Production of Adenoviral Vectors

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This protocol explains how to produce adenoviral vectors in a easy way. **Adenoviral vectors can be harmful!** Take care of the security issues and read more about it then only this protocol. I don't stress safety issues and basic things in this protocol, I expect that you know how to work with viruses and with tissue culture.

Production of Adenoviral Vectors from Plasmids

Preparation: Digestion of Plasmid

At the moment the adenoviral vector is part of a bacterial plasmid. To release the adenoviral ends it is necessary to digest with Pac I. The best is to digest 12 µg of plasmid in 100 µl total volume for 3 hours. Then the enzyme is inactivated by heating to 65 °C for 20 minutes. I use this mixture directly without purification, I never noticed any problem. The rest can be stored at -20 °C.

Preparation: Transformation of 293A Cells

Here you need 293A cells. Alternatively you could use 293 cells or 911 cells. It is important that they produce the adeno-E1 proteins, because they are deleted in the vector. The 293A cells should be splitted 1:3 on Monday, Wednesday and Friday. If they grow slower the cells are probably too old and should be replaced with a fresh stock. The best is to start the following protocol on a Monday, otherwise you need to work on the weekends.

Monday, day 1: Split 293A Cells

Split the 293A cells that they are 50% confluent on the next day. I split usually 1:3 into small 25 cm² flasks.

Tuesday, day 2: Transform 293A Cells

Transform the 293A cells using Lipofectamine 2000. Alternatively $CaPO_4$ can be used, but Lipofectamine gives more constant results.

First refresh the medium on the 293A cells.

Prepare the two mixes:
DNA Mix:
- 25 µl of the digest containing 3 µg DNA
- 0.1 µg of a GFP expressing plasmid (to control transformation efficiency)
- 500 µl Opti-Mem I Medium

Lipofectamin Mix:
- 9 µl of Lipofectamin 2000
- 500 µl Opti-Mem I Medium

Incubate for 5 minutes.

Add the DNA Mix slowly to the Lipofectamin Mix.

Incubate for 20 minutes.

Add the combined mix **dropwise** to the cells.

Wednesday, day 3: Refresh Medium

Refresh the medium on the 293A cells. You can already check transformation efficiency. If you see a few green cells you are in the game, the more the better, 30% would be quite good.

Thursday, day 4: Split 293A Cells

Today we split the 293 cells. The complete content of the small 25 cm² flask is spread on a bigger 175 cm² flask. The cells are then widely spread, but they manage to grow confluent till Monday. Now the interesting time starts when the infected cells start producing virus and infect the uninfected cells.

Monday, day 8: Refresh Medium

Refresh the medium on the 293A cells. Freeze the old medium at -80 °C, just to be on the save side.

Wednesday, day 10: Refresh Medium, Prepare new 293A Cells

Refresh the medium on the 293A cells. If there are holes in the cell layer or the cell layer detaches from the plastic, we harvest the cells today. See Friday for details.

Prepare also 175 cm² flasks with 293A cells for Friday!

Friday, day 12: Harvest the virus

You probably can keep the cells much longer, but I always harvested latest on this day. To harvest you flush off the cells and freeze them with the medium at -80 °C. The harvested medium/cell mix is freeze/thawed and aliquoted as described below. This is the F1 adenovirus stock. Use it directly to infect new 293A cells.

Freeze / Thaw

After the production up to 90% of all the adenoviral vectors are still in the cells. They can be released by repeated freezing and thawing cycles. Unfortunately, freezing and thawing also destroys the vectors, so we only do three cycles to get an optimum of active particles. Therefore we freeze them at -80 °C or in liquid nitrogen and thaw them in a 37 °C waterbath. This we repeat two times (so in total it was done three times). Then the debris is spin down at 4000 RPM for 10 min. To avoid freezing and thawing in the future we aliquot the supernatant which is our adenovirus stock.

Amplifcation of Adenoviral Vectors

Wednesday, day 1: Prepare new 293A Cells

Prepare 175 cm² flasks with 293A cells.

Friday, day 3: Amplify Adenoviral Vectors

The prepared big 175 cm² flasks should be 80% confluent today.
Discard the old medium.

If you use a F1 stock, it may not have a sufficient concentration of infectious particles, therefore amplification is necessary. Add 15 ml of the F1 adenovirus stock and incubate for 30 min. Then add to the F1 adenovirus stock 10 ml of fresh medium.

If you use an old and titrated stock, use a 5-10 infectious particles per cell (1-2 x 10^8 IU per flask) in 25 ml fresh medium.

Incubate at least for 3 hours, but not over night.

Remove the adenoviral vector mixture and replace it by fresh medium.

Monday - Friday, day 5 - 9:

Depending on the concentration of adenoviral vectors, the cells may detach 48-72 hours post infection or still stick to the plastic on Friday.
At the moment they detach, but latest on Friday, day 9, I would harvest the cells. The harvested medium/cell mix is freeze/thawed and aliquoted as before. This is the F2 adenovirus stock. If they do not detach on Monday, day 5, or on Wednesday, day 7, the medium is replaced by fresh medium.
Repeat this procedure till a good concentration is reached and the cells detach not after day 4 post infection. I usually need not more than one passage, at maximum I needed 3 passages.

Known Issues:

- Adenoviruses have a size limit in what they can package into their capsid. A maximum of 10% more than the wildtype genome size fits in, than things get tricky. Take care that your construct is within this size limits.
- Too many freeze/thaw-cycles damage the adenoviral particles. Aliquot you stocks and store them at -80°C. Glycerol is not required.

References and Comments:

I developed this protocol based on the instructions provided with the gateway adenoviral vectors and the AdEasy protocol. I have done it many times and optimized it along the way. I guess my protocol gives a better overview, but have a look at the provided protocol from gateway for details and instructions were to buy the things. With this protocol I produced adenoviral vectors without any problems.

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