

## Sequencing Requirements

### Requirements for DNA sequencing:

1. Only submit cleaned samples
  - a. PCR products need to be purified before submitting for sequencing.
  - b. Please submit purified templates and primers diluted in dH<sub>2</sub>O (avoid buffers that contain EDTA or other metal chelating reagents, as they can interfere with the sequencing reaction)
2. Please submit your samples in a **final volume of 10 µL**.
3. We accept samples in a 96 well light plate, or individual or strip PCR tubes
  - a. If you are submitting a fully prepared plate, please make sure that your samples begin on an **Odd Numbered Column**- the sequencer can only begin a read on odd numbered columns.
  - b. The sequencer reads injections in multiples of 16- ie one injection is 16 wells/2 columns (1&2, 2&3, etc). If you do not have samples to fill an entire injection, please make sure to put 10 uL of Hi-Di Formamide in the "blank" wells, so that the capillaries are still in fluid during the run.
  - c. Fully prepared plates **MUST** be in Light Labs 96 well, semi-skirted plates (catalog #A3150). These are sold in the ERB and LS Light Labs Stores by the box, we can also sell you an individual plate. Our instrument is calibrated to these plates.
4. Plate layout
  - a. If you are submitting a full plate's worth of samples for full service sequencing, please submit a maximum of 95 samples so that a control can be run.
  - b. If you are submitting already prepared plates for sequencing, both full and partial, please place a pGEM control in one well within the first two columns/first injection of your plate. (Columns 1 & 2 are one injection; columns 3 & 4 are one injection, etc.)
  - c. If you need a pGem control/m13 primer, Melissa can provide you with one.
5. How much sample should you submit?

As a general rule of thumb, please submit 50 ng of PCR product and 10 pmol of primer per sample in 10 uL total volume.

Plasmid	PCR	Primer
~1000 ng	50 ng	10 pmol

6. DNA concentrations are best estimated by comparison with the bands of a DNA mass ladder on an agarose gel or a NanoDrop spectrophotometer.
  - a. Purified DNA should run as a single band on an agarose gel
  - b. For NanoDrop, the A260/A280 ratio should be 1.8. The NanoDrop only requires 1-2 uL of sample.

7. Do you supply primers?

-The GCF **DOES NOT** supply primers. Please submit **ONE** primer (fwd or rev) and sample in one tube at the appropriate concentration upon submitting your sequencing request

8. How do I choose a primer?

- a. Primers should be at least 18 bases long, and no longer than 27 bases long with an optimum length of 20-22 bases.
- b. Avoid runs of an identical nucleotide, especially guanine, to prevent slippage.
- c. Primers should ideally be around 50- 55% GC.
- d. Our annealing temperature is 50°C. Make sure your T<sub>m</sub> is above 45°C and below 55°C.
- e. Avoid primers that have secondary structure or that form dimers (4 or more bases that compliment either direction of the primer should be avoided)

9. How do I dilute my primer to get 10 pmol?

\*please note that you will need to check your individual primers for initial concentration\*

100 μM primer in solution (starting concentration) = 100 nmol/ml or 100 pmol/μl

You need 10 pmol per one sequencing reaction:

- Do a 1:10 dilution: (10 μl of 100 μM solution: 90 μl of water) to get a solution of 10 pmol/μl.
- Add 1 μl of this primer to the sequencing mix

10. Please label the samples clearly and with the same name that will be used to identify the electropherogram.

- a. Sequence names should contain less than 18 characters and cannot contain spaces.
- b. You may also label your sample PCR tubes 1, 2, 3 etc if your sequencing request form correlates the number to the sample name.

11. Please submit the completed Sequencing Request form with the samples. The form can be found under the "Resources" tab on [gcf.uta.edu](http://gcf.uta.edu) and is on the left side of the page under "Request"

12. When will I get my results?

- Results will be emailed no later than two business days after the order is received.

13. How much does sequencing a sample cost?

- a. Sequencing will be performed at a cost of \$5.00 per sample\*.
- b. Sequencing of more than 48 samples will be performed at a cost of \$4.00 per sample.
- c. Fully processed and prepared sequences (plates are dropped off ready to be sequenced) are run at a cost of \$0.50 per sample. *\*Non UT prices are slightly higher, please inquire.*