Plasmid purification using Qiagen Kit

* After overnight culture
  + Combine 2 x 25 mL overnight cultures into one 50 mL falcon tube
  + If necessary, make glycerol stock of overnight culture
    - In 2 mL cryovial, add 750 uL of 50% glycerol and 750 uL of overnight culture
    - Pipette up and down gently
    - Store in -80C for future use
  + Centrifuge 50 mL overnight culture at max speed for 20 minutes
    - There should be a large bacterial pellet at the bottom, and the media remaining should not be cloudy
  + Decant the remaining media
    - Pause point: store pellet in -20C
  + Add 5 mL of P1 resuspension buffer, vortex pellet
    - Can pause here for about an hour at most
    - Here you can set up the vacuum filtration manifold
      * Spin column, tube extender, QIA filter
  + Add 5 mL of P2 lysis buffer, flip up and down until solution is blue
    - Wait no longer than 2 minutes
  + Add 5 mL of S3 neutralization buffer, flip up and down until blue color disappears
  + Centrifuge for 5 minutes at max speed
  + Add liquid to QIA filter
  + Using plunger, plunge liquid through to tube extender and spin column
  + Remove filter, and add 2 mL binding buffer to each tube extender
    - Shake a little to homogenize binding buffer
  + Turn on vacuum, and after liquid is filtered, turn off vacuum and remove tube extender so all that’s left is the spin column
  + Wash by adding 700 uL of ETR buffer to each spin column and turning on the vacuum
  + Once filtered, turn off vacuum and repeat wash step with PE buffer (700 uL, turn vacuum on then off)
  + Transfer each spin column to a collection tube, centrifuge for 1 minute at 10,000g
  + Transfer spin column to new 1.5 mL Eppendorf tube, elute DNA with 100 uL dw, and spin for 1 minute at 10,000g
    - You can adjust your elution volume based on the size of your pellet from the beginning
      * Large pellet: 100 uL, medium pellet: 50 uL, etc.
  + Repeat elution step with same volume containing DNA
  + Measure concentration on nanodrop

**Common Pitfalls**

* After adding P2 buffer, wait no longer than 2 minutes before adding S3. Waiting too long could dramatically decrease your plasmid yield
* Do not forget to add binding buffer
* The vacuum manifold is old, so make sure you use the holes that don’t leak (leakage will decrease your yield)