Transfection of [T47D] Cells

Day 1 (8/20/02):

1) Replace media with fresh media (10% FBS + Pen/St).
2) Split 1 plate's worth into a 6-well plate
   e.g.- 3mls cell suspension into each 10-cm plate
   -500µl cell suspension into each well of the 6-well plate

3) Incubate for 3-4 hours at 37°C
4) Dilute the adenovirus as follows:

Tube #	Virus	DMEM*	Dilution
1	5ul stock	45ul	1:10
2	5ul Tube 1	5ml	1:10^5
3	500ul Tube 2	5ml	1:10^6
4	500ul Tube 3	5ml	1:10^7
5	500ul Tube 4	5ml	1:10^8
6	500ul Tube 5	5ml	1:10^9

*DMEM F12 (50/50 Mix) + 10%FBS + Pen/St

5) Add 1.5 mls of each virus/DMEM solution to each well as follows:

Control (no virus)	**1:10^5** (Tube 2)	**1:10^6** (Tube 3)
1:10^7 (Tube 4)	**1:10^8** (Tube 5)	**1:10^9** (Tube 6)

6) Incubate overnight.

Day 2 (8/21/02):

1) Remove media
2) Add SERUM-FREE DMEM and incubate for 30 minutes.
3) Prepare a solution of serum-free DMEM containing 5µg/ml heparin
   --1.5ml will be needed for each well, so make ~10ml
4) Remove the media
5) Add 1.5ml DMEM + heparin solution to each well
6) Incubate overnight.

Day 3 (8/22/02):

Harvest the media.