Sep 06, 2022

Genotyping by next generation sequencing

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dx.doi.org/10.17504/protocols.io.b4n3qvgn

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ABSTRACT

This collection describes a standard genotyping procedure using next generation sequencing (NGS) in the Hockemeyer lab.

Collection overview

Preparing of genomic DNA from in vitro cultured cells
- 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

Amplifying the target genomic region by PCR
Preparing samples for NGS
CRISPResso analysis

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.

DOI

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COLLECTION CITATION

Hanqin Li, Yogendra Verma, Dirk Hockemeyer, Frank Soldner 2022. Genotyping by next generation sequencing. **protocols.io** https://dx.doi.org/10.17504/protocols.io.b4n3qvgn

FUNDERS ACKNOWLEDGEMENT

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

KEYWORDS

ASAPCRN

CREATED

Feb 03, 2022

LAST MODIFIED

Sep 06, 2022

COLLECTION INTEGER ID

57787

Citation: Hanqin Li, Yogendra Verma, Dirk Hockemeyer, Frank Soldner Genotyping by next generation sequencing https://dx.doi.org/10.17504/protocols.io.b4n3qvgn

MATERIALS TEXT

Item	Vendor	Catalog #
DMEM/F12	Thermo Fisher	11320082
DPBS w/o Calcium and magnesium	Corning	MT21031CV
Newborn Calf Serum	Sigma	N4762
Penicillin & Streptomycin (100X)	Thermo Fisher	15140163
0.25% Trypsin with EDTA	Thermo Fisher	25200114
dNTP	NEB	N0447L
10xHF buffer	NEB	B0518S
Phusion DNA polymerase	NEB	M0530S
Wizard SV Gel and PCR clean-up system	Promega	A9301
AMPure XP beads alternative	UC Berkeley	
Microseal® 'B' Adhesive Seals	Biorad	MSB1001
Proteinase K	Sigma	P6556
Exact N Amp Blood PCR Kit	Sigma	XNAT2-1KT

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FILES

Preparing of genomic DNA from in vitro cultured cells
Version 1
by Devin E Snyder

Amplifying the target genomic region by PCR
Version 1
by Devin E Snyder

Preparing samples for NGS
Version 1
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