

Adenovirus-mediated gene transfer

- 1) Grow cells to a 90-95% confluence in a 10 cm² dish.
- 2) Aspirate the medium to leave behind approximately 300ul of medium, and add 100-200ul of adenovirus. Incubate the virus at 37°C for 1 hour, gently rocking every 15 minutes to ensure that the plate is evenly infected.

Note 1: Alternatively, if you want to be more precise, you may wish to aspirate all the media and add back 200 ul of adenovirus with 300ul of DMEM. The main point is to use the achieve a very high initial concentration of virus to maximize the adenovirus binding to cells. Consequently, you may wish to play around with conditions to optimize them for your specific cell type.

Note 2: Also note that these numbers are for a 10cm² dish: adjust the volumes accordingly for smaller or larger dishes.

- 3) Add back the appropriate amount of media and culture the cells for 1-2 days. Typically you can see expression 24 hours post-infection, but 48 hours is usually optimal.

Every cell will respond differently to different adenovirus constructs. Therefore, it is imperative that you optimize your infection conditions, especially if you are trying to achieve a dominant negative effect.

Adenovirus Amplification

- 1) Grow cells to 90-95% confluence in a 162cm² flask.
- 2) Aspirate the media and add ~1.5ml of DMEM+5% containing ~10⁸ virus particles (1 ml medium + 0.5ml crude virus lysate).
- 3) Rock the flask every 15-20 minutes for about 1hr.
- 4) Add 10-12ml of DMEM+5% and let the flask incubate at 37°C until all the cells have been lysed

** The media usually turns yellow from cell lysis, but does not have to.

**Lysis should take between 2-5days. If it takes longer the viral titer is low, if it is faster than two days, than this usually means you've added too much virus, and it is likely that the amplification has failed.