Preparing of genomic DNA from in vitro cultured cells

Sep 06, 2022

In 1 collection

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dx.doi.org/10.17504/protocols.io.b4ntqven

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ABSTRACT

This protocol describes a standard procedure for preparing crude cell lysate which can be further analyzed by PCR.

Protocol overview:
A. Preparing crude cell lysate directly from hPSCs culture
B. Preparing crude cell lysate from dissociated cells
C. Preparing cell lysate from FACS-sorted cells (small cell number)

General notes

1. Throughout this protocol, 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.

DOI

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PROTOCOL CITATION

Hanqin Li, Yogendra Verma, Dirk Hockemeyer, Frank Soldner 2022. Preparing of genomic DNA from in vitro cultured cells. **protocols.io** https://dx.doi.org/10.17504/protocols.io.b4ntqven

FUNDERS ACKNOWLEDGEMENT

Aligning Science Across Parkinson's
Grant ID: ASAP-000486

COLLECTIONS

Genotyping by next generation sequencing

KEYWORDS

ASAPCRN

CREATED

Feb 03, 2022

LAST MODIFIED

Sep 06, 2022

PROTOCOL INTEGER ID

57779

PARENT PROTOCOLS

Part of collection

Genotyping by next generation sequencing

MATERIALS TEXT

Item	Vendor	Catalog #
DMEM/F12	Thermo Fisher	11320082
DPBS w/o Calcium and magnesium (DPBS)	Corning	MT21031CV
Newborn Calf Serum	Sigma	N4762
Penicillin & Streptomycin (100X)	Thermo Fisher	15140163
0.25% Trypsin with EDTA (Trypsin)	Thermo Fisher	25200114
Proteinase K	Sigma	P6556
Exact N Amp Blood PCR Kit	Sigma	XNAT2-1KT

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A. Preparing crude cell lysate directly from hPSCs culture 15m

1 Add 500 µl crude lysis buffer (Proteinase K added) to a fully confluent well of a 6 -well plate (~1.5 million cells)

1.1

Crude lysis buffer

A	B
KCl	50 mM
MgCl2	2 mM
NP-40	0.45%
Tween-20	0.45%
Tris	10 mM
Proteinase K (add before use)	250 µg/ml

2 Incubate at **37 °C** **00:10:00** 10m

3 Collect the crude lysate into a 1.7 ml Eppendorf tube.

4 Incubate at **50 °C** **Overnight**

5 Transfer 50 µl to a 200 µl microcentrifuge tube

6 Heat inactivate at **95 °C** for **00:05:00** in a thermocycler. 5m

7 Chill **On ice**

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8 The crude cell lysate is ready for PCR

B. Preparing crude cell lysate from dissociated cells 10m

9 Wash cells with DPBS once

10 For each well in a 6-well plate, use 0.5 ml Trypsin

11 Incubate **37 °C** **00:05:00** 5m

12 Inactivate Trypsin using 2 ml wash medium

12.1 Wash medium

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

Final volume; 500 ml

13 Centrifuge at **200-300 x g, Room temperature, 00:05:00** 5m

14 Remove supernatant

15 For a fully confluent well of a 6-well plate, mix the cell pellet with 500 µl crude lysis buffer (Proteinase K added). Reduce the amount of crude lysis buffer if the culture is not fully confluent.

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16 Transfer the crude lysate into a 1.7ml Eppendorf tube.

17 Incubate at **50 °C** **Overnight**

18 Transfer 50 µl to a 200 µl microcentrifuge tube

19 Heat inactivate at **95 °C** **00:05:00** in a thermocycler 5m

20 Chill **On ice**

21 The crude cell lysate is ready for PCR

C. Preparing cell lysate from FACS-sorted cells (small cell number)

22 For small cell numbers, we usually use a direct cell to DNA extraction kit (Exact N Amp Blood PCR Kit [Sigma, # XNAT2-1KT]) according to the manufacturers' instructions.

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