Restriction Analysis

Restriction analysis to confirm the Biobricks cloning requires a little amount of plasmidial DNA. We performed double digest reaction with the standard enzyme pairs *EcoRI*, *Spe*I, *Xba*I, *Pst*I.

Materials

- Microtubes
- About 300 - 500 ng plasmidial DNA
- Enzyme 1: *EcoR*I or *Xba*I (Fast Digest Fermentas/Thermo Scientific)
- Enzyme 2: *Spe*I or *Pst*I (Fast Digest Fermentas/Thermo Scientific)
- 10X Fast Digest Buffer (Fermentas/ Thermo Scientific)
- Water, nuclease free

Apparatus

- Centrifuge
- Incubator

Method

1. On ice, prepare the following mixture:

300-500 ng of plasmidial DNA	X µL
Enzyme 1 (1FDU/µl)	0,5 µL
Enzyme 2 (1FDU/µl)	0,5 µL
10X Fast Digest Buffer	1µL
Water, nuclease free	to 10 µL

2. Spin the mixture.
3. Incubate at 37°C for 5-1 hour.
2. Run an agarose gel **(see the section Agarose Gel Electrophoreis and DNA purification).** [http://2014.igem.org/wiki/images/a/af/Agarose_Gel_Electrophoresis_and_DNA_Gel_Purification.pdf]
4. Verify the result and take a picture.

Considerations:

If you want to use enzymes of other company, always read the manufacturer's instructions and be careful with star activity, temperature, incubation time of reaction and also the ideal buffer for double digestion. If you prefer, you can perform sequential digestions rather than double digestions.

Digestion in large scale

After confirming the cloning, select a positive clone and digest the plasmidial DNA in large scale to get a good final amount of insert. We performed double digest reaction with the standard enzyme pairs *EcoRI*, *Spe*I, *Xba*I, *Pst*I.

Materials

- Microtubes
- About 2-5 µg plasmidial DNA
- Enzyme 1 (Fast Digest Fermentas/Thermo Scientific)
- Enzyme 2 (Fast Digest Fermentas/Thermo Scientific)
- 10X Fast Digest Buffer (Fermentas/ Thermo Scientific)
- Water, nuclease free

Apparatus

- Centrifuge
- Incubator

Method

1. On ice, prepare the following mixture for each 1 µg of plasmidial DNA (scale the reaction according to the initial quantity of DNA):

1 µg of plasmidial DNA	X µL
Enzyme 1 (1FDU/µl)	1 µL
Enzyme 2 (1FDU/µl)	1 µL
10X Fast Digest Buffer	2 µL
Water, nuclease free	to 20 µL

3. Spin the mixture.
4. Incubate at 37°C for 1-16h.
5. Run an agarose gel.
6. Excise the band of interest with expected size.
7. Purify de DNA fragment from gel **(see the section Agarose Gel Electrophoreis and DNA purification).** [http://2014.igem.org/wiki/images/a/af/Agarose_Gel_Electrophoresis_and_DNA_Gel_Purification.pdf]

Considerations: If you want to use enzymes of other company, always read the manufacturer's instructions and be careful with star activity, temperature, incubation time of reaction and also the

ideal buffer for double digestion. If you prefer, you can perform sequential digestions rather than double digestions.