**Plates & Strip-tubes –** purifying DNA **Templates** (04 June 2025)

See “**Science Aid Center**” on Core website for additional information as indicated below.

* ***DNA recovery****: Should be >80-90%; for safety factor, assume 70%. Estimate required downstream volumes based on brightness of raw PCR products in a gel. For example: Assume you ran 5 ul of a 25-ul PCR in an agarose gel, and estimated by the band brightness (see images at* “How much DNA to use in a sequencing reaction?”*) that you need 2-ul of raw product for a BigDye DNA Sequencing reaction. If pre-aliquoting raw PCRs for sequencing, clean ~2.9 ul product per reaction. Alternatively, if cleaning & post-aliquoting templates, resuspend in ~14-ul (i.e., 70% \* 20 ul* *– i.e., remaining PCR volume) of buffer to aliquot 2 ul of cleaned templates for sequencing. (Note: consider verifying concentrations by new gel.)*
* ***Templates for DNA Sequencing****: when calculating volumes, assume 70% recovery of template (or, ~50% for 2X cleanings).*
* *Option #1: Pre-aliquot desired amount of PCR template into wells for each sequencing reaction.*
* *Option #2: Clean entire PCR volume for all samples, resuspend samples in TVLE (see* “Choice of primer resuspension buffer?”*), and aliquot desired amount of cleaned PCR template into wells for individual sequencing reactions.*
* *PCRs* *with high primer input or “difficult-to-remove” primers: If suspected, repeat cleaning & resuspension steps to ensure sequencing reactions will contain just 1 primer (i.e., no detectable levels of opposite PCR primer in sequencing reactions). Before recleaning, vortex resuspended samples briefly, touch-spin samples, and resuspend (>15 min).*
* ***Miscellaneous notes****:*
* *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.*
* *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.*
* *Steps 9 & 10: It is important to fully flush out the ethanol as any residual ethanol may contain high levels of the original PCR primers; if present in a sequencing reaction, residual primers may dramatically reduce read quality.*

1) To settle volumes, briefly spin plate (thaw first, if needed) in Plate Centrifuge.

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 clean Kimwipe for later re-use); otherwise, remove caps and switch to a silicone mat for Steps 6-8 and Step 10. (Do NOT use adhesive seals; EtOH leaks!)

3) For 10 ul volumes, proceed to Step 4; otherwise, it is simplest to first fully dry samples in SpeedVac (*50-ul reactions, takes ~45 min @ 70oC*) vs. adjusting EDTA & ethanol volumes.

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

 – If Dried: add 15 EDTA (30-40 mM); seal and vortex plate; after 10 min, vortex & touch-spin plate.

5) Add 30-33 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). Finally, touch-spin (i.e., 1 min, ~150 rcf) to settle contents.

 *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 as the liquid flies about in the wells.*

7) Incubate samples @ room temperature *(better than 4oC)* for4 min. Then, if using tight-fitting caps, remove & set caps aside for re-use (*prevents jostling pellets in Step 8*); with mats, just go to Step 8.

8) Spin plate (2500 rcf, 20oC, 15 min). When done, remove mat & proceed to Step 9 within 2 min; otherwise,

 re-spin plate (2 min) prior to Step 9.

9) Cover plate with “*foil cap*” (*thick pad of Kimwipes inside ‘cap’ to minimize EtOH discharge into centrifuge; see image in* “How to clean my sequenced templates?”), invert plate in centrifuge bucket, and spin plate (1 full minute, 150 rcf).

10) Add 33 μl 70% EtOH to each well; seal & mix plate as in Step 6. Centrifuge (2500 rcf, 20oC, 5 min); repeat Step 9.

11) Dry plate to remove traces of EtOH in thermal cycler (70oC, 1 min).

12) For pre-aliquoted templates, leave the wells dry and add your reaction mix directly to the dried templates. If aliquoting the cleaned DNA templates, resuspend in the right amount of TVLE to achieve your desired concentrations. *(For a BigDye sequencing reaction, see How much DNA to use in a sequencing reaction?)*