Sample preparation for aCGH karyotyping

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ABSTRACT

This protocol describes the standard procedure preparing cell pellets from human pluripotent stem cells (hPSCs) cultured on MEFs for outsourced aCGH karyotyping.

General Notes:

1. Throughout these protocols, the term hPSC is used to collectively refer to both hiPSCs and hESCs. All described procedures have been tested and work equally well for hiPSCs and hESCs.

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MATERIALS TEXT

A	B	C
Item	**Vendor**	**Catalog #**
DMEM/F12	Thermo Fisher	11320082
Knockout Serum Replacement	Thermo Fisher	10828-028
Newborn Calf Serum	Sigma	N4762
L-Glutamine	Sigma	G8540
Penicillin & Streptomycin (100X)	Thermo Fisher	15140163
MEM Non-Essential Amino Acids (100X)	Thermo Fisher	11140050
Collagenase type IV	Thermo Fisher	17104019
40 micron Cell Strainer	Corning	352340
DPBS w/o Calcium and magnesium (DPBS)	Corning	MT21031CV

Collecting hPSCs colonies from MEFs 30m

1 Use one, almost confluent, 6-well plate of hPSCs to prepare cell pellet

2 Wash once with DPBS

3 Add 1 ml of 1 mg/ml collagenase solution into each well to separate hPSC colonies from MEFs. Incubate **00:30:00** at **37 °C** . 30m

3.1 Collagenase solution

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Collagenase type IV	10 mg
KSR medium	10 ml

1 mg/ml
Final volume: 10 ml

KSR medium

DMEM/F12	385 ml
Knockout Serum Replacement	100 ml
L-Glutamine (200 mM)	5 ml
Penicillin & Streptomycin (100X)	5 ml
MEM Non-Essential Amino Acids (100X)	5 ml

Final volume: 500 ml

4 Add 2 ml Wash Medium to each well

4.1 Wash Medium

DMEM/F12	470 ml
Newborn Calf Serum	25 ml
Penicillin & Streptomycin (100X)	5 ml

Final volume: 500 mL

5 Pipette repeatedly with 5 ml pipette to lift colonies. Be careful not to carry over too many MEFs

6 Transfer all colonies into a 15 ml tube

7 Add 7 ml Wash Medium into the 15 ml tube. Pipette with 10 ml Stripette for 5 times to separate MEFs that are attached to hPSCs colonies

8 Place the 15 ml tubes on a tube rack and gravity settle colonies at **Room temperature** for 5m **00:05:00** .

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9 Aspirate supernatant and add 10 ml Wash Medium. Invert the tube 3 times to mix.

10 Gravity settle at **Room temperature** for **00:05:00** . 5m

11 Aspirate supernatant and re-suspend colonies in 10 ml Wash Medium

12 Place a 40 µm cell strainer onto a 50 ml tube

13 Strain the colony suspension from step 11. Keep the strainer since colonies are trapped on it.

14 Wash the strainer with 10 ml Wash Medium (x2)

15 Revert the strainer and place it in a 6-well plate, bottom-up

16 Apply 5 ml Wash Medium to the bottom of the strainer, twice. This will separate colonies from the strainer and wash them into the 6-well plate.

17 Collect colonies from the 6-well plate to a new 15 ml tube

18 Centrifuge the 15 ml tube at **300 x g, 00:05:00** 5m

Aspirate most of the medium. Leave 1 ml of medium

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20 Re-suspend colonies in the remaining medium, then transfer them to a 1.8 ml Eppendorf tube

21 Centrifuge the 1.8 ml tube at **300 x g, 00:05:00** 5m

22 Remove the supernatant and cap the tube

23 Snap freeze the 1.8 ml tube by placing it in liquid nitrogen for more than 5 min.

24 Store the snap frozen samples at **-80 °C** , and ship it to Cell Line Genetics on dry ice

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