**Plates & Strip-tubes –** purifying DNA **Templates** (01 May 2022)

* ***DNA recovery****: Should be >80-90%; for safety factor, use 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 reaction in the initial agarose gel, with* [*band brightness*](https://genomics.lsu.edu/mages/genomics/1_vs_3-ul_DNA_mixed-bands.jpg) *suggesting that 1-ul of the raw product would be sufficient for a BigDye DNA Sequencing reaction. If pre-aliquoting templates and ~70% of original product remains post-cleaning, ~1.4 ul of raw PCR product/reaction should be sufficient. Alternatively, if cleaning & post-aliquoting templates for sequencing, resuspending in ~14-ul (i.e., 70% \* 20 ul remaining)* *should give original concentration so that you would then aliquot 1 ul of cleaned templates for sequencing. (Note: consider running a new gel to verify new concentrations.)*
* ***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*](https://genomics.lsu.edu/genomics_SACKs_main.php#Primer-1)*, 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, the 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 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 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*](https://genomics.lsu.edu/images/genomics/Foil_cap.jpg)” (thick pad of Kimwipes inside ‘cap’) and invert plate in centrifuge bucket. Spin plate for 1 full minute at 150 rcf. *(The ‘foil cap’ minimizes how much ethanol is spun out into the centrifuge.)*

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](https://genomics.lsu.edu/genomics_SACKs_main.php#Primer-1) to achieve your desired concentrations. *(For a BigDye sequencing reaction, see* [*How much DNA to use in a sequencing reaction?*](https://genomics.lsu.edu/genomics_SACKs_main.php#Template-6)*)*