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Irradiation and single-cell dissociation of hESCs and cortical spheroids

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We use this protocol and it's working

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Abstract

This protocol describes the procedure of irradiation and single-cell dissociation of hESCs and cortical spheroids for MULTI-Seq barcoding and sequencing.

Protocol Overview

A. Irradiation of hESC

B. hESCs derived cortical spheroids

Note

A list of reagents and relevant vendor information can be found in the table listed under the materials tab.

Attachments

PDF

Irradiation of hESCs...

62KB

Materials

Reagents

	Item	Vendor	Catalog Number
	10x HBSS (Ca and Mg Free)	Invitrogen	14185-052
	Sodium Pyruvate 100mM	Life Tech	11360070
	D-Glucose	Sigma	G8769-100ml
	HEPES pH 7.3	Invitrogen	15630-080
	Y-27632 – ROCK Inhibitor	Chemdea	CD0141
	Accutase	Thermo Fisher Scientific	SCR005
	Ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA)	Sigma	E6635-100G
	2-Mercaptoethanol	Sigma	M3148
	L-Cystine solution	Sigma-Aldrich	C7352-100G
	Papain suspension	Worthington Biochemical	LS003126
	PBS- (without Ca and Mg)	Corning	MT21031CV
	PBS+ (with Ca and Mg)	Thermo Fisher	14040-182
	Trypsin Inhibitor	Sigma	T9253-5G
	Costar‰ 6-well Clear Flat Bottom Ultra-Low Attachment 6-well plate	Corning	3471

Irradiation of hESCs

10m

1 Culture hESCs with feeder-free system (dx.doi.org/10.17504/protocols.io.b4mcqu2w)

2 3 days before irradiation, passage cells and let them grow to approximately 50% confluence.

3 On the day of irradiation, change media to hESC media + 10uM Rock Inhibitor (RI).

4 Using a discrete cesium source, irradiate cells in plates at desired dosage. We used 0, 0.5, 2, 5 and 10 Gy.

5 24 hours post irradiation, dissociate cells by aspirating media and adding 1 mL of accutase per well of a 6-well plate. Return to incubator for 00:05:00 to 10 min. 5m

6 Resuspend cells using 10 mL of PBS- to dilute accutase. Spin down for 00:05:00 at 300 x g . 5m

7 Aspirate supernatant and resuspend cells in 1 mL PBS- per well.

8 Label cells with MULTIseq oligos as described in MULTI-Seq Barcoding and Library Preparation protocol (dx.doi.org/10.17504/protocols.io.kxygx3xzkg8j/v1) and proceed with sequencing.

hESCs Derived Cortical Spheroids

2h

9

Note

Dissociation Media (500mL):

- 48.9 mL 10x HBSS (Ca and Mg Free)
- 440 mL H2O
- 5 mL Sodium Pyruvate 100mM
- 1.1 mL D-Glucose
- 5 mL HEPES pH 7.3

Differentiate hESCs to Cortical spheroids as described in Cortical Spheroid Differentiation protocol (https://doi.org/10.17504/protocols.io.5jyl8po57g2w/v1.).

10 At 25 days in differentiation, two organoids per condition were transferred with 1.5 mL of media into a 1.5mL Cryovial with screw top. Using a discrete cesium source, irradiate organoids in vials at desired dosage. We used 0, 0.5, 2, 5 and 10 Gy.

11 1. Post-irradiation, transfer both organoids per condition into one well of a 6-well low adherence plate with 2.5 mL additional media. Add Rock Inhibitor (RI) to final concentration of 10uM.

12 24 hours post irradiation, prepare 5 mL of Activated Papain Solution per irradiation condition:

12.1 To each 5mL of Dissociation Media, add 12 µL of 0.5M EDTA, 23ul of 0.1uM β-mercaptoethanol, 5 µL of 5.5mM L-Cystine solution, and 172 µL of papain suspension.

12.2 Transfer the solution to the 37 °C water bath for ~ 00:20:00 to 30 minutes to activate. The solution will gradually go from cloudy to transparent. 20m

12.3 Sterilize by passing the solution through a 0.22 µm syringe filter.

13 Collect cortical spheroids and sediment. Aspirate the media and rinse once with PBS+.

14 Add 5 mL Activated Papain Solution to each well and return to the incubator for ~ 00:45:00 . 45m

15 While dissociating, prepare 15mL Trypsin Inhibitor solution per 5 mL of Activated Papain Solution.

15.1 For each 15mL Trypsin Inhibitor Solution: Add 15 mg Trypsin Inhibitor to 15 mL of usual organoid media. Mix thoroughly and sterilize by filtering through a 0.22 µm syringe filter.

16 After 00:45:00 of dissociation, add 5 mL of Trypsin Inhibitor solution to each well and very gently triturate with a 5mL strippette to dissociate. Avoid excessive pipetting and bubbles. 45m

17 Strain mixture through a 70µm cell strainer into a 50mL conical tube.

18 Use remaining 10 mL Trypsin Inhibitor Solution to rinse the well to collect any cells left behind, then pass this through the same 70µm strainer to release any cells still stuck in the mesh.

19 Spin down cells at 300 x g for 00:05:00 and aspirate the media. 5m

20 Resuspend dissociated cells in 4 mL 0.2% BSA in PBS+ with RI per condition, strain through a 40um mesh cell strainer and FACS sort for desired number of live cells and to remove debris.

21 Spin down live cells at 300 x g for 00:05:00. Resuspend in 0.2% BSA in PBS+ with 10uM RI in appropriate volume according to single-cell sequencing protocol, count cells, and proceed to single-cell sequencing according to manufacturer protocol. 5m