Plasmid-reprogramming of human fibroblasts

In 4 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 Sigma
  - Aldrich Catalog #303410

Plasmids (from Addgene):

- pCXLE-hOCT3/4 addgene Catalog #27076
- pCXLE-hSK addgene Catalog #27078
- pCXLE-hUL addgene Catalog #27080

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Protocol status: Working

We use this protocol and it’s working

Created: Apr 26, 2022
Nucleofection (Day 0)- with Amaxa Nucleofector I/II

1. Prepare nucleofection solution:

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<tr>
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<th>A</th>
<th>B</th>
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<tbody>
<tr>
<td></td>
<td>Human Dermal Fibroblast Nucleofector solution</td>
<td>82 µL</td>
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<tr>
<td></td>
<td>Supplement</td>
<td>18 µL</td>
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<tr>
<td></td>
<td>Plasmid</td>
<td>10 µg</td>
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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 + 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.