Transformation assays: growth in semi - solid medium

Because of their loss of anchorage dependance transformed cells are able to grow and form colonies in a semi-solid medium such as agarose. This is a truer test of transformation than the focus assay as growth in agarose correlates well with tumoregenicity in animals.

A layer of 4mls 0.8% agarose is put into 60mm dishes and then overlaid with a suspension of cells in 6mls 0.4% agarose. After 2 weeks the dishes are examined under the microscope for the presence of colonies. Low melting temperature agarose (FMC Sea Plaque) is used because once liquified it will stay liquid at 37oC, solidify at room temperature and then remain solid at 37oC again.

All manipulations must be done in the hood to maintain sterility.

Preparing the bottom layer (0.8% agarose)

1. Weigh out 0.8gm Sea Plaque LMP agarose into a sterile bottle or flask, add 85ml à-MEM and dissolve by heating in the microwave on the LOW setting. Place in the 37o water bath to cool.
2. Add 10mls prewarmed Calf Serum, 2ml Fungizone and 1ml Pen-Strep, swirl gently to mix and avoid making bubbles.
3. Pipette 4mls each into 60mm dishes, just as many as you need for the assay.
4. Let the agarose harden at room temperature - leave them in the hood.

Preparing the top layer (0.4% agarose)

1. Weigh out 0.4gm agarose and dissolve in 70ml à-MEM as above, place in the 37oC water bath to cool.
2. Add 8ml prewarmed Calf Serum, 2ml Fungizone and 1ml Pen-Strep.
3. Trypsinize and count the cells to be tested, it's a good idea to do each sample in duplicate. Dilute the cells to 104 cells/ml in complete medium.
4. For each dish, pipette 1ml cell suspension into a sterile tube eg Falcon 12ml round bottom tubes (#2057). If you have a lot, cap the tubes and place at 37oC.
5. Add 5mls agarose to each tube, mix gently by pipetting up and down and transfer to the dish with the bottom layer in. Do each sample separately to avoid having the agarose solidify before you get it into the dish.
6. After the agarose has solidified place the dishes in the incubator.
7. After one week feed the cells by adding 1ml complete medium (liquid) to each dish.

Remember to include positive and negative controls

An estimate of transformation efficiency can be calculated by counting the colonies and the single cells in several microscope fields.

transformation efficiency = # colonies x 100%

colonies + # single cells

You may also want to take photographs of the cells for publication.