

Amplification and Purification of Adenovirus

Amplification of adenovirus and creation of crude stock

- 1) Culture 293 cells in 162 cm² flask with DMEM+10%FBS, and grow them to 80-90% confluence.

Note about 293 cells: do not use trypsin to dissociate 293 cells, but instead use warm PBS+ 1mM EDTA to dissociate cells, this is gentler and increases cell viability.

- 2) Aspirate the medium to leave behind approximately 1ml of DMEM, and add 250-500ul of adenovirus (crude prep) or 50-150ul of purified virus. Rock the flask to ensure that the entire surface area is covered, then incubate the flask for 60 minutes, rocking the flask every 15-20 minutes ensure that the whole flask is covered.

Alternatively, aspirate all the medium, then add back 500ul of crude adenovirus with 500ul of DMEM, and rock for 1 hour.

- 3) Add back 10-12 ml of DMEM+5% FBS (note the decreased amount of FBS), and culture the cells until all the cells become floaters (around 3-6 days).
- 4) Collect the cells and medium in a 50 ml conical tube, and store at -80°C, or proceed immediately to next step.
- 5) Thaw viral prep at RT if frozen, then sonicate the cells for 10 minutes (alternatively, you can freeze/thaw 5 times). Spin the conical tubes at 3000rpm for 10 minutes to pellet the cellular debris. The supernatants are now your high titer stock that can now be purified, or used in cell culture experiments.

NOTE: Since there are ~10⁸ 293 cells in a 162 cm² flask, and adenovirus can grow up to 10,000 copies per cell, the theoretical final titer can be up to ~10¹⁰-10¹¹ pfu/ml.

Purification for *in vivo* use

Buffers: 10mM HEPES (1.3g HEPES in 500ml)
2.2M CsCl (38g CsCl in 100ml of 10mM HEPES)
4.0M CsCl (67g CsCl in 100ml of 10mM HEPES)
Saturated CsCl (67g CsCl in 50ml of 10mM HEPES)

All of the above should be autoclaved and pH adjusted to 7.4

PBS+ 10% glycerol

Equipment: SW28 tubes
SW41 tubes
SW28 and SW41 rotors and adapters
50ml conical tubes, syringes, needles

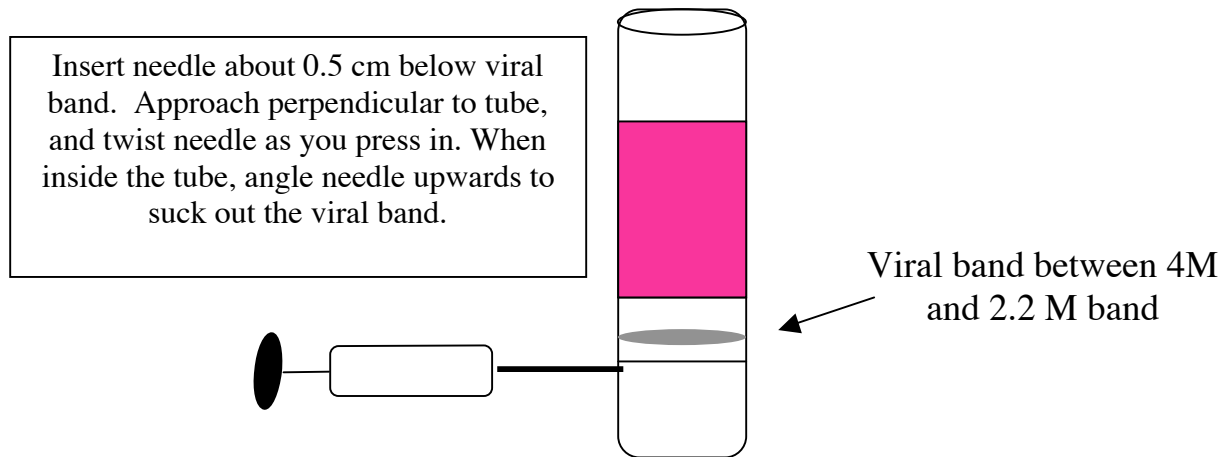
- 1) Create a CsCl gradient in a SW28 tube as follows:

20ml viral lysate →
5 ml of 2.2M CsCl →
10ml of 4.0M CsCl →

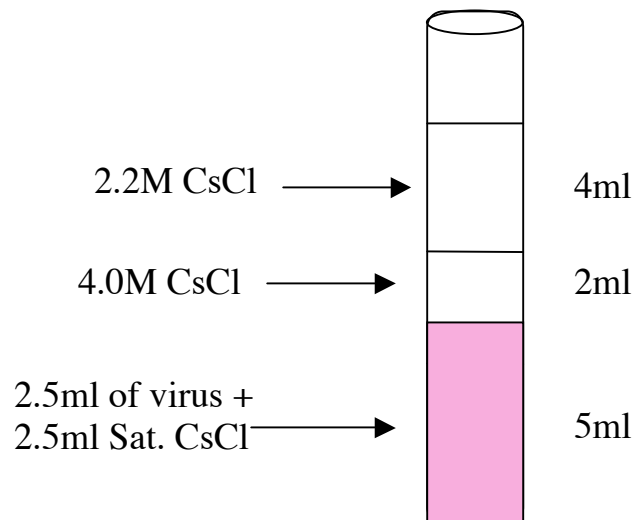


Make sure that you *gently* create each layer. The better your layers, the higher your yield.

- 2) Centrifuge at 25,000 rpm for 2 hrs to overnight at 4°C. After the spin, you should find the viral band between the 4M and 2.2M layer.
- 3) To retrieve the band, you can either aspirate the media from the top to reach the viral band, or you can insert a needle directly into the tube to retrieve the band. I recommend the latter. Use an 18 gauge needle attached to a 3ml syringe and poke the needle into the tube about 0.5 cm below the white viral band. Push the needle upwards and take out 2.5 ml of virus.



- 4) Mix the 2.5 ml of virus with 2.5ml of saturated CsCl, then add to a new SW41 tube. Layer on top of this 2ml of 4M CsCl followed by 4 ml of 2.2M CsCl.



Again, *gentle* layering results in higher viral yields.

- 5) Centrifuge at 35,000rpm for 3 hours to overnight at 4°C. After the spin, you should find the viral band between in the middle of the 2.2M layer.

- 6) Again, retrieve the band with either by pipetting off the top layers or by sucking out the virus with a needle. A 1 ml syringe and 21 gauge needle should be all you need to remove the viral band. Repeat as directed above to remove the viral band with a needle.
- 7) Transfer the virus solution into a dialysis cassette (Pierce# 66110, 3.5K MWCO), and dialyze in PBS+10% glycerol for 1-2 hours.
- 8) Change the buffer to fresh PBS+10% glycerol after the first dialysis and dialyze overnight.
- 9) Quantify virus before storing at -80°C indefinitely.

Quantifying viral amount:

Adenovirus Titering

- 1) Take a 5ul aliquot of the dialysate as a blank and dilute 1:100 in 500ul (TE/0.1%SDS). You can use 10ul if you think the viral titer is low.
- 2) Dilute 5ul of viral sample into TE/0.1%SDS and put into a UV spec. If you took 10ul for your blank, also use 10ul here.
- 3) Measure the OD_{260} . An $\text{OD}_{260}=1 \times 10^{12}$ pfu/ml, but remember that you diluted your sample 1:100.

Thus, if you have an OD_{260} of 0.0534:
 $0.0534 * (100) * (10^{12}) = 5.34 \times 10^{12}$ pfu/ml

- It is typical to make aliquots of 1×10^{11} pfu/ml aliquots at this stage, since freezing/thawing the purified virus decreases its biological potency.
 - **REMEMBER:** It is easy to overlook, but when you make your aliquots, use the adenovirus storage buffer (below) for dilutions!
 - Good luck with the adenoviral work!
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Buffer for Adenovirus:

20mM Tris pH=8 (20ml in L from 1M stock) 25mM NaCl (5ml/L from 5M stock) + 5% glycerol (can also be 10%)

50ml=250ul NaCl, 1ml Tris