Sample Submission Guidelines

Sanger Sequencing: Single Pass Primer Extension (SPPE) and High Throughput SPPE

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

Single Pass Primer Extension (SPPE)

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.**

- If you are planning to send a large volume of samples in 96-well plates, please consider placing a High Throughput SPPE order(a minimum of 48 or more reactions).
- If you have less than 48 reactions, but still plan on sending samples in a 96-well plate, the reactions will be performed according to the following orientation–A1-H1, and so on. (See diagram 1-0 on the right)

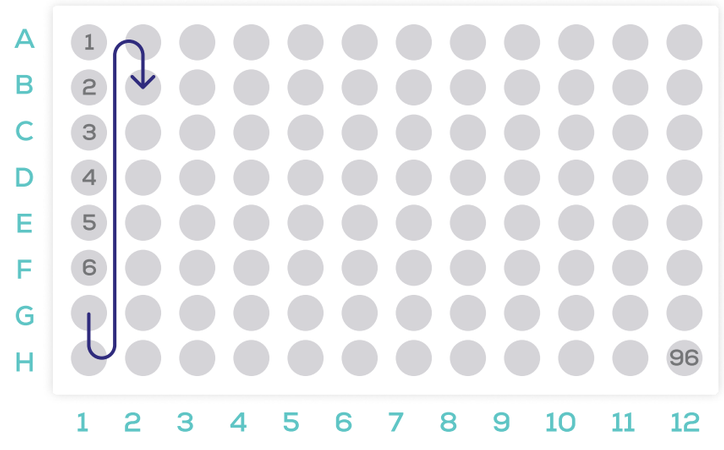

Diagram 1-0: 96-well plate

High Throughput SPPE:

Samples must be submitted in 96-well plates or PCR strip tubes. We recommend that plates be sealed using strip tube caps, rather than plate seals and foils, to avoid leakage and cross-contamination.

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.

Volume Requirements

A minimum of 10uL of sample per reaction is required, regardless of the service type. To accommodate quality control (QC) checks and potential repeats, we recommend submitting a larger volume. If your are requesting banking services or intend to use the same sample for a future order, please provide a minimum of 25uL per sample.

Sample Concentration Requirements

Please note: if you are submitting a High Throughput SPPE order, concentration guidelines are the same as any SPPE order but we ask that all of the sample concentrations are consistent across the plate for ease during processing. Concentrations should not vary across the plate.

LCO (Low Cost Option)

Template DNA Type	Size of DNA	Template Concentration	Primer Concentration (pmol/µL = µM)	Volume per Reaction (in same tube)
Plasmid	Up to 10 kb	20 ng/µL	33 – 66 ng/µL *or* 10 pmol/µL	10 µL Template + 2 µL Primer
Purified PCR Product	100 bps – 200 bps	0.2 – 0.6 ng/µL		
	200 bps – 500 bps	0.6 – 1 ng/µL		
	500 bps – 1 kb	1 – 2 ng/µL		
	1 kb – 2 kb	2 – 4 ng/µL		
	Over 2 kb	4 – 10 ng/µL		

- Each tube should contain the appropriate amount of template and primer needed for that reaction, mixed using the concentrations specified above. For example, if you have a plasmid at 100 ng/µL, please dilute this down to 20 ng/µL. Mix 10 µL of this plasmid dilution with 2 µL of primer (10 pmol/µL) in a single tube. You must do this for all template/primer combinations.
- We do not recommend sending PCR products that are less than 150 bps in length as the first 100 bps of sequencing data following the primer binding site is often unreliable.
- If you are requesting the use of a free ACGT Universal Primer, you will only send the template DNA at the recommended concentration above. ACGT will add the primer according to your selection on the order form. *If you are pre-mixing your primer and it has a name that matches one of our Universal Primers, please state in the comments section that ACGT does not need to add the primer, since the samples already contain it*.
- Banked primers cannot be used for this service. If you would like to use a primer you have banked at our facility, you will need to select the Standard service option.

Sample Concentration Requirements – Continued

Standard

Template DNA Type	Size of DNA	Template Concentration	Primer Concentration (pmol/µL = µM)	Volume per Reaction (in separate tube)
Plasmid	**Up to 10 kb**	**25 – 50 ng/µL**	**33 – 66 ng/µL *or* 10 pmol/µL**	**10 µL Template 7 µL Primer**
Purified PCR Product	**100 bps – 200 bps**	**1 – 3 ng/µL**		
	200 bps – 500 bps	**3 – 5 ng/µL**		
	500 bps – 1 kb	**5 – 10 ng/µL**		
	1 kb – 2 kb	**10 – 20 ng/µL**		
	Over 2 kb	**20 – 50 ng/µL**		
Large DNA*	**Over 10 kb**	**100 – 200 ng/µL**		

*Large DNA may include: lambda, BACs, PACs, YACs, P1 clones, Cosmids, Fosmids, etc. We recommend requesting the use of our BAC protocol for these larger inserts to ensure high-quality data (additional charges apply).

- You must record the known concentration of your templates and primers on the order form.
- Templates and primers must be sent in separate tubes. If you have multiple reactions using the same template or primer, you may send the total volume needed in the same tube. For example, for 2 reactions using the same template, you will send 20 µL of DNA.
- We do not recommend sending PCR products that are less than 150 bps in length as the first 100 bps of sequencing data following the primer binding site is often unreliable.
- If you are requesting the use of a free ACGT Universal Primer, you will only send the template DNA at the recommended concentration above. ACGT will add the primer according to your selection on the order form.
- Previously banked primers must be indicated on the order form for use.

Premium

Template DNA Type	Size of DNA	Template Concentration	Primer Concentration (pmol/µL = µM)	Volume per Reaction (in separate tube)
Plasmid	**Up to 10 kb**	**25 – 50 ng/µL**	**33 – 66 ng/µL *or* 10 pmol/µL**	**10 µL Template 7 µL Primer**
Purified *or* Unpurified PCR Product	**100 bps – 200 bps**	**1 – 3 ng/µL**		
	200 bps – 500 bps	**3 – 5 ng/µL**		
	500 bps – 1 kb	**5 – 10 ng/µL**		
	1 kb – 2 kb	**10 – 20 ng/µL**		
	Over 2 kb	**20 – 50 ng/µL**		
Large DNA*	**Over 10 kb**	**100 – 200 ng/µL**		

*Large DNA may include: lambda, BACs, PACs, YACs, P1 clones, Cosmids, Fosmids, etc. We recommend requesting the use of our BAC protocol for these larger inserts to ensure high-quality data (additional charges apply).

- These are *recommended* concentrations by template type. Concentrations do not need to be known for the Premium service, but we will send you an email notification if our QC checks show that the concentrations of your templates or primers are too low to perform the reactions.
- Templates and primers must be sent in separate tubes. If you have multiple reactions using the same template or primer, you may send the total volume needed in the same tube. For example, for 2 reactions using the same template, you will send 20 µL of DNA.

Sample Concentration Requirements – Continued

- If your PCR products are un-purified, ACGT lab personnel will purify your templates prior to performing the reactions (available at an additional charge).
- We do not recommend sending PCR products that are less than 150 bps in length as the first 100 bps of sequencing data following the primer binding site is often unreliable.
- If you are requesting the use of a free ACGT Universal Primer, you will only send the template DNA at the recommended concentration above. ACGT will add the primer according to your selection on the order form.
- Previously banked primers must be indicated on the order form for use.

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