Taq PCR Protocol

Taq buffer	2.5 µL
10 mM dNTP	0.5 µL
10 mM forward primer (enzyme 4941)	0.5 µL
10 mM reverse primer (enzyme 4941)	0.5 µL
Taq enzyme	0.5 µL
DNA template	0.5 µL
H_2O	20.375 µL
or 1ng/µL DNA	20.875 µL

		30 cycles			
Initial denaturation	denaturation	Annalnation	Extension	Final extension	
95℃	95℃	52℃	68℃	68℃	4℃
30s	15s	30s	3min	5min	forever

Seamless + Transformation Protocol

1. Seamless Reaction
    a. Adjust the DNA amount there is no need to add water
    b. Add sample (~3 µL)
    c. Add digested vector (~0.3-0.5 µL)
    d. Add buffer (~1.0 µL) (iced, not for negative control)
    e. Add enzyme (~0.5 µL) (iced, not for negative control)
    f. Vortex, spin, and incubate at 25℃ for 30 minutes in BioRad PCR machine

2. Transformation
    a. Thaw the competent cell on ice (OneShot TOP10) (Do this while incubating the DNA)
    b. Add all of the DNA samples to the competent cells

c. Heat shock at 42℃ for 30 seconds
d. Place back on ice for 3 minutes
e. (By flame) Add 200 uL SOC liquid
f. Incubate at 37℃ for 1 hour in a rotating incubator
g. (By flame) Plate the colony
h. Culture at 37℃ overnight

Mini Prep Protocol

1. Take the glass tubes out of the incubator and transfer everything into new plastic tubes by pouring
2. Spin the new plastic tubes at 5000 rpm for 10 minutes
3. Discard all the liquid inside the plastic tubes, leaving the pellet
4. Resuspend the pellet using 250 µL of P1 buffer
5. Transfer all the resuspended E. coli into new 1.5 mL tubes
6. Add 250 µL of P2 buffer into each tube (lysis)
7. Mix the liquid by inverting the tubes upside down a few times
8. Add 250 µL of N3 buffer into each tube (neutralization)
9. Mix the liquid very well by inverting the tubes upside down ~50 times
10. Spin at 13k rpm for 10 minutes
11. Transfer the supernatant into columns
12. Spin at 13k rpm for 1 minute
13. Discard flow through by pouring
14. Add 700 µL PE (wash) buffer into the columns
15. Spin at 13k rpm for 1 minute
16. Discard flow through and dry the tubes well by tapping on dry paper towels
17. Spin at 13k rpm for 1 minute
18. Transfer columns into new 1.5 mL tubes
19. Add 35 µL of water into each tube
20. Elute for 1 minute
21. Measure concentration of DNA for each tube using Nanodrop
22. Store the DNA at -20

Digestion Analysis Protocol

1. Find out the cut sites
2. Go to NEB Double Digest Finder to find out what buffer to use
3. Do the mini prep following the chart below

Mini Prep (DNA)	3µL
Enzyme	0.5µL

Buffer	2µL
Water	14.5µL
Total	20µL

4. Culture at 37℃ for 1 hr
5. Run a gel electrophoresis

3A Assembly Protocol

Master Mix

Enzyme Master Mix for **Plasmid Backbone** (10 ul total, for 2 rxns)

- 2.2 ul NEB Buffer 2.1
- 0.2 ul EcoRI-HF
- 0.2 ul PstI
- 0.2 ul DpnI (Used to digest any template DNA from production)
- 7.2 ul dH20

Enzyme Master Mix for **Part A** (10ul total, for 2 rxns)

- 2.2 ul NEB Buffer 2.1
- 0.2 ul EcoRI-HF
- 0.2 ul SpeI
- 7.4 ul dH20

Enzyme Master Mix for **Part B** (10ul total, for 2 rxns)

- 2.2 ul NEB Buffer 2.1
- 0.2 ul XbaI
- 0.2 ul PstI
- 7.4 ul dH20

Digestion

Digest **Plasmid Backbone**

- Add 4 ul linearized plasmid backbone (25ng/ul for 100ng total)
- Add 4 ul of Enzyme Master Mix

Digest **Part A**

- Add 4 ul Part A (25ng/ul for 100ng total)
- Add 4 ul of Enzyme Master Mix

Digest **Part B**

- Add 4 ul Part B (25ng/ul for 100ng total)
- Add 4 ul of Enzyme Master Mix

Digest all three reactions at 37C/30 min, heat kill 80C/20 min

Ligation

- Add 2ul of digested Plasmid Backbone (25 ng)
- Add equimolar amount of Part A (EcoRI-HF SpeI digested) fragment (< 3 ul)
- Add equimolar amount of Part B (XbaI PstI digested fragment) (< 3 ul)
- Add 1 ul T4 DNA ligase buffer. Note: Do not use quick ligase
- Add 0.5 ul T4 DNA ligase
- Add water to 10 ul
- Ligate 16C/30 min, heat kill 80C/20 min
- Transform with 1-2 ul of product

Transformation

- Thaw the competent cell on ice (OneShot TOP10) (Do this when incubating the DNA)
- Add the all the DNA samples to the cells
- Heat shot at 42oC for 30sec
- Place back on ice for 3min
- (By flame) Add 200uL SOC liquid, incubate at 37oC for 1hr in a rotating incubator
- (By flame) Plate the colony
- Culture at 37oC overnight