**General PCR Protocol**

**Final PCR reaction will be 100 l total volume with:**

* + 100 ng of template DNA
  + 1 mM final concentration of each primer (forward and reverse)
  + 0.2 mM dNTPs
  + 1X DNA polymerase buffer
  + 0.5 ul of PFU (heat-resistant DNA polymerase)

**Ahead of time:**

Dissolve lyophilized primers in water to make a 500 M stock and store at -20C

Quick Tip: Find nmole on label and add 2X the volume of water in microliters (e.g. if it is 18 nmole then dissolve the lyophilized primer in 36 l of water)

**On day of PCR prepare working solutions:**

Prepare 20 mM working solution of forward and reverse primers by diluting the 500 mM stock 1:25 into water.

Dilute your template (plasmid) to 100 ng/ul in water (**Note:** this will come from your minipreps)

**Reaction mixture:**

* 1 l DNA template
* 5 l forward primer
* 5 l reverse primer
* 5 l of 4 mM dNTP mix
* 10 l of 10X PFU buffer
* 73.5 l of water (will now be a volume of 99.5)
* 0.5 l PFU enzyme – MIX WELL

**Thermocycler (PCR machine) protocol:**

1. 2 minutes at 95C
2. 45 seconds at 95C (Denaturation)
3. 45 seconds at 55C (Primer annealing)
4. 60 seconds at 72C (Extension)
   * Repeat steps 2-4 30X
5. 10 minutes at 72C
6. Forever at 4C

Extension time rule of thumb for PFU: 2 minutes for first KB and 1 minute for each additional KB

* Since our PCR product will be 500 bp we set extension to 60 seconds