[Protocol 4.04] Chromosome preparation for Karyotyping of iPSCs

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Version: 1.0

1. Introduction and Purpose

This protocol describes the preparation of iPSCs for karyotyping at the institute of human genetics at Universitätsklinikum Jena. Karyotyping is an important tool in quality control of iPSCs, since chromosomal abnormalities and changes in gene expression and cellular functions can occur during long-term culture. This can even increase the risk of tumorigenic iPSCs. Chromosomes will be stained during the metaphase of mitosis and analyzed by light microscopy (in Jena). The number of chromosomes in a cell as well as their formation, relative and absolute size, location of the centromere, secondary constrictions, specific banding patterns, and chromatin distribution in the chromosome can be determined. Based on these features the genomic integrity of the iPSCs, e.g. deletion and duplication, can be determined.

2. Media and Reagents

Reagents

- Colcemide (Biochrom AG, L6221)
- Potassium chloride (PCL) (Merck, 1049360500)
- Methanol
- Acetic Acid
- Fixative
- TrypLE-Select (1x) (Thermo Fisher, 12563011)
- PBS w/o Ca2+Mg2+ (Thermo Fisher, 14190250)
- Y-27632 ROCK inhibitor (Stem Cell Technologies,72305 or Selleck Chemicals, SEL-S1049-10mM)
- KnockOut™ Serum Replacement (Thermo Fisher, 10828010)

Media

Prepare maintenance medium in regarding protocol 1.07, 1.08 or 1.09
Y-27632 ROCK inhibitor (Stem Cell Technologies,72305 or Selleck Chemicals, SEL-S1049-10mM)
Add ROCK inhibitor regarding protocol 1.03.1

3. Safety notes

- Methanol, acetic acid as well as fixative must be used oder fume hood
- Supernatant of fixative must be discarded into a glas covered with aluminum foil under fume hood
- After step 9 cells can leave Cell culture lab

4. Preparation Solutions

Potassium chloride solution (1000ml)

Mix 5.59 g (PCL) with 1000 ml dH2O

- Store at 2-8 °C for 6 month

96% Methanol (1000ml)

Mix 960 ml 100% Methanol with 40ml dH2O

- Work under fume hood
- Store at RT

Fixative (one sample)

Mix 24ml 96% Methanol with 8ml acetic acid

- Prepare on day of preparation,
- Work under fume hood

10% KSR media (one sample)

Mix 1ml KSR with 9ml cell media + ROCKI 1:2000

Media + 0.1µg/ml colcemide (one sample)

Mix 100µl colcemide with 10ml medium + ROCKI 1:2000

5. Materials and Equipment:

- Geltrex coated T25 Flasks (2 per cell line) *refer to protocol 1.05*
- Aspiration Pump
- 2ml/5ml/10ml pipettes
- Cell counting equipment
- 15/50 ml Falcon tube
- Pipette-boy
- Big centrifuge
- Aspiration pipettes
- Sterile 1ml pipette tips

6. Prearation Cells

1. Split cells one day before preparation using *[Protocol 1.03.0] Passaging of iPSC into single cells using TrypLE/Accutase* to reach 50% confluence the next day.
    - One well on 6 well plate with 80 % confluence per T25 flask
    - If cells are thawed fresh they need to be passaged minimum twice before preparation

7. Procedure

1. Discard culture media and add 5 ml of 0.1 µg/ml colcemide media to the Flask.
2. Incubate 2.5 h at 37 °C
3. Wash cells with 6 ml PBS
    - do not touch cells
4. Discard PBS and add 2.5 ml/flask TrypLE-select (1x)
5. Incubate 4-5 min at 37 °C
6. Detach cells with 5 ml/flask 10 % KSR media and pool cells from both flasks to one 50 ml Falcon
    - KSR is used to inactivate TrypLE
7. Centrifuge at 1500 rpm for 7 min
8. Discard supernatant leaving 1 ml to resuspend cell pellet
9. Resuspend cell pellet and transfer to 15 ml Falcon Tube
10. Add 10 ml PCL solutionincubate 20 min. at 37 °C
11. Add 1 ml ice cold fixative and invert to mix
12. Centrifuge at 1500 rpm for 7 min
13. Leave 1 ml supernatant and discard rest
14. Resuspend cell pellet in the rest of the supernatant
15. Add 7 ml fixative 1 ml at a time
    - invert between each step
16. Centrifuge at 1500 rpm for 10 min
17. Discard supernatant leaving 1 ml
18. Repeat Steps 13-15 two times
19. Finally discard supernatant and resuspend cell pellet in 2 ml fresh fixative
20. Transfer cell suspension to safe lock Tube 2 ml
21. Seal with parafilm and store at -20 °C

8.Shipping of cells

- After the cells were prepared they need to be sent to the address below.
- The cells need to be packed well so nothing leaks and can then be shipped at room temperature by normal post.
- Contact person at the institute for human genetics for questions regarding the protocol or the shipping is Dr. Anja Weise (Anja.Weise@med.uni-jena.de)

Shipping address

Dr. Anja Weise
Institut für Humangenetik
Universitätsklinikum Jena
(FUI E20.058)
Am Klinikum 1
D-07747 Jena
Germany

Relevant applicable documents:

Protocol 1.03.1Reconstitution, aliquoting and use of Y26732 ROCK inhibitor
Protocol 1.05 Geltrex coating of culture vessels
Protocol 1.07 Preparation of E8 medium
Protocol 1.08 Preparation of mTeSR1 medium
Protocol 1.09 Preparation of stemMACS/UPPS medium