Transformation assays: focus forming assay

Cells that contain a transforming oncogene will grow without contact inhibition and on a confluent monolayer of non-transformed cells will form dense, raised foci which can be visualized by fixing and staining the cells.

By transfection

1. Transfect cells according to Tissue Culture - 9 and 10 but do not change medium to that containing selection antibiotic, maintain in medium + serum + pen-strep only.
2. Continue to change the medium every 3 to 4 days for 3 weeks.
3. Stain the dishes with Geimsa stain, see below.

Testing established cell lines

1. Trypsinise the test cell line and a dish of untransformed Rat 2 cells (Tissue Culture - 5), count both cell suspensions (Tissue Culture - 6).
2. Plate out 102 test cells with 5 x 105 Rat 2 cells onto a 10cm dish in non- selective medium.
3. Change the medium every 3 to 4 days for 3 weeks.

It is very important to include both a positive and negative control with these assays.

A crude value for transformation efficiency can be determined by counting the number of foci obtained as a percentage of the number of cells plated.

Staining foci with Geimsa stain

1. Rinse plates with PBS.
2. Add 5ml/plate of 10% formaldehyde in PBS, leave at room temperature 30 min.
3. Remove and add 5ml diluted Geimsa stain, leave 2hrs room temperature.
4. Remove and rinse the plates with 18mê water, leave upturned to dry.
5. Seal plates with parafilm and store in a cool, dark place. Cells will remain stained indefinitely.

Geimsa stain

Dilute 4mls Geimsa stain into 100mls PBS.