**Plasmid Purification (Isopropanol Method)**

* 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, 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
* Here’s where the protocol diverges from Qiagen protocol
  + Transfer 15 mL sample to new 50 mL falcon
  + Add 12 mL isopropanol, keep in -20C to precipitate plasmid for 1-2 hr
    - Or overnight, can also be left in -20C for weeks
  + After precipitation, spin down for 10-15 minutes as max speed
    - You should see a thick plasmid pellet on the bottom
  + Decant, add 25mL 80% ethanol to wash
    - After adding the ethanol, vortex to break the pellet a bit, but you don’t need to reconstitute the pellet
  + Spin down again for 5-10 minutes at max speed
  + Decant and keep the tube upside down on a kim wipe so no residual ethanol flows back down to the pellet
  + Leave to dry for 20 minutes
  + Once dry, reconstitute the pellet in 500uL – 1mL dw, store in 4C
  + 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

**QIAGEN method**

Alcohol precipitation is commonly used for concentrating, desalting, and recovering nucleic acids. Precipitation is mediated by high concentrations of salt and the addition of either isopropanol or ethanol. Since less alcohol is required for isopropanol precipitation, this is the preferred method for precipitating DNA from large volumes. In addition, isopropanol precipitation can be performed at room temperature, which minimizes co precipitation of salt that interferes with downstream applications.

1. Adjust the salt concentration if necessary, for example, with sodium acetate (0.3 M, pH 5.2, final concentration) or ammonium acetate (2.0–2.5 M, final concentration).
2. Add 0.6–0.7 volumes of room-temperature isopropanol to the DNA solution and mix well.   
   **Tip:**Use all solutions at room temperature to minimize co-precipitation of salt.  
   **Tip:** Do not use polycarbonate tubes for precipitation as polycarbonate is not resistant to isopropanol.
3. Centrifuge the sample immediately at 10,000–15,000 x g for 15–30 min at 4°C.  
   **Tip:** Centrifugation should be carried out at 4°C to prevent overheating of the sample. (When precipitating from small volumes, centrifugation may be carried out at room temperature.)  
   **Tip:** Genomic DNA can alternatively be precipitated by spooling the DNA using a glass rod following addition of isopropanol. The spooled DNA should be transferred immediately to a microfuge tube containing an appropriate buffer and redissolved (see step 9).
4. Carefully decant the supernatant without disturbing the pellet.   
   **Tip:** Marking the outside of the tube before centrifugation allows the pellet to be more easily located. Pellets from isopropanol precipitation have a glassy appearance and may be more difficult to see than the fluffy salt-containing pellets resulting from ethanol precipitation.  
   **Tip:** Care should be taken when removing the supernatant as pellets from isopropanol precipitation are more loosely attached to the side of the tube.  
   **Tip:** Carefully tip the tube with the pellet on the upper side to avoid dislodging the pellet.  
   **Tip:** For valuable samples, the supernatant can be retained until recovery of the precipitated DNA has been verified.
5. Wash the DNA pellet by adding 1–10 ml (depending on the size of the preparation) of room-temperature 70% ethanol. This removes co-precipitated salt and replaces the isopropanol with the more volatile ethanol, making the DNA easier to redissolve.
6. Centrifuge at 10,000–15,000 x g for 5–15 min at 4°C. Tip: Centrifuge the tube in the same orientation as previously to recover the DNA into a compact pellet.
7. Carefully decant the supernatant without disturbing the pellet.
8. Air-dry the pellet for 5–20 min (depending on the size of the pellet).  
   **Tip:**Do not overdry the pellet (e.g., by using a vacuum evaporator) as this will make DNA, especially high-molecular-weight DNA, difficult to redissolve.
9. Redissolve the DNA in a suitable buffer.   
   **Tip:** Choose an appropriate volume of buffer according to the expected DNA yield and the desired final DNA concentration.  
   **Tip:** Use a buffer with a pH of 7.5–8.0, as DNA does not dissolve easily in acidic buffers. (If using water, check pH.)  
   **Tip:** Redissolve by rinsing the walls to recover all the DNA, especially if glass tubes have been used. To avoid shearing the DNA, do not pipet or vortex.  
   **Tip:** High-molecular-weight DNA, such as genomic DNA, should be redissolved very gently to avoid shearing, e.g., at room temperature overnight or at 55°C for 1–2 h with gentle agitation.