

Digestion protocol C3 Vector and BioBricks

Restriction enzyme concentrations

EcoR1: 12 U/ μ l

Pst1: 10 U/ μ l

Xba1 10 U/ μ l

Alternatively, to digest 1 μ g use 5U and for 2 μ g DNA use 10U of enzyme

Materials

- DNA sample
- Restriction enzymes
- Buffer H
- BSA
- Enzymes
- dH₂O

Protocol

1. Take DNA - thaw on ice
2. Prepare restriction enzyme mixture according to pipetting tables found below.
 - Add in listed order.
 - Use dH₂O to top up the reaction volume to 20 μ l and as negative control.
 - Do not exceed maximum reaction volume of 20 μ l!
2. Mix gently by pipetting up and down and then spin down the sample.
3. Incubate at 37°C for 1- to 4 hours. (200 ng = 2 hours, 1 μ g = 3 hours, 2 μ g = 5 hours)
4. 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.

Reagent	Vector C3	BioBrick 1	Biobrick 2	Negative ctrl
dH ₂ O μ l μ l μ l	16.88 μ l
Buffer H	2.0 μ l	2.0 μ l	2.0 μ l	2.0 μ l
Acetyl BSA	0.2 μ l	0.2 μ l	0.2 μ l	0.2 μ l
DNA (up to 2 μ g) μ l μ l μ l	x
EcoR1	0.42 μ l	0.42 μ l	0.42 μ l	0.42 μ l
Pst1	0.5 μ l	0.5 μ l	0.5 μ l	0.5 μ l
Final volume	20.0 μ l	20.0 μ l	20.0 μ l	20.0 μ l

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 μ l 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.