High efficiency BsaI-BsmBI cloning

1. Digest insert with BsaI. Run in PCR machine: 2 hr @ 37C, then 20 min @ 65C.

45 ul	water
10 ul	10X NEBuffer 3
10 ul	10X BSA
30 ul	~20 ng/ul insert
5 ul	BsaI enzyme
100 µl	BsaI digest rxn

2. Digest vector with BsmBI. Run in PCR machine: 2 hr @ 55C, then 20 min @ 80C.

55 ul	water
10 ul	10X NEBuffer 3
30 ul	~50 ng/ul vector (~400 fmol for a 5.6 kb vector)
5 ul	BsmBI enzyme
100 µl	BsmBI digest rxn

3. PCR purify both cut insert and vector. If using the QIACube, use 0 ul fillup volume and 30 ul elution with IDTE.

4. Run 5 ul of each purified cut vector on a 0.8% agarose gel, and 5 ul of each purified cut insert on a 2% agarose gel. Compare each to uncut sample. Can proceed with rest of protocol while this is running.

5. Prepare 0.1 U/ul T4 DNA ligase on ice.

78 ul	water
20 ul	5X T4 ligation buffer (Invitrogen)
2 ul	5 U/ul T4 DNA ligase (Invitrogen)
100 µl	0.1 U/ul T4 DNA ligase

6. Perform 50 ul ligation reaction. Remember to do a no-insert control. Incubate at room temperature for 1 hr. Add 5 ul of 0.5M EDTA to stop rxn. Can store at 4C. Use equimolar amounts of insert and of vector; aim for roughly 200 fmol of each per rxn.

9.5 ul	5X T4 ligation buffer (Invitrogen)
_____ ul	purified digested vector (~10 ul)
_____ ul	purified digested insert
_____ ul	water (so above sums to 47.5 ul)
2.5 ul	0.1 U/ul T4 DNA ligase
50 ul	Ligation rxn

7. Dialyze 40 ul of ligation rxn on ~20 ml MilliQ for 90 min.

8. Electroporate as much of dialyzed ligation rxn as can be recovered from dialysis into DH5α-E electrocompetent cells. Recover transformed cells in SOC at 37C for 60 min.

9. Plate 10^{-5}X of each transformation to quantify library diversity; using 200 fmol of vector and of insert should give ~10^{8} transformants.

10. Dump rest of transformation rxn in 50 ml LB with appropriate antibiotics. Let grow overnight.

11. Miniprep as much of each 50 ml library as desired.

Reagents

BsmBI enzyme; BsaI enzyme; Invitrogen T4 DNA ligase (5 U/ul); 0.5 M EDTA; dialysis filters; electrocompetent cells

Source

Demonstrated by JBK on 5/28/2011