Cloning of Adenoviral Vectors

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This protocol explains how to clone 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.

Adenoviral vectors are produced from plasmids of ca. 35000 bp in size. Plasmids bigger than 10 kbp are difficult to handle. All restriction enzymes you would like to use are already several times in the vector and UV light and pipetting damages the DNA over proportional. That's why I would recommend to use the Gateway technology for the construction of the plasmids. I don't explain the details of gateway here.

You will work only on the small ENTR clone. Assemble the plasmid with classical cloning steps or with BP-cloning as you like. Because of it's small size it is very easy. Depending on your choice of the DEST vector you need to clone a promoter, an open reading frame and a terminator or only an open reading frame.

Take care that your sequences do not contain a Pac I restriction site! Pac I is required later to release the adenoviral ends.

The adenoviral vector plasmid is obtained by a Classic LR-Reaction II. Have a look at this protocol for details. The destination vectors are ampicilin resistant. Digest the recombined plasmids with Hind III and check the band pattern to avoid working with incomplete vectors (I have never seen one). Make a maxiprep of the DNA because several µg are needed for transformation. I would recommend to use columns to get very clean DNA which is not toxic to the cells.

Materials needed:

ViraPower Adenoviral Expression System (# K4930-00 or K4940-00) by Invitrogen

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.

References and Comments:

I developed this protocol based on the instructions provided with the gateway vectors. 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.

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