Azenta Life Sciences & Sanger Sequencing Overview

Kayla Martin, PhD
Process Development Manager/Technical Support
South Plainfield, New Jersey

Michael Bullard
Sr. Account Manager
Michael.Bullard@Azenta.com
St. Louis, Missouri
WHAT YOU KNOW
Brooks Life Sciences Services, GENEWIZ® and Products businesses have rebranded under the creation of a new identity

OUR NEW NAME
Azenta Life Sciences

Signals our commitment to helping customers reach new heights in their pursuit of scientific progress.
We unite all our solutions and offerings under one Azenta Life Sciences company.
Our Brand Promise

We enable life sciences companies and academic research to **bring impactful breakthroughs and therapies** to market faster.

We provide unrivaled genomics services, sample exploration and management solutions to help **accelerate discovery, development and delivery**.

Today, we have thousands of employees around the world working across our leading capabilities of **genomics, cryogenic storage, automation and informatics**.
At the Forefront of the Technology Curve
GENEWIZ Legacy

- 1999: LAUNCHED SANGER SEQUENCING
- 2000: Human Genome Project Completed
- 2009: First Synthetic Living Organism
- 2010: LAUNCHED SANGER SEQUENCING
- 2012: LAUNCHED NEXT GENERATION SEQUENCING
- 2015: First Human in vivo CRISPR Performed
- 2017: LAUNCHED SINGLE CELL SEQUENCING SERVICES
- 2017: LAUNCHED NGS-CRISPR SCREENING SERVICE & CLIA SANGER
- 2018: LAUNCHED CLIA/CAP WGS & WES
- 2019: LAUNCHED SPATIAL TRANSCRIPTOMICS SERVICES
- 2020: LAUNCHED AAV SOLUTIONS FOR SYNTHETIC PRODUCTS AND SEQUENCING

GENEWIZ Acquired by Brooks Life Sciences Nov 2018
Serving the life sciences and academic community for over 20 years

- 2011-2012
  - RTS
  - Nexus

- 2013-2014
  - Matrical
  - 22% of BioCision

- 2015-2016
  - FluidX
  - BioStorage Technologies

- 2017-2018
  - BioCision
  - PBMMI
  - FreezerPro
  - 4titude Ltd.
  - BioSpeciMan

- 2019-2020
  - GENEWIZ
  - RURO
  - Trans-Hit Biomarkers

- 2021
  - Azenta Life Sciences Launch
Serving an Impressive Roster of Global Customers

<table>
<thead>
<tr>
<th>1 in 3</th>
<th>18,500</th>
<th>33</th>
</tr>
</thead>
<tbody>
<tr>
<td>US molecular biologists use Azenta*</td>
<td>Citations in scientific journals</td>
<td>Nobel laureate labs use Azenta</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>20 of 20</th>
<th>13/15</th>
<th>Top 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Top pharma/biotech served by Azenta</td>
<td>Top pharmas trust Azenta with their samples</td>
<td>Best-selling pharma products: clinical samples managed by Azenta</td>
</tr>
</tbody>
</table>

Azenta Life Sciences | Proprietary and confidential.
OUR PORTFOLIO
Research & Discovery

For customers focused on R&D challenges, we help you to source, store, transport, and analyze samples.

<table>
<thead>
<tr>
<th>Offer Categories</th>
<th>Azenta Solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genomics &amp; Analytical Services</td>
<td>Sequencing</td>
</tr>
<tr>
<td></td>
<td>DNA &amp; RNA synthesis</td>
</tr>
<tr>
<td>Sample Sourcing</td>
<td>Custom global sample sourcing for R&amp;D workflows</td>
</tr>
<tr>
<td></td>
<td>Biospecimen Collection Consultation</td>
</tr>
<tr>
<td>Storage, Automation &amp; Logistics</td>
<td>On-site &amp; off-site storage</td>
</tr>
<tr>
<td></td>
<td>Automated storage systems</td>
</tr>
<tr>
<td>Consumables &amp; Instruments</td>
<td>2-D barcoded consumables</td>
</tr>
<tr>
<td></td>
<td>Cryogenic storage consumables</td>
</tr>
<tr>
<td>Data &amp; Informatics</td>
<td>Full Sample Lifecycle Management Platform</td>
</tr>
<tr>
<td></td>
<td>Full Product Lifecycle Management Platform</td>
</tr>
<tr>
<td>Consultative Services</td>
<td>Workflow assessment &amp; optimization</td>
</tr>
</tbody>
</table>
Well-Positioned in Key Global Life Science Markets
Research & Discovery

GENOMICS & ANALYTICAL SERVICES

Expedite your research objectives with Azenta’s comprehensive portfolio of genomics services.

• Sequencing Solutions
  - Sanger Sequencing
  - Next-Generation Sequencing
    • Illumina Certified Service Provider
    • PacBio Sequel Certified Service Provider
    • 10xGenomics CRO
    • Nanostring GeoMx™ Digital Spatial Imaging

• DNA & RNA Synthesis
  - Gene Synthesis
  - Oligo Synthesis
  - Gene Editing
  - Plasmid preparation
Sanger Sequencing
Sanger Sequencing Agenda

- SERVICE TYPE OVERVIEW
- UN-PURIFIED PCR PRODUCT SEQUENCING
- BACTERIAL COLONY, GLYCEROL STOCK AND PHAGE SEQUENCING
- DIFFICULT TEMPLATE SEQUENCING
- AZENTA WORKFLOW
- HOW TO CREATE AND LOGIN TO ACCOUNT
- HOW TO PLACE AN ORDER
- REVIEWING AND INTERPRETING SEQUENCING RESULTS
- TROUBLESHOOTING
Why Azenta?

- Largest Sanger Sequencing provider in the world
- Virtually unlimited capacity with backup lab capacity
- Tech Support: personalized customer support
- 25 universal primers: [Universal Primers](#)
- Proprietary difficult template Protocols: GC rich, hairpin
- 24 hour and “Same Day” Service – Monday - Friday
- High Quality Data, Quality Score >40
- ~750 - 1000bp read length
- Easy data retrieval through Azenta account
Wide variety of sample types accepted

Start from:
- Plasmid (<50 kb)
- PCR Product
- Glycerol Stock
- Bacterial Colony
- Phage

End Result:
- DNA
- Primer
- Reaction Mix
- Seq CAGCTCAGAT
Sanger Service Overview

**Pre-Mixed**
- Plasmids or purified PCR products
- Submit template and primer mixed in the same tube according to Azenta Sample Submission Guidelines

**Pre-Defined**
- Plasmids or purified PCR products
- Submitting template and primer in separate tubes or using Azenta universal primers
- Azenta will add primer
  - No additional charge when using Azenta universal primers

**Custom**
- Plasmids, purified PCR products, un-purified PCR products, bacterial colonies, glycerol stock, phage
- Submit template and primer in separate tubes
- Azenta will:
  - Adjust the concentrations of samples and primers
  - Clean up crude PCR products containing one single band using enzymatic clean-up
  - Amplify templates using rolling circle amplification
    - Bacterial colonies, glycerol stock, phage plaque, or phage supernatant
- Additional charges apply
ENZYMATIC PURIFICATION

• Ideal for PCR products with one band
• Determine approximate concentration by gel image
  - Compare samples to ladder with known concentration
• Concentration not measured after cleanup as nanodrop readings are inaccurate
• Note: Gel purification not performed at Azenta
Bacterial Colonies, Glycerol Stock, Phage Sequencing & AAV-ITR Sanger Sequencing

ADVANTAGES

• Rolling Circle Amplification
  - Amplify circular DNA without the need of a DNA preparation
  - Advantageous for large screenings

• Bundle with Plasmid Preparation service
  - Request to “save for prep”
  - Plasmid Prep order can be submitted after analyzing Sanger Sequencing data
  - Samples retained for 2 weeks

• Proprietary AAV-ITR Sequencing
  - Research Use Only (RUO)
  - GLP Compliant
Difficult Template Sequencing with Proprietary Protocols

• GC-rich
  - A template of > 60% GC content or high GC-content concentrated in a small region
• Hairpin Structures
  - A sequence containing two inverted repeats, separated by at least three nucleotides
• Repeat Regions
  - Certain di- or tri-nucleotide repeats
• Templates with secondary structures or ITRs (Inverted Terminal Repeats)
  - ITR through Molecular Genetics
Sample Submission Guidelines

PREMIX AND PRE-DEFINED:

<table>
<thead>
<tr>
<th>DNA Type</th>
<th>DNA Length (include vector)</th>
<th>Template Concentration in 10uL</th>
<th>Template Total Mass</th>
<th>Your Primer Total Picomoles</th>
<th>Premixed Volume (Template + Primer)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasmid (10ng/uL/kB)</td>
<td>&lt;6 kb</td>
<td>~50 ng/µL</td>
<td>~500ng</td>
<td>25 pmol</td>
<td>15µL</td>
</tr>
<tr>
<td></td>
<td>6-10 kb</td>
<td>~80 ng/µL</td>
<td>~800ng</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt;10 kb</td>
<td>~100 ng/µL</td>
<td>~1000ng</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Purified PCR Products (2ng/uL/kb)</td>
<td>&lt;500 bp</td>
<td>~1 ng/µL</td>
<td>~10ng</td>
<td>25 pmol</td>
<td>15µL</td>
</tr>
<tr>
<td></td>
<td>500-1000 bp</td>
<td>~2 ng/µL</td>
<td>~20ng</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1000-2000 bp</td>
<td>~4 ng/µL</td>
<td>~40ng</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2000-4000 bp</td>
<td>~6 ng/µL</td>
<td>~60ng</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt;4000 bp</td>
<td>Treat as plasmid</td>
<td>Treat as plasmid</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Primers: For sequencing: 5pmol/µl (5µM)
Tm of the primer should be between 50°C-60°C (our Annealing Temp is 50°C)

* https://www.genewiz.com/Public/Resources/Sample-Submission-Guidelines/Sanger-Sequencing-Sample-Submission-Guidelines
Optimizing for high quality reads

PRIMER DESIGN

- 18-21 nucleotides in length
- ~50% GC content
- Tm between 50 °C-60 °C
- Include a G or C anchor
- No secondary structures or primer dimer

CLEAN TEMPLATE

- 260/230: ~2.0 (1.8-2.2)
- 260/280: ~2.0 (1.8-2.2)
- <0.1mM EDTA, little to no EtOH, etc.
- Use water or Tris to resuspend DNA, do not use TE

CORRECT AMOUNT OF TEMPLATE

- Plasmid: 10ng/µl per kb of your construct (5kb = 50ng/µl of template)
- PCR products: ~ 2ng/µl per kb
Azenta Sample Processing

Order is Submitted in CLIMS

Courier receives notification for sample pick-up

Sample pick-up occurs and samples are sent to Azenta

Samples are received and sorted

Samples run on ABI 3730xl DNA analyzer

Sequencing reaction is set up and performed

Samples are processed in 96 well format (2 wells contain internal controls)

Data analyzed by default ABI analysis Protocol

Data is reviewed by Technical Support

Data is reported into customer account

Data is reviewed by Technical Support
Creating an Azenta Online Account

Register at: https://www.genewiz.com
Registration

Basic Information

- Email
- First Name
- Last Name
- Create Password
- Confirm Password
- Institution Name
- Institution Type

Address

- Country/Area
- Street
- City
- State/Province
- Zip/Postal Code
- Phone

Email Updates

- I acknowledge I will receive communications about Azenta/GENEWIZ services including service and laboratory updates, new technology developments, and promotions.
- No, I would not like to receive these communications from Azenta/GENEWIZ.

Privacy

- I have read and understood Azenta/GENEWIZ’s Privacy Policy. I consent to the processing of my personal data by Azenta/GENEWIZ. I can withdraw my consent at any time by contacting Azenta/GENEWIZ at DNAseq@azenta.com.

Submit

Captcha

Submit
Complete Profile

Lab/Mailroom address to receive Oligo/GS constructs

PI/purchasing department's address to be included on the invoice
CREATING A SANGER ORDER
Choose Sanger Sequencing and Click Desired Order Type

1. Login to Account

2.

3.
Creating an Order
Complete Form → Save and Review

Use “NC-COUPON100” promo code
$100 discount - first order only!
**Confirm Primer Details**

*Note: PreDefined primers must be submitted at 5uM concentration. If Azenta/GENEWIZ is storing the primer for you, 5uM is also appropriate.*

<table>
<thead>
<tr>
<th>Primer Name</th>
<th>Primer Source</th>
<th>Stored At: Azenta</th>
<th>Conc. (µM)</th>
<th>E-Library</th>
<th>Save to E-Library</th>
<th>Order Primer</th>
<th>Sequence</th>
<th>Scale (nmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test</td>
<td>My Primer</td>
<td>□</td>
<td>5</td>
<td>My Library</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Note: Oligo for Retail Orders*
A 25nmol scale oligo will yield enough primer for 500 Sanger sequencing reactions.
A 50nmol scale oligo will yield enough primer for 1000 Sanger sequencing reactions.
Add Order to Cart

Home / Order Form / Order(s) Review

Order(s) Information

<table>
<thead>
<tr>
<th>DNA Type</th>
<th>Service Type</th>
<th>Service Priority</th>
<th>Order Name</th>
<th>Order Comments</th>
<th>Samples Submitted</th>
<th>Number of Reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasmid</td>
<td>Predefined</td>
<td>Standard</td>
<td></td>
<td></td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

Estimated Price Information

<table>
<thead>
<tr>
<th>Service</th>
<th>Unit Price</th>
<th>Quantity</th>
<th>Itemized Subtotal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-Defined Sanger DNA sequencing</td>
<td>$0.00</td>
<td>2</td>
<td>$0.00</td>
</tr>
</tbody>
</table>

Order Subtotal: $0.00
Shipping/Handling: $0.00
Estimated Total*: $0.00

*Pricing is estimated. Actual charges will reflect work done. In cases where tax is applicable but not estimated, it will be applied on the invoice.

IMPORTANT: Selecting multiple primers per template may impact template/primer grouping required for sample submission. Please review the sample well/tube number assignments below prior to organizing your samples for submission.

Submitted Samples

- Sample 1 To 2

Resulting Reactions

- Tracking Number [30-703228036] (Test_1-Test - Test_2-Test)
Select Sample Submission Method
Azenta Drop-box Selection

Sample Submission/Pickup method:

- General Dropbox FAQs

Locate a box:

2 dropboxes found for your institution (click here to select)

Locate a dropdown

- Search by address, city, state, zip
- My Default State/Province (Minnesota)
- All States

Sample pickup at University of Minnesota-Minneapolis - MCB 6-113 - TEMPORARILY 2PM CUTOFF

Sample pickup at University of Minnesota-Minneapolis - CCRB Rm 4-230B, bench 66 by 1...

View box

CANCEL SELECT THIS BOX
Current Dropboxes

University of Minnesota-Minneapolis:
• CCRB Rm 4-230B, bench 66: 1:30PM
• MCB 6-113: 2:00PM

University of Minnesota-St. Paul:
• Gortner Laboratory Building, Room 142: 5:00PM

Mayo Clinic:
• Guggenheim Bldg Room 16-18: 4:00PM
• Gonda Bldg 19th Fl, outside of Room 306: 4:00PM
• Stabile 13-21 Genome Analysis Core: 4:00PM

https://www.mayo.edu/research/core-resources/genome-analysis-core/services/sequencing
Select & Enter Payment Information

• **Purchase Order#:**
  - Invoice created with 1 week of order completion
  - Emailed to billing email addresses provided in account profile

• **Credit Card** charged on order completion
Agree to Terms and Conditions and Complete Checkout
Print Order Receipt to include with samples & deposit in drop-box
Tubes or Plates?

• < 48 samples - **tubes**
  - 8-strip 0.2mL PCR tubes with caps

• 48 samples or more - **plates**
  - 96-well, semi-skirted plate
  - Cap wells with 8-strip caps
  - **Seal tightly!**
  - $1/rxn price break @ 48 – 93
  - $2/rxn price break @ 94 or more

• Label with initials and sample number found on reaction column on order receipt
  - Ex. TA01, TA02, etc.
VIEW RESULTS & TROUBLESHOOTING
Login to Account to Review Results
**Note:** All Sanger Sequencing Results are accessible for 2 years. After 2 years, the data will no longer be available.
Common Result File Types

DOWNLOAD OR VIEW:

Seq

> A_1-AmpR239_A01.ab1
NNNNNNNNNNNCCNNTCGGCTGGCTGGTATTGGCTGATAATTCTGGACGTTGCCTCGGACGTTCTCCGGGTATTT
GCAGCCTGCTGGCCGATGATGTAAGCCCTCCGTATCGTAGTTATCTACACGACGAGAGTCAGGCAACTATGGATGAACG
AAATAGACAGATCGTGATAGAGGTGGCTCACCTGATTAAGCTTTGGTAACCTGTCAGCAGATGTTACTCTATATATCTTT

Trace
Quality Control

TO PASS OUR QUALITY CONTROL:

• QS: Quality Score
  - >40

• CRL: Continuous Read Length
  - >500bp when applicable

• Include controls on sequencing plate when possible
Request Repeats

- Number of free repeats for failed reactions based on order size
  - 1-16: 2 free repeats
  - 17-47: 3 free repeats
  - 48+: 4 free repeats
  - \textit{If free repeats improve, we will repeat remaining similarly failed reactions free of charge}
- Can submit request for modified repeats for charge
- Sample retention
  - Plasmid/PCR: 5 business days (1 week)
  - Bacterial Colonies/Glycerol Stock/Phage: 10 business days (2 weeks)
Troubleshooting Help Sheets

<table>
<thead>
<tr>
<th>QS</th>
<th>CRL</th>
<th>Analysis Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>54</td>
<td>685</td>
<td></td>
</tr>
<tr>
<td>54</td>
<td>683</td>
<td></td>
</tr>
<tr>
<td>36</td>
<td>585</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>64</td>
<td>High Background</td>
</tr>
<tr>
<td>54</td>
<td>685</td>
<td>Poor Quality</td>
</tr>
<tr>
<td>33</td>
<td>685</td>
<td>Poor Quality</td>
</tr>
<tr>
<td>16</td>
<td>47</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>14</td>
<td></td>
</tr>
</tbody>
</table>

Description

Poor quality results are typically due to an inefficient reaction. In general, some or all of the trace before position 500 is close to background signal, which makes base calling in these regions unreliable. Furthermore, results are scored as “poor quality” if they fail to pass quality control and cannot be well classified as another failure type. Please note that the distinction between “poor quality” and “no priming” results is sometimes arbitrary; however, the former may provide some useful data whereas the latter usually does not.

POOR QUALITY – TYPE 1
Dominant trace is present but near background signal; average signal intensity* is usually <200

POOR QUALITY – TYPE 2
Trace starts strong but gradually diminishes to near background signal before position 500

GOOD RESULT
Well defined peaks with low background signal at least 500 bp

*The average signal intensity of each base (G, A, T, C) is displayed at the bottom of the online trace viewer.

Troubleshooting

There are several possible causes for a poor quality result: Select your DNA type below to jump to the appropriate section in the guide. The list of potential causes is not exhaustive but represents the most common issues. For more assistance, please contact Technical Support at 877-436-3849 ext. 2 or dnaseq@geneviz.com.

DNA Type | Page
---|---
1. Plasmids | 2
2. Purified PCR Products | 4
3. Unpurified PCR Products | 7

Page 49
Technical Support
7 AM to 7 PM CT
DNAseq@Azenta.com

- Personalized Ph.D. - level customer support
  - +1-877-GENEWIZ (436-3949) ext. 2
  - Email: DNAseq@azenta.com
  - Live Chat
- In-depth troubleshooting at no extra cost
- Free & ½ price repeats
- 5 day sample storage or longer on request
Sanger FAQ on Website

SANGER SEQUENCING FAQS

Customer Accounts | DNA Sequencing | Sample Submission | DNA Sequencing Results | About Primers | Troubleshooting | Regulatory DNA Sequencing Services

CUSTOMER ACCOUNTS

1. I am a new customer. How do I set up an account?
2. How can I try out your service?
3. What is the price of my order?
4. Can I open an account and submit samples if I live outside the US?
5. Why should I choose GENEWIZ?

DNA SEQUENCING SAMPLE SUBMISSION

1. What is the difference between Pre-Mixed, Pre-Defined, and Custom?
2. How do I prepare my samples so I will get good DNA sequencing results?
OLIGO RETAIN FOR SANGER

- Bundled with Sanger and Prep Services

OLIGO-RAPID SERVICES

- Standard, unmodified oligos for 15 - 60mer lengths, 2nd day delivery
- Expedited, Next day delivery option (15 – 30mer)
- Synthesized and ships from Indianapolis, IN

OLIGO-FLEX

- Customized oligos with purification (PAGE/HPLC, desalted) and modification options
  - 1 umol – 200nmol
  - 15 – 150mer
- Synthesized and shipped from our China facility
Our reputation:

“Excellent data quality, effective communication, flexibility, and regular updates. I wish every CRO had such a high level of customer service.”

• Principle Investigator from a major institution
Thank you