

Irradiation and single-cell dissociation of hESCs and cortical spheroids.

Reagents

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

hESCs

1. Culture hESCs with feeder-free system ([dx.doi.org/10.17504/protocols.io.b4mcqu2w](https://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 1mL of accutase per well of a 6-well plate. Return to incubator for 5-10 min.
6. Resuspend cells using 10mL of PBS- to dilute accutase. Spin down for 5 min at 1000 RPM.
7. Aspirate supernatant and resuspend cells in 1mL PBS- per well.
8. Label cells with MULTIseq oligos as described in MULTI-Seq Barcoding and Library Preparation protocol (<https://doi.org/10.17504/protocols.io.kxygx3xzkg8j/v1>) and proceed with sequencing.

hESCs Derived Cortical Spheroids

Dissociation Media (500mL):

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

1. Differentiate hESCs to Cortical spheroids as described in the Cortical Spheroid Differentiation protocol found at: <https://doi.org/10.17504/protocols.io.5jyl8po57g2w/v1>.

2. At 25 days in differentiation, two organoids per condition were transferred with 1.5mL 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.
3. Post-irradiation, transfer both organoids per condition into one well of a 6-well low adherence plate with 2.5mL additional media. Add Rock Inhibitor (RI) to final concentration of 10uM.
4. 24 hours post irradiation, prepare 5mL of Activated Papain Solution per irradiation condition:
 - a. To each 5mL of Dissociation Media, add 12uL of 0.5M EDTA, 23uL of 0.1uM β -mercaptoethanol, 5uL of 5.5mM L-Cystine solution, and 172uL of papain suspension.
 - b. Transfer the solution to the 37C water bath for ~20-30 minutes to activate. The solution will gradually go from cloudy to transparent
 - c. Sterilize by passing the solution through a 0.22 μ m syringe filter.
5. Collect cortical spheroids and sediment. Aspirate the media and rinse once with PBS+.
6. Add 5mL Activated Papain Solution to each well and return to the incubator for ~45 minutes.
7. While dissociating, prepare 15mL Trypsin Inhibitor solution per 5mL of Activated Papain Solution.
 - a. For each 15mL Trypsin Inhibitor Solution: Add 15mg Trypsin Inhibitor to 15mL of usual organoid media. Mix thoroughly and sterilize by filtering through a 0.22 μ m syringe filter.
8. After 45 min 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.
9. Strain mixture through a 70 μ m cell strainer into a 50mL conical tube.
10. Use remaining 10mL Trypsin Inhibitor Solution to rinse the well to collect any cells left behind, then pass this through the same 70 μ m strainer to release any cells still stuck in the mesh
11. Spin down cells at 300 x g for 5 min and aspirate the media.
12. Resuspend dissociated cells in 4mL 0.2% BSA in PBS+ with RI per condition, strain through a 40 μ m mesh cell strainer and FACS sort for desired number of live cells and to remove debris.
13. Spin down live cells at 300 x g for 5 min. 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.