

## Amplification of Purified Plaques

About 10 days after plaque assay was performed, (see "viral plaque assay" procedure) plaques can be observed clearly. This is the time to pick them up and go for plaque purification and amplification!

1. Check under the light, to detect relatively isolated visible plaques
2. Draw a circle on the bottom of the plate, around the plaques, with a waterproof marker.
3. Open the plate in a sterile hood, and place the tip of sterile pasture pipette directly over the plaque.
4. Dip the tip in a tube containing 1ml medium.
5. Seed  $4 \times 10^6$  cells in  $25\text{cm}^2$  T-flask (in 4-5ml of medium)
6. Incubate for 1h/27°C.
7. After cells have attached, aspirate most of medium and add 0.5-1ml of the plaque virus to the flask. Screw the cap tightly and incubate for 1h/27°C .
8. Add 4ml of fresh medium. Leave for 72h till cells seem swollen. If cells don't look at least 40% detached and swollen, harvest virus and repeat the infection from step 4 with this new virus.
9. Collect the sup and spin 2000 rpm/5 min/22°C. Take the sup into a new tube.
10. Take 0.5-1ml from viral sup into 10 ml SF9 cells at a density of  $2.5\text{-}3 \times 10^6/\text{ml}$ .
11. Count cells every day. After 3 days ~40% are supposed to be dead, when counted using Trypan blue.

12. Collect supernatant when cells seem between 40-50% dead. At this point cells can be harvested and used for expression analysis.