A52. Procedure for Sanger DNA Sequencing Services by AZENTA Life Sciences

MOLab Online Order Steps:

- 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 BuySite system.
- 3). Create your account at https://clims4.genewiz.com/ or use the MOLab login account (tche@uchicago.edu // UCmolab@2024).
- 4). Complete the online submission process (see pages 4 to 7).
- 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

- o Pre-defined: \$5.51 per rxn
- Custom: \$8.82 per rxn
- PCR clean-up: \$3.31 per rxn
- o Same-day processing: FREE

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

Account Contact:

Emily Fish, MS Account Manager – Genomic Services +1 763.923.1267 Emily.Fish@azenta.com azenta.com

Genomics & Analytical Services | Sanger Sequencing

Sample Submission Guidelines

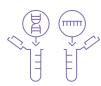




Pre-Mixed Service: Plasmid & Purifed PCR

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 Type | DNA Length (include vector) | Template Concentration in 10 µl | Template Total Mass | Your Primer Total Picomoles | Pre-Mixed Volume (Template + Your Primer) | |
|-------------------------|--------------------------------|---------------------------------------|------------------------|-----------------------------------|---|--|
| Plasmids | <6 kb | ~50 ng / µl | ~500 ng | | 15 µl | |
| | 6 - 10 kb | ~80 ng / µl | ~800 ng | 25 pmol | | |
| | >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 µl | |
| | 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 µM (pmol/µl) | Your Primer Volume Per Reaction |
|--------------|-----------------------------------|---------------------------|---|------------------------------------|--|---------------------------------------|
| Plasmids | Any size | Unknown | At least 500 ng | 10 µl | 5 μΜ | 5 μΙ |
| PCR Products | Any size | Unknown | At least 40 ng | 10 µl | 5 μΜ | 5 μΙ |

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



azenta.com

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

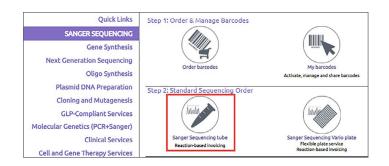
If you have not logged into your account before, your username will be your email address. Use the 'Forgot Password' function to reset your password. A link will be sent to email address to enable you to reset it.

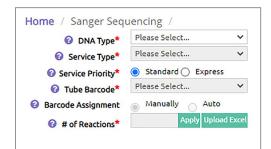
After you have logged into your CLIMS account you will be directed to the homepage.

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

3 Complete Sample and Primer Details

| Order | # | Reaction Barcode | Sample Name * ③ ④ ③ | Primer Barcode | My Primer * | library | ary Azenta/GENEWIZ Primer ^① <u>Selection Too</u> ⑦ ③ | |
|-------|---|------------------|------------------------|----------------|---------------------|---------|--|--|
| 1 | 1 | | EXAMPLE | | ex:Your_Primer_Name | | ex:T7 | |
| 1 | 1 | T0000051 V | AZENTA1 | 00133957 🛨 | primer-1 | + | v | |
| 1 | 2 | T0000052 V | AZENTA2 | + | | + | CMV-Forward 🔻 | |

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."

| Order # Reaction Barcode Sample Name Primer Barcode My Primer Ubrary Azenta/GENEWIZ Primer © Selection Tool 0 | Notes |
|---|-------|
| | 0 0 |
| 1 1 EXAMPLE ex:Your_Primer_Name ex:T7 | |
| 1 1 T00000051 V AZENTA1 00133957 V primer1 4 | |
| 1 2 T0000052 Y AZENTA2 + CMV-Forward Y | |

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

| Primer Name | Primer Source | Stored A Azenta €⊗ | t Conc. (µM) €⊗ | E-Library | Save to E-Library O⊗ | Order Primer ©⊗ | Sequence | Scale (nmol) ♥ ⊗ |
|------------------------------|--------------------------------|--------------------------|-----------------------|---------------|----------------------------|-----------------------|----------|------------------------|
| CMV-Forward | Azenta/GENEWI. | | | | | | | |
| primer-1 | My Primer 🗸 | | 10 | My Library | | | | |
| | | | | | | | | |
| nte: Oligo for Petain Orrigo | lars | | | | | | | |
| ste: Oligo for Retain Orc | jers ield enough primer for | 500 Sandi | er sequenci | ng reactions. | | | | |

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.''

| order(s) Infor | | | | | | | |
|------------------------------|---------------------------------------|-----------------------------|------------------------------------|---|---------------------------|---|------------|
| DNA Type | | PCR Product - Purified | | Order Name | | Samples Subr | |
| Service Typ Service Price | | Predefined Standard | | Order Comments | | Number of Re | eactions 2 |
| Purification | | Column | | | | | |
| | | | | | | | |
| stimated Pi | Price Informatio | n | | | | | |
| | | | | | | | |
| Service | | | | | Unit Price | Quantity | |
| Tube Sange | er DNA sequen | cing, Prepaid | | | Prepaid | 2 | |
| | | | | | | Order Subtotal: | |
| | | | | | | | |
| | | | | | | Shipping/Handling: | |
| *Pricing is e | estimated. Actu | ual charges will reflect we | ork done. In cases where tax is ap | plicable but not estimated, it will be | applied on the invoice | Shipping/Handling: Estimated Total*: | |
| esulting Re | | - | ork done. In cases where tax is ap | pplicable but not estimated, it will be | applied on the invoice | | |
| esulting Re | eactions | - | DNA Name | plicable but not estimated, it will be | Primer | Estimated Total*: Primer Details | Notes |
| esulting Re | eactions Imber [50-6684 | 409924] | DNA Name AZENTA1 | | Primer primer-1 [10µM] | Estimated Total*: | |
| racking Nur Reaction | eactions Imber [50-668 Sample # | 409924] Reaction Barcode | DNA Name | Primer Barcode | Primer | Estimated Total*: Primer Details | |

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

| 2: Sample Submission/Delivery Options | | | |
|--|--|-----------------------------|--|
| Sample Submission/Pickup method: General Dropbox FAQs | Ship Samples to Azenta/GENEWIZ L | Lab ~ | + Map Satellite Entspannung |
| Please ship your samples to: | GENEWIZ Europe Attn: GA Bahnhofstraße 86 04158 Leipzig Germany 877-(436-3949) | | Construction of the standardstrade Crosswer Centre of the standardstrade Ce |
| Save as default method: | | | Googlehenus Data Office Keyboard shortcas Martenator |
| 3: Shipping Address | | | |
| ۲ | <i>ø</i> Edit − | + Add New Address | |
| | | | |
| 4: Payment Type | | | |
| 🔿 Credit Card 🗕 PO 🔿 Multiple POs | Upload PO/VAT Certificate | | |
| PO Number Please note: must provide valid PO | | Save this PO for Future Use | |

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.

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.)

| | | Order F | Receipt - Tra | cking # 50-66771 | 7407 | | | |
|---|-------------|--|---------------|--|-------|--------------------------|--|--|
| ú ú | | | | ce Type - Premix | | 50-667717407 | | |
| NSTRUCTIO | | int this form and include a online account. For Regul | | | | ervices, please submit a | | |
| GENEWIZ Eu Attn: GA | | Name Phone | ation | Payment Details Payment Type PO Number Submitted Time | Custo | mer Shipping Address | | |
| Bahnhofstraße 86 PI 04158 Leipzig Company Germany 877-(436-3949) Email | | Company | | Account Manager Account Manager Email | | | | |
| | Summary | 12 2 3 | | 1 | | | | |
| Order typ | | Sanger Sequencing | | | | | | |
| Service Pr | | Standard | | | | | | |
| Service Ty | | Premix | | | | | | |
| DNA Type | | PCR Product - Purifi | ea | | | | | |
| Purificatio | | Enzymatic | | | | | | |
| interio en o | f Reactions | 1 | | | | | | |
| Layout | | Vertical View | | | | | | |
| Reaction | | Barcode | DNA Name | N | lotes | | | |
| | | | 001 | | | | | |

5 Track Your Order Status

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

| | PLACE AN OF | RDER | | Choose an Ico | n Below to Start Your Order | | | | | | ⊘ |
|------------|---|--|--|---------------|-----------------------------|--|----------|-----------------------------------|-------------------------------------|----------------------|-------------------|
| | Sanger Se Gene ! Next Generation Se Oligo ! Plasmid DNA Pre Cloning and Mut GLP-Compliant GLP-Compliant Molecular Genetics (PCR | Synthesis quencing Synthesis eparation cagenesis : Services +Sanger) I. Services rocessing | Wy barcode Activete, manage and sh Mutation/SNP Ar | are barcodes | Order barcodes | Sanger Sequencing Reaction based live Standard Prep Per run service for all p |) | Plate- Oligo Modified and p | Sequencing plate based invoicing | | 며 Fraesoock |
| MY ORDERS | My Shared Projects | C | GeneGroup® | My Tools | | | | | | | |
| Search | Show Advanced Filt | er | | | | | | Edit View I | Results Print Receipt Downloa | d Add to Project Del | ete Bulk Download |
| Ŧ | Tracking # | Order Name | | Project Name | Service Line 🛩 | Service Type | Priority | Qty | Action ~ | | |
| Order Date | Tracking # | Order Name | • • | Project Name | 🛊 Service Line 🗧 | Service Type | Priority | \$ Qty | Order Status | Action | \$ |
| | 0 50-668409924 | | | | Sanger Sequencing | PCR Product - Purified | Standard | 2 | Waiting for Sample | Summar | У |

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



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