

Standard Ligation and Transformation
NDJ V.1 1.26.04

Ligation

Used Roche Rapid Ligation kit:

Follow company protocol.

Add 2ul of 5x DNA diluent

Total 150ng DNA with Vector: Insert (1:0 , 1:3 , and 1: 5+)

Let sit RT for 15 minutes

Ligation mixture can be stored at -20°C.

Transformation of DH5a E. coli

1. Add 1ul of ligation mixture to 50ul of subcloning grade DH5a
2. Mix with pipet tip and incubate on ice for 30 minutes
3. Incubate in 37°C for 20 seconds
4. Incubate 2 minutes on ice
5. Add 950ul LB media RT
6. Incubate in microfuge tube 37°C in 225rpm shaker for 1 hour
7. Spread 100ul on LB plate with appropriate selector (AMP)