BANANA GUARD

Protocols

Restriction and Ligation

Restriction Digestion

(Adjusted protocol based on New England Biolabs NEB Biobrick Assembly Kit protocol)

COMPONENT	20 µl REACTION
Plasmid or DNA fragment to digest	1µg
Restriction Enzyme I	1 µl
Restriction Enzyme II	1 µl
10X NEBuffer 2.1*	5 µl
Water, nuclease-free	to 20 µl

* double digestions specific buffer has to be checked on line in the New England Biolabs website

- Incubate restriction digestion at 37°C for at least 1 hour.
- Deactivate the enzymes at 80°C for 20 minutes.

Ligation

(Adjusted protocol based on New England Biolabs Biobrick Assembly Kit protocol)

- Set up the following reaction in a microcentrifuge tube on ice.
- Gently mix the reaction by pipetting up and down and microfuge briefly.
- For cohesive (sticky) ends, incubate at least 20 minutes, optimally overnight at room temperature.

COMPONENT	20 µl REACTION
10X T4 DNA Ligase Buffer*	2 µl
Vector DNA (4 kb)	50 ng (0.020 pmol)
Insert DNA (1 kb)	62 ng (0.060 pmol)
Water, nuclease-free	to 19 µl
T4 DNA Ligase	1 µl (dilute 5 times)

T4 DNA Ligase should be added last. Note that the table shows a ligation using a molar ratio of 1:3 vector to insert for the indicated DNA sizes. Use NEBioCalculator to calculate molar ratios.