Restriction enzyme digestion

Description

The protocol is designed to produce linearized plasmid vector (AAV plasmid) for recombination with target insert. Two enzymes from different manufacturer (New England BioLabs and Thermo Scientific) are chosen for the experiment. Due to the difference in buffer used for these enzymes, two single restriction digestion is performed.

Materials and equipment
-autoclaved pcr tubes
-ultrapure H_2O
-rCutSmart™ Buffer
-FastDigest Buffer/FastDigestion Green Buffer
-MfeI (New England BioLabs)
-FastDigest HindIII (Thermo Scientific)
-thermal cycler
-1 kb DNA ladder
-1% agarose gel (12 well, comb thickness: 1.5mm)
- QIAquick Gel Extraction Kit (cat. nos. 28704)
Method

First single enzyme digestion

1. Prepare the sample mix according to the table below:

Component	100 µL reaction
ultrapure H_2O	Add to make 100 µL
MfeI (New England BioLabs)	5 µL
rCutSmart™ Buffer	10 µL
DNA plasmid to be digested	X µL (10 µg in total)

2. Incubate the sample mix in the thermal cycler at 37°C for 1 hr.
3. Heat the sample mix at 80 °C for 20 minutes to deactivate the enzyme (optional).
4. Perform gel electrophoresis using 1% agarose gel according to the table below:

Lane 1	Lane 2	Lane 3-6	Lane 7	Lane 8
1 kb DNA ladder	1 µL restriction digestion product+ 4 µL ultrapure H_2O+ 1 µL 6X loading dye	Remaining restriction digestion product + 20 µL 6X loading dye	100 ng undigested plasmid+ X µL (add to make 5 µL before adding loading dye)+ 1 µL 6X loading dye	4 µL ultrapure H_2O+ 1 µL 6X loading dye

5. Identify and cut out the correct band of the vector in the gel.

6. Perform gel extraction using QIAquick Gel Extraction Kit to purify the desired linearised plasmid. Elute the DNA with 30 µL ultrapure H_2O.

Second single enzyme digestion

7. Prepare the sample mix according to the table below:

Component	70µL reaction
ultrapure H_2O	36 µL
FastDigest HindIII (Thermo Scientific)	7 µL
FastDigest Buffer/FastDigestion Green Buffer	7 µL
DNA obtained from gel extraction	20 µL

8. Repeat step 2 and 3.
9. Perform gel electrophoresis using 1% agarose gel according to the table below:

Lane 1	Lane 2	Lane 3-6	Lane 7	Lane 8	Lane 9
1 kb DNA ladder	1 µL restriction digestion product digested with both enzymes+ 4 µL ultrapure H_2O+ 1 µL 6X loading dye	Remaining restriction digestion product digested with both enzymes+ 14 µL 6X loading dye	5uL restriction digestion product digested with only MfeI+ 1 µL 6X loading dye	100 ng undigested plasmid+ X µL ultrapure H_2O (add to make 5 µL before adding loading dye)+ 1 µL 6X loading dye	4 µL ultrapure H_2O+ 1 µL 6X loading dye

10. Repeat step 5 and 6. The linearised DNA vector obtained are ready for ligation with target insert.