



NEW YORK CONSORTIUM ON MEMBRANE PROTEIN STRUCTURE

**Ligation Independent Cloning
January 2010**

Preparation of vector:

Digest plasmid pNYCOMPS-LIC-ccdB-FH10T+ (N-term.) with restriction enzyme *Sna*BI. Gel purify vector fragment using Qiagen Gel Extraction Kit. Elute into Buffer EB. Ideally, the resulting DNA concentration is >50ng/ul.

For 120 LIC reactions:

- 12ul 10X Buffer 2
- 1.2ul 100X BSA
- 60ul LIC C-term./*Sna*BI (at 50ng/ul, 25ng/ul final)
- 3ul 100mM dGTP (2.5mM final)
- 1.5ul T4 DNA Polymerase (0.0375Units/ul final)
- 42.3ul H₂O
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- 120ul

For large-scale cloning, it is best to assemble the reaction in a 96 well PCR plate.

Incubate at 22°C for 60 minutes. Heat inactivate enzyme at 75°C for 20 minutes.

Preparation of inserts:

Perform PCR and clean-up products on the 96- or 384-well scale as usual.

Prepare a reaction mix for inserts (120):

- 120ul 10X Buffer 2
- 12ul 100X BSA
- 30ul 100mM dCTP (2.5mM final)

15ul T4 DNA Polymerase (0.0375Units/ul)
785ul H₂O

960ul

Combine 8ul of the above mixture with 2ul of each purified PCR product.

Incubate at 22°C for 60 minutes. Heat inactivate enzyme at 75°C for 20 minutes.

Combine 2ul of vector with 4ul of each insert. Incubate at 22°C for 60 minutes

Add 2ul 25mM EDTA and incubate at 22°C for 5 minutes.

Use 2ul of each LIC reaction to transform 20-50ul competent cells.

Plate transformations on to LB+Kan agar in 24 well blocks containing glass balls. Shake plates in orbital shaker until all liquid is adsorbed on to plate.

Incubate overnight at 37°C