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ES cells general methods

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ABSTRACT

general methods for mouse ES cell culturing

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BEFORE STARTING

All media should be warmed up to **37 °C** in a water-bath before use.

Spray all the bottles (except new culture flasks or tubes) before putting into the hood.

Reactivating cells from liquid nitrogen stock

1 Reactivating cells for liquid nitrogen stock

1.1 Have a 15ml Falcon tube (for resuspending cells) and T25 flask ready.Add ~2ml of0.1% gelatine to cover the bottom of T25.Let the gelatine set for **00:15:00** 15m

ES cells need the gelatine layer for proper attachment. To make 0.1% gelatin add 0.5g of gelatin powder to 500ml of PBS and autoclave. Keep at RT afterward

1.2 Thaw cells in a **37 °C** water bath (in a beaker with a float).

37 °C water: heat ~150ml of water in microwave for **00:00:15**

1.3 Spray a tube with 70% EtOH and wipe off. Transfer all thawed cells (~1ml) into the 15ml Falcon tube.

Carefully open the cap of the cryo tube. Cells at the screwing “neck” may spill out when liquid nitrogen trapped in the tube is released suddenly.

1.4 Drop by drop, and swirl from time to time, add 9ml ES complete medium or EC10 medium. Final volume of cells is 10ml. (DMSO is diluted by 10 times, from 10% in the freezing medium to 1%).

1.5 Spin the cells down in the centrifuge at room temperature, 1000rpm for **00:05:00**

1.6 While the cells are spinning, aspirate the gelatine from the T25. Then, add 1-2ml of ES complete medium to wet the bottom surface so that it is ready to receive the cells.

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Cells do not like dry surfaces.

1.7 When cells are spun down, aspirate all the supernatant. Be careful not to suck in the cell pellet! Resuspend cells in 4-5ml of ES complete medium.

1.8 Transfer ALL resuspended cells from Falcon tube to the flask.

1.9 A proportion of cells die when frozen, so it is better to transfer them all to the new flask, ensuring we got enough cells to seed the new culture.

Splitting confluent cells

2 Splitting confluent cells

2.1 Add gelatine to a new flask for receiving split cells.

2.2 Aspirate all medium in the flask. Wash the cells with 5ml PBS. Shake gently, then aspirate all the liquid.

2.3 Add 0.7ml of trypsin (for a T25) to the cells. Incubate the flask at 37°C for 5 minutes. Shake vigorously to split clumps of cells if necessary.

(Sometimes over-trypsinised cells become even stickier than before. Check how the cells look under the microscope from time to time to decide the optimal incubation time. Do not incubate at 37°C or leave at RT after shaking – add meadium immediately to prevent secondary stickiness!)

2.4 While the cells are trypsinising, aspirate gelatine from the new flask and replace with 1-2ml of ES complete medium.

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2.5 Inactivate the trypsin by resuspending the cells in 4-5ml of ES complete medium. Make sure an even cell suspension is obtained by gently pipetting up and down. Then, using the same pipette, transfer a proportion of resuspended cells from the old flask to the new one.

cells require splitting 1:6-1:8 every second day on average

Freezing cells in liquid nitrogen (from a confluent T75)

3 Freezing cells in liquid nitrogen

3.1 Split cells as described. Transfer resuspended cells to a Falcon tube, and spin cells down at 1000rpm for 3-5 minutes at room temperature (🌡 **25 °C**).

3.2 While the cells are spinning, prepare 5ml of foetal calf serum (FCS) with 20% DMSO.

i.e. 4ml FCS + 1ml DMSO.

3.3 Resuspend cells in 4ml FCS. Gently but quickly add in an equal volume (4ml) of FCS + DMSO. (DMSO is toxic to cells.) The final volume of cells will be 8ml and DMSO concentration will be 10%.

DMSO reduces the formation of ice crystals in a culture so that cell membranes do not burst.

3.4 Aliquot 1ml of resuspended cells into each freezing vial (cryo tubes).

3.5 Immediately transfer vials to Mr Frosty (box with a sponge soaked with

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isopropanol) and then to -80 °C freezer. Cells will be frozen gradually to avoid bursting (temperature drops by 1 °C per minute).

3.6 Transfer vials to liquid nitrogen canister the next day. When thawing the vial, plate cells into T25 flask.

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