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

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# Plasmid-reprogramming of human fibroblasts

In 6 collections

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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 µL |
| | Supplement | 18 µl |
| | Plasmid | 10 µg |

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 ng/mL FGF2 (Peprotech) with 1% P/S (Millipore).

## Day 3/4

6 Change medium to E8 medium + [M] 100 micromolar (µM) 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.