



# Sequencing Sample Submission Guidelines

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**Following these submission guidelines allows for efficient processing of your order to deliver your sequencing data as early as possible.**

## Submitting Templates & Primer Samples: Concentrations & Volumes

### I. Concentrations

**Table 1: Prepared Template and Primer Submissions**

Template Type	Template Size	[Template] (ng/uL)	[Primer] (pmols/uL)
Plasmid	<20 Kb	100-200	2-10
Purified PCR	100-300 bp	10-20	2-10
Purified PCR	301-1000 bp	20-40	2-10
Purified PCR	>1000 bp	40-60	2-10
Large Constructs (BAC)	>20 kb	250-500	10-15

### II. Volumes

#### Separate Template and Primer

- Your template DNA is being submitted separately from your primer samples.
- Sample and primer will be mixed by our technicians prior to sequencing.
- One primer per tube (Sanger reactions are unidirectional)

**Table 2: Volume Required for Separate Template and Primer Submissions\***

Single Aliquot	Volume (1 <sup>st</sup> Rxn)	Additional Volume (Each Additional Rxn)
Template DNA	10 uL	5 uL
Primer	10 uL	5 uL
<a href="#">Power Read DNA</a>	10 uL	10 uL

\*Please see Table 1 for relevant concentrations

#### Pre-mixed Template and Primer

- Your template DNA is being submitted already mixed with your primer (1 primer per tube).
- Adhere to concentrations set-forth in Table 1 for template DNA and primer solutions prior to mixing.

**Table 3: Pre-mixed Sample Submissions**

Template Volume (uL)	Primer Volume (uL)	Total Volume (uL)
8	4	12

### Samples for Preparation / Purification

- Your template DNA requires further preparation or purification prior to sequencing.
- Primer must always be submitted separate from sample in this instance.
- See Table 3 for further volume and primer specifications.

Template Type	[Primer] (pmols/uL)	Submission Volumes (uL)
Crude PCR For Purification (>100 bp)	2-10	Entire Reaction Volume (15-30)
Bacterial Cultures for RCA Plasmid Preparation	2-10	10 uL Glycerol Stocks using a 50% glycerol solution

### Shipping Address & Information - How to Ship Samples

Enclose your tubes in our Blue Bag and ship in the provided padded UPS envelope to the following address:

**Eurofins Genomics**  
**DNA Sequencing Services**  
**12701 Plantside Drive**  
**Louisville KY 40299**  
**USA**

\*A copy of the online order confirmation page must be enclosed with every shipment to ensure proper identification, tracking, and result retrieval.

### Free Overnight Shipping Kits Available!

Your FREE Overnight Kit will contain:

- 16 UPS shipping envelopes personalized for your lab



- 96 bar-coded labels for unique template and primer ID
- 8 sequencing sample bags

## Overnight Sequencing Orders as early as 12pm local time

The lab prioritizes orders based on overnight versus normal orders. To qualify for overnight service, your order must meet the following:

1. **Be less than 192 reactions**
2. **Contain only standard reactions (No Power Read or BAC reactions. No preparations or purifications)**
3. **Not contain any non-barcoded tubes or plates**
4. **Not contain any tubes smaller than 1.5ml**
5. **Not contain any tubes with screw caps**
6. **Be housed in a Eurofins “Blue Bag”**
7. **Not be sealed with parafilm**

## New! SimpleSeq™ Webless™ Pre-Mixed Reaction Submissions ⓘ

1. Prepare aqueous template and primer solutions at the concentrations listed in Table 1.
2. Add 8 µL template + 4 µL primer to each tube by inserting your pipet tip through the split seal (total volume = 12 µL). See Table 2.

## SimpleSeq and Tube Submissions ⓘ

1. Prepare aqueous template and primer solutions at the concentrations listed above
2. A barcoded label must be placed on any tubes not provided by Eurofins.
3. Aliquot templates and primers as follows (per Table 2)
4. Free standard primers can be added by our lab
5. Enter the details of your reactions in our [online order form](#)

Additional information can be entered into the comments section during order submission.

## Plate Submission

### (Standard, Express, Ready to Load)

1. Samples should be shipped using 96-well, v-bottom, semi-skirt PCR plates
2. Two wells on each plate should be left empty for our internal quality controls at positions H11/H12 for row layouts and G12/H12 for column layouts
3. Enter the detail for your reactions in our online order form. Additional information should be added in the comments section during order submission.
4. Seal the plates using only strip caps, which provide the most secure shipping conditions. (we do not recommend foil or tape-seals as cross-contamination and sample loss can frequently occur)
5. ~~Please attach~~ One barcode label must be attached to each plate for unique identification

### Standard Plate Sequencing

- Full length analysis protocol
- Minimum of 48 reactions per plate
- Online order required

### Express Plate™ Sequencing

- Prepare aqueous template and primer solutions at the concentrations listed above in Table 2
- Using 96-well, v-bottom, full-skirt PCR plates, add 8 µL template + 4 µL primer to each well (total volume = 12 µL)
- Note that Express plates are processed using an abbreviated analysis protocol (for improved turnaround time) and read lengths are 500-700 bp

### Ready to Load Plate Sequencing

- Only semi-skirted, ready to load plates are accepted for analysis
- Your BigDye v3.1 reaction must arrive post-purification and after the order has been placed online.
- Note that RTL plates are processed using an abbreviated analysis protocol (for improved turnaround time) and read lengths are 500-700 bp
- Dried samples are preferred

### Power Read Service\*

Utilizes a proprietary chemistry, geared towards resolving sequence across difficult stretches of template DNA

*Ideal for Sequencing the Following:*

*GC-rich templates*  
*Difficult secondary structures*  
*Homopolymeric sequences*  
*Repetitive Sequence*  
*Bisulfite-treated DNA*  
*shRNA*

Includes one automatic repeat read for reactions which fail or generate insufficient sequence quality on the initial run

As part of our ongoing efforts to provide you with the highest quality results as quickly as possible, the template size of your Power Read reactions must be provided in the online order form. Feel free to contact our technical support team if you have any questions.

\*Repeat reads may not be possible if samples are submitted outside the recommended minimum volume conditions

## RCA Plasmid Preparation Service

- Glycerol stocks are highly recommended
- Send a minimum of 10 µL for each sample (50% glycerol solution)
- Prepare stocks from overnight cultures
- Glycerol stocks, pelleted cells, overnight cultures, and agar plates are all accepted submission methods and all should be shipped overnight on cold packs

## Primer Selection

### Free standard primers

- Over 81 standard vector primers are available for sequencing
- See our [Primer Library](#) for a comprehensive list

### Primer Design & Storage

- Primers synthesized or shipped to us for sequencing should be between 18 and 22 bp in length, avoid stretches of identical nucleotides, display about 50% GC content, and exhibit an annealing temperature of 50 – 60°C
- Primers that are synthesized for your sequencing order remain available to use for six months
- Primer walking, primer design, and primer synthesis services are also available

## For Best Results:

- Suspend templates in water only as other buffers may inhibit the sequencing reaction (specifically excessive concentrations of EDTA)
  - Sequencing templates should be provided as purified plasmid, BAC, or PCR product (RCA Plasmid prep and PCR purification by requesting “Template Prep” within your order submission – BAC preparation services are available by e-mail order form)
- **Need help troubleshooting your sequencing data?** Feel free to contact our Technical Support team at 1-800-688-2248 (option2), e-mail us at [dnatechsupport@eurofins.com](mailto:dnatechsupport@eurofins.com), or review our [Troubleshooting Guide online](#).



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