**Tubes** (0.5 or 1.5 ml) – 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, and estimated by the apparent* [*band brightness*](https://genomics.lsu.edu/mages/genomics/1_vs_3-ul_DNA_mixed-bands.jpg) *that 1-ul of the raw product would be sufficient for a BigDye DNA Sequencing reaction.*
* *Resuspending cleaned templates in ~14-ul (i.e., 70% \* 20 ul) of buffer 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 concentrations.)*
* ***Templates for DNA Sequencing****:*
* *Clean entire PCR volumes, resuspend in*[*TVLE*](https://genomics.lsu.edu/genomics_SACKs_main.php#Primer-1)*, and aliquot templates 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****:*
* *This protocol is based on an ABI* [*96-well plate*](https://genomics.lsu.edu/documentation/3130_Sequencing_EtOH_Precip_Plates_01May2020.docx) *DNA Sequencing protocol. Better results are easier to obtain with plates (or 8-strip tubes in a 0.2-ml 96-place rack) and a plate centrifuge; however, with careful technique, this works too.*
* *These speeds & times were designed for a low-speed plate-centrifuge; you can reduce spin times with the higher speeds achievable in traditional bench-top models.*
* *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.*
* *Steps 6 & 9: It is important to fully remove 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) 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.

2) For 10 ul samples, add 5 μl EDTA (70-150 mM, pH 8), ensuring droplet enters sample before Step 3.

 *– Alternative #1: For 20 μl samples, use 5 μl @ 150 mM EDTA & increase EtOH volumes, including wash, to 60 μl.*

 *– Alternative #2: SpeedVac samples dry; add 15 EDTA (30-40 mM); incubate ~10 min; seal, vortex and touch-spin.*

3) Add 30-33 μl 100% EtOH to each tube; cap & shake tubes to shift EtOH top-to-bottom (repeat 4X).

4) Incubate samples @ room temperature (>4 min – *RT works better than 4oC*).

5) Orient tubes identically in rotor so DNA will pellet in same location in each tube; spin tubes (minimum speed = 2500 RCF, 20oC, 15 min). Proceed to Step 6 immediately; otherwise, first briefly re-spin tubes (2 min).

6) Remove supernatants by either (A) aspirating with a pipette tip (sliding pipette tip down wall opposite pellet) or (B – *for large volumes*) using the ‘dump-blot-spin-aspirate’ process *(i.e., dumping supernatant; blotting tubes on a clean Kimwipe; centrifuging tubes to collect residual EtOH; and, aspirating remaining EtOH)*.

7) Add 30-33 μl 70% EtOH to each tube; cap tubes; shake tubes as in Step #3.

8) Spin tubes as in Step 5, but centrifuge for only 5 min (*to ensure pellet is stuck to tube*).

9) Remove supernatant by aspiration; consider doing a second 70% wash to remove more residual PCR primers.

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

 DNA in sufficient [TVLE](https://genomics.lsu.edu/genomics_SACKs_main.php#Primer-1) to achieve desired concentrations.  *(e.g., For sequencing 500-750 bp fragments, plan*

 *to use 1-5 ul of template, giving a total of 2-6 ng template/reaction; see* [*How much DNA to use in a sequencing reaction?*](https://genomics.lsu.edu/genomics_SACKs_main.php#Template-6)*)*