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Differentiation of KOLF2.1J hIPSC to midbrain dopaminergic neurons

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Guochang Lyu[1], Ernest Arenas[1]

[1]Karolinska Institute

Ernest Arenas: deceased 15.09.2024

SOX6 mDA differentiation

Roberto Garcia Swinburn
Karolinska Institute Stockholm

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

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Keywords: representative markers for ventral midbrain dopaminergic neuron, ventral midbrain dopaminergic neuron, mature midbrain dopaminergic neuron, dopaminergic neuron, dopaminergic neurons this protocol, mature midbrain, resultant cell, neuronal acitivity, sox6

Abstract

This protocol was used to differentiate KOLF2.1J cells into mature midbrain dopaminergic neurons. The resultant cells do not show representative markers for ventral midbrain dopaminergic neurons such as EN1 or SOX6, although they showed neuronal acitivity and other canonical markers.

Protocol materials

- Stemolecule LDN-193189 **Stemgent - Bio-connect Catalog #**04-0074
- Stemolecule™ SB431542 **Reprocell Catalog #**04-0010-10
- Purmorphamine **Tocris Catalog #**4551
- CHIR99021 **R&D Systems Catalog #**4423
- Y-27632 dihydrochloride **Tocris Catalog #**1254
- Laminin-511 **Biolamina Catalog #**LN511
- Poly-L-Ornithine **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**P4957
- Ascorbic Acid 500 mg **STEMCELL Technologies Inc. Catalog #**72132
- Dibutyryl-cAMP **STEMCELL Technologies Inc. Catalog #**73884
- Neurobasal™ Medium **Gibco - Thermo Fisher Scientific Catalog #**21103049
- B27 supplement minus vitamin A **Gibco - Thermo Fisher Scientific Catalog #**12587010
- L-Glutamine **Gibco - Thermo Fisher Scientific Catalog #**25030024
- Human Recombinant BDNF **STEMCELL Technologies Inc. Catalog #**78005
- Human Recombinant GDNF **STEMCELL Technologies Inc. Catalog #**78058
- Tgf beta 3 (human) Recombinant Protein **Invitrogen - Thermo Fisher Catalog #**RP8600
- PD0325901 1 mg **STEMCELL Technologies Inc. Catalog #**72182
- SU5402 1 mg **STEMCELL Technologies Inc. Catalog #**73912
- Geltrex™ hESC-Qualified, Ready-To-Use, Reduced Growth Factor Basement Membrane Matrix **Thermo Fisher Catalog #**A1569601
- Essential 8 Flex complete medium (E8) **Gibco - Thermo Fisher Scientific Catalog #**A2858501
- N2 supplement **Gibco - Thermo Fisher Scientific Catalog #**17502048

Troubleshooting

Pre-Differentiation (iPSC Expansion & Seeding)

1 Day -2 (Two Days Before Differentiation Start):
Coat culture plates with:

- Geltrex (Life Technologies)
  Geltrex™ hESC-Qualified, Ready-To-Use, Reduced Growth Factor Basement Membrane Matrix **Thermo Fisher Catalog #**A1569601
- Laminin-511 (Biolamina) Laminin-511 **Biolamina Catalog #**LN511

2 **Seed iPSCs:**

- Plate **adapted iPSCs at a density of 200,000 cells/cm²** onto the coated plates. Maintain cells in Essential 8 Flex (Thermo Fisher Scientific) medium
  Essential 8 Flex complete medium (E8) **Gibco - Thermo Fisher Scientific Catalog #**A2858501
  . Continue until cells reach ~80% confluency.

Initiation of Differentiation

3 Day 0: Start of Differentiation:
Culture Medium: Switch to **Neurobasal/N2/B27 medium** supplemented with **2 mM L-glutamine** (Invitrogen).

Neurobasal™ Medium **Gibco - Thermo Fisher Scientific Catalog #**21103049

N2 supplement **Gibco - Thermo Fisher Scientific Catalog #**17502048

B27 supplement minus vitamin A **Gibco - Thermo Fisher Scientific Catalog #**12587010

L-Glutamine **Gibco - Thermo Fisher Scientific Catalog #**25030024

4 **Small Molecule Additives (Day 0–11):**

- LDN193189 – 250 nM (Day 0–7)
  Stemolecule LDN-193189 **Stemgent - Bio-connect Catalog #**04-0074
- SB431542 – 10 µM (Day 0–7)
  Stemolecule™ SB431542 **Reprocell Catalog #**04-0010-10
- Purmorphamine – 12 µM (Day 0–7) Purmorphamine **Tocris Catalog #**4551
- CHIR99021 – 0.7 µM (Day 0–3), increase to 7.5 µM from Day 4–11
  CHIR99021 **R&D Systems Catalog #**4423
- Y27632 – 10 µM (Day 0–1 only) Y-27632 dihydrochloride **Tocris Catalog #**1254

Replating Plates

5 Day 11: First Replating. Dissociate into single cells.

6 Replate at **800,000 cells/cm²** on **LN511-coated plates**. Add **10 µM Y27632** for **48 hours** post-replating. Laminin-511 **Biolamina Catalog #**LN511

Y-27632 dihydrochloride **Tocris Catalog #**1254

7 Day 16: Second Replating. Dissociate into single cells.

8 Replate at **800,000 cells/cm²** on **Poly-L-ornithine+LN511-coated plates**. Add **10 µM Y27632** for **48 hours** post-replating. Laminin-511 **Biolamina Catalog #**LN511

Poly-L-Ornithine **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**P4957

Y-27632 dihydrochloride **Tocris Catalog #**1254

Differentiation Media Transition

9 Day 10–28: Switch to Differentiation Media.

- Neurobasal / B27 / L-glutamine base, plus:
  - Neurobasal™ Medium **Gibco - Thermo Fisher Scientific Catalog #**21103049
  - B27 supplement minus vitamin A **Gibco - Thermo Fisher Scientific Catalog #**12587010
  - L-Glutamine **Gibco - Thermo Fisher Scientific Catalog #**25030024
- BDNF – 20 ng/mL
  - Human Recombinant BDNF **STEMCELL Technologies Inc. Catalog #**78005
- GDNF – 20 ng/mL
  - Human Recombinant GDNF **STEMCELL Technologies Inc. Catalog #**78058
- TGF-β3 – 1 ng/mL
  - Tgf beta 3 (human) Recombinant Protein **Invitrogen - Thermo Fisher Catalog #**RP8600
- Ascorbic acid – 0.2 mM
  - Ascorbic Acid 500 mg **STEMCELL Technologies Inc. Catalog #**72132
- Dibutyryl cAMP – 0.2 mM
  - Dibutyryl-cAMP **STEMCELL Technologies Inc. Catalog #**73884

Note on Supplements Withdrawal:

- **LDN193189, SB431542, and Purmorphamine** are withdrawn after **Day 7**.
- **N2 supplement** is withdrawn after **Day 10**.

Additional Small Molecule Treatments

10 Day 12–15: Add **GW3965** at **10 µM** to promote mDA identity.

11 Day 16–21: Administer Dual Inhibitor Cocktail.

- **PD0325901** – 1 µM
  PD0325901 1 mg **STEMCELL Technologies Inc. Catalog #**72182
- **SU5402** – 5 µM SU5402 1 mg **STEMCELL Technologies Inc. Catalog #**73912

12 Day 26–28: **Neurogenesis Promotion.**
Add **DAPT** at **10 µM** to promote neuronal maturation.