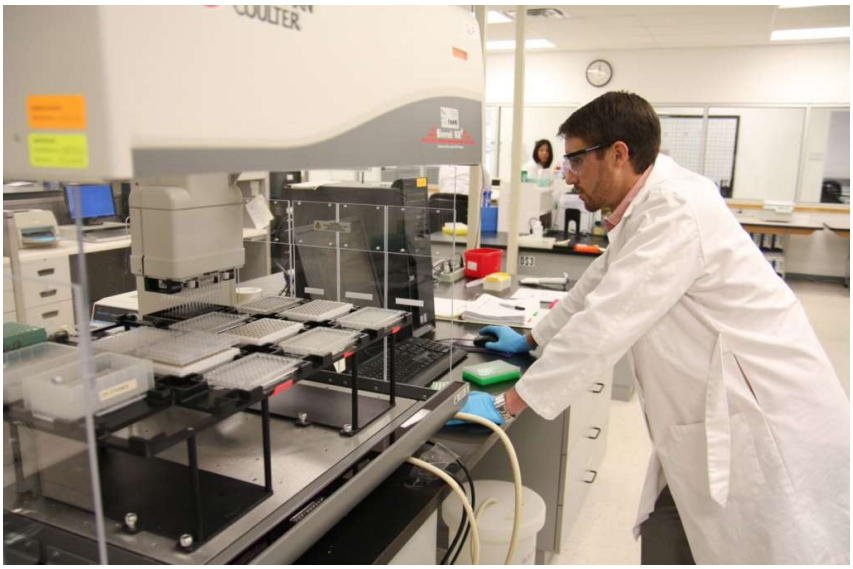
SAMPLE SUBMISSION REQUIREMENTS



To yield quality sequencing data on the initial reaction (i.e. without having to repeat reactions which result in delay), your submitted sample(s) must meet our DNA Sample Submission Requirements. A failure to conform with these minimum requirements as set forth below, may render ACGT's performance of service(s) behind our turnaround time schedule and/or order completion unattainable.

Important:

- *Template DNA should be eluted in de-ionized water and not in elution buffer. Elution buffer will result in "noisy" or unusable data.*
- 260/280 value should be 1.8
- 260/230 value should be 2
- For LCO service, the template should be premixed with only one primer per tube. Example: If a template must be analyzed in both directions using the LCO service, pre-mix the template with forward primer in one tube and with the reverse primer in another tube.

LCO Service:

NOTE: ***Please do not use the Premium/Standard table to determine the concentration and volume for LCO orders.***

	Template DNA type	Size of DNA	Template Concentration (***ng in 5 ul***)	Volume of Template per reaction	Primer concentration per ul	Volume of Primer per reaction
LCO service	Plasmid	up to 10kb	100	10ul	10pMol/ulOr33-66ng/uL	2ul
	PCR DNA	100-500bps	1- 3			
		500bps-1kb	3- 5			
		1kb-2kb	5- 10			
		Over 2kb	10- 15			
	Large DNA	Over 10kb	500-1000			

For example, if you have a plasmid at 100ng/ul, please dilute down to 20ng/ul. Mix 10ul of this plasmid at a concentration of 20ng/ul and 2ul of primer at a concentration of 10pmol/ul in a single tube. Correspondingly, PCR products must be diluted down to the concentration provided above, and then premixed with primers.

If you are using an ACGT universal primer, you may send only the templates optimized at the concentration provided above. ACGT will then add the primers for you. If, however, a universal primer has already been added, please indicate this in the additional comments section on our online order form. Banked primers cannot be used for this service.

Standard Or Premium Service:

The amounts provided below are the minimum concentrations required per reaction. To ensure quality results, ACGT strongly advises using the higher end amounts of concentration.

	Template DNA type	Size of DNA	Template Concentration (in ng/ul)	Volume of Template to send per reaction	Primer concentration (pmol/uL) or in (ng/uL)
Premium/Standard service	Plasmid	up to 10kb	25- 50	10ul	10 pmol/ulOr33-66 ng/uL
	PCR DNA	100-200bps	1.5- 3		
		200-500bps	3- 7.5		
		500bps-1kb	7.5- 15		
		1kb-2kb	15- 30		
		Over 2kb	30- 50		

	Large DNA	Over 10kb	100- 200		

For example, if you intend on submitting plasmid A which must be sequenced with one forward and one reverse primer, then the number of reactions required is two. Thus, if the concentration of plasmid A is 25ng/ul, you must send us 20ul.

For PCR products, apply the following rule of thumb: *For every 100bp → add 1.5ng of PCR product.*

For example, if the size of the PCR product is 500bp, then the concentration of the sample (per ul) is 5 x 1.5ng = 7.5ng/ul. ACGT would require a total volume of 10ul. Therefore, the sample submitted must be 75ng of PCR product in 10ul.

1. Samples can be submitted in microfuge tubes (e.g. 1.5 ml, 650µl, or other tubes) or 8-strip microtubes with the appropriate amount of template and primer.

2. Quantitation of template DNA: For the most accurate quantitation, ACGT recommends using gel electrophoresis (where the band intensity of a sample DNA is compared to a standard ladder). For example, the 1.6 kb band of the common Invitrogene 1 KB plus ladder contains 8% of the total amounts of DNA present in the ladder mixture, and can be used as a quick reference for estimating the concentration of your template. We also recommend using Nanodrop Spectrophotometer that measures 1 µl samples with high accuracy and reproducibility.

OPTIONAL: Please include a gel photograph of the template DNA with the quantity of the DNA loaded including a molecular marker.

3. **Important: Elute the template DNA after purification in de-ionized water, not in elution buffer. Buffer components have a tendency to inhibit the sequencing reaction and result in failed runs.**

4. Please provide an extra amount of DNA so we have enough samples to repeat a reaction in the event the initial reaction fails.

5. You must accurately indicate the DNA concentration on the order form, especially for Standard service orders. For Premium service orders, DNA sample quality and quantity are randomly checked by Nanodrop or agarose gel electrophoresis.

6. To sequence the PCR product, the DNA concentration must be checked following PCR clean up. ACGT can also clean your PCR DNA prior to DNA sequencing. (Extra charges will apply.)

7. If your DNA sequences include high GC content, hairpin or secondary structures, please select the appropriate protocol on our online order form so we may adjust the sequencing reaction conditions to yield good quality results from the initial run.

8. Please indicate if your DNA was purified by CsCl or large scale purification.

9. If a template must be analyzed in both directions, pre-mixed samples of the template with forward and reverse primers in separate tubes must be submitted.

Sample Primer Naming

1. The sample name on the tube and the sample name provided on the order form must be identical.

2. Do not make your sample name too long. Combined template and primer names with more than 23 characters will be automatically truncated.

3. When naming your sample, ACGT accepts only the following symbols: underscores (_) and dashes (–). Do not use any other symbols or spaces in the primer and/or template names.

4. If using tubes with screw-on-caps, label both the tube and caps.

5. Use BLACK marker only to label your tubes. Colored markers smear and are difficult to read.

6. Templates and primers should be clearly identified on our online sample submission form.

7. To avoid sample mix-up, different samples submitted in 8-strip microtubes should be clearly indicated or referenced to the corresponding template descriptions.

+ Shipping

+ Additional Requirements

(HTTP://WWW.CAP.ORG)

Headquarters and Laboratory: 35 Waltz Drive Wheeling, IL 60090
Germantown, MD Laboratory: 12321 Middlebrook Road, Suite 125
Germantown, MD 20874
Toll-Free: 800-557-2248 | P: 847-520-9162 | F: 847-520-9163