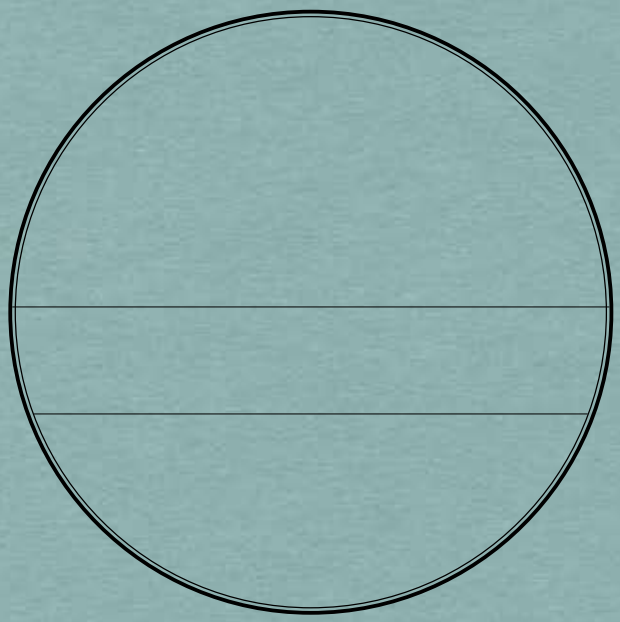
iCeMS - CiRA Classroom

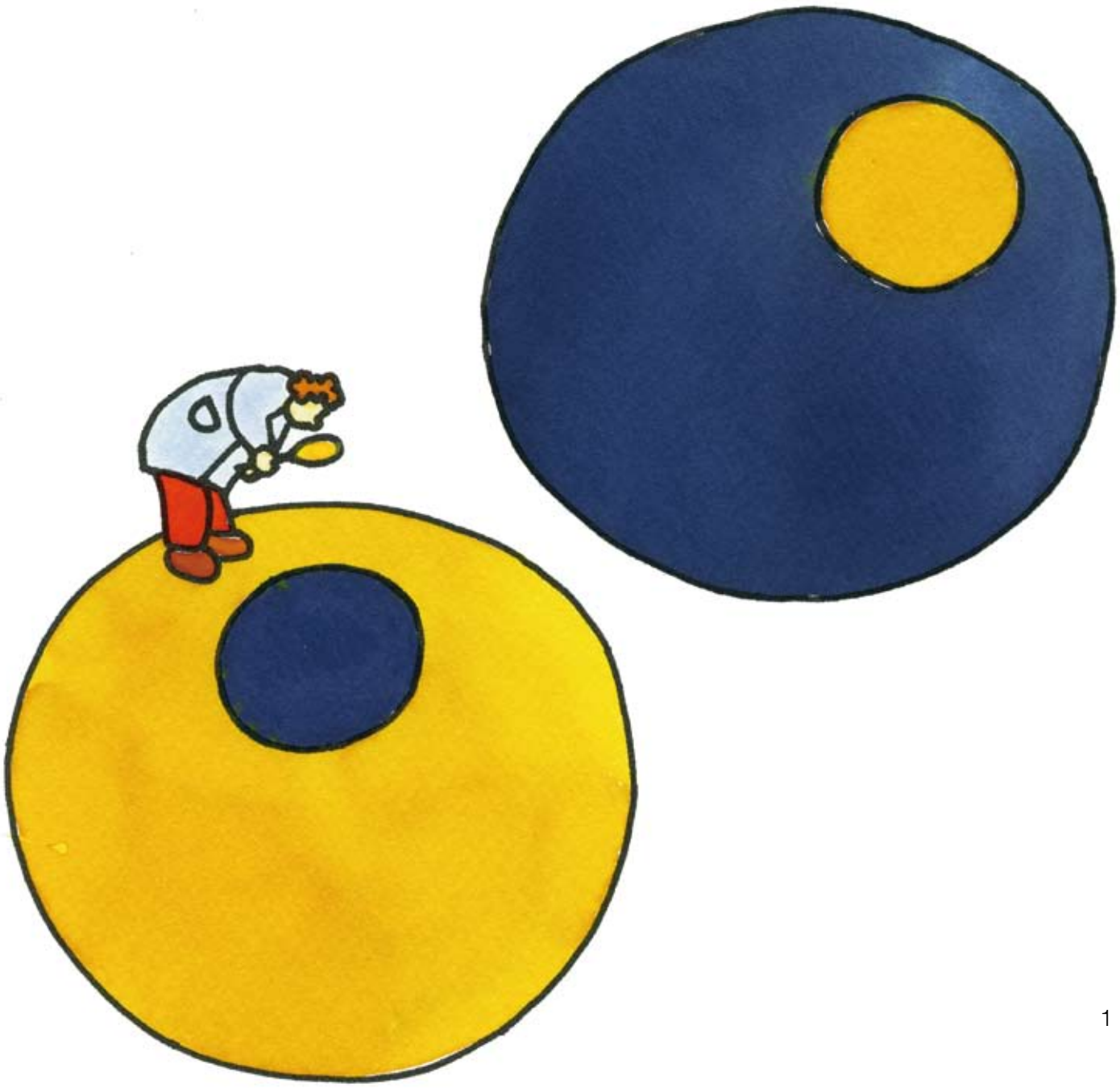
Hands-on with Stem Cells!

WORK BOOK

Institute for Integrated Cell-Material Sciences, Kyoto University (iCeMS)
Center for iPS Cell Research and Application (CiRA), Institute for Integrated Cell-Material Sciences, Kyoto University



Are ES cells the same as iPS cells generated from the skin cells?



Flow chart

1. Propose a mission using stem cells
↓
2. Design a hypothesis around the agreed mission
↓
3. Examine the hypothesis through carefully planned experiments

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Contents

To ensure your safety and enjoyment during the laboratory exercises, we ask for your cooperation in carefully observing some basic regulations and guidelines:

1 Keep valuables in your possession at all times.
2 Wear your name tag at all times.
3 Do not bring any food or drink into the laboratory.
4 Wash your hands before entering and exiting the laboratory.
5 Do not touch any equipment without being instructed to do so.
6 If you are injured or sick, please notify the staff immediately and follow their instructions.

Please contact the laboratory staff if you have any questions.

TODAY'S **Mission!**

To determine whether ES cells are the same as iPS cells generated from skin cells

Factors to assume that ES cells are the same as iPS cells

How to investigate

List as many as you can. Ask your friends.

Hypothesis

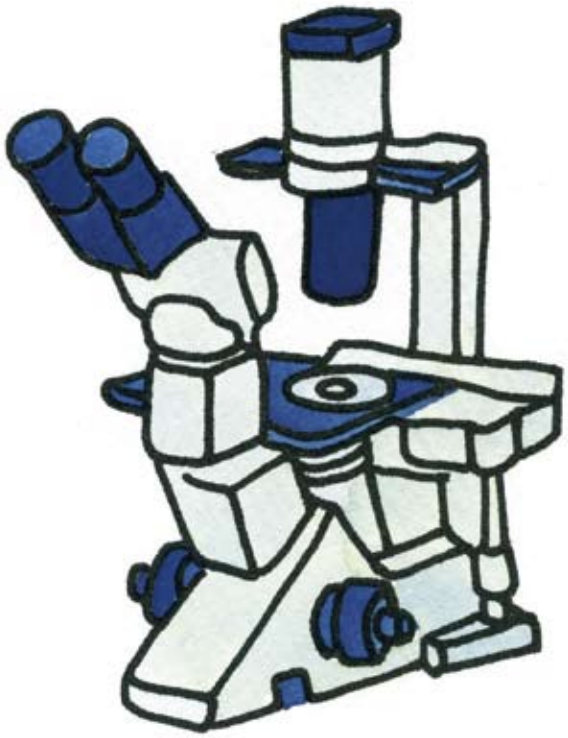
Experiment 1

Comparing pluripotent cells with fibroblasts

What you will use in this experiment

Equipment

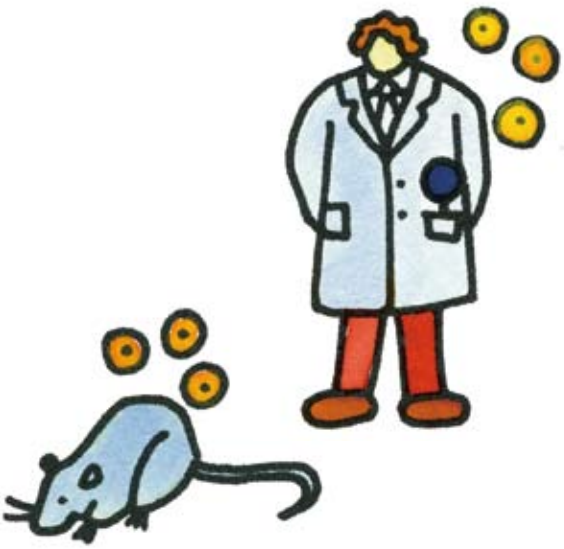
- Phase-contrast inverted microscope
- Fluorescence microscope



Cells

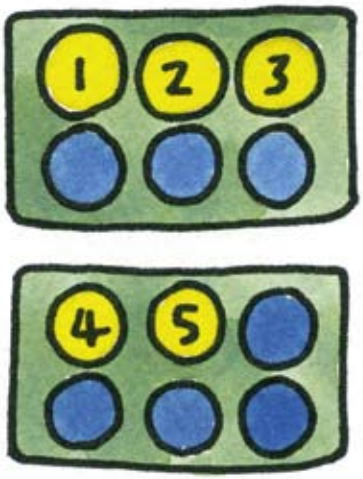
- Mouse fibroblasts (skin cells)
- Mouse ES cells
- Mouse iPS cells
- Human fibroblasts (skin cells)
- Human iPS cells

✳All of the above MOUSE cells have the Nanog reporter gene, a marker for pluripotent stem cells.



Preparation for the experiment

Wash your hands and wear latex examination gloves at all times.



1 Firstly, observe the cells in the (2) of the 6-well plate.

Observation and notes

Q. How many kinds of cells are there in this well?

A. _____ kinds of cells

2 Compare the mouse fibroblasts(1) with mouse ES cells(2).

Observation and notes

Q. What's the difference in the shape of these cells?

Q. What's the difference in their size?

Q. What's the difference in their color?

3 Compare the mouse ES cells (2) with mouse iPS cells (3).

Observation and notes

Q. What's the difference in the shape of these cells?

Q. What's the difference in their size?

Q. What's the difference in their color?

4 Observe the mouse fibroblasts (1), mouse ES cells (2), and mouse iPS cells (3) under the fluorescence microscope.

Observation and notes

Q. Do the cells glow green What's the difference in the light between these cells?

Q. What do these results show?

5 Compare the human fibroblasts (4) with human iPS cells (5).

Observation and notes

Q. What's the difference in the shape of these cells?

Q. What's the difference in their size?

Q. What's the difference in their color?

6 Compare the human fibroblasts (4) with human iPS cells (5) under the fluorescence microscope.

Observation and notes

Q. Do they glow green? What's the difference in the light among these cells?

Q. What do these results show?

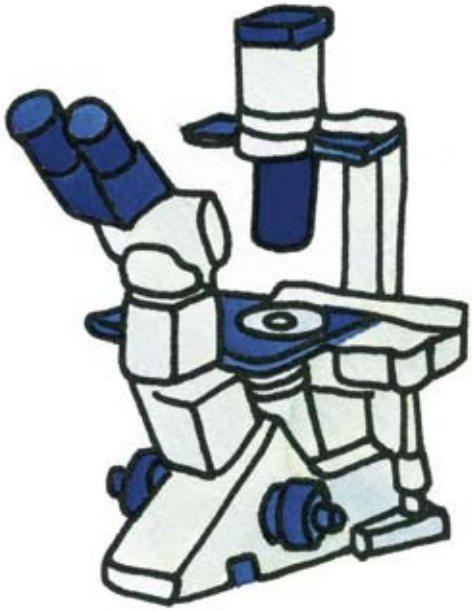
Experiment 2

Comparing stained pluripotent cells with stained fibroblasts

What you will use in this experiment

Equipment

- Fluorescence microscope

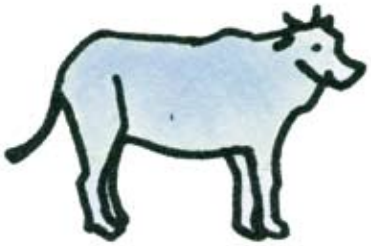


Instruments

- 6-well plate
- Micropipette (2-20μL)
- Micropipette (100-1000μL)

Reagents

- PBS (Phosphate Buffered Saline)
- 1% BSA (bovine serum albumin) in PBS
- 5% BSA in PBS
- 0.2% Triton in PBS
- 4% PFA (paraformaldehyde) in PBS



Antibodies

- First antibody
  Anti-Nanog antibody
- Second antibody
  Alexa488 anti-rabbit IgG

✳Alexa488: Green fluorescen

Cells

- Human fibroblasts
- Human iPS cells

Flow chart

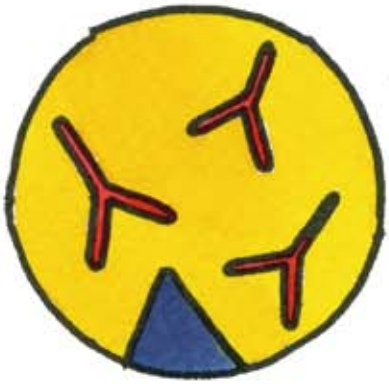
1 Cell preparation

2 Cell fixation

3 Breaking down the cell membranes

4 Blocking

5 First antibody response



6 Second antibody response

7 Observation

Experiment 2

Protocol

1. Prepare the cells.
2. Wash each well with 2mL PBS.
3. Fix the cells for 20 min in 4% PFA in PBS.
4. Wash each well with 2mL PBS.
5. Add 2mL of 0.2% Triton in PBS for 5 min to break (permeabilize) the cell membranes.
6. Wash each well with 2mL PBS.
7. Add 5% BSA in PBS and then incubate at room temperature for 60 min.
8. Dilute the anti-Nanog antibody by 1/500 with 1% BSA in PBS.
9. Add the prepared dilution into each well.
10. Incubate overnight at 4°C.
11. Suck and discard the solution from each well using a micropipette (100-1000μL).

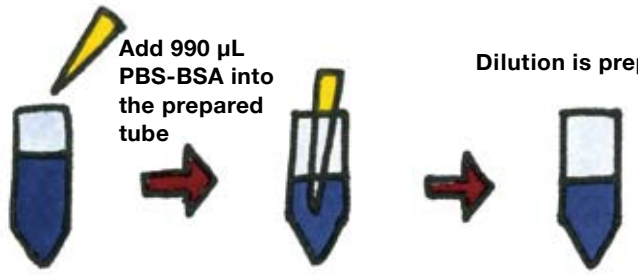
    CAUTION! Proceed immediately to the next step, to prevent drying of the wells.
12. Add 2mL PBS into each well by micropipette (100-1000μL) and incubate at room temperature for 5 min.
13. Repeat steps (11) and (12) twice.

——Staff have previously prepared these

—— YOU will implement the following procedures:

14. Dilute the Alexa 488 anti-rabbit IgG antibody to 1% with PBS containing BSA.
    (Add 990 µL PBS-BSA into the prepared tube, which includes 10 µL Alexa 488 anti-goat IgG)
    Prepare this dilution a second time.

    CAUTION! Mix gently, preventing the formation of foam.

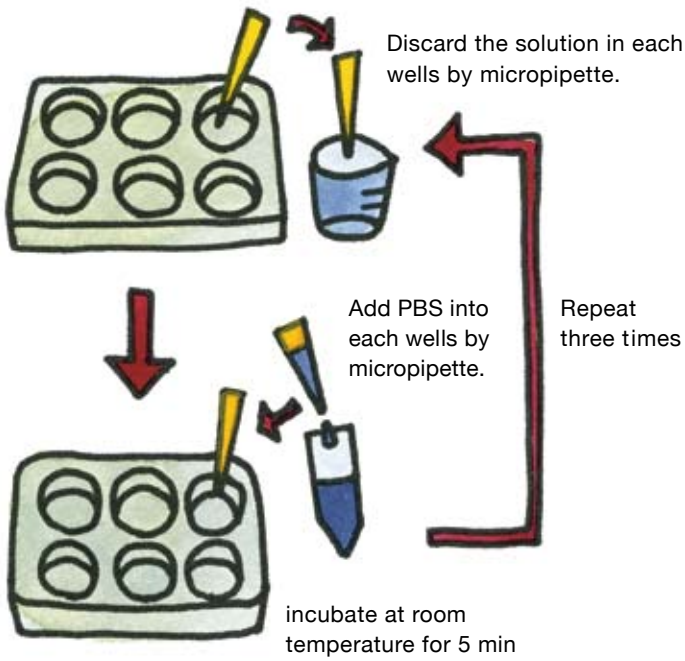


15. Suck and discard the solution from each well using a micropipette (100-1000µL).
16. Add 900µL of the dilutions prepared in step (14) to each well.
17. Cover the 6-well plate with alminium foil and then incubate at room temperature for 60 min.

    Start ____ : ____ Finish ____ : ____

18. Repeat steps (11) and (12) three times.

    CAUTION! While incubating for 5 min at room temperature, cover the 6-well plate with aluminum foil to prevent the discoloration.



Observe under the fluorescence microscope, your heart should be racing!

Experiment 2

1 Compare the human fibroblasts (4) with the human iPS cells (5) under the fluorescence microscope.

Observation and notes

Q. Do the cells glow green? What is the difference in the light among these cells?

Q. What do these results show?

? QUESTIONS!

Think about the following questions on the differentiation capabilities of ES/iPS cells.

Q1. Can ES/iPS cells differentiate into muscle cells?

How do you determine this?

Q2. Can ES/iPS cells differentiate into nerve cells?

How do you determine this?

Q3. Can ES/iPS cells differentiate into each cell type in your body?

How do you determine this?

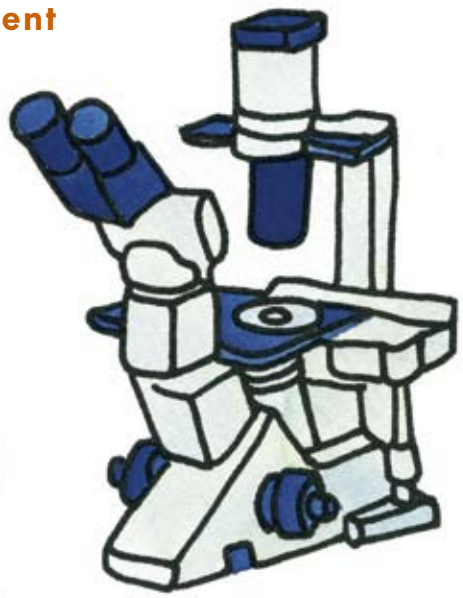
Experiment 3

Comparing human iPS cells with nerve cells differentiated from human iPS cells

What you will use in this experiment

Equipment

- Phase-contrast inverted microscope



Cells

- Human iPS cells
- Nerve cells differentiated from human IPS cells

1 Observe human iPS cells and nerve cells differentiated from human iPS cells.

Q. What is the difference between the two?
Record as many of your observations as possible.

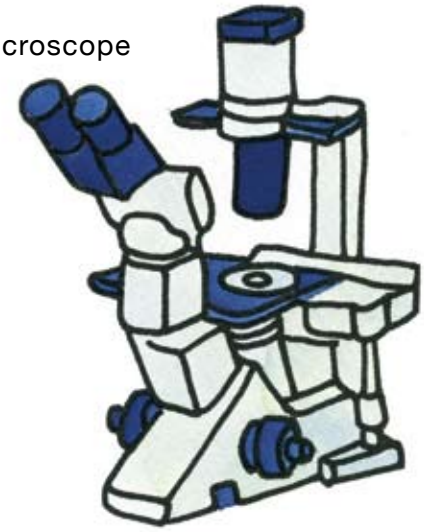
Experiment 4

Observing stained nerve cells, differentiated from human iPS cells

What you will use in this experiment

Equipment

- Fluorescence microscope



Cells

- Stained nerve cells differentiated from human iPS cells
  ✳ Staff have pre-stained the cells.

Antibodies

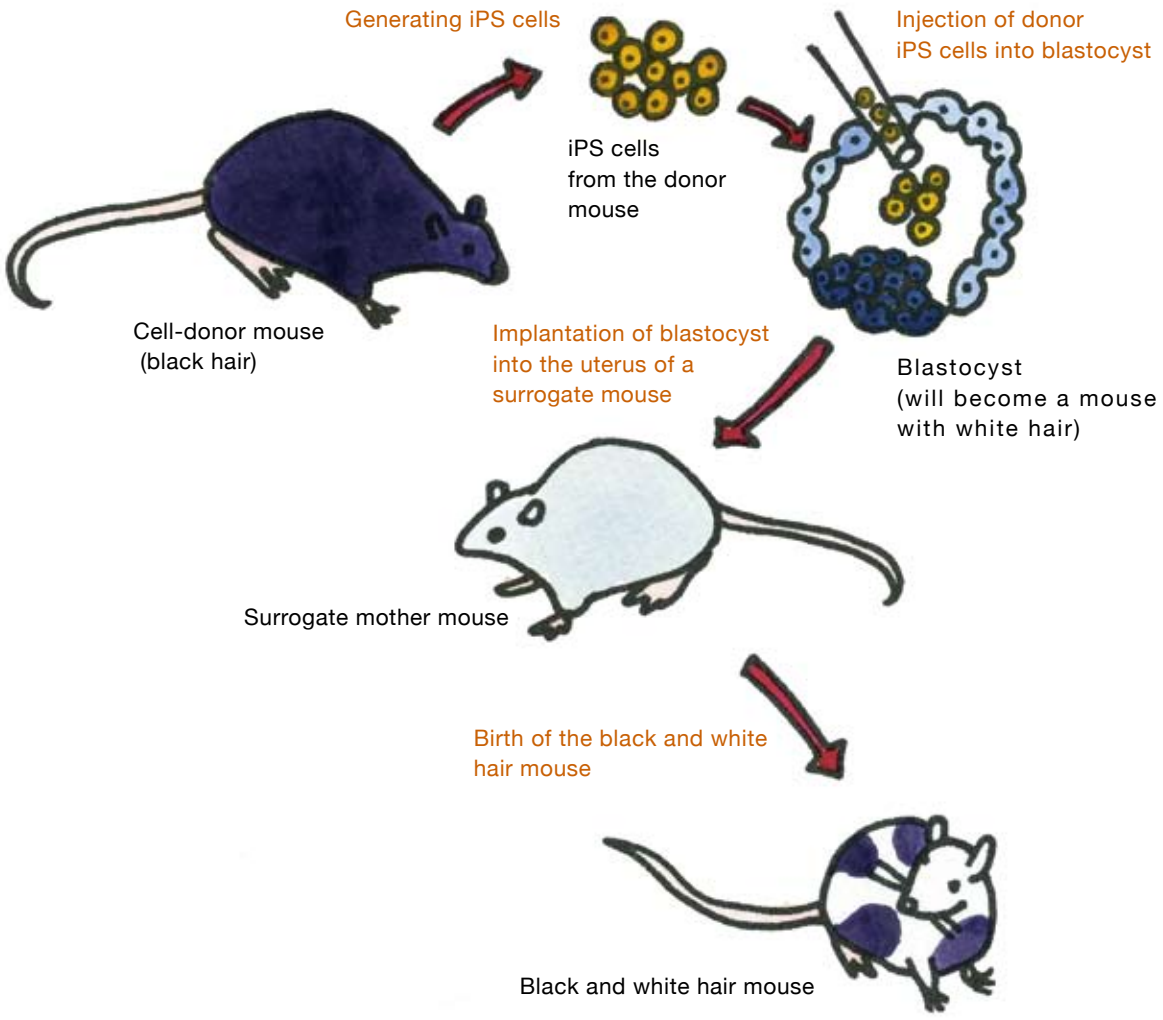
- First antibody
  Anti-TuJ1 antibody
  ✳ TuJ1: nerve cell marker
- Second antibody
  Alexa488 anti-mouse IgG
  ✳ Alexa488: Green fluorescent dye molecule

1 Observe the stained nerve cells, differentiated from human iPS cells, under the fluorescence microscope.

Q. Can human iPS cells differentiate into nerve cells?
Record as many of your observations as possible.

Let's think…

Let's think about the experiments using mouse iPS cells.



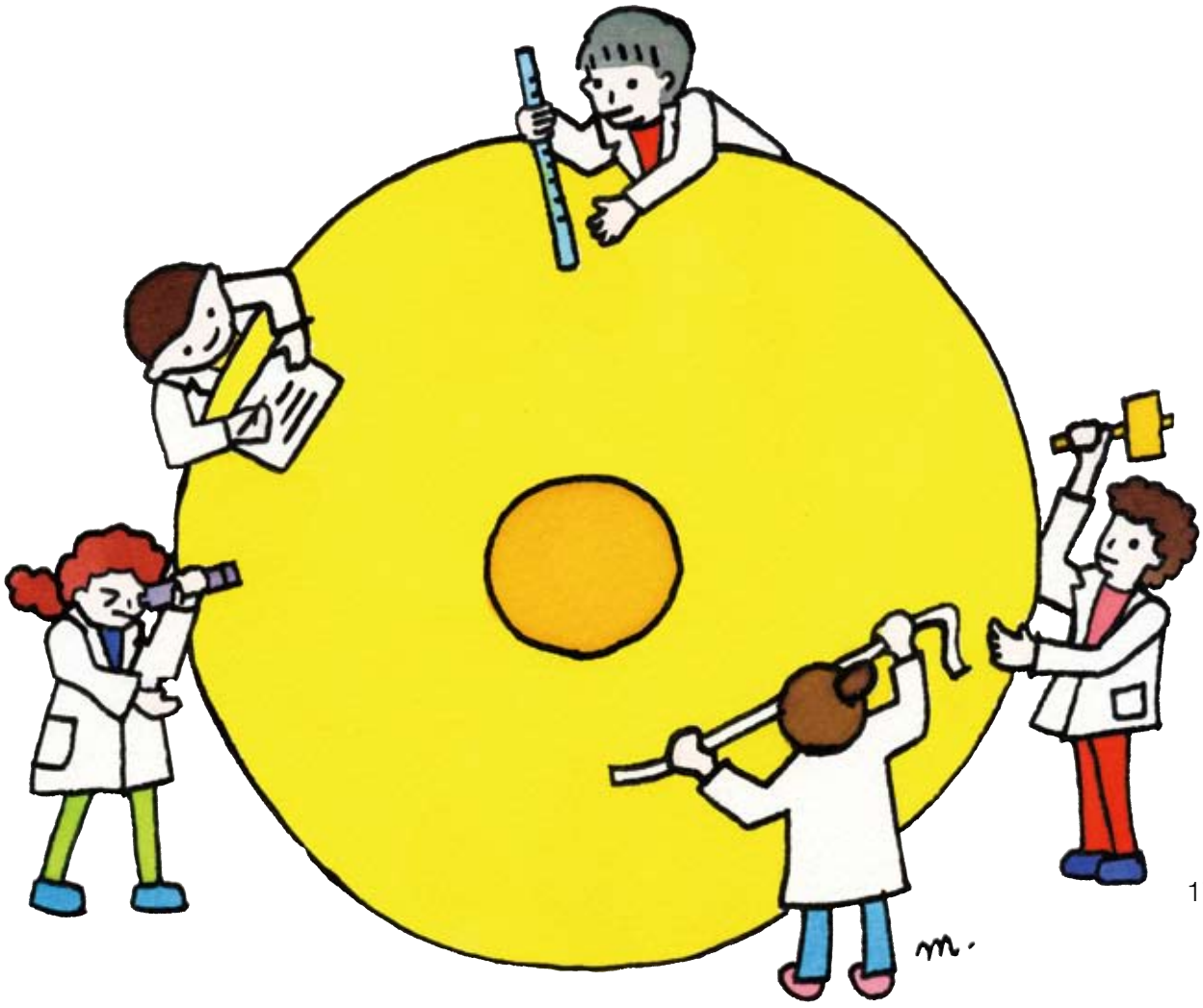
Answer the following question, taking into consideration the fact that a black and white hair mouse was born.

Q. What types of cells did iPS cells differentiate into?

Quick Review

Now you can ...

1. Make a hypothesis.
2. Give it a try.
3. Explain and organize results from experiments.



Glossary

Feeder Cells

- feed the neighbor cells.
- are modified not to grow.
- help ES/iPS cells to grow.

Nanog

- An essential protein in maintaining the pluripotency of pluripotent stem cells.
- A marker of pluripotent stem cells as its gene is always switched ‘ON’ in pluripotent stem cells.
- Named after the Irish term ‘Tir nan Og’, meaning ‘Land of Youth’.

TuJ1

- Frame nerve cells.
- A marker of nerve cells as its gene is always switched 'ON' in nerve cells.

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