A52. Procedure for DNA Sequencing Services by AZENTA Life Sciences

SANGER SEQUENCING SERVICES ONLINE ORDER STEPS (pages 3-8)

- 1). Prepare Plasmid and Purified PCR Products as Instructed (see below):
 - a). **Pre-Mixed Service: Plasmid & Purified PCR (Preferred):** Provide **your template** and **your primer mixed** in the **SAME TUBE**. Refer to table for recommended concentrations and volumes (see next page).
 - b). **Pre-Defined Service: Plasmid & Purified PCR (Optional)**: Submit your template(s) and primer(s) in <u>SEPARATE TUBES</u> or submit your template(s) and select AZENTA UNIVERSAL PRIMERS. Refer to table for recommended concentrations and volumes per reaction (see next page).
- 2). Ask TC to create a ONE-TIME Purchase Order (PO) number or Standing Purchase Order (PO)number through the FST system.
- 3). Create your account at https://clims4.genewiz.com/ or use the MOLab login account (tche@uchicago.edu/ // UCmolab@2024) (Standing PO# X0025429)
- 4). Complete the online submission process (see pages 5 to 8).
- 5). Drop your samples in **GCIS W403M** in the box with a printed copy of your order receipt before the 4PM cutoff time.

Azenta turnaround time

- Standard orders: noon the day following order delivery at Azenta
- *Same day: the day the order is received (by 10am) at Azenta

Pricing Information (as of May 1, 2024)

Please see pricing below. You can also coupon code **GA20-NEW50** at checkout for \$50.00 towards your first Sanger sequencing order.

Pre-mixed: \$4.41 per rxn

• Pre-defined: \$5.51 per rxn

Custom: \$8.82 per rxn

o PCR clean-up: \$3.31 per rxn

Same-day processing: FREE

FAQ: https://www.genewiz.com/en/Public/Resources/FAQs/FAQs-DNA-Sequencing

WHOLE PLASMID SEQUENCING SERVICES (pages 9-10)

GENEWIZ by Azenta is offering whole plasmid sequencing (Plasmid-EZ) to verify the integrity and fidelity of whole plasmids using long-read technology. With our new end-of-year promotion, you will receive \$10 per sample using promo code GA24-PLASMID-EZ10 when you place an order through your online account! There is no minimum requirement and the promo can be used multiple times through 12/31/24. The turnaround time is 24 hours, and you can conveniently submit samples through one of your local drop-boxes.

- **Price**: \$13.99 for 24+ samples, \$14.99 for 1-23 samples
 - o **PROMO**: use promo code GA24-PLASMID-EZ10 for \$10/sample through 12/31/24
 - o Same rate for all plasmids, regardless of size or complexity

- Turnaround time: 1 business day after samples are received
- **Starting Material**: Purified plasmid DNA; minimum amount of 500ng and concentration normalized to 50 ng/μL.
- **Sample Submissions**: Can ship samples directly to our NJ facility, or can leave samples in your dropbox with a printed copy of your order receipt (received at checkout)
- **Deliverables:** An interactive data report, consensus sequence of your plasmid(s) in FASTA format, raw reads in FASTQ files, GenBank files, and annotation files, including plasmid map images
- **Ordering Process**: Place orders directly through your online account by selecting "NGS" and "Plasmid-EZ" and filling out your order form. I've attached an ordering guide for your reference.
- **FAQ Page**: https://web.genewiz.com/ngs-plasmid-ez-faq? ga=2.16424958.957779649.1730400912-1781756750.1725401839

Dropbox Location

Address: 929 E 57th Street Chicago, IL 60637

Location: GCIS W403M on bench near window-please tell lab members when dropping off

Pickup: Mon-Fri @ 4PM

Account Contact:

Emily Fish, MS

Account Manager - Genomic Services

+1 763.923.1267

Emily.Fish@azenta.com

azenta.com

Sample Submission Guidelines

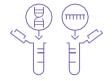




Pre-Mixed Service: Plasmid & Purifed PCR

Provide your template and your primer mixed in the SAME TUBE

Refer to table for recommended concentrations and volumes.



Pre-Defined Service: Plasmid & Purified PCR

Submit your template(s) and primer(s) in SEPERATE TUBES or submit your template(s) and select AZENTA UNIVERSAL PRIMERS.

Refer to table for recommended concentrations and volumes per reaction.

Pre-Mixed and Pre-Defined Samples

DNA Tvne	Δ Tyne DNA Length (include vector)		Template Total Mass	Your Primer Total Picomoles	Pre-Mixed Volume (Template + Your Primer)	
	<6 kb	~50 ng / µl	~500 ng			
Plasmids	6 - 10 kb	~80 ng / µl	~800 ng	25 pmol	15 μΙ	
	>10 kb	~100 ng / µl	~1000 ng			
	<500 bp	~1 ng / µl	~10 ng			
	500 - 1000 bp	~2 ng / µl	~20 ng			
Purifed PCR Products	1000 - 2000 bp	~4 ng / µl	~40 ng	25 pmol	15 μΙ	
	2000 - 4000 bp	~6 ng / µl	~60 ng			
	>4000 bp	Treat as plasmid	Treat as plasmid			



Sample Submission Guidelines





Custom Service: Plasmid, Unpurified PCR & More

Azenta will determine template concentration, optimize for sequencing, and mix with primer.

Refer to table for recommended concentrations and volumes.

DNA Type	DNA Length (include vector)	Template Concentration	Template Total Mass (recommended)	Template Volume Per Reaction	Your Primer Concentration μΜ (pmol/μl)	Your Primer Volume Per Reaction
Plasmids	Any size	Unknown	At least 500 ng	10 μΙ	5 μΜ	5 μΙ
PCR Products	Any size	Unknown	At least 40 ng	10 μΙ	5 μΜ	5 µІ

Note: Please send primer in a separate tube.

Azenta Life Sciences will perform the following depending on your template:

- Plasmid Measure and optimize template concentration for sequencing
- Unpurified PCR Clean up crude PCR products containing single band products with ExoSAP-IT
- Bacterial Colony / Glycerol Stock / Phage Plaque / Phage Supernatant Amplify template from clones
- BAC DNA Perform specialized chemistry reactions to overcome BAC DNA sequencing challenges



Welcome to the improved Azenta Life Sciences Laboratory Information Management System (CLIMS). This At-A-Glance will walk you through placing a Sanger Sequencing order.

Getting Started

Go to https://clims4.genewiz.com

If you have not logged into your account before, your username will be your email address. Use the 'Forgot Password' function to reset your password. A link will be sent to email address to enable you to reset it.

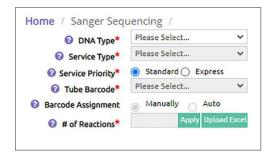
After you have logged into your CLIMS account you will be directed to the homepage.

Select "Sanger Sequencing" on the lefthand tab, then select whether you will be submitting your samples in tubes or plates.



2 Select Service Type

- 1. Please choose the DNA type and Service type from the drop-down menus.
- 2. Choose the service priority: Standard (or Express if available at your location).
- Select the type of barcodes you wish to use Prepaid or Non-Prepaid.
 (Please note: Barcodes must be available in your account to use see separate guide on ordering, activating and assigning barcodes).
- 4. Select whether the barcodes labels will be assigned manually or automatically.
- 5. Fill out the "# of reactions" (not the number of samples) and click "apply" and an order template will load.



Pre-Mixed: Your primer and sample have been mixed in the **SAME TUBE**

Pre-Defined: Your primer and sample are in **SEPARATE TUBES** or an Azenta Universal primer is selected

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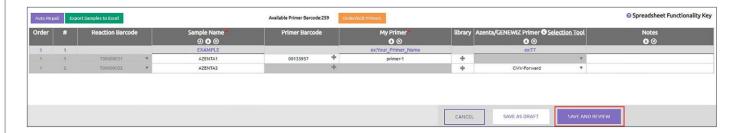
3 Complete Sample and Primer Details



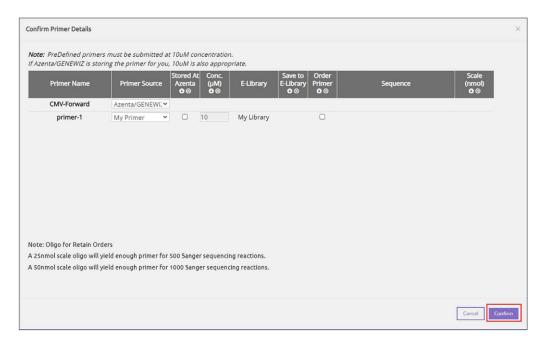
Select barcodes for each sample and primer and fill in the sample and primer names. Each line represents a reaction.

Please note that if you have a large number of samples, to save you rewriting, you can click on to fill sequentially or on to fill all values.

Once all the details are correctly entered, click on "Save and Review."



A primer review page will then appear. If correct, click on "Confirm."



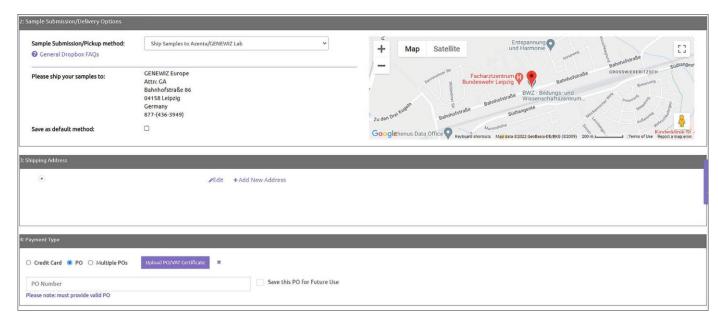
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3 Complete Sample and Primer Details (Continued)

Following this, a summary of your order will be displayed with the number of Samples Submitted, and the resulting reactions; if the details are correct, click on 'Add to Cart.'



You will be re-directed onto another page, which will ask you to confirm your shipping option, addresses and payment method.



If you use Purchase orders as your method of payment, please select "PO" and enter your "purchase order number" in the box; if you are using a prepaid account as your method of payment, please select "PO" and enter your "prepaid account number" in the box.

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4 Submit Order

Once all details are entered, click on "Submit Order." This will display another window with your "Order Receipt" along with your "Tracking #." Please print the Order Receipt and attach it to your samples (please note that pop-ups need to be enabled on your browser to allow this window to be displayed.)



5 Track Your Order Status

To review the status of your order, this can be accessed from your dashboard/home page:



You can also opt in to receive 3 different emails at different stages of the order: Order Placed, Order Received, Delivery of Results.

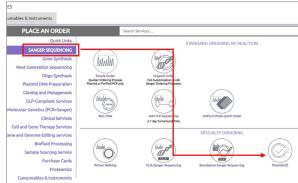


Plasmid-EZ Order Guide

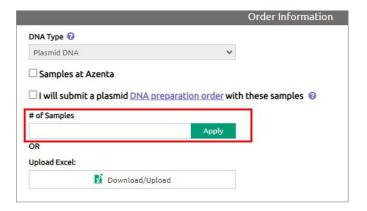


Log into your account and select Plasmid-EZ under Next Generation Sequencing or Sanger Sequencing.

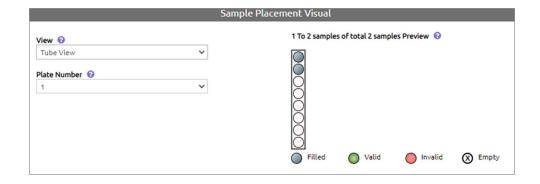




Enter general information about your order, including sample number, to pre-populate the samples table.



Complete sample tube/





Plasmid-EZ Order Guide



4 Enter sample information in the table. Required fields are marked with asterisks (*).

Plate	Tube	Sample #	Sample Name	Sample Buffer	Method of DNA Measurement	O Normalized 50 ng/uL	DNA Conc.	Sample Vol.	Total DNA Amount(ng)	O Host	O Plasmid Size	• Reference	Comments
		⊕ ⊙ ⊗	0.0	Measurement O ⊗	O ⊗	(ng/uL)	(ш)	Amount(ng)	00	00	Sequence ② ③	00	
1	A:1	1	EXAMPLE	Tris/Eb	Qubit	Yes	50	25	500	Zmobilis, E Coll	<25 kb	ATCTAG_	
1	121	4			*	*						+	
1	177	2			*	*						+	
3	373	3		· · · · · · · · · · · · · · · · · · ·	¥	v						+	
3	574	4		¥	¥	¥						+	
	475	16										- 1	

- 5 Click SAVE AND REVIEW
- 6 Review the order information and click ADD TO CART
- 7 Click PROCEED WITH CHECKOUT
- 8 Select sample submission method; you can choose to submit your samples at your local dropbox location or ship to us directly at the provided address.



9 Complete payment information and checkout your order. Make sure to print out your order receipt and include it with your samples when submitting at your local dropbox or shipping directly to us.

