

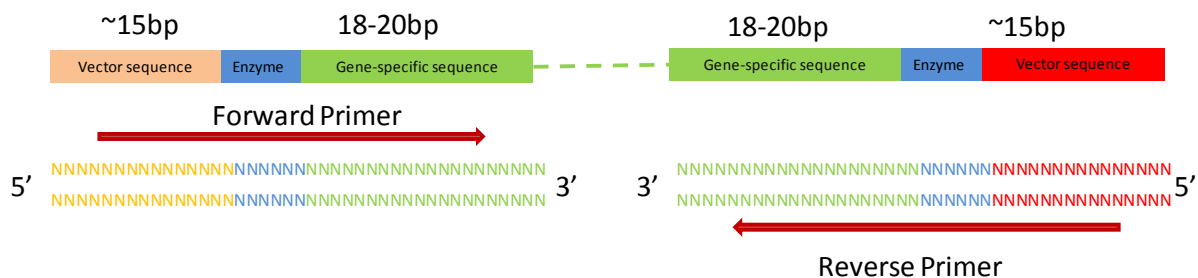
Cold Fusion Protocol

A. Preparation of Linearized Vector

The linearized vector can be generated by PCR or restriction enzymes (single or double cut)

B. PCR Amplification of Target DNA

The PCR fragments can be generated by Taq DNA polymerase or other high fidelity DNA polymerase, with primers that are designed to have at least 15 bases of homology at their linear ends.



Note: For both linearized vector and PCR product, we recommend gel purification.

C. Reaction Set up

Linearized destination vector (10-100ng/μl)	1μl*
PCR insert(s) (20-200ng/μl)	1μl* for each PCR product
dH ₂ O	_μl
5x master mix	2μl
total	10μl

D. Reaction Incubation

1. 5 minutes at room temperature
2. 10 minutes on ice

E. Transformation

1. Add 50µl Cold Fusion competent cell to the cloning mixture
2. Incubate on ice for 20 minutes
3. Heat shock at 42°C for 50 seconds
4. Transfer on ice for 2 minutes
5. Add 250µl S.O.C medium or LB broth
6. Incubate at 37°C for an hour
7. Take 100µl culture and spread on pre-warmed (37°C) culture plate containing selected antibiotic
8. Incubate the plate at 37°C to grow colonies overnight.