

## **DNA sequencing protocol**

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- 1.) Start with fresh plasmid DNA purified with Qiagen plasmid kits.
- 2.) Resuspend DNA in H<sub>2</sub>O only.
- 3.) Adjust concentration to 250 ng/uL.
- 4.) Set up primers to a concentration of ~ 2-4 pM/uL.
- 5.) Set up reaction as follows:
  - 4 uL H<sub>2</sub>O
  - 4 uL 2.5X Buffer
  - 4 uL DNA
  - 4 uL Big-Dye Enzyme Mix
  - 4 uL Sequencing Primer
- 6.) Run on PCR machine using following conditions
  - 95° C for 2:30 minutes.
  - 96° C for 20 sec.
  - 50° C for 20 sec (adjust annealing temp for your primers) (this is optimal for pUC sequencing primers).
  - 60° C for 5:00 minutes.
  - 50 cycles
  - 4° C HOLD
- 7.) Clean PCR reactions with Qiagen DyeEX removal kit.  
Alternatively ethanol precipitation is acceptable.  
50ul 95% EtOH + 2.1 ul 3M NaOAc pH 4.6 add 20ul of PCR reaction, mix well let sit on bench.  
5 minutes at room temperature spin at max for 20 minutes. decant  
Wash pellet with 70% EtOH, spin at max for 15 minutes.
- 8.) Speed-vac reactions to dry.
- 9.) Take to Biotech center for sequencing (Expect a two day wait).

NOTE: If you have lower DNA concentrations adjust primer accordingly (ex. 200ng of PCR product you 3-5 pM of primer).