**DNA Templates: Precipitation of PCR Reactions (plate centrifuge)**

 (20 April 2020)

Note: Protocol refers to ‘plates’; however, 8-strip tubes can be processed by the same protocol

as long as they are stabilized in a 96-place rack for 0.2-ml tubes.

1) Spin plate (thaw first, if needed) briefly to settle volumes.

2) If plate is sealed with caps, decide whether to reuse caps or switch to a silicone mat. If reusing caps, mark them and remove (storing carefully on a clean Kimwipe for later re-use); otherwise, remove caps and switch to a silicone mat for Steps 6-8 and Step 11. (Do NOT use adhesive seals!)

3) For 10 ul reactions, you may proceed to Step 4; otherwise, fully dry samples in SpeedVac (for 50-ul reactions, takes ~70 min @ 45oC or ~45 min @ 70oC).

4) – For ~10 ul samples: add 5 μl EDTA (70-150 mM); ensure droplet enters sample before Step 5.

 – For dried samples: add 15 EDTA (30-40 mM); incubate ~10 min; seal plate & vortex; spin plate.

5) Add 33 μl 100% EtOH to each well.

6) Seal plate (caps or 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: Maintain a firm seal by compressing the mat/caps onto the plate with an inverted 96-well plate plastic rack – otherwise, cross-contamination of wells and sample losses might occur.

7) Incubate @ room temperature (RT; 10 min). If using caps, touch-spin (i.e., ≤1’ @ ~2500 rcf) to settle volume; remove caps (store carefully for re-use); if using mats, just proceed.

8) Spin plate (2500 RCF, 20oC, 20 min). When complete, proceed to Step 9 immediately; otherwise, re-spin plate for additional 2 min prior to Step 9.

9) Cover plate with “*foil cap*” (thick pad of Kimwipes inside cap) and put upside-down in centrifuge plate bucket. Spin plate (Program 2: 150 RCF, 1 full minute to completely remove ethanol).

10) Dry plate to remove traces of EtOH (in thermocycler @ 70oC, 1 min).

11) Resuspend **PCR products**: Add [TVLE](https://biosci-batzerlab.biology.lsu.edu/Genomics/genomics_faq_page.php#Primer-1) to desired volume (see Note below). If bringing to >20 ul, use sets of ≤25 ul to avoid splash-up onto tips and potential cross-contamination on the plate; seal plate. *(Note: Consider running gel to determine if additional dilution is needed for sequencing.)*

12) PCRs with high primer input or “difficult-to-remove” primers: If this situation is suspected, repeat the cleaning and resuspension steps above to ensure that sequencing reactions will contain just one primer (i.e., that there won’t be detectable levels of the opposite PCR primer in the sequencing reactions). Before recleaning, vortex samples briefly, touch-spin samples, and allow to resuspend for at least 15’.

***Note****: DNA recovery should be >80-90%; thus, estimate resuspension volume based on brightness of raw PCR products in a gel, keeping in mind that some of the original reaction has already been consumed. For example: Assume you ran 5 ul of a 25-ul PCR reaction in the initial agarose gel, with* [*band brightness*](https://biosci-batzerlab.biology.lsu.edu/Genomics/images/genomics/1_vs_3-ul_DNA.jpg) *suggesting that 1-ul of the raw product would be sufficient for a BigDye reaction. If ~80% of original product remains, resuspending in ~17-ul (i.e., ~85% \* 20 ul) should retain same concentration; if we want to add 2-ul to each BigDye reaction, resuspend in ~34-ul.*