**Tubes** (0.5 or 1.5 ml) – purifying **Sequencing** reactions (01 May 2022)

This protocol is based on an ABI [96-well plate protocol](https://biosci-batzerlab.biology.lsu.edu/genomics/documentation/3130_EtOH_Precipitation_plate.pdf). Better results are easier to obtain with plates and a centrifuge capable of spinning 96-well plates; however, good results are possible with tubes and a regular centrifuge.

1) Remove tubes from thermocycler; spin tubes to consolidate all fluids at the bottom. Transfer contents to tubes large enough to fit in your centrifuge rotor. [***Note #1***]

2) For each 10-ul reaction, add 5 μl EDTA (70-150 mM, pH 8); ensure that EDTA droplet enters sample before Step 3. (*For 20 μl reactions, use 5* μl *@ 150 mM & increase EtOH volumes, including wash, to 60 μl.*)

3) Add 30-33 μl 100% EtOH to each tube; cap tubes and mix by vigorously shaking tubes to shift EtOH from top-to-bottom (repeat 4X). [***Note #2***]

5) Incubate samples @ room temperature (RT; >4 min).

6) Orient tubes identically in rotor so that DNA will pellet in the same location in each tube; spin tubes (minimum speed = 2500 RCF, 20oC, 15-30 min) [***Notes #3 & #4***]. Proceed to Step 7 immediately; otherwise, if >2 min have passed, re-spin tubes for additional 2 min prior to Step 7.

7) Remove supernatants by either (A) aspirating with a pipette tip (sliding pipette tip down wall opposite pellet) or (B) dumping and blotting tubes on a clean Kimwipe. Then, spin tubes briefly to collect residual EtOH at bottom of tubes, and use a pipette tip (do NOT try to dump-&-blot) to aspirate remaining EtOH. [***Note #5***]

8) Add 30-33 μl 70% EtOH to each tube; cap tubes and mix as in Step 3.

9) Spin tubes as in Step 6, but for only 5-10 min [***Notes #3 & #4***].

10) Repeat Step 7(A) or 7(B); consider doing a second 70% wash to remove more UDT’s.

11) Dry tubes (caps open) to remove traces of EtOH (RT, >15 min; 70oC, >1 min; or SpeedVac).

 *Caution: Chilling open tubes at this point can cause severe degradation of the C-fluorophore.*

12) Add 15 ul ABI Hi-Di formamide *[****Note #6****]* to each tube; cap tubes; briefly vortex & spin.

13) Refrigerate tubes (>30 min); vortex samples well, and centrifuge to collect contents. [***Note #7***]

14) Transfer samples to strip tubes or a plate for sequencing.

***Note 1****: If placed in a 96-tube rack, 0.2-ml tubes can be processed faster & more effectively with the ‘Plate Protocol’.*

***Note 2****: Store EtOH solutions in a low-humidity environment (e.g., fridge or freezer) to prevent absorption of water from the air; your DNA won’t precipitate if the % of EtOH drops too low.*

***Note 3****: These spin speeds & times were designed for a plate-centrifuge; you can reduce spin times with the higher speeds achievable in traditional bench-top models.*

***Note 4****: At these speeds, signal strengths from 15 vs. 30 min spins are ~ identical; but, a 15-min spin loses ~10-20 bp of sequence close to the primer (vs. a 30-min spin) – in general, 20-min spins ensure optimal results. Signal strength drops with shorter spin times (e.g., 10 min). ABI recommends 4oC spins; RT actually works better.*

***Note 5****: Residual EtOH contains numerous unincorporated dye terminators (UDT’s). Drying the EtOH does not remove UDT’s; if not removed, UDT’s degrade sequence data (see* [*Why do massive peaks occur ~50-70 bp into my data?*](https://biosci-batzerlab.biology.lsu.edu/Genomics/genomics_faq_page.php#Troubleshooting-5)*).*

***Note 6****: Other* [*formamide*](https://biosci-batzerlab.biology.lsu.edu/Genomics/genomics_faq_page.php#Post-sequencing_reaction-6) *brands may have water, contaminants, or improper buffering, leading to poor signal.*

***Note 7****: Vortexing is critical if transferring samples from clean-up tubes shortly after adding formamide (i.e., <30 min).*