**Full-Service DNA Sequencing** (Printer-Friendly version, 11Jul2025)

Briefly, after a client submits DNA templates for sequencing, the Genomics Core performs the BigDye sequencing reactions and cleanup procedures, followed by electrophoresis on the 3130xl. For a more complete description of this service, see the following topics at the Core’s webpage for **Services** under **DNA Sequencing or Fragment Analysis**:

– **Overview – Sequencing vs. FA**; and,

– **Service Levels: DNA Sanger-Sequencing**.

* 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 the protocols outlined below, contact Genomics Core (GC) – for further details, click on the ‘links’ below or see Science Aid Center.

**Authorization**: Before using this option, consult with the Genomics Core to ensure you understand the process.

**Number of Reactions**

* **'Standard Pricing’**: no minimum number of reactions; minimum BigDye volume = 0.5 ul/rxn.
* ‘**Bulk Discounts’**: ≥47 reactions; reduced rates; can request reduced BigDye/rxn for more savings.

**Purified vs. Non-Purified Templates**

* **Purified-&-Aliquoted’ templates**: strongly preferred option, with rapid, lowest-cost results.
* **Non-purified’ templates**: contact GC staff for options and instructions. (Note: Along with a request for DNA Sequencing, this option requires a concurrent Service Request for template purification.)

**Purified Template Requirements**

* **‘Purified-&-Aliquoted’ Templates**: must have been processed for use in a BigDye sequencing reaction.
* **Purification**: PCR & Plasmid products must be ‘purified’, either by a commercial product or by a GC protocol for 96-well Plates (or 8-tube PCR Strips) or for 1.5-ml Tubes.
* **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).

**Preferred Buffer**: Resuspend DNA in low TE (TVLE: 10 mM Tris; 0.05 mM EDTA; a GC supply item).

**Single Templates**: Only 1 template/sample (i.e., a single PCR product or a pure clone).

**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).

* **PCR products**: ‘Gel-estimation’ is strongly recommended. Do not use spectrophotometer absorbance readings (e.g., NanoDrop); unless PCR products have been thoroughly purified, NanoDrop readings will be grossly inflated by the presence of primers and residual dNTPs.
* **Plasmids**: Unlike PCR products, this type of DNA is typically produced without primers and dNTPs; further, the resulting molecules are usually mostly intact. Thus, as long as the DNA produces an electropherogram that is consistent with purified DNA and is mostly RNA-free, spectrophotometers which scan from ~220-350 nm (e.g., see Nanodrop Quick Tips) are usually an acceptable means of quantification. However, spectrophotometers which take readings at just 260/280 nm will not show the presence of contaminating compounds (e.g., bacterial residues, EDTA, or phenol) which might be inflating the values at 260/280 nm; thus, while it is always advisable to assess a subset of plasmid DNA samples (linearized) by agarose gels, that is especially critical when using a simple 260/280-nm spectrophotometer.
* **Alternative assays**: There are various fluorometric assays which measure only dsDNA. However, fluorometric assays are relatively expensive and they are not suitable for circularized plasmid DNA (the dye offloads and onloads randomly as supercoiling shifts). Further, if the assay does not display fragment sizes (e.g., Qubit dsDNA), then the estimate may be inflated by the presence of extraneous dsDNA products; this is very common in the form of primer-dimers for PCR products.
* **Sub-sampling**: Quantification of a subset of all samples (i.e., ≥10-30%) may be ok if variability is low.

**Pre-Aliquoted Template Requirements**

* Aliquoted DNA must be submitted in 8-Tube Strips or a 96-well plate; both must have standard 0.2-ml wells.
* 3130XL Plate Assembly: Must pre-verify that your plate or tube style will fit properly.
* Caution: Plates and tubes cannot be a ‘fast’ style (i.e., 0.1-ml wells) because the short wells will destroy the capillary array on the DNA sequencer.

**Plates**: Easily-cut ‘rim-less’ style plates are preferred (e.g., VWR 82006-636, a GC supply item).

– **Sealing wells**: use caps, clear 3M packing tape, or PCR seals (Note: avoid foil seals as they are difficult to remove);

– **Partial plates**: if plate is cut, include an even-number of columns.

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

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

**Volume**: Use same volume for all reactions; if needed, add nuclease-free water or TVLE to more concentrated templates.

– 1-5 μl template (if reactions will use 1 μl primer); or,

– 1-4 μl template (if reactions will use 2 μl primer).

**Nanograms (proxy for copy number)**: Values shown are rough approximations – scale ng for different fragment sizes.

– **PCR products** (~500-bp): 2-6 ng/reaction; and,

– **Plasmids** (~5-kb, including insert): ~50-300 ng/reaction.

**Positive Control templates**: including ≥1 p-ctrl (up to 3-5% of total samples) is highly recommended.

**Primers:** Only 1 primer can be used in each reaction. GC Primers: GC 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´).
* **Other primers**: must be provided by the client by one of the following options.
  + **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 product (e.g., TVLE... a GC supply item).
* For PCR templates, provide 10-20 μM stocks (additional primer helps to outcompete residual PCR primers).
  + **Pre-pipetted**: primer included with each template (1 μl @ 2-5 μM [10-20 μM for PCR], or 2 μl @ 0.5X concentration).
  + **Multiple primers (per submission)**: If <4 rxns/primer or >8 primers, contact GC to see if primers need to be pre-aliquoted.

**Submission Process – 2 steps**

* **Online submission**: Login, upload Excel “Names” file, complete form, and click ‘Submit’.
  + For inclusion of primers in Master-mix(es), samples needing same primer must be in contiguous wells.
  + On Excel sheet, identify controls by inserting “p-ctrl” or “n-ctrl” in sample names.
* **Physical Submission**:
  + See ***https://genomics.lsu.edu/genomics-sacks-main.php#submitting-samples-1***.
  + Ensure you provide entire 8-strips [tubes] or an even number of ‘columns’ [partial plates], even if extra wells are empty.
  + Transfer tubes (or plate) to a GC 96-place rack and take your rack back to your lab.
  + Put samples in in “Mini-Fridge” by sink in the Genomics Core (Rm. A628); if Core is locked, put your samples in the clear plastic "Drop-Box" by the sink in the Cold Room (A650).

**Failed Reaction Policy:** The Genomics Core is the sole arbiter regarding sample re-processing or other adjustments; however, if you receive sub-standard results which you believe might be due to actions by the Core, please request a review of the data and sample processing.