Sample Submission Guidelines

Sanger Sequencing: Full Sequence Analysis (FSA)

ACGT DNA Sequencing Services

Naming and Labeling Protocol

Character Limit

ACGT order forms on your account dashboard can only accept a total of 23 characters for both template name and primer name combined. Special characters are limited to numbers, underscores (_), and hyphens (-). No spaces.

Sample Labels

- Please make sure that the labeling on your tubes **matches** what you have written on the order form **exactly**.
- Use **BLACK** permanent marker to label your tubes, as colored markers tend to smear and are difficult to read.
- ACGT **does not** recommend tubes with screw-on caps, however, should you send samples in tubes with screw-on caps, you must label both the tube and cap.
- If you prefer to number your tubes instead of writing the full name, add the corresponding numbers in front of the template name according to the numbering protocol as follows:
  - 1_Template1 with PrimerA
  - 2_Template2 with PrimerB; etc.

  Our order forms will provide the option to autofill these numbers as well. This is to ensure that the samples can be correctly identified.

Primers

Only one primer may be used per Sanger reaction. If you need a template analyzed using multiple primers, those will need to be listed as separate reactions on the order form:

- Template1 with PrimerA
- Template1 with PrimerB
- Template2 with PrimerA
- Template2 with PrimerB; etc.

Acceptable Tubes

Samples should be submitted in 1.5 ml or 0.5 ml microfuge tubes with the appropriate amount of template and/or primer. You may also send PCR strip tubes or 96-well plates. **Please do not send individual PCR tubes.**

Quality Check

- ACGT recommends that you run your samples on agarose gel and nanodrop your samples Before submission to check the quality of your DNA and concentration. You should observe a single clear band on the gel and an A260/230 ratio of around 1.7-1.9. The A260/280 value should be around 2-2.2. Any value higher or lower than this could indicate contamination. If your purity ratios are not within these ranges, you may need to add an additional wash step prior to elution to your protocol.
- Template DNA should be eluted in nuclease-free water. Some kits recommend buffers for eluting DNA, but there are components in various buffers that may interfere with sequencing reactions and result in noisy or unusable data.

Sample Concentration Requirements

Order Number	Size of DNA	Amount of DNA	Template Concentration
Plasmid	› 2kb	200 ng per kb	50 ng/mL
Purified *or* Unpurified PCR Product	› 2kb	50 ng per kb	20 ng/mL

- **Primers** (if providing) should be around 33 – 66 ng/µL or 10 pmol/µL (pmol/µL = µM). We recommend sending at least 10 µL of each primer.
- The reference sequences should be provided in FASTA format. If needed, primers may be designed and synthesized based on this sequence.
- Please include a photograph of the template DNA with the quantity of the DNA loaded and a molecular marker with the order.

Bacteria With BAC Or Cosmid For Large Insert Sequencing

- Submit at least 100 µl of glycerol stock grown in LB media with 10% glycerol for large insert sequencing.
- Although different types of containers are accepted for sample submission, a 1.5 mil or 650 µl microfuge tube is recommended. Glycerol stocks should be submitted via frozen package.
- The reference sequence for each sample should be provided in FASTA format (if available).

Purified BAC Or Cosmid For Large Insert Sequencing

- Each sample should contain at least 50 µl of purified DNA, preferably processed with the QIAGEN Large-Construct kit.
- DNA templates should be of high molecular weight. Sample(s) can be submitted as a pellet or re-suspended in water or alcohol.

Please visit here for a map of nationwide ACGT drop-boxes.
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