Date:

Protocol for subcloning cap into pAAV_RC _containing four mutations and synthesized rep part

Investigator:

Comment: Since BspMI is a type IIs endonuclease we have to figure out another protocol. According to the publication from Gormley *et al*. (2002) plasmid DNA with only one recognition site is not cut very efficiently from this type of restriction enzyme. Linearized DNA and lower salt concentration conditions increase the activity of BspMI for plasmids with only one recognition site as it is be present in our pAAV_RC construct. Linearized DNA will serve as the second recognition site in trans. In order to have the best cutting results the plasmid will be digested first with BsiWI at 55°C and then BspMI and 1,25µL H20 will be added for reducing the salt concentration. Further details can be found in Gromley *et al.* "The Type IIs Restriction Endonuclease BspMI is a Tetramer That Acts Concertedly at Two Copies of an Asymmetric Sequence" (2003).

Digestion:

Plasmid used:

First step

Enzyme used: ***BsiWI***

Second step

Enzyme used: ***BspMI***

Step 1

Mix	**/µL**
DNA	1500ng
Buffer 3 (10x)	2
Enzyme ***BsiWI***	1
H2O	
Total volume	**20**

Incubate at 55°C for 60 minutes

Step 2

	/µL
Mix from step 1 which was incubating for 60 minutes	20
Enzyme ***BspMI***	1
H2O	1,25
Total volume	**22,25**

Incubate at 37°C for another 60 minutes