Development of Plaque assay for PV on PV transformed cells.

9/19/67

Used PY-6 - these are 3T3 (mouse cells) transformed with PV by T. Benjamin.

How done by him?

Took 1 large Petri plate of "confluent" PY-6 cells - removed the medium and washed plate 1x with 5 mL of trypsin-TD. Add 4 mL of trypsin-TD to cover cells and incubate ~ 5' at 37° (actually, should watch to see when holes in the layer begin to form). Added 1 mL serum to plate (to stop trypsin action) and suspend cells by forcing up and down in pipette

Cells were diluted ~~in~~ 1:10 in Eagles - 10% serum and 5 mL of cell suspension put down on each of 6 plates

Incubate at 37° for 2 days. After several hours one could already see cells sitting down on plate and attaching

Harvested cells on 9/21/67.

PB2

9/21

Media removed from each of plates rinsed 1x ~ 5 ml of trypsin-TD add then detached with 2.5 ml of trypsin-TD for 5' at 37°. Added 0.5 ml serum per plate and cells were then removed from plate.

Total volume cell suspension ~ 16 ml.

Above suspension distributed 2-out 3 small petri plates ~5 ml each. and X-rayed.

Distance 20.5 cm.

① 30" ~300-320 R

② 90" 1500-1500 R

③ 240" ~3000-5000 R

Cells were then centrifuged for 5' at 1/3 setting in desk top centrifuge. Resuspended in ~ 4 ml of medium (Eagle's + 10% CS). Did aliquot 1:10 for cell count.

~ 90 cells per large square = 9×10^5 cells/ml of 1:10 dil

= 9×10^6 of cells/ml

∴ ~2×10^7 cells/original large plate.

Took 1.8 ml of each suspension (~ 1.6×10^7 cells) and diluted to 5 ml (~ 3×10^5 cells/ml). Put down 5 ml on each of 5 plates. ∴ ~ 1.5×10^6 cells/plate. Incubate at 37°. About 6-7 hours later looked like cells were attaching to plates

YSV-4213

9/22

On 9/22 visual inspection of plates cells looked like they were growing. In evening of 9/22 cells placed at 33° C.

9/23

Sample	Color of medium	State of cells
①	Yellow	Confluent - some mitotic figures
②	yellow → pink	Nearly confluent
③	pink	not confluent - more mitotic figures but these were not entirely normal

~~Cells were placed at 33° C~~

Changed medium on all cells and kept at 33° until 9/25

9/25

Removed medium - washed plates with 5 ml of Tris buffer.

Used two virus strains for infection

LP 147 (large plaque). 10^{-7} pfu dil 10^{-4} and 10^{-5}

P16 (small plaque). 10^{8} pfu. dil 10^{-5} and 10^{-6}

Infected as follows

Two plates for each X-ray dose received 0.1 ml 10^{-4} dil of LP140 (Lo) or 0.1 ml 10^{-5} dil of LP140 (Hi).

Total 12 plates

Two plates for each X-ray dose received 0.1 ml 10^{-5} dil of P16 (Lo) or 0.1 ml of 10^{-6} dilution of P16 (Hi).

Total plates

The virus suspension was spread over surface by tipping plates and then incubated at 37° for 1-1.5 hours.

Preparation of agar overlay

Flask of 1.8% agar (50 ml) — cooled to 43° + equal volume of 2x Eagle's medium + 3.5 ml of horse serum.

Put 6 ml of this over ~~flask~~ cells taking care not to disturb monolayer.

→ should have added bicarbonate to agar overlay.

Incubate at 37° after agar hardens.

9/26

Plates with 30" & 90" dose looked yellow to ~~yellow~~ yellow-pink but 4' plates still looked good.

9/29.

Overlayed the 30" & 90" plates with ~2 ml of agar medium with extra bicarbonate and put back in incubator.

The 4' plate was not overlayed.

10/2

Made up staining agar mixture

1 flask neutral red-agar mixed with flask of 2x Eagles & added horse serum to 3.5%. Should add tube of bicarbonate whenever overlaying to wet cells (but did not do this now).

Overlayed all plates