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RETINOIC ACID DIFFERENTIATION OF ES CELLS

(modified Edith Heard protocol (11/12/03), based on Austin Smith, J. Tiss. Cult. Meth. 13:89-94, 1991):

DAY 0

ES cells should be at 70-80% confluency

- Wash cells once with PBS
- Trypsinise as usual
- Seed cells onto gelatinised flasks or coverslips or slides at a density of **4-5 x10^4 cells/cm^2** in ES cell medium.
- Allow cells to attach overnight (or for 8 hours minimum)
- Next day, wash twice with DMEM (minus FCS) and once with PBS, and then add differentiation medium:

  DMEM/10% FCS (ie "Fibroblast" medium; NO LIF)

  β-mercaptoethanol

  Retinoic Acid (RA) (FINAL conc. of RA on the cells should be **10^{-7}M**)

{NB if there is extensive cell death, the concentration of RA (for inducing differentiation but not causing too much cell death) may have to be adjusted for different ES cell lines, and for different batches of serum.}

- Change medium with fresh RA every day for 3 days (or more). Substantial cell death is usually seen around day 2. After day 3 the majority of cells are differentiating and the continued presence of retinoic acid is not essential. If RA is no longer added, the cells can then be changed every 2-3 days.

Retinoic Acid preparation

[**With this method RA is not dissolving – do not use!**

1M stock made by dissolving 500mg of All-trans-retinoic acid (Sigma) powder in 1.67 ml of ethanol

Take 1 µl of this into 1 ml of ethanol for 10^{-3}M stock in dark tubes; keep small aliquots at –20^{o}C
Use at 10^{-7} M (final conc.) (ie 1/10 000 dilution)]

Method from A.Smith, 1991 J Tis Cult Meth: (use this one!)

- Protect from direct light.
- Dissolve 50mg retinoic acid in 1.67 ml dimethyl sulphoxide -> **0.1M stock solution**
- Store in the dark at -20^{o}C
- Dilute stock retinoic acid to 10^{-6}M or 10^{-7} in EC10 medium

Working stocks:

10^{-3}: 10µl 0.1M per 1ml DMSO

10^{-4}: 100µl 10^{-3}M per 1ml DMSO

Working concentrations:

Prepare EC10/RA 10^{-6}M: 25µl 10^{-3}M working stock/25ml EC10

EC10/RA 10^{-7}M: 25µl 10^{-4}M working stock/25ml EC10