**‘Template-Service’ DNA Sequencing REQUIREMENTS** (01 May 2022)

* For Templates submitted before noon, data will typically be posted online by end of the following 1-2 business days.
* Processing may take longer during busy periods or if submissions also require template cleanup and aliquoting.
* Before deviating from these protocols, contact Genomics Facility (GF)... for further details, click on ‘links’ or see [Science Aid Center](https://genomics.lsu.edu/genomics_SACKs_main.php).

1. **Authorization**: Before using this option, consult with the Genomics Facility to ensure you understand the process.
2. **Number of Reactions:**
   1. ‘Templates – Standard Pricing’: no minimum number of reactions; minimum BigDye volume = 0.5 ul/rxn.
   2. ‘Templates – Bulk Discounts’: ≥47 reactions; reduced rates; can request reduced BigDye/rxn for more savings.
3. **‘Purified-&-Aliquoted’** vs. **‘Non-Purified’ Templates**:
   * + 1. ‘Purified-&-Aliquoted’ templates: strongly preferred option, with rapid, lowest-cost results.
       2. ‘Non-purified’ templates: contact GF staff for options and instructions.
4. **‘Purified-&-Aliquoted’ Templates**: must have been [processed](https://genomics.lsu.edu/genomics_SACKs_main.php#Template-5) for use in a BigDye sequencing reaction.
   1. Purification: PCR & Plasmid products must be ‘purified’, either by a commercial product or by GF protocol ([96-well Plate or 8-Tube Strips](https://genomics.lsu.edu/documentation/3130_EtOH-EDTA-Precip_Sequencing_Plates_01May2022.docx); or, [Tubes](https://genomics.lsu.edu/documentation/3130_EtOH-EDTA-Precip_Sequencing_Tubes_01May2022.docx)). PCR primers can be removed by ExoSAP-IT; alternatively, we have developed a “proprietary” EtOH-EDTA protocol specifically designed to remove primers and primer-dimers *(not always accomplished by standard protocol)*.
   2. Preferred Buffer: Resuspend DNA in a [low TE](https://genomics.lsu.edu/genomics_SACKs_main.php#Primer-1) (TVLE: 10 mM Tris; 0.05 mM EDTA; available as a GF supply).
   3. Single Templates: Only 1 [template/sample](https://genomics.lsu.edu/genomics_SACKs_main.php#Template-3) (i.e., a single PCR product or a pure clone).
5. **Template Quantification**: If you choose to skip quantification, template input may be either too low or too high... leading to poor sequencing data; document any quantification results in your Excel file (photos must be <200 Kb).
   1. PCR products: Do *not* use a [spectrophotometer](https://genomics.lsu.edu/genomics_SACKs_main.php#Template-6); ‘[gel-estimation](https://genomics.lsu.edu/images/genomics/1_vs_3-ul_DNA_mixed-bands.jpg)’ is highly preferable.
   2. Plasmids: Spectrophotometers (e.g., see [Nanodrop](https://genomics.lsu.edu/documentation/3130_NanoDrop_tips.pdf) tips) are ok, if samples are mostly RNA-free; however, it’s best to assess a subset of the samples ([linearized](https://genomics.lsu.edu/genomics_SACKs_main.php#Template-7)) by agarose gel.
   3. Alternative assays: e.g., Qubit assay (measures only dsDNA); generally not suitable for circularized plasmid DNA.
   4. Sub-sampling: Quantification of a subset of all samples (i.e., ≥10-30%) may be ok if variability is low.
6. **Pre-Aliquoted Templates**: Aliquoted DNA must be submitted in [8-Tube Strips](https://genomics.lsu.edu/genomics_SACKs_main.php#Submitting_samples-2) (0.2-ml) or a [96-well plate](https://genomics.lsu.edu/genomics_SACKs_main.php#Submitting_samples-3).
   1. 3130XL Plate Assembly: Must [*pre-verify*](https://genomics.lsu.edu/genomics_SACKs_main.php#Submitting_samples-3) that your plate or tube style will fit (*cannot be a ‘fast’ style as wells are short*).
   2. Plates: Easily-cut ‘rim-less’ style plates are preferred (*GF supply item = VWR 82006-636*).

– use PCR seals, caps, or clear 3M [packing tape](https://genomics.lsu.edu/genomics_SACKs_main.php#Submitting_samples-8) to seal plates; if plate is cut, include an even-number of columns.

* 1. Tubes: Styles with detachable caps (i.e., non-hinged) are strongly preferred.

– submit the entire strip of 8 tubes, even for 1 sample.

* 1. Volume: Use same volume for all reactions; if needed, add nuclease-free water or [TVLE](https://genomics.lsu.edu/genomics_SACKs_main.php#Primer-1) to more concentrated templates.
     1. **1-5 μl** template (if reactions will use 1 μl primer); or,
     2. **1-4 μl** template (if reactions will use 2 μl primer).
  2. Nanograms (proxy for copy number): Values shown are rough approximations – scale **ng** for different fragment sizes.
     1. PCR products (~500-bp): 2-6 ng/reaction; and,
     2. Plasmids (~5-kb, including insert): ~50-300 ng/reaction.
  3. [Positive Control](https://genomics.lsu.edu/genomics_SACKs_main.php#Sequencing_reaction-4) templates: including ≥1 p-ctrl (up to 3-5% of total samples) is highly recommended.

1. **Primers**: Only [1 primer](https://genomics.lsu.edu/genomics_SACKs_main.php#Primer-4) can be used in each reaction.
   1. GF Primers: The GF will provide M13 and T3/T7 primers free-of-charge.

**M13-F** (5´-GTAAAACGACGGCCAG-3´); **M13-R** (5´-CAGGAAACAGCTATGAC-3´);

**T3** (5´-ATTAACCCTCACTAAAGGGA-3´); **T7** (5´-TAATACGACTCACTATAGGG-3´); **T7**-Terminator (5´-GCTAGTTATTGCTCAGCGG-3´).

* 1. Other primers: must be provided by the client by one of the following options.
     1. Stock tube (typically 2-5 μM): 1 primer/tube, for inclusion in the Master-mix(es) @ 1 μl/reaction.
        + Minimum volume: the larger of (**a**) 40 μl, or (**b**) [# samples \* 1.25-ul/sample].
        + Resuspend primers in a [low TE](https://genomics.lsu.edu/genomics_SACKs_main.php#Primer-1) product (e.g., TVLE... *a GF supply item*).
        + For PCR templates, provide 10-20 μM stocks (additional primer helps to outcompete residual PCR primers).
     2. Pre-pipetted: primer included with each template (**1 μl** @ 2-5 μM [10-20 μM for PCR], or **2 μl** @ 0.5X concentration).
  2. Multiple primers (per submission): If <4 rxns/primer or >8 primers, contact GF to see if primers need to be pre-aliquoted.

1. **Submission Process... 2 Steps**:
   1. Online submission: [Login](https://genomics.lsu.edu/genomics_login.php), upload Excel “Names” file, complete form, and click ‘*Submit*’.
      1. For inclusion of primers in Master-mix(es), samples needing same primer must be in contiguous wells.
      2. On Excel sheet, identify controls by inserting “**p-ctrl**” or “**n-ctrl**: in sample names.
   2. Physical Submission:
      1. Label samples according to website [*instructions*](https://genomics.lsu.edu/genomics_SACKs_main.php#Submitting_samples-1); transfer tubes (or plate) to a GF 96-place rack; and,
      2. put samples in in “Mini-Fridge” by sink in the Genomics Facility (Rm. A628); if facility is locked, put your samples in the ice chest by the sink in the Cold Room (A650).
      3. Ensure you provide an even number of ‘columns’ (partial plates) or entire strip-tubes, even if extra wells are empty.
2. **“Failed Reactions Policy”**: Genomics Facility is **sole** arbiter regarding sample re-processing or other adjustments.