Transformation

MiniPrep

Start a culture.
Use the available kits.

Digestion

1. Measure DNA concentration, and adjust the volume to fit the amount of DNA recommended in the protocol [plasmid 2 µl 0.5 g/ml, PCR 10 µl 0,02 g/ml] . (p.43 Fermentas enzyme catalogue).

2. Prepare for (5 + vector) digestion reactions, using a double digestion. (p.43 Fermentas enzyme catalogue). Restriction enzymes to use are EcoR1 and Pst1 and do the digestion.
   Calculate amount of vector needed for ligation …….. µl

3. Purify by running on column according *Nucleo Spin® Extract 2 PCR cleanup protocol*

Ligation

1. Combine 38 fmol of vector with a three time molar excess of insert, adjust volume to 10 µl with dH_2O.
2. Add 10 µl of 2X Quick ligation buffer and mix.
3. Add 1 µl of T4 DNA ligase and mix.
4. Spin down and incubate at room temperature for 5 min.

Transformation

Use either the transformation protocol from Open wet ware 1 (meticulous) or the one included from Quick Ligation™ Kit (Fast]

1. **psb1a3**