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

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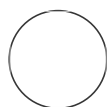
Keywords: Nucleofection, Plasmid-reprogramming, human fibroblasts

Plasmid-reprogramming of human fibroblasts

In 1 collection

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ABSTRACT

This protocol details about plasmid-reprogramming of human fibroblasts.

ATTACHMENTS

[404-873.docx](#)

GUIDELINES

Adapted from Okita et al PMID: 21460823.

MATERIALS

Materials

- DMEM
- GlutaMAX supplement (Gibco)
- FBS (Gibco)
- FGF2 (Peprotech)
- Nucleofection Kit for Amaxa Nucleofector: Normal Human Dermal Fibroblasts

⊗ Human Dermal Fibroblast Nucleofector™ Kit **Lonza Catalog #VPD-1001**

⊗ Sodium butyrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #303410**

Plasmids (from Addgene):

⊗ pCXLE-hOCT3/4 **addgene Catalog #27076**

⊗ pCXLE-hSK **addgene Catalog #27078**

⊗ pCXLE-hUL **addgene Catalog #27080**

Nucleofection (Day 0)- with Amaxa Nucleofector I/II

1 Prepare nucleofection solution:

A	B
Human Dermal Fibroblast Nucleofector solution	82 μ L
Supplement	18 μ l
Plasmid	10 μ g

2 Nucleofect 700.000 fibroblasts (resuspended in the nucleofection solution) with the P-022 program.

3 Distribute nucleofected fibroblasts on a 6-Well plate, coated with Matrigel.

4 Culture cells in DMEM + GlutaMAX supplement (Gibco) + 10 % FBS (Gibco) without P/S.

Day 1

5 Change medium to DMEM + GlutaMAX supplement + 10% FBS + 2 undetermined FGF2 (Peprotech) with 1% P/S (Millipore).

Day 3/4

6 Change medium to E8 medium + 100 micromolar (μ M) Sodium Butyrate (Sigma Aldrich 303410-100G) + 0.1 % P/S and change medium every other day.

Day 21-28

- 7 Pick iPS cell colonies.

Note

Note: First colonies should appear around Day 14.