Purpose: To cut at restriction sites and remove inserted DNA from plasmid

Restriction Digest Protocol

30 µL Fast Digest Restriction Digest

1. Prepare a Fast Digest concentration cocktail with the following proportions: 1 µL Restriction Enzyme #1, 1 µL Restriction Enzyme #2, 3 µL of 10X Fast Digest Buffer, and 15 µL of diH2O.
2. Add 20 µL of this cocktail to a clean 1.5 Eppendorf tube and then add 10 µL of DNA
3. Incubate at 37° C for 30 minutes.

Purpose: To digest large amounts of plasmid for gel extraction

Restriction Digest Protocol

250 µL Fast Digest Restriction Digest

4. Prepare a Fast Digest concentration cocktail with the following proportions: 25 µL Restriction Enzyme #1, 25 µL of 10X Fast Digest Buffer, and 100 µL of diH2O.
5. Add 20 µL of this cocktail to a clean 1.5 Eppendorf tube and then add 100 µL of DNA
6. Incubate at 37° C for 3 hours.

Purpose: To inactivate the restriction enzymes before a ligation is performed

Heat Kill

1. Place digested DNA in water bath at 65° C for 20 minutes