**Plates & Strip-tubes** – purifying **Sequencing** reactions (01 May 2022)

1. Spin plate (*Note 1*) briefly to settle volumes (thaw samples first, if needed).
2. Add 5 μl EDTA (70-150 mM, nuclease-free); ensure each droplet enters sample before Step 3.
3. Add 30-33 μl 100% EtOH to each well (bringing total volume/well to ~45-48 μl; *Note 2*).
4. Seal plate (PCR mat); mix by vigorously shaking plate (~2-3 s), then invert plate & repeat shaking.
	* Repeat the “shake-invert-shake” process a total of 4X. (*Note 3*)
	* Maintain a firm seal by compressing the mat onto the plate with an inverted 96-well plate plastic rack; otherwise, cross-contamination of wells and sample losses might occur.
5. Incubate samples ≥4 min (@ room temperature, RT).

1. Balance rotor and centrifuge plate (2500 rcf, 20oC, 20 min [range: 15-30 min]; *Note 4*). After centrifuge stops spinning, proceed to Step 7 immediately; otherwise, if >2 min have passed, re-spin plate (2 min) first.
2. Prepare to spin the plate inverted (150 rcf, 1 **full** minute for 100% supernatant removal; *Note 5*)
	* Put 1 clean Kimwipe over existing Kimwipe pad (≥5 wipes) in “[*foil cap*](https://biosci-batzerlab.biology.lsu.edu/Genomics/images/genomics/Foil_cap.jpg)” (*Note 6*);
	* Gently remove PCR mat and cover plate with “foil cap” (kimwipe-side down);
	* Put covered plate upside-down in centrifuge bucket, balance rotor, and centrifuge (150 rcf, 60 s).
3. Wash step: Add 30-33 μl 70% EtOH to each well (*Note 7*). Seal plate and mix as in Step 4; repeat Step 6 & Step 7 once (except, reduce centrifugation to only 5-min in Step 6); and, continue to Step 9.
4. To remove trace EtOH, dry plate in thermal cycler (70oC, 1 min; hold @ 30oC; *Note 8*).
5. Resuspend samples: add 15 ul ABI Hi-Di formamide to each well; seal plate; briefly vortex; spin plate @ 2,500 rcf (1 min). Refrigerate plate until samples are placed on 3130XL sequencer. [*Note 9*]
* ***Note 1****: To process 0.2-ml Strip-tubes, put them in a 96-place tube rack (0.2-ml); if needed, put blank tubes on the other end of the rack for balance. For 1.5-ml tubes, see alternative “tube” protocol.*
* ***Note 2****: For 20-µl reactions, use 150 mM EDTA and double EtOH volumes throughout protocol. Also, 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 percentage of EtOH drops too low.*
* ***Note 3****: Effective mixing will not occur if you shake too gently. Also, PCR mats or caps are the most reliable seals; ethanol leaks past adhesive seals. If using caps, touch-spin samples & remove caps prior to Step 6 so as not to disturb pellets when preparing to centrifuge plate inverted. Caps may be reused during protocol if kept clean & restored to same wells (or tubes).*
* ***Note 4****: ABI recommends 4oC spins; RT actually works better. Signal strengths from 15 vs. 30 min spins are ~ identical; however, a 15-min spin loses ~10-20 bp of sequence close to the primer (vs. a 30-min spin) while a 20-min spin loses only ~5-10 nt. Signal strength drops with shorter spin times (e.g., 10 min).*
* ***Note 5****: Pellets might remain stuck to the plastic even if high rcf is accidentally used; by contrast, inadequate spin time may leave residual EtOH in wells. Flexible plates (i.e., lacking partial skirt) should be supported in a rigid 96-place rack to ensure that centrifugation cannot twist the wells and dislodge pellets.*
* ***Note 6****: The “Foil Cap” is a doubled-sheet of aluminum foil shaped (~11 x 21 x 3 mm) to fit like a cap over the 96-well plate. The kimwipes absorb EtOH spun out when plate is centrifuged while inverted; the foil helps to keep the EtOH from dispersing into the centrifuge during the spin. Alternatively, turn the centrifuge plate bucket upside-down (with Kimwipe insert), insert plate face-up, flip assembly over, and spin... but, more EtOH will escape.*
* ***Note 7****: With weak sequencing reactions, Step 8 improves result by more fully removing unincorporated dye terminators (UDTs), thereby further minimizing interference with basecalling. By contrast, with strong sequencing reactions, Step 8 (wash) might not be critical because there are usually fewer UDTs in the first place.*
* ***Note 8****: Chilling open tubes at this point can cause severe degradation of the C-fluorophore. Also, residual EtOH contains UDT’s. Drying the EtOH does not remove UDT’s; if not removed, UDT’s* [*degrade sequence data*](https://biosci-batzerlab.biology.lsu.edu/Genomics/genomics_faq_page.php#Troubleshooting-5)*).*
* ***Note 9****: Other formamide brands may have water, contaminants, or improper buffering, leading to poor signal. Vortexing is not essential, but may be helpful if the plate will be loaded immediately on sequencer.*