Digestion protocol AraC3 Vector and BioBricks

Restriciton enzyme concentrations

PstI 10 U/µl
SpeI 10 U/µl
XbaI 10 U/µl
Alternatively, to digest 1 µg use 5U and for 2 µg DNA use 10U of enzyme

Materials

- DNA sample
- Restriction enzymes
- Buffer B/H
- BSA
- Enzymes
- dH_2O

Protocol

1. Take DNA - thaw on ice
2. Prepare restriction enzyme mixture according to pipetting tables found below.
    - Add in listed order.
    - Use dH_2O to top up the reaction volume to 20 µl and as negative control.
    - Do not exceed maximum reaction volume of 20 µl!
3. Mix gently by pipetting up and down and then spin down the sample.
4. Incubate at 37°C for 1- to 4 hours. (200 ng = 2 hours, 1 ug= 3 hours, 2 ug = 5 hours)
5. After incubation heat inactivation at 65°C to inactivate the restriction enzymes or proceed with a Gel- or PCR purification Kit.
    - For gel purification, follow instructions below table before proceeding with kit.

Table for up to 1 µg DNA reaction. Use double values for 2 µg.

Reagent	**Vector AraC3**	**BioBrick 1**	**Biobrick 2**	**Negative ctrl**
dH_2O	….. µl	….. µl	….. µl	16.80 µl
Buffer B/H	**B** 2.0 µl	**H** 2.0 µl	**H** 2.0 µl	**B/H** 2.0 µl
Acetyl BSA	0.2 µl	0.2 µl	0.2 µl	0.2 µl
DNA (up to 2 µg)	….. µl	….. µl	….. µl	x
SpeI	0.5 µl	x	x	0.5 µl
PstI	0.5 µl	0.5 µl	0.5 µl	0.5 µl
XbaI	x	0.5 µl	0.5 µl	0.5 µl
Final volume	20.0 µl	20.0 µl	20.0 µl	20.0 µl

Gel purification preparation

1. Make an agarose purification gel.
2. Mix the total volume of the digestion with 5X loading dye.
3. Load max 25 ul in each well. Do not run gel above 120 V.
4. After about 45 minutes stop the run and cut the bands out under UV light
5. After the bands have been cut out, use the Gel extraction kit to re-elute the DNA,
6. Measure the DNA concentration on nanodrop.