

Infection of primary MECs with Adenovirus (MR)

Rijnkels, M. and Rosen, J.M. (2001). Adenovirus-Cre-mediated recombination in mammary epithelial early progenitor cells. *J Cell Sci.* **114**: 3147-3153.

Infection can be done any time after the mammary epithelial cells (MECs) have been plating for 2 days. More cells have spread-out after 3 days, which seems to improve infection rates.

Get an estimate of the number of cell on the plate/wells.
(many cells initially plated do not attach/survive; count!)

Use an MOI (multiplicity of infection) of 50= 50 pfu/cell.
(there is room for some improvement but MOI 100 will kill many cells)

Dilute the virus in growth media such that MOI 50/volume to be added to plate (see below).

Wash plates with F12/gentamycin media.

Add virus (MOI 50) in growth media to plate/well in smallest volume possible without the plate/well drying out.

100mm plate: ~2.5-5 ml

Well/6well plate: ~0.3-0.5 ml

Control: same volume of media without virus or with control virus

Incubate at RT 1 hour (make sure plates are leveled and do not dry out) and incubate 30-60' at 37°. *(I have also taken the virus off after 1-2 hr and that seems to work too)*

Add growth media to desired culture volume.

Place at 37°C for 2-12 hours.

Wash 3-5x F12/gent.

Add fresh growth media change the next day again.

Protein expressed by Adenovirus under CMV promoter is supposedly expressed within 6 hours of infection and levels increase up to 24 hrs.

Adenovirus DNA (and thus potential expression) is still present 3 days after infection and might stay around much longer depending on the proliferation rate of the cells.