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Plasmid-reprogramming of human fibroblasts

 In 2 collections

DOI

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Pascale Baden¹, michela.deleidi¹

¹German Center for Neurodegenerative Diseases (DZNE), Tübingen, 72076 Germany



Federico Bertoli

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Protocol status: Working

We use this protocol and it's working

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Abstract

This protocol details about plasmid-reprogramming of human fibroblasts.

Attachments



[404-873.docx](#)

16KB

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 μ L 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.