ cells
    a. Thaw CD34+ cells using Lonza's protocol and culture for 4-5 days (http://www.lonzabio.com/uploads/tx_mwaxmarketingmaterial/Lonza_ManualsProductInstructions_Procedure_for_Thawing_Poietics_Cells.pdf)
2. Day 1: nucleoporate 1x10^6 hCD34+ cells with single (up to 10 µg), or combination of plasmids (8 µg C5 + 2 µg Tg) by Amaxa using program U-008

3. Days 1 and 2: culture nucleoporated cells in one well of a 12-well plate in the $CD34^+$ medium with cytokines
4. Day 3: transfer nucleoporated cells to 3 wells of MEF coated 12-well plate and culture in MEF medium for one day
    a. Once cells are seeded into wells, spin plates at 100xg for 30 min to help cells attach to MEF coated wells
5. Day 4: replace MEF medium with hESC medium (supplemented with 10 ng/ml FGF2)
    a. OPTIONAL: collect MEF medium and spin it down at 100xg for 5 min; aspirate medium and resuspend cell pellet in hESC medium with 10 ng/ml FGF2 – some $CD34^+$ cells may not attach during the first day, so save them and replate them
    b. Change hESC medium every other day for total of 6 days
    c. OPTIONAL: add valporic acid (0.5 mM) or Na-butyrate (0.25 mM)
6. Switch to MEF-CM with 10 ng/ml FGF2 one week after transfer onto MEF coated wells
7. Two weeks after nucleoporation, perform TRA1-60 staining on live cells to identify most likely iPSC clones
    a. With cord blood $CD34^+$ cells expect to see colonies appearing 7-11 days post-nucleoporation
    b. With adult bone marrow and peripheral blood $CD34^+$ cells colonies start appearing 11-14 days post-nucleoporation
8. Manually dissect each TRA1-60 positive colony and transfer to a separate well of a 12-well plate: each colony becomes a clone
    a. OPTIONAL: add 10 μM ROCK inhibitor and/or hESC cloning and recovery supplement to improve survival and attachment of dissected colonies
9. For the first 2 – 3 passages keep clones in 12-well plates, then expand to 35 mm dishes
10. Manually passage clones for the first 6 – 10 passages, then switch to 1 mg/ml collagenase (depending on whether clones remain undifferentiated when enzymatically passaged). In instances when less than 10% of colonies are differentiated, remove differentiated cells manually and proceed to enzymatic passage; if more than 10% colonies are differentiated, continue with manual passaging
11. Gradually reduce FGF2 concentration in MEF-CM to 4 ng/ml and switch to hESC medium by mixing MEF-CM and hESC medium in order to adopt iPSC clones to hESC medium with 4 ng/ml of FGF2.

## ❖ Materials:

| **Product** | **Company** | **Catalogue number** |
|---|---|---|
| MEF, mitomycin C treated | Millipore | PMEF-N |
| DMEM, high glucose | Gibco | 11995 |
| FBS | | |

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|---|---|---|
| KNOCKOUT™ DMEM/F12 | Gibco | 12660 |
| NEAA | Gibco | 11140 |
| Anti-Anti | Gibco | 15240 |
| KNOCKOUT™ Serum Replacer | Gibco | 10828 |
| 2-mercaptoethanol | Gibco | 21985 |
| GlutaMAX™-1 | Gibco | 35050 |
| $CD34^+$ cells | Lonza | 2C-101 |
| HPGM™ | Lonza | PT-3926 |
| DNase I | Sigma | D4513 |
| SCF | Peprotech | AF-300-07 |
| TPO | Peprotech | AF-300-18 |
| FL | Peprotech | AF-300-19 |
| FGF2 | Stemgent | 03-0002 |
| Nucleofector kit for $CD34^+$ cells | Lonza | VPA-1003 |
| ROCK inhibitor, Y27632 | Stemgent | 04-0012 |
| hESC cloning and recovery supplement | Stemgent | 01-0014-500 |
| Na-butyrate | Stemgent | 04-0005 |
| Valporic acid | Stemgent | 04-0007 |
| TRA1-60 antibody | eBioscience | 13-8863-83 |
| C5 – EBNA1 carrying OCT4, SOX2, KLF4, LIN28, cMYC | Addgene | http://www.addgene.org/28213/ |
| TG – EBNA1 carrying SV-40 Large T antigen | Addgene | http://www.addgene.org/28220/ |

MEF medium
90% DMEM
10% FBS
1% Anti Anti

hESC/hiPSC medium
KNOCKOUT™ DMEM/F12
20% KNOCKOUT™ Serum Replacer
1% GlutaMAX™-1
1% NEAA
1% Anti/Anti
4 – 10 ng/ml FGF2
0.1 mM 2-mercaptoethanol

CD34+ cell medium (recommended by Lonza)
HPGM™ Hematopoietic Progenitor Growth Medium supplemented with the following concentrations of cytokines:
FL – 50 ng/ml

TPO – 50 ng/ml
SCF – 25 ng/ml
All cytokines are from Peprotech and are diluted in trechalose at concentration of 100 ng/µl.

Abbreviations
MEF = mouse embryonic fibroblasts
FBS = fetal bovine serum
NEAA = non-essential amino acids
FL = Flt3 ligand
SCF = stem cell factor
TPO = Thrombopoietin
MEF-CM = hESC medium conditioned for 24 hrs on MEF

- Troubleshooting:

- **References:**