

Sample Preparation Guidelines

The preparation of various samples for ordering a sequencing service depends on the type of sample being submitted, the type of sequencing chemistry being ordered and the additional services being requested. Prepare your samples based on the guidelines below.

Concentration of Stock Solutions

Prepare stock solutions of purified/prepared templates and primers in ddH₂O based on the size of the template being sequenced. Use these stock solutions to assemble samples to be submitted in tubes or plates. These guidelines do not apply to samples for Ready2Load Sequencing service.

Table 1: Concentrations of the stock solutions of primers and prepared templates

Template Type	Size (kilo basepairs)	[Template] (ng/μL)	[Primer] (pmol/μL)
PCR Products, Purified	0.100–0.300	10–20	2–10
PCR Products, Purified	0.301–1.000	20–40	2–10
PCR Products, Purified	>1.000	40–60	2–10
Plasmid, Prepared	< 1 .000	30 - 60	2–10
Plasmid, Prepared	1.0 << 6 .0	60-150	2–10
Plasmid, Prepared	6.0 << 20.0	150-250	2–10
Large Constructs (BAC), Prepared	>20	250–500	10–15

Note that the concentration of the Template is in ng/μL while the concentration of Primers is in pmol/μL.

Volumes of Stock Solutions for Assembling Samples

The volumes of the Sample stock solution(s) and the Primer solution(s) depend on the type of the sequencing services required. Ensure that the stock solutions are prepared according to the guidelines in table 1 before proceeding to section below.

'Premixed' Samples:

When you submit your samples as 'premixed', **ensure that the primer is mixed with your purified/prepared template**. After assembling the Templates and Primers as described in the table 1 and prepare your sample by mixing the following volumes of the two stock solutions.

Table 2: Assembling Premixed samples

Component	Volume (μL)
Template DNA	8
Primer	4
Total (Sample)	12

Note that only the premixed samples must be submitted for **ShortSeq** plate and the **SimpleSeq Premixed** sequencing services. Ensure that the samples for 'ShortSeq plates' or 'SimpleSeq Kits - Premixed' are prepared based on the guidelines set up here for 'Premixed' samples.

'Templates Only' Samples

When you do not have the standard or custom primers handy or ready for use, you can submit 'Templates Only' Samples. For the primer required in this sequencing reaction, you can

- Use one of our >75 universal primers to be added to your sample before sequencing (free), or
- Order the synthesis of a custom primer that will then be used for sequencing your samples and subsequently stored in our facility for your future sequencing orders

Table 3: Assembling 'Templates Only' Samples.

Sequencing chemistry	Template Stock solution — Volume (µL)	
	First Reaction	Each additional reaction
Standard Read	10	5
Power Read	10	10

'Templates and Primers' Samples:

When you submit 'Templates and Primers' Samples, our sequencing technicians will mix the primer(s) with your sample(s) prior to sequencing. The ability to send primers and templates separately allows you to submit samples for multiple sequencing reactions or order additional services such as PCR purification/DNA Preparation for your template sample. The following table provides guidelines for the submission of prepared templates and primers. Use the concentration of templates and primers in table 1 to prepare the stock solutions. When you submit 'Templates and Primers' samples:

- You must submit your primers and templates in separate containers,
- Concentrations of template DNA and Primers must adhere to the recommended values (see table 1).
- Provide sufficient quantities of samples and primers (see table below). You can order multiple reactions for a template submitted in 1 tube or a single well of the 96-well plate. Please note that the volume requirements vary for standard and power read sequencing services.
- Use barcode labels (optional) on the primer and sample containers,
- Indicated on the tube sequencing (check box) or plate sequencing (mention in notes) order editor if you want the sent primer to be stored at our facility for your future orders. On request, these primers will be stored in our facility for 1 month or till they are exhausted, which ever is earlier.

Table 4: Assembling 'Templates and Primers' samples

Sequencing chemistry	Template Stock solution — Volume (µL)		Primer Stock Solution — Volume (µL)	
	First Reaction	Each additional reaction	First Reaction	Each additional reaction
Standard Read	10	5	10	5
Power Read	10	10	10	5

Samples Requiring 'Additional Services':

When your sample requires further preparation or purification prior to sequencing, the following conditions should be met:

- The template(s)/Sample(s) and primers(when enclosed) should be submitted in separate containers
- The volume of the sample based on its type should meet the following requirements (see table).

Table 5: Assembling samples which require additional services

Sample Type	Type of service	Sample Solution — Volume (µL)	[Primer] (pmol/µL)	Primer(s) stock solution — Volume (µL)
Crude PCR Products (>100 bp)	PCR Purification	15–30 (Entire Reaction Volume)	2–10	10 (5 for each addition rxn)
Bacterial Cultures	RCA Plasmid preparation	10 (Glycerol stock recommended)	2–10	10 (5 for each addition rxn)

Preparation of 'Ready2Load' Samples

Ready2Load sequencing services is provided for samples that have a single requirement: to be run on the sequencing gel. Samples being submitted as Ready2Load samples must meet the following requirements:

- The BigDye v3.1 reaction must be completed.
- The purification steps that remove unincorporated dye molecules must have been completed.
- It is recommended that these samples are sent as dry pellets.

Note that the Ready2Load plates are processed using an abbreviated protocol which typically have a read length of 500–700 bp.

Types of Services and accepted Samples

We have tailored our DNA sequencing services using the Sanger method considering the diverse natures of the projects and requirements of the researchers. Researchers can send different types of samples in different formats with or with out including the primers or requiring additional services on the samples.

Sample/Services Feature	Description
Services Provided	<p>The following services are provided:</p> <ul style="list-style-type: none"> • DNA Sequencing using the Sanger Method* <ul style="list-style-type: none"> • for Research samples • for Clinical Samples • PCR Product purification, and • DNA Preparation
Accepted Sample types	<p>The following types of templates are accepted:</p> <ul style="list-style-type: none"> • Plasmids <ul style="list-style-type: none"> • as Clones • Purified or crude • PCR Products <ul style="list-style-type: none"> • Crude • Purified • BACs/Fosmids/Cosmids • Post BigDye samples (Read2Load samples) • Clinical Samples (See CLIA/CAP page)
Sequencing Chemistries	Standard Read - Suitable for most standard templates requiring read lengths of upto 700 bps

	<p>Power Read - Suitable for templates with hard to read segments in the sequence. These can include:</p> <ul style="list-style-type: none"> • GC rich segments • Sequences with nucleotide repeats • shRNA constructs • Sequences with secondary structures
Accepted Sample containers	<p>These include:</p> <ul style="list-style-type: none"> • Custom Tubes (with or without* free barcodes from Eurofins Genomics) <ul style="list-style-type: none"> • 1.5 mL Centrifuge tubes • 2.0 mL centrifuge tubes • Barcoded SimpleSeq Tubes • Custom 96-well plates • Barcoded 96-well plates from Prepaid Plate kits

Sequencing Services provided for various Sample Types based on the inclusion of Sequencing primers

Each of the sequencing reaction requires a Sequencing primer that is specific to the template being read. We provide the most widely used sequencing primers for no additional cost. However, if you do need custom primers, our DNA synthesis lab will synthesize and deliver the same to the sequencing lab with remarkable turnaround times.

Sample Types	Description
Premixed	Submit the template of interest premixed with sequencing primer to receive the results from a standard read in the shortest time.
Templates Only	<p>Submit only your template. You can choose from a wide range of services for samples submitted as templates only. These include:</p> <ul style="list-style-type: none"> • Use of over 75 free standard sequencing primers • Synthesis of custom primers specifically for your use • Use of stored primers (Custom primers are stored for 6 months after their synthesis at our facility) • Upgrade to power read for difficult to read template segments.*
Templates and Primers	<p>When you send your sample templates and primers in separate containers, we can provide the following additional services:</p> <ul style="list-style-type: none"> • Mix the primer supplied by you and then sequence the template • Store the sent/enclosed primer for upto a month (you need to tick the box during the tube sequencing order or incate in notes in the plate sequencing order) • Upgrade to power read.* • Provide additional services such as DNA preparation (for Plasmids) or PCR Purifications (for PCR Products).*
Custom	<p>Research Services: Whether you need the sequence of a large construct such as BACs or Fosmids, crude PCR product, or you just need the sequencing gel run after having performed the BigDye® reaction, we have a service tailored for your need. If you do not find something for your specific need, please contact your sales representative or write to us at genomicsUS@eurofins.com.</p> <p>Clinical Research Services: Our CLIA and CAP accredited sequencing lab provides services</p>

suited to your specific needs. Contact our [CLIA specialist](#) for more information.

#For more details on Next Generation Sequencing Services, click [here](#).

*Additional charges apply.

Prepaid Plate Kits and Custom Plate Sequencing services

The Prepaid Plate kit sequencing service and custom Plate sequencing services provide a high level of customization in terms of the sequencing services and additional services offered. The table below highlights the various sample types, sequencing services offered for samples submitted in 96-well plates.

Accepted Sample Types based on		Types of Sequencing Chemistry Offered	Number of Sequencing reactions per sample in a well	Additional Services Offered
Sequencing Service	Nature of the template			
'Premixed' Samples 'Templates Only' Samples 'Templates and Primers' Samples	Plasmids Purified PCR Products Crude PCR Products	Standard read Power Read	Single Multiple	DNA Preparation PCR Purification Custom Primer Synthesis Sent Primer Storage

Please note that different Prepaid Plate kits have different redeemable services. Additional services will incur additional costs.

To order sequencing services for samples submitted in plates:

- Stock Solutions:** Prepare stock solutions as described in Table 1
- Label Plates:** Label your 96-well plates
 - Prepaid Plates are pre-labeled with Barcodes.
 - Paste the barcoded labels procured previously from Eurofins Genomics at the bottom side of the plate (These barcoded labels are shipped to you free of cost when ordered through Eurofins Genomics).
 - Use a marker to label the plate in clearly legible letters.
- Assemble Samples:** Prepare your sample in the 96-well plate based on the type of sequencing services required (refer to Sample Preparation Guidelines, Tables 2, 3, 4 or 5 for the relevant information)
- Provide Sample Related Details:** Provide the details of your sample and required services in the Plate Sequencing excel upload template. Read the general guidelines (Plate Sequencing Service) before editing the excel templates.
- Plate Level Details:** Provide the details of the samples in each plate in each excel sheet. These entries describe the general properties of the samples submitted for the plate sequencing order.

Entry	Description
Plate Barcode:	<p>If you are using a Prepaid Plate kit, enter the barcode attached to the side of the plate here to redeem those services during checkout.</p> <p>For custom 96-well plates, you can paste the free barcoded-labels from Eurofins Genomics at the</p>

	bottom of the plate; enter that barcode here. If you do not have a barcode label, leave this field empty.
Sample Type:	Choose the sample type based on the nature of the template being submitted in the plate. Accepted templates include Plasmids, PCR Products or a Mix of Both. Please note that you can submit different samples types requiring different additional services using different sheets of the excel file.
Construct Size/Product Length:	Select the approximate range of the size of the samples. ^a If the range of the sample falls out the predefined ranges, you may indicate that on the plate notes.
Add. Service:	PCR purification is available for PCR products and DNA Preparation is available for Plasmids. ^b
Advanced Options:	By default, the Standard Read chemistry is used to sequence your samples. We offer Power Read services for difficult to read segments of the template.
Template Specification:	Choose this only when you request for Power Read options. Select the feature which you have identified is the primary reason why the sequencing of the template can be challenging.
Plate Layout:	Simply by choosing the two options in the dropdown, you can populate the well positions based on the way you decide to load your samples. Choose "By Rows" if you want to fill your samples sequentially from A1 to A12, before moving on to B1.

^aConstruct Size/Product length is used in the lab for two independent purposes: 1) Power Read services, and 2) Quality Control (particularly when the size of the template is smaller than the typical read length).

^bAdditional charges may apply

- **Sample Level Details:** You can provide the sample level details in each of the rows in the excel template. Please follow the recommendations below to provide this information.

Well Position	Sample Name	Primer 1	Sequence to synthesize or Barcode for enclosed primers	Additional Columns
A1, A2 ... or A1, B1 ... will be filled based on your plate layout choice	Enter a unique sample name for each sample-containing well in the 96-well plate.	<p>Critical: you must provide a primer name. You can either:</p> <p>select the desired universal primer/'Premixed' from the dropdown</p> <p>Type in the name of the custom primer to be synthesized</p> <p>Type in the name of the enclosed primer</p> <p>Type in the name of the Stored primer (please note that the name of the stored primer is case sensitive and should be typed in correctly to avoid any confusion).</p>	<p>The entries in these fields depend on the Primer column:</p> <p>For 'premixed', stored or standard primers, Leave this empty.</p> <p>For ordering a custom primer synthesis, enter the sequence of the primer.</p> <p>For sent/enclosed primer, enter the barcode number (if applicable) or leave empty. Caution: If you enter a sequence, the same will be ordered.</p>	information for upto 4 sequencing reactions for sample in each well. Follow the guidelines as described in the left.

You can also place the order using the Excel upload form. Download the form, provide information in the required fields based on the 'Helpful Tips' page and upload the form at the order editor. The data provided by will be captured by the plate sequencing order editor. Provide any additional information in the following steps and then proceed to checkout.

- 6. Upload Excel template:** follow the general guidelines when entering the data in the excel template and upload the same at the order editor. Please note that you can enter data in multiple sheets.
- 7. Verify Information:** Once the excel template is uploaded on the order editor, you can provide any information that is not already provided. On step 3, you can verify the details of the sample and edit the samples in the third step, if required. You can proceed to the checkout. You will not be charged for any standard redeemed services (Prepaid Plate Kits), all other services will be priced.
- 8. Checkout and Shipping:** When you proceed to checkout, you will be prompted with Eurofins Genomics Dropboxes, within 30 miles of your labs location. Where available, you can drop your samples in the drop box before the specified time. Otherwisem, you can use the UPS location to ship the samples to Eurofins Genomics, DNA Sequencing Lab.
- 9. Download Sequence Data:** once the QA team has verified the processed data, these sequencing results will be available from your login account. Please note that you can share these results with the members of your group using the Group Admin Tool. Please note that when you order multiple sequencing results for the sample in each well, the sequence data will be delivered with a 1, 2, ..., n appended to the name of the sample.

Custom Services and Other Projects

Please contact our [CLIA/CAP specialists](#) for sequencing projects related to clinical samples. Please contact our [sales team](#) for all other projects.

General recommendations for Shipping samples in Tubes and Plates.

Samples shipped by you are recieved in our lab and immediately set for porcessing. The barcodes on tubes and plates help us track the samples through all stages of the sequencing workflow. For receiving high-quality data efficiently and economically, adhere to the following guidelines:

- Ensure that your samples are closed tightly to prevent sample cross contamination. We, however, do not recommend applying parafilm to the tubes, since processing such samples will take longer.
- Send your samples in padded shipping envelops. We offer pre-addressed, pre-paid overnight shipping packages with our kits or on order through our ecommerce site ([free shipping supplies](#)). These shipping supplies are free to you and will be delivered via 2-day shipping service after we receive your order. When shipped using these packages, your samples will be delivered to us using the overnight shipping service. Please use these free supplies judiciously and avoid shipping packet with a single sample.
- With the new sequencing lab in Canada, customers in Canada must ship their samples to the new address (see below). Customers in the USA will continue to ship their samples to Louisville, KY.
- Please note that there might be a Eurofins Genomics dropbox near you. Deposit your samples in these dropboxes in a timely manner.

Guidelines for Overnight Sequencing Service

Overnight Sequencing service from Eurofins Genomics allows you to advance your research project in an uninterrupted manner within a limited budget. To avail the Overnight Sequencing service from Eurofins Genomics, your order/samples must meet the following requirements:

- The samples are shipped using 'Overnight Shipping' service
- The order must require less than 192 sequencing reactions
- This services applies only to samples requiring 'Standard Read' sequencing chemistry (this does not include other services such as power reads, BAC, PCR purification or RCA)

- Samples must be supplied in one of the following containers
 - 1.5-mL or 2-mL centrifuge tubes with barcode labels* pasted on them
 - 96-well plates with a free barcode label pasted at the bottom of the plate
 - Use barcode-labeled tubes or semi-skirt Plates supplied by Eurofins Genomics, e.g., as tubes from SimpleSeq Kits or 96-well plates from the PrePaid plate Kits
 - Use of Screw Caps or Sealing using parafilms prevents cross contamination, but can extend the turnaround time.

Shipping Addresses

Eurofins Genomics Dropboxes

When available, the location of a Eurofins Genomics Dropbox near you will be indicated during checkout. Please deposit your samples before the cut-off time.

Customers in United States	Customers in Canada (new lab)
Eurofins Genomics DNA sequencing services 12701 Plantside Drive Louisville, KY 40299	Eurofins Genomics DNA Sequencing Services 700 bay street, Suite #1100 Toronto, ON M5G 1Z6

If you have submitted an online order, please enclose a copy of the online order confirmation page with every shipment to ensure proper identification, tracking, and result retrieval.