

# Azenta Life Sciences & Sanger Sequencing Overview

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# ABOUT US





#### 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







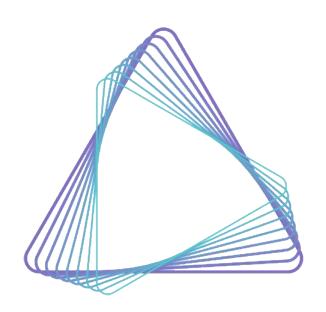














#### Our Brand Promise



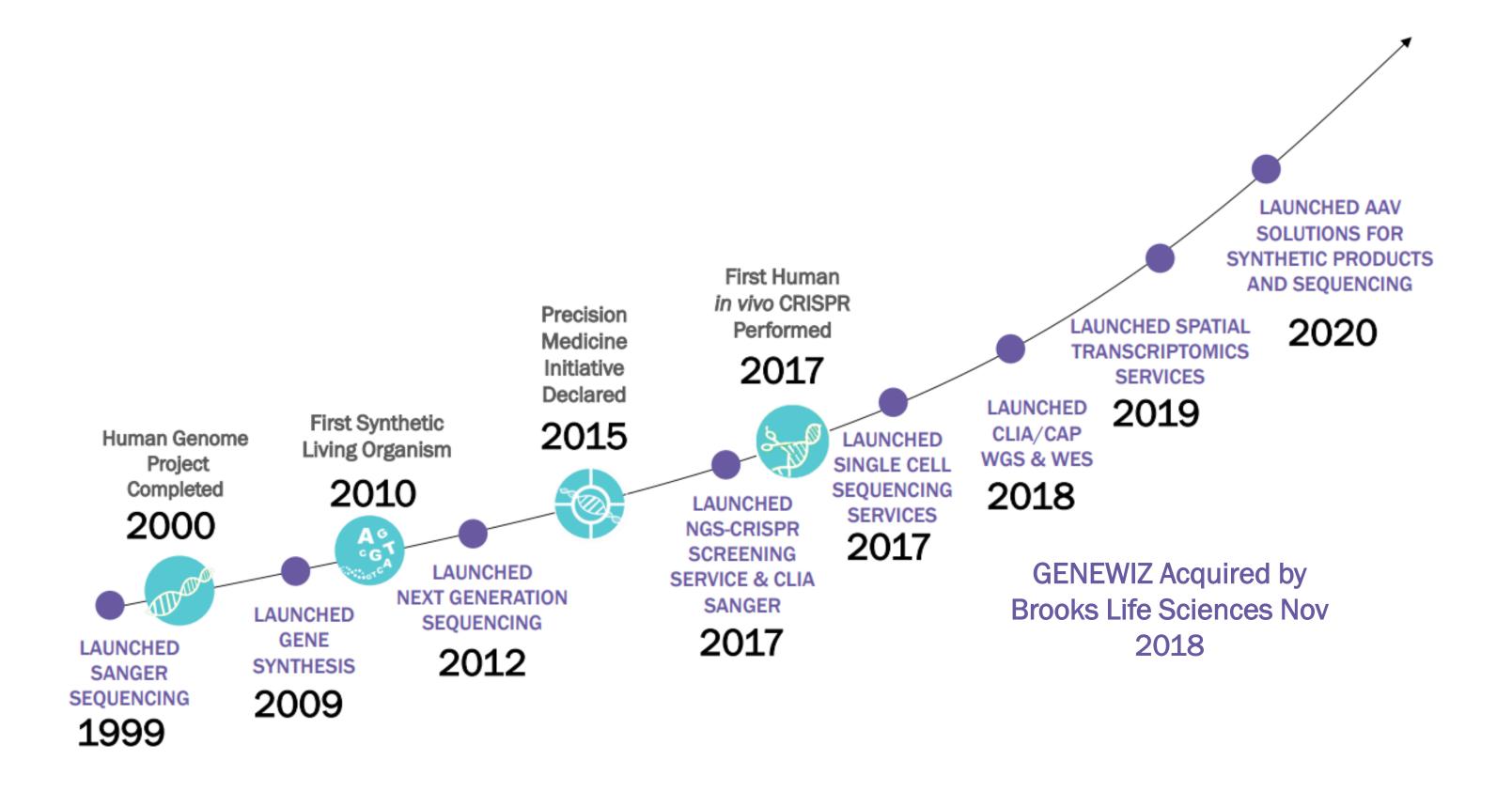
We enable life sciences companies and academic research to bring impactful breakthroughs and therapies to market faster.

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.

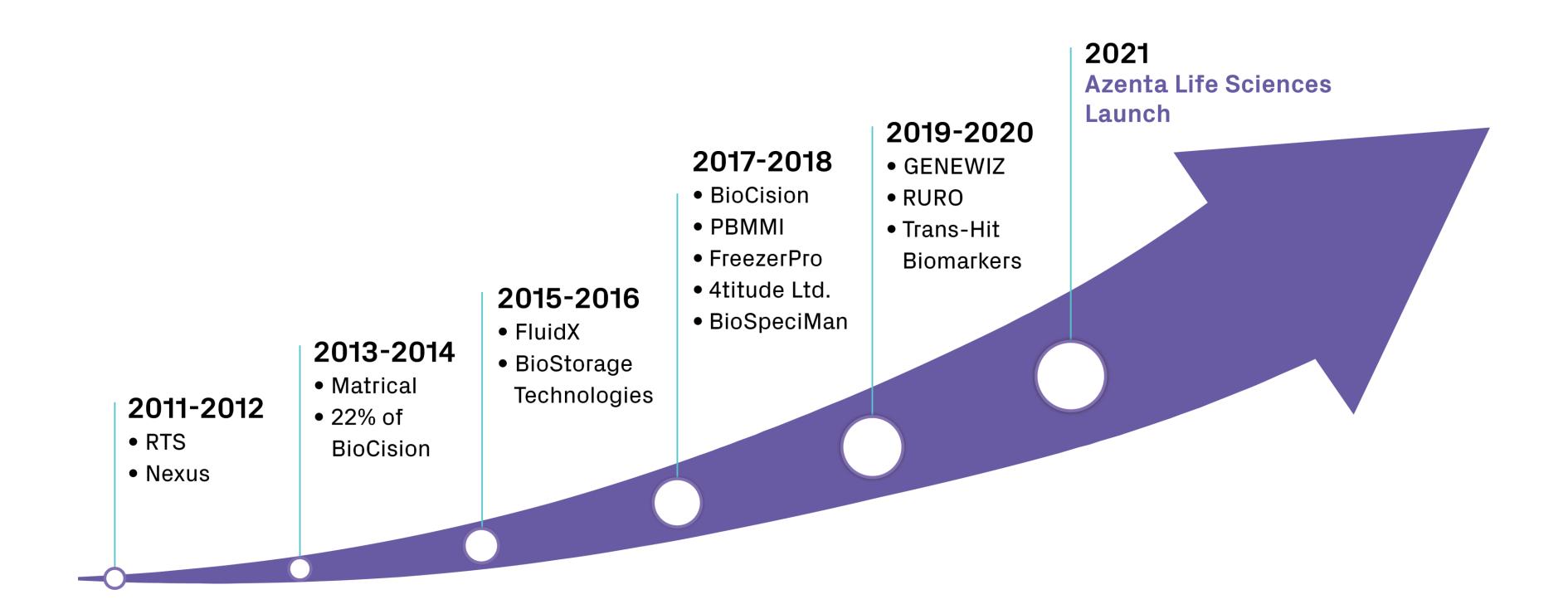
# At the Forefront of the Technology Curve GENEWIZ Legacy





# Serving the life sciences and academic community for over 20 years





# Serving an Impressive Roster of Global Customers



1 in 3

US molecular biologists use Azenta\*

18,500

Citations in scientific journals

33

Nobel laureate labs use Azenta

20 of 20

Top pharma/biotech served by Azenta

13/15

Top pharmas trust Azenta with their samples

Top 5

Best-selling pharma products: clinical samples managed by Azenta



# OUR PORTFOLIO



# Research & Discovery

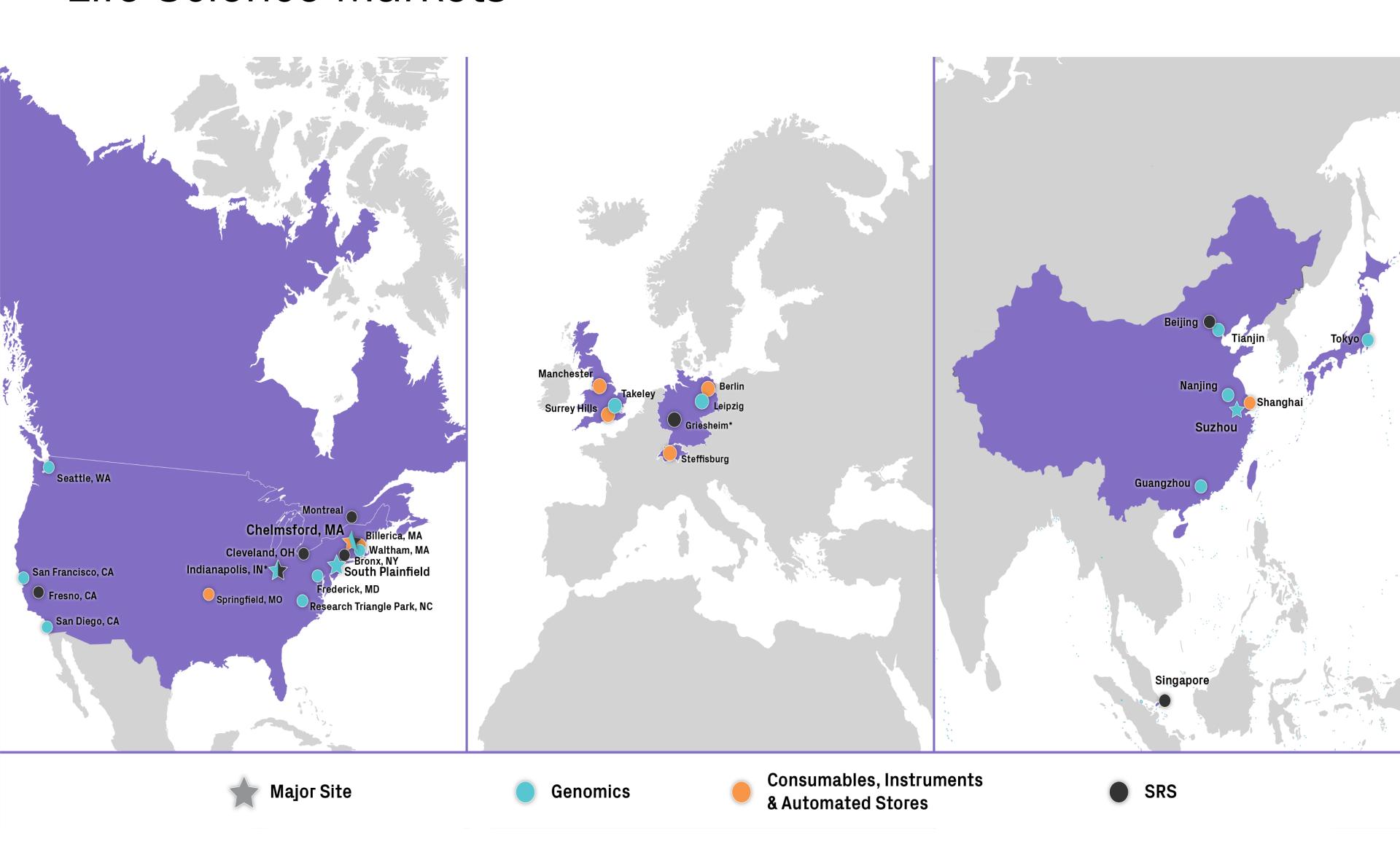


For customers focused on R&D challenges, we help you to source, store, transport, and analyze samples.

Offer Categories	Azenta Solutions		
Genomics & Analytical Services	Sequencing DNA & RNA synthesis		
Sample Sourcing	Custom global sample sourcing for R&D workflows Biospecimen Collection Consultation		
Storage, Automation & Logistics	On-site & off-site storage Automated storage systems		
Consumables & Instruments	2-D barcoded consumables Cryogenic storage consumables		
Data & Informatics	Full Sample Lifecycle Management Platform Full Product Lifecycle Management Platform		
Consultative Services	Workflow assessment & optimization ————————————————————————————————————		

# 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<sup>™</sup> 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?

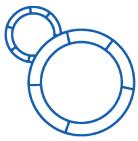


- Largest Sanger Sequencing provider in the world
- Virtually unlimited capacity with backup lab capacity
- Tech Support: personalized customer support
- 25 universal primers: <u>Universal Primers</u>
- Proprietary difficult template Protocols: GC rich, hairpin
- 24 hour and "Same Day" Service Monday 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:



(<50 kb)

Plasmid





Product



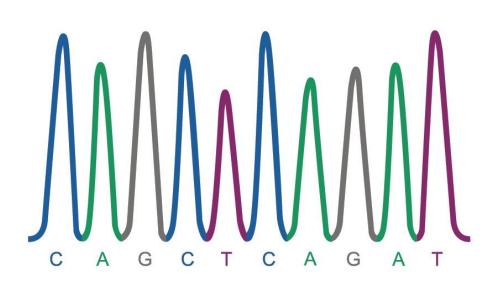
Glycerol Stock

Bacterial Colony

Phage



#### End Result:



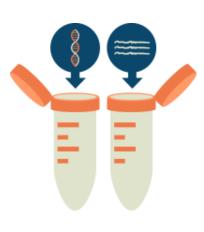
# Sanger Service Overview





#### **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**

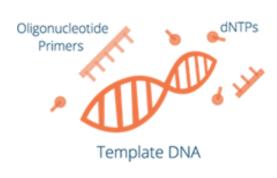
- Plasmids, purified PCR products, un-purified PCR products, bacterial colonies, glycerol stock, phage
- Submit template and primer in separate tubes
- Azenta will:
  - Adjust the concentrations of samples and primers
  - Clean up crude PCR products containing one single band using enzymatic cleanup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

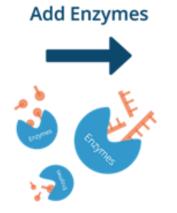
## Un-purified (crude) PCR Sequencing



#### **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





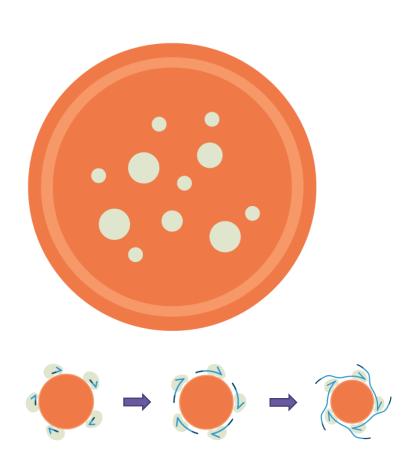


# Bacterial Colonies, Glycerol Stock, Phage Sequencing & AAV-ITR Sanger Sequencing



#### **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:

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

Primers: For sequencing: 5pmol/µl (5µM)

Tm of the primer should be between 50°C-60°C (our Annealing Temp is 50°C)

<sup>\*</sup> 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**

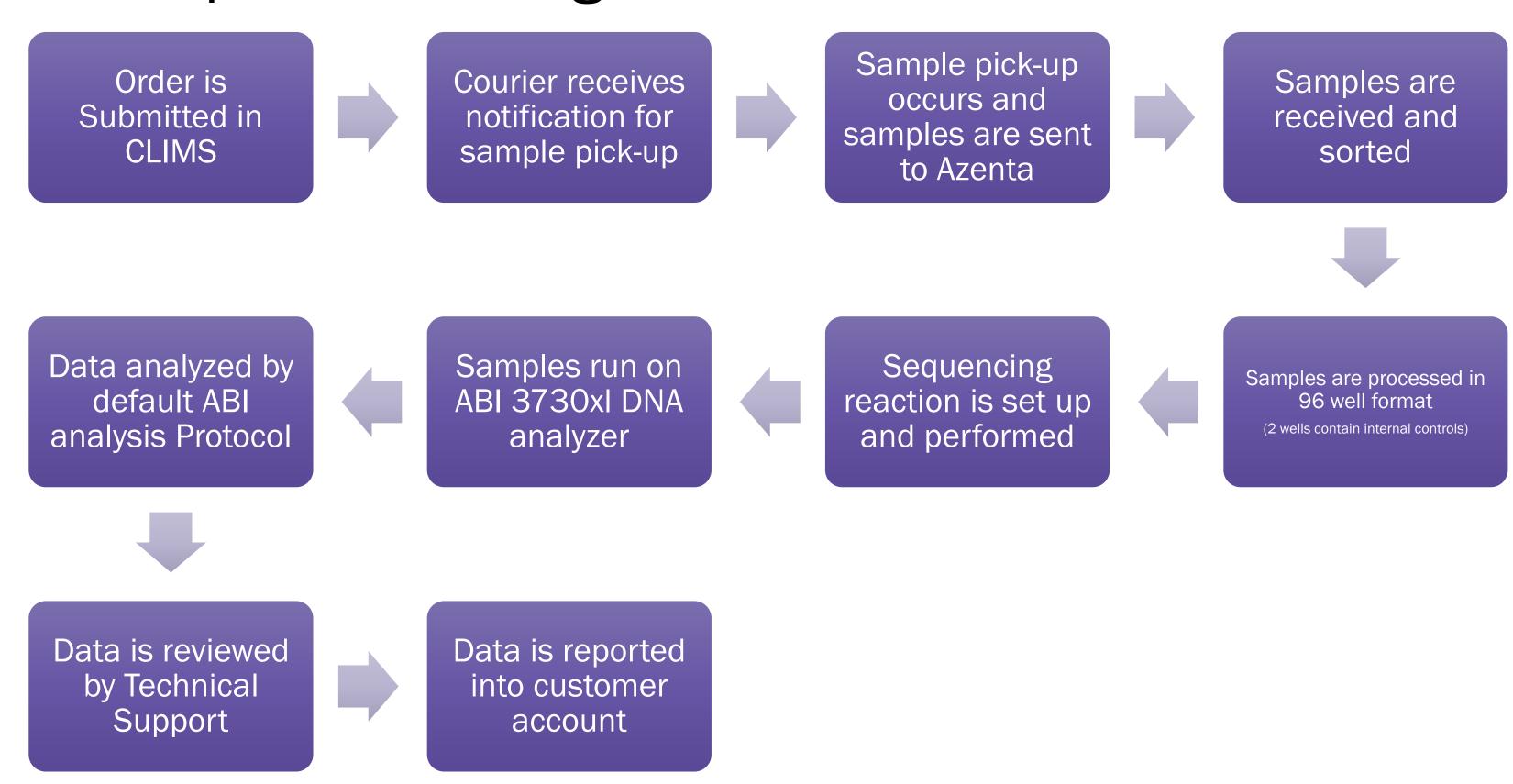
- 260/230: ~2.0 (1.8-2.2)
- 260/280: ~2.0 (1.8-2.2)
- <0.1mM EDTA, little to no EtOH, etc.
- Use water or Tris to resuspend DNA, do not use TE

#### **CORRECT AMOUNT OF TEMPLATE**

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

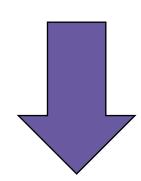


## Azenta Sample Processing



# Creating an Azenta Online Account





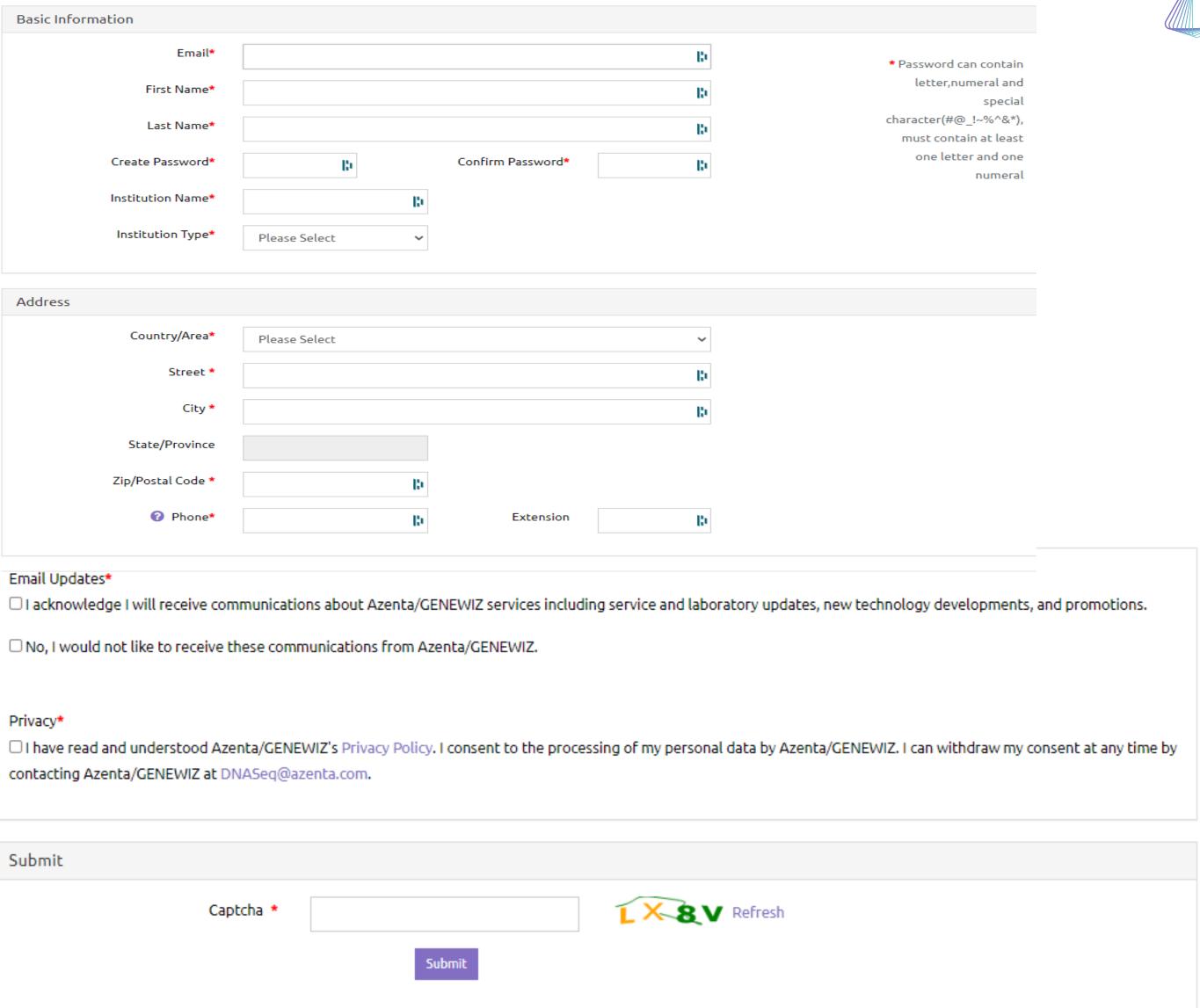
#### Register at: https://www.genewiz.com



# Welcome Back! User name Password Forgot Password? Remember me? LOGIN New customer? Sign up now

# Registration

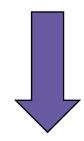




## Login to Account



https://www.genewiz.com





**SERVICES** RESEARCH AREAS RESOURCES

PRODUCTS

COMPANY Q

Login | Order

#### Home / GENOMICS SERVICES

Consumables & Instruments Genomics Services

#### **PLACE AN ORDER**

Search Services ...

Q

#### **Quick Links**

#### **SANGER SEQUENCING**

Gene Synthesis

**Next Generation Sequencing** 

Oligo Synthesis

**Plasmid DNA Preparation** 

Cloning and Mutagenesis

**GLP-Compliant Services** 

Molecular Genetics (PCR+Sanger)

Clinical Services

**Cell and Gene Therapy Services** 

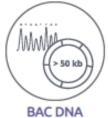
**Biofluid Processing** 

Sample Sourcing Service

Consumables & Instruments



Simple Order Quicker Ordering Process Plasmid or Purified PCR only

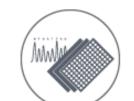




Original Order Full Customization on All Sanger Ordering Processes



**AAV-ITR Sequencing** 5-7 day Turnaround Time



STANDARD ORDERING BY REACTION

Uniform Plate Quick Order

#### SPECIALTY ORDERING



MY ORDERS

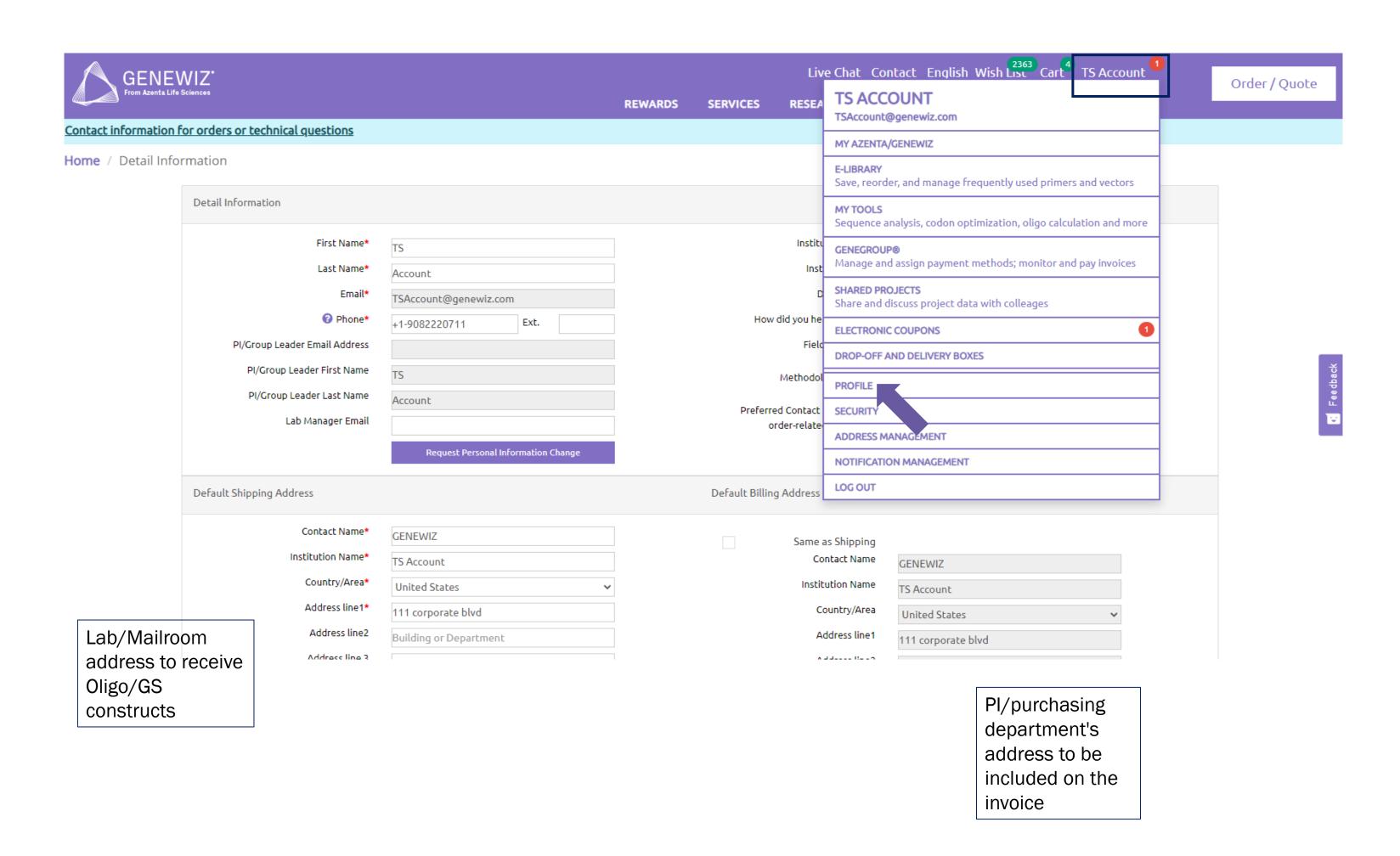
**My Shared Projects** 

GeneGroup®

My Tools

# Complete Profile





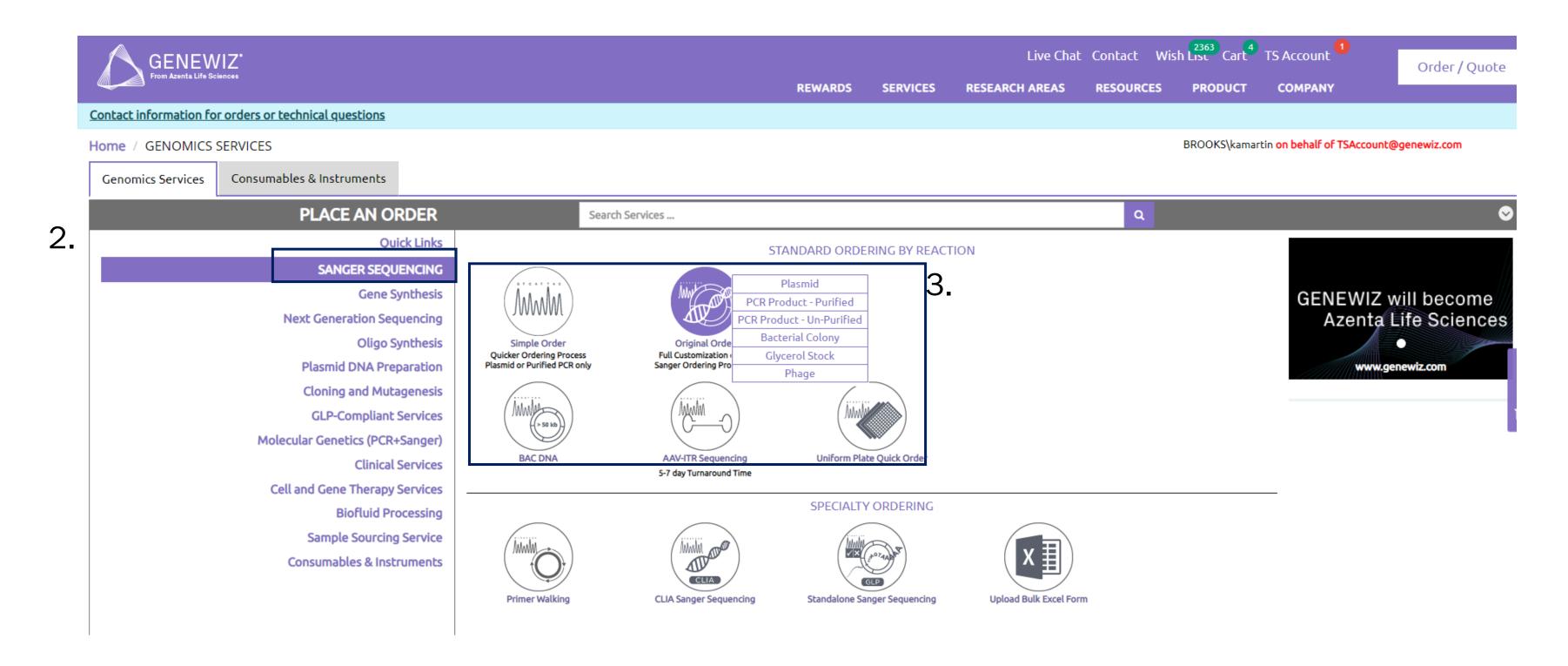


# CREATING A SANGER ORDER

# Choose Sanger Sequencing and Click Desired Order Type

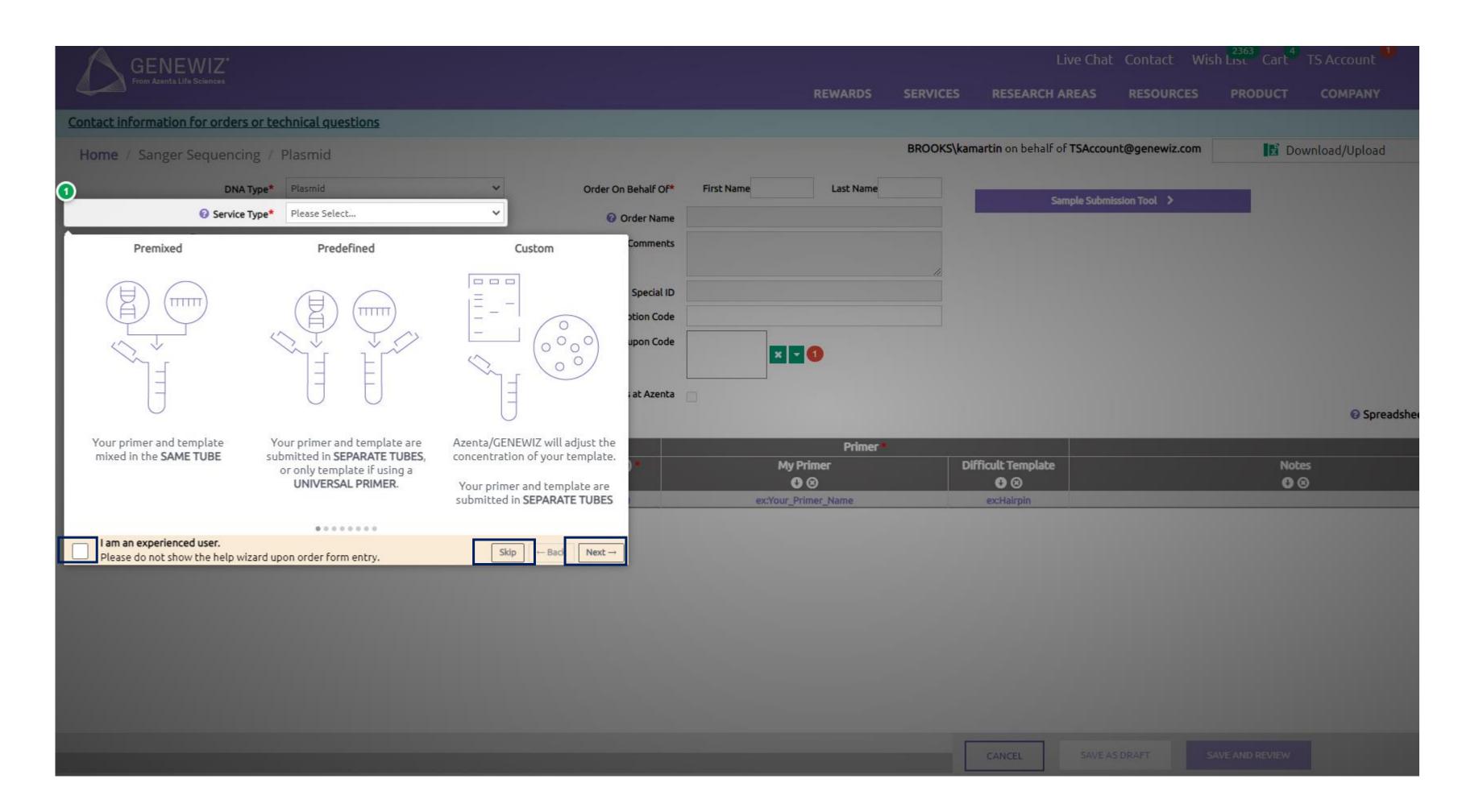


#### 1. Login to Account



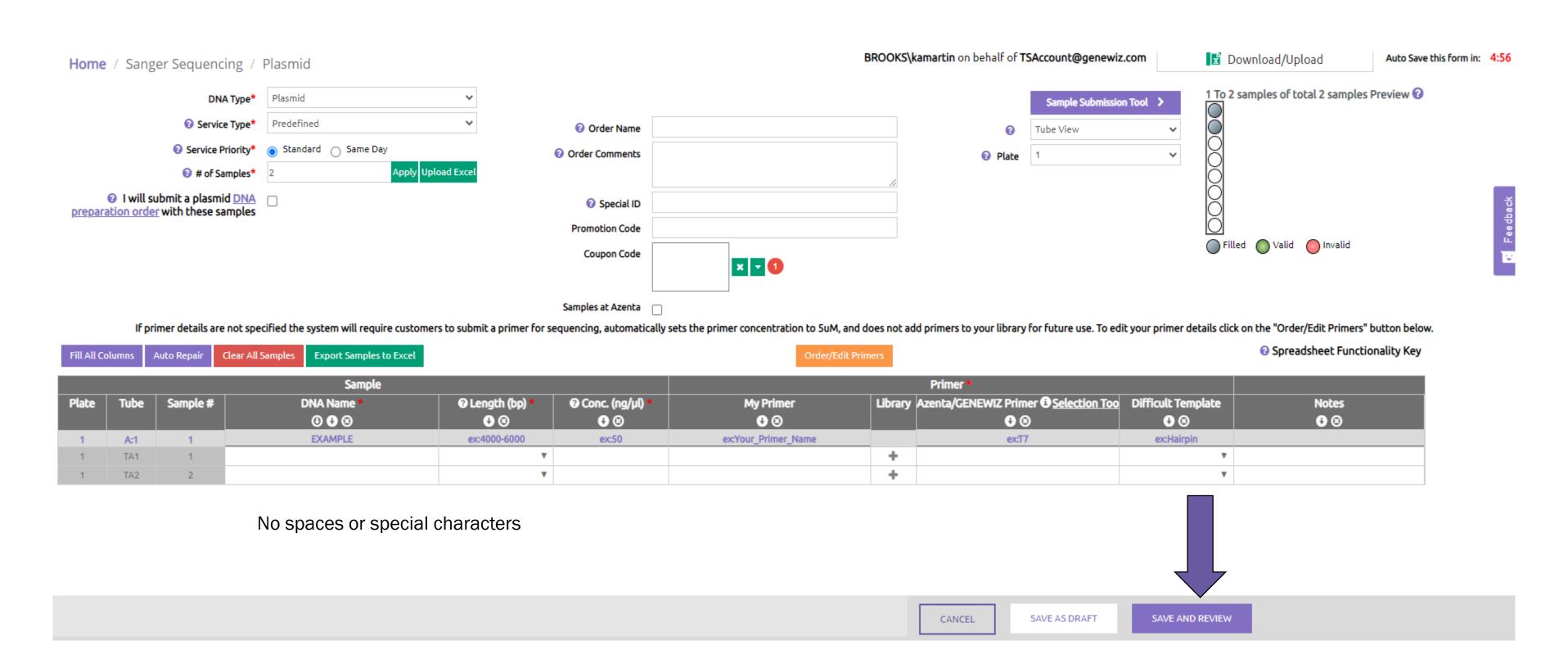
## Creating an Order





## Complete Form → Save and Review

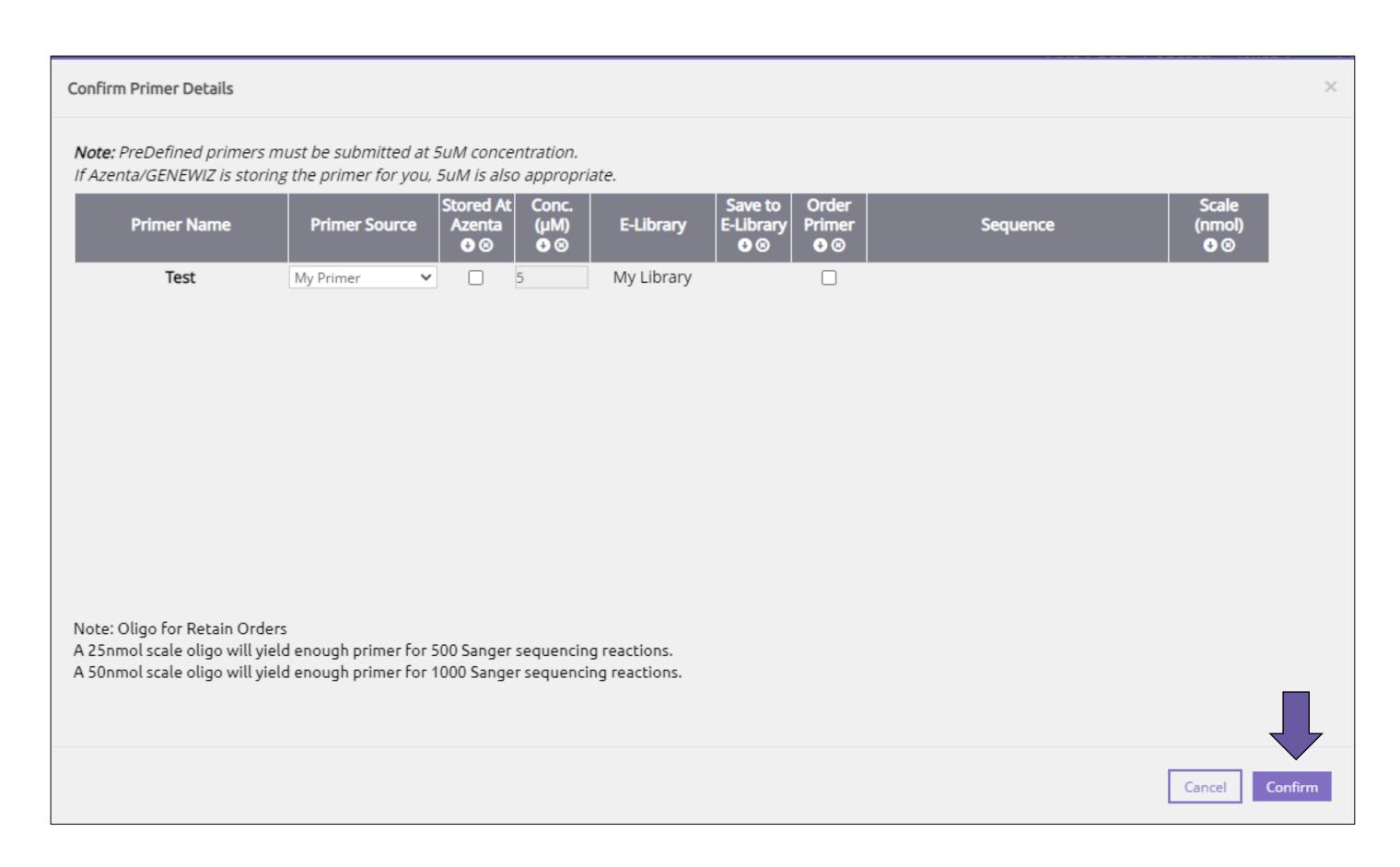




Use "NC-COUPON100" promo code \$100 discount - first order only!

#### Confirm Primer Details

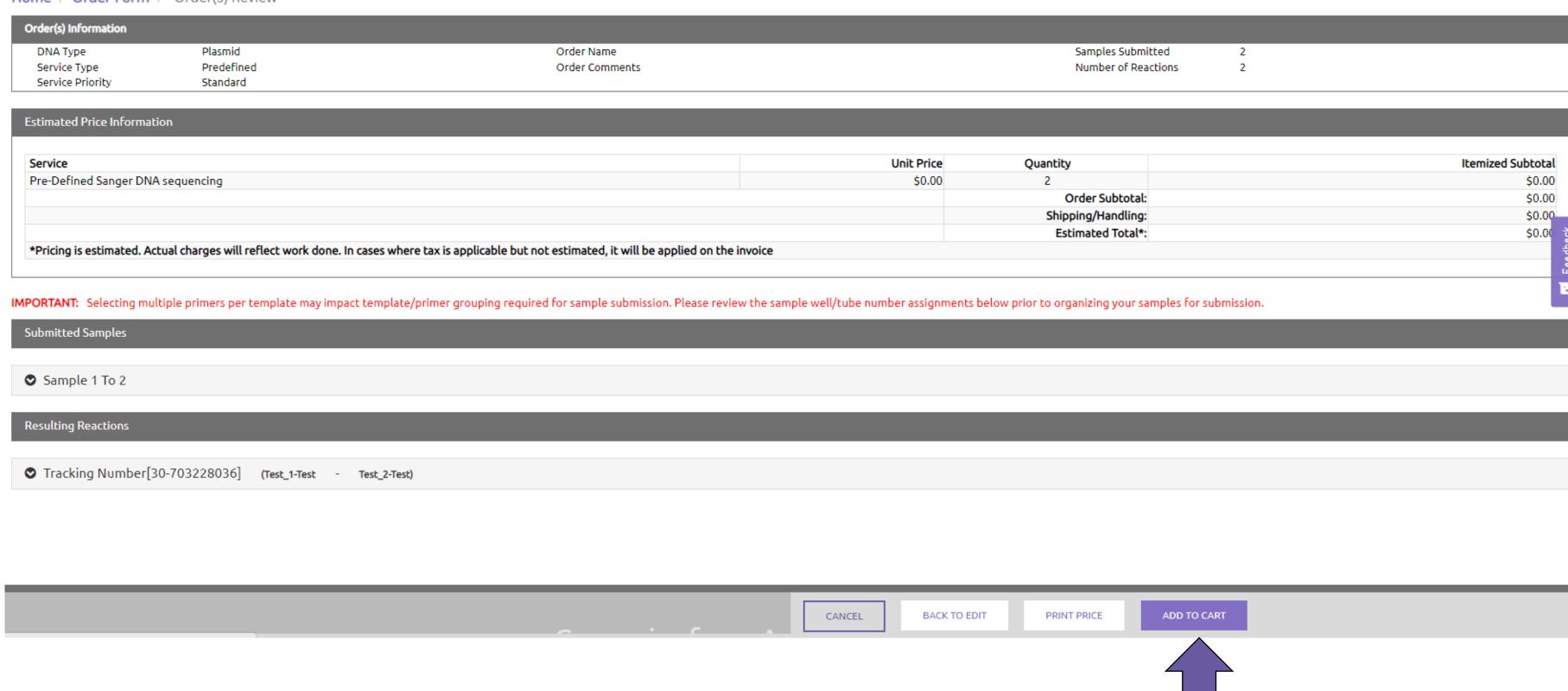




#### Add Order to Cart

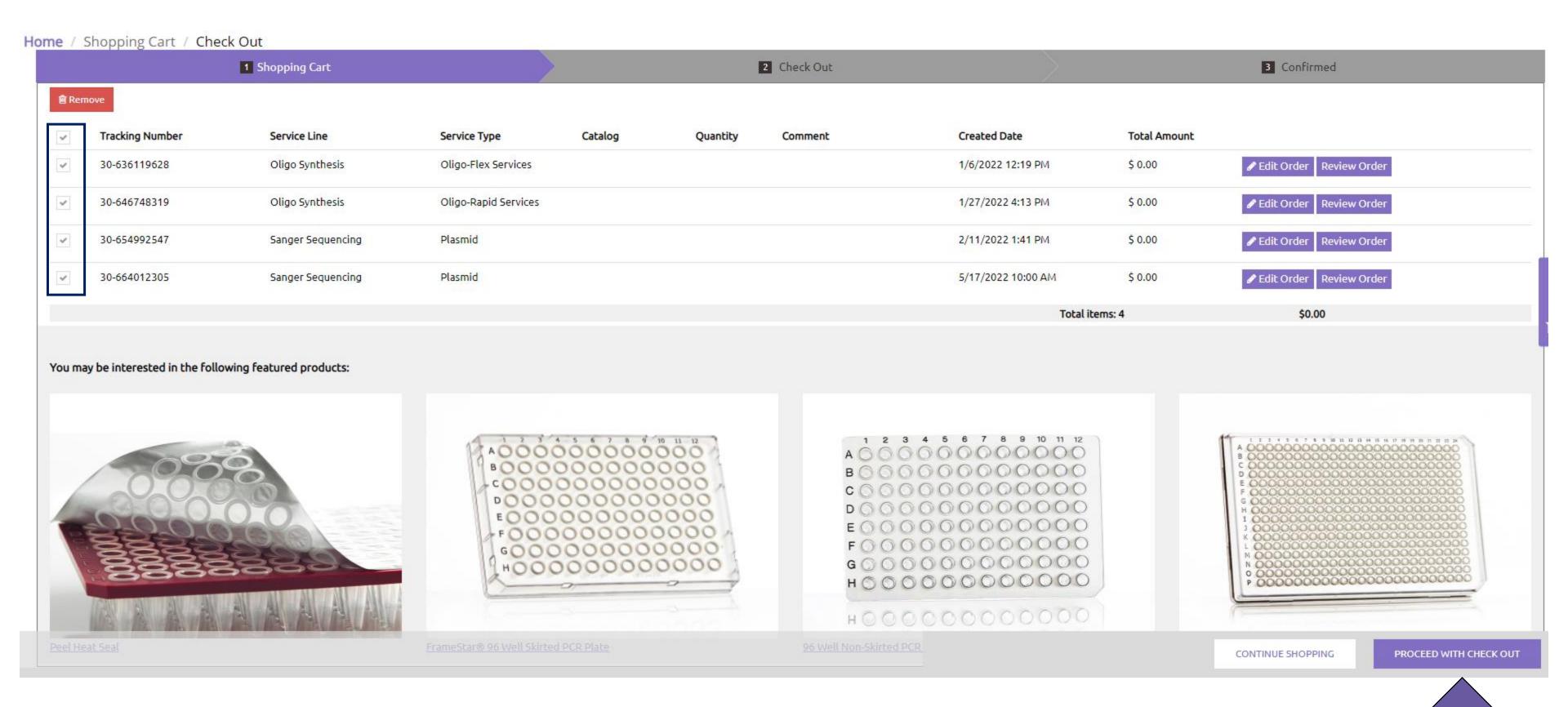


Home / Order Form / Order(s) Review



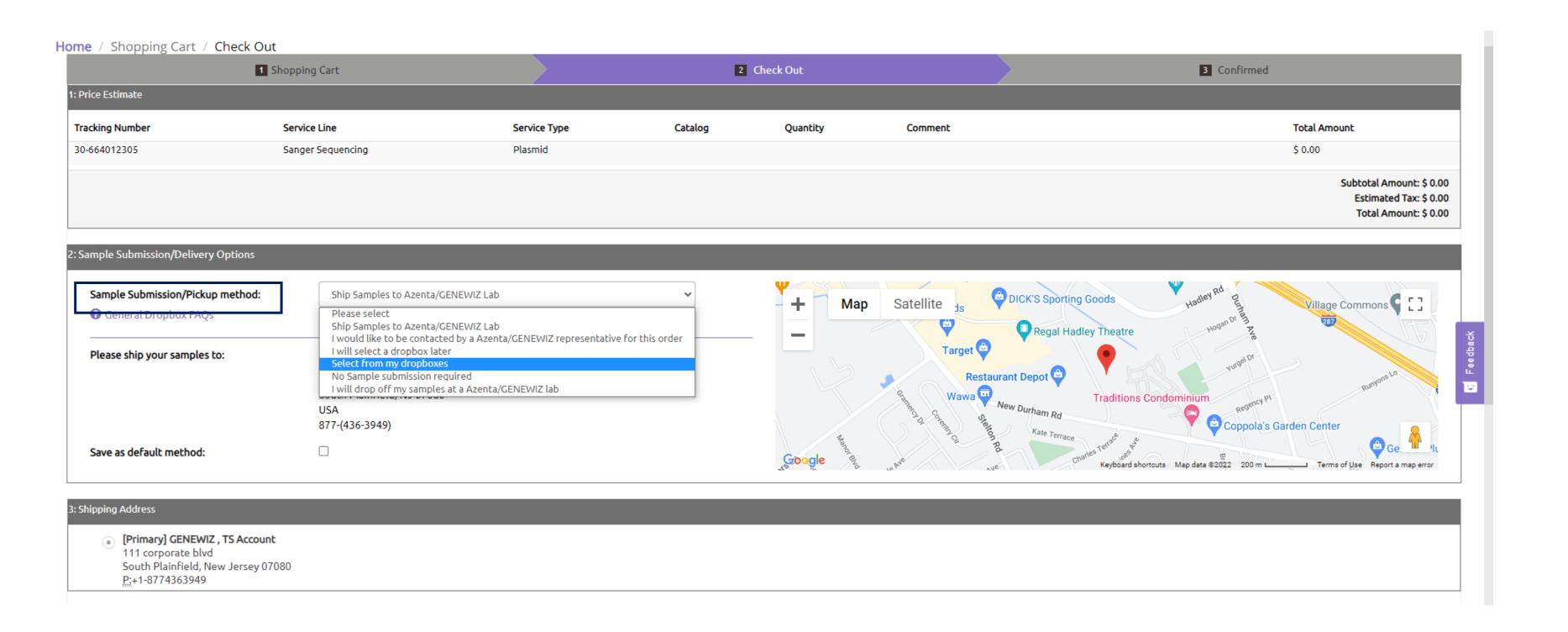
#### Proceed with Checkout





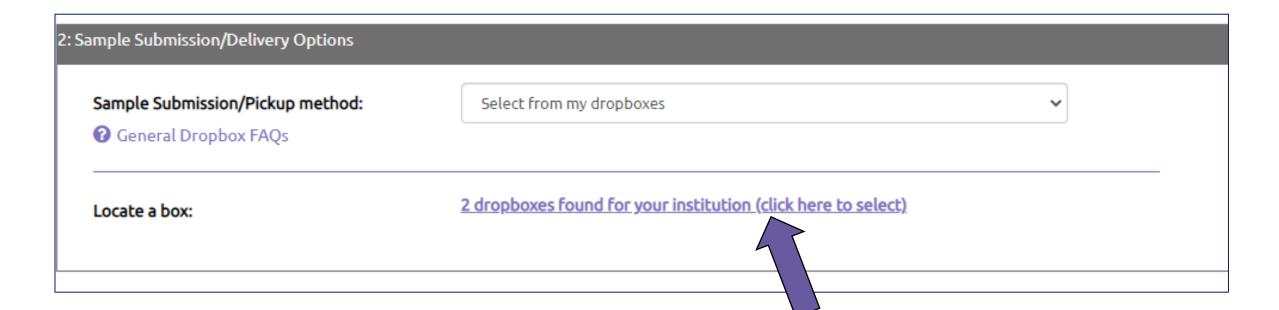
# Select Sample Submission Method

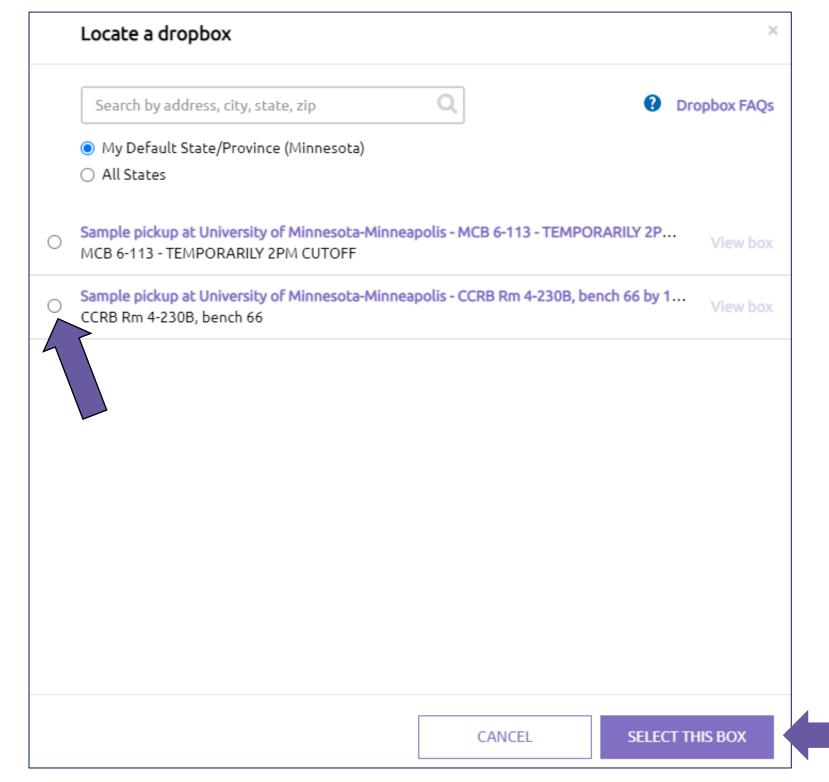




# Azenta Drop-box Selection







## **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

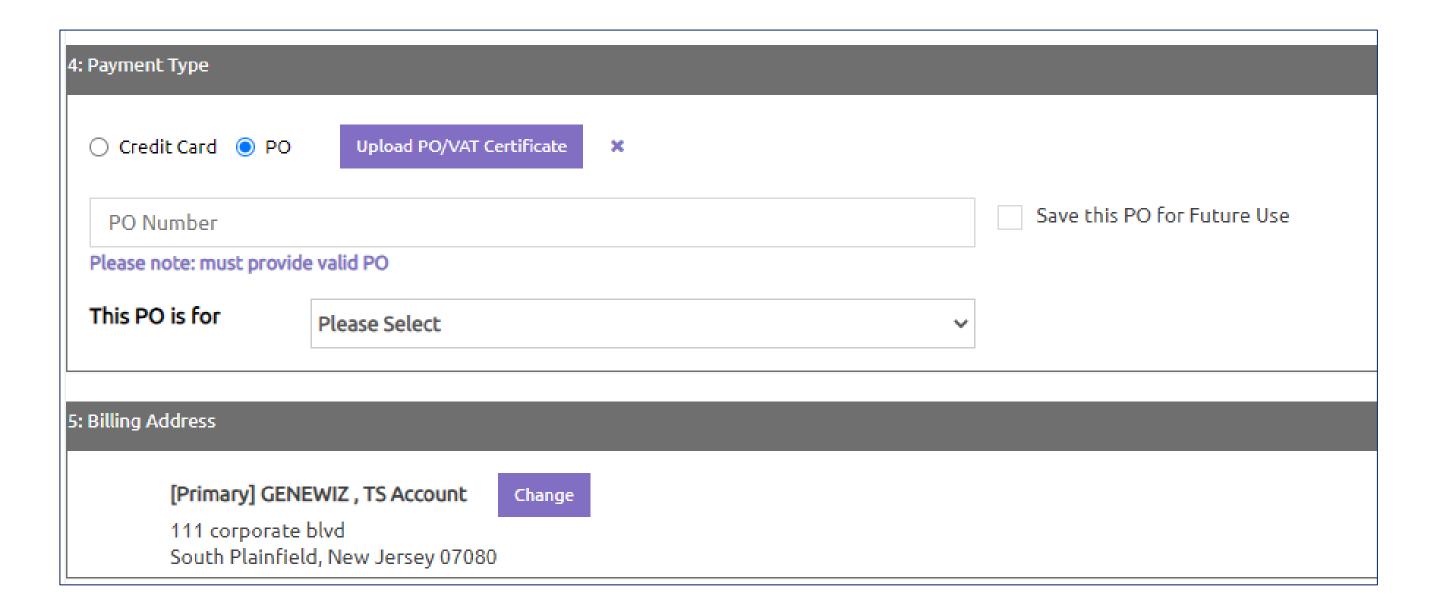
• Gonda Bldg 19th Fl, outside of Room 306: 4:00PM

• Stabile 13-21 Genome Analysis Core: 4:00PM

https://www.mayo.edu/research/core-resources/genome-analysis-core/services/sequencing

## Select & Enter Payment Information

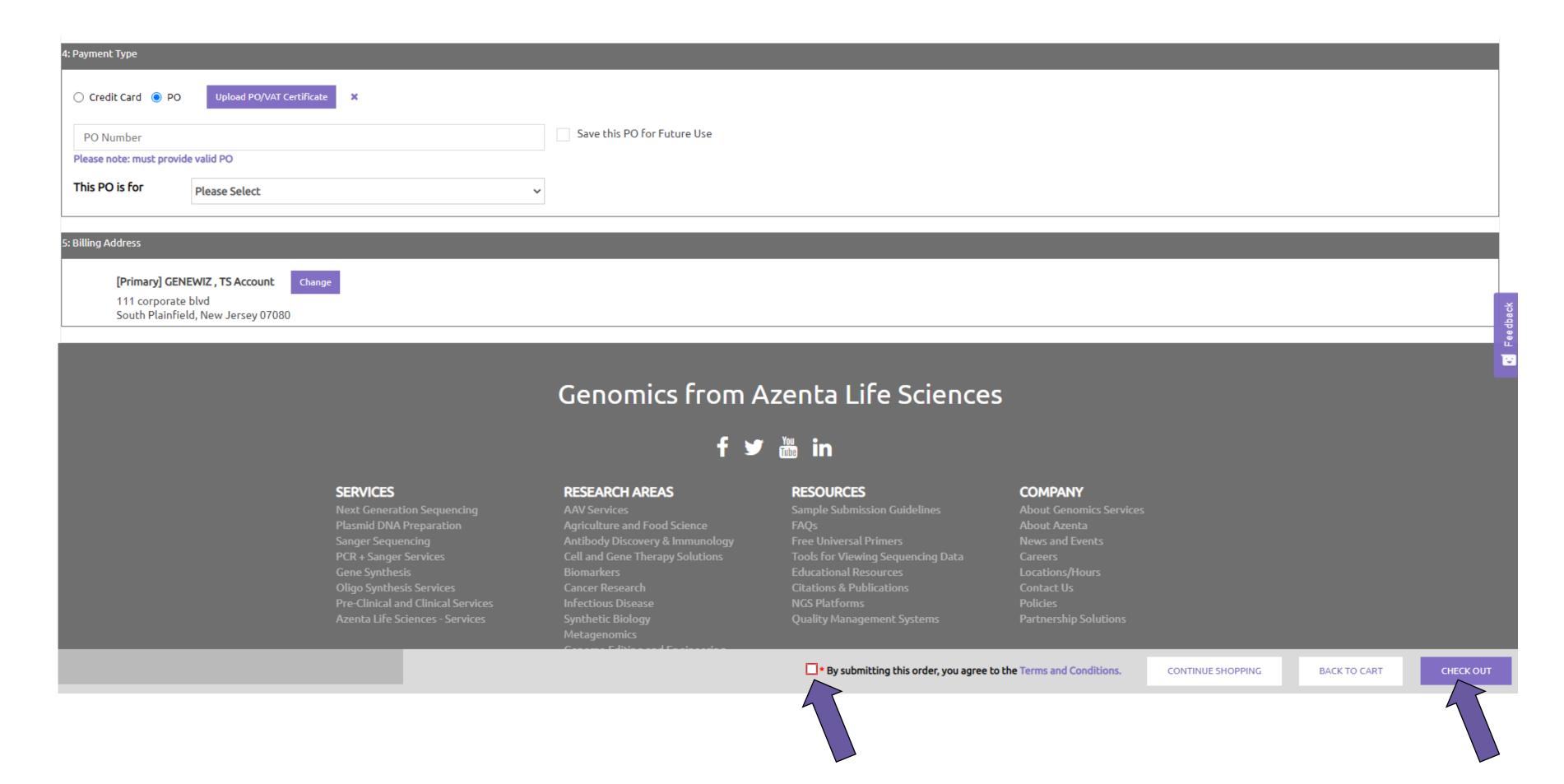




- Purchase Order#:
  - Invoice created with 1 week of order completion
  - Emailed to billing email addresses provided in account profile
- Credit Card charged on order completion

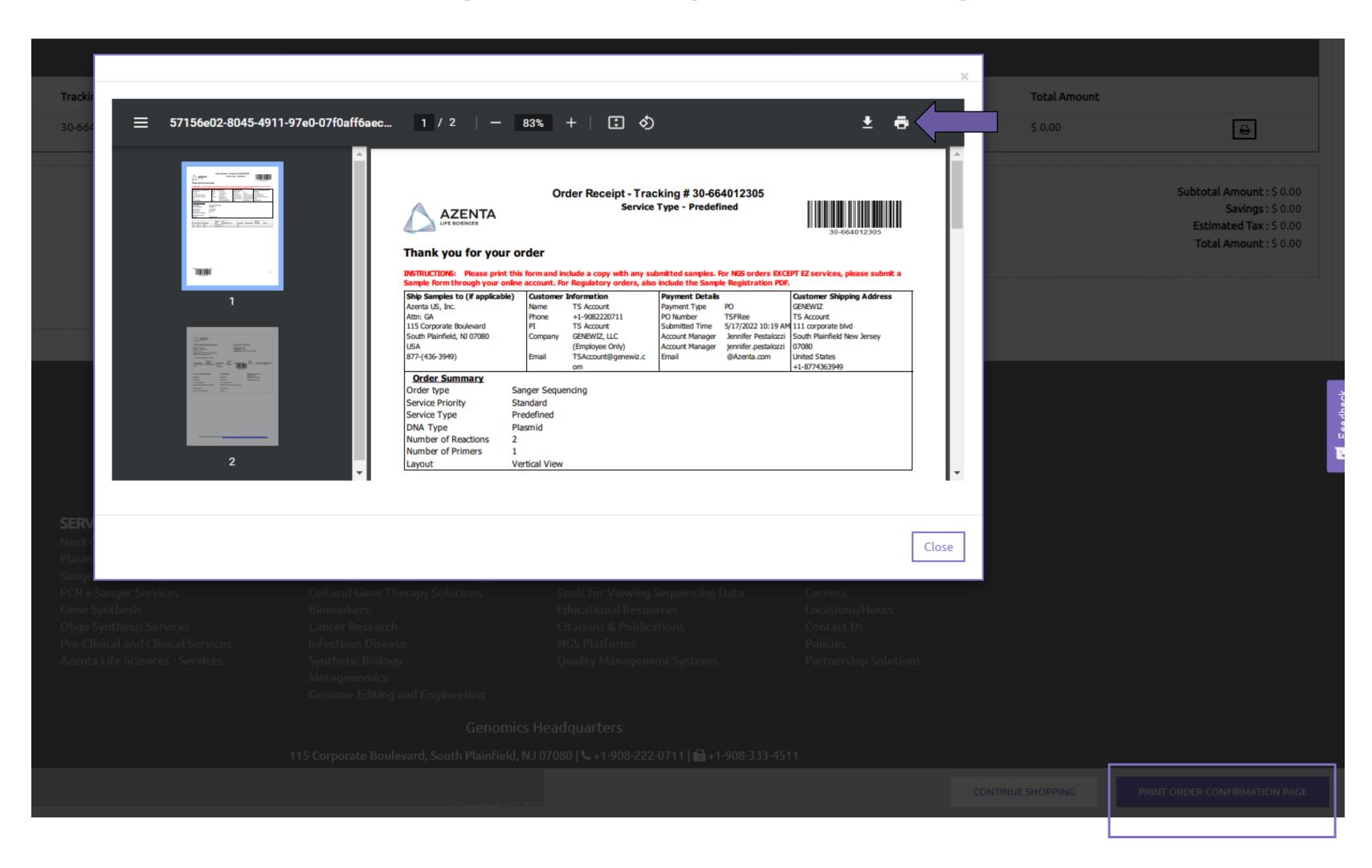
## Agree to Terms and Conditions and Complete Checkout





# Print Order Receipt to include with samples & deposit in drop-box





### **Tubes or Plates?**



- < 48 samples tubes</li>
  - 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. TAO1, TAO2, etc.

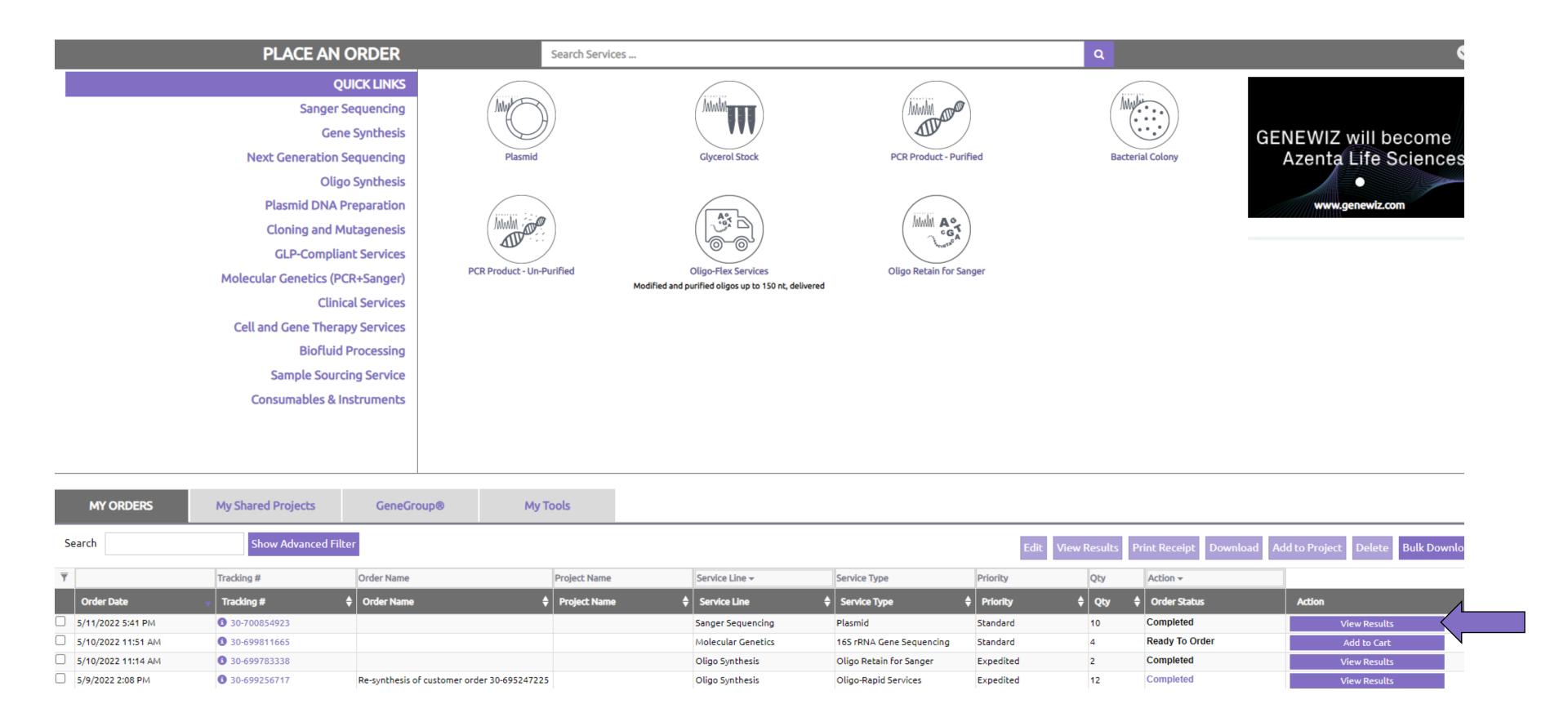
Reaction	Well	#	DNA Name	Length (bp)	Conc. (ng/µl)	My Primer	GW Primer
TA01	A:1	1	A_1	6001-8000		AmpR239 [5μM]	
TA02	B:1	2	A_2	6001-8000		AmpR239 [5μM]	
TA03	C:1	3	A_3	6001-8000		AmpR239 [5μM]	
TA04	D:1	4	A_4	6001-8000		AmpR239 [5μM]	
TA05	E:1	5	A_5	6001-8000		AmpR239 [5µM]	
TA06	F:1	6	A_6	6001-8000		AmpR239 [5μM]	
TA07	G:1	7	A_7	6001-8000		AmpR239 [5μM]	
TA08	H:1	8	A_8	6001-8000		AmpR239 [5µM]	



# VIEW RESULTS & TROUBLESHOOTING

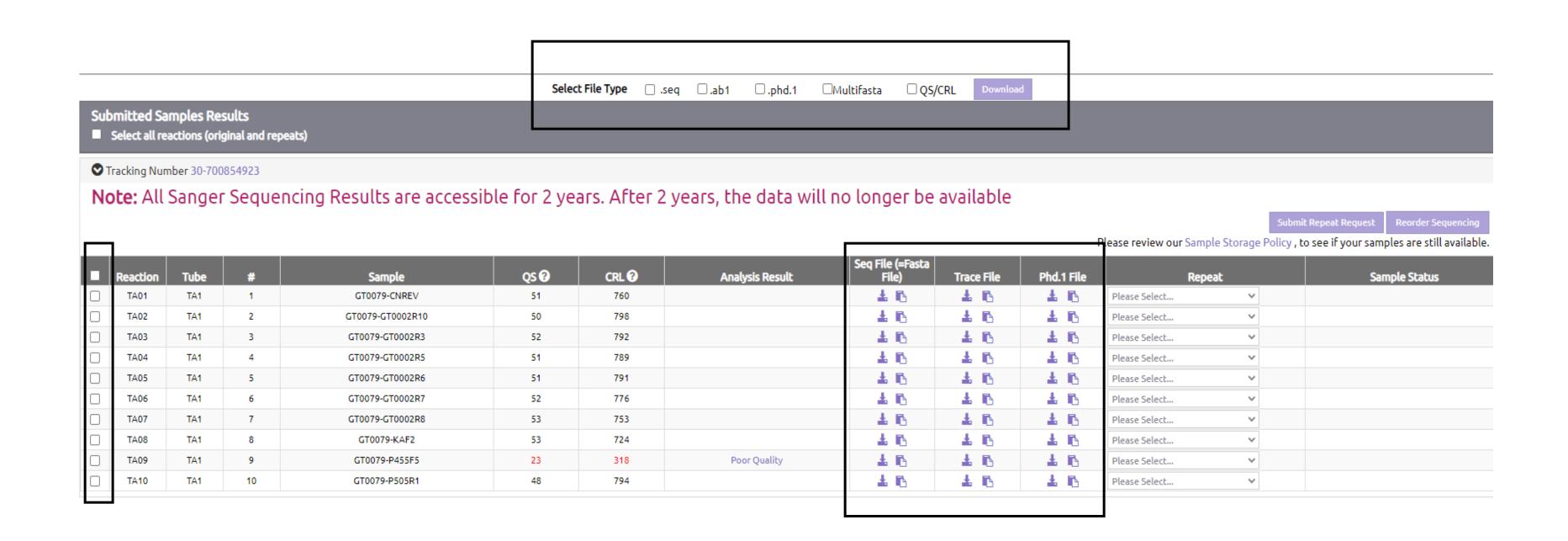
## Login to Account to Review Results





### View or Download Results





## Common Result File Types



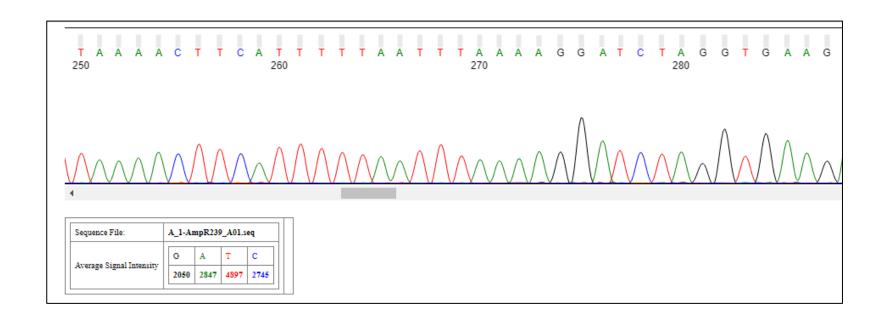
#### **DOWNLOAD OR VIEW:**

#### Seq

>A\_1-AmpR239\_A01.ab1

NNNNNNNNNNNNNNNCNNGNCNTCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATT
GCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGAGGGAGTCAGGCAACTATGGATGAACG
AAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAGACCAAGTTTACTCATATATACTTT

#### Trace

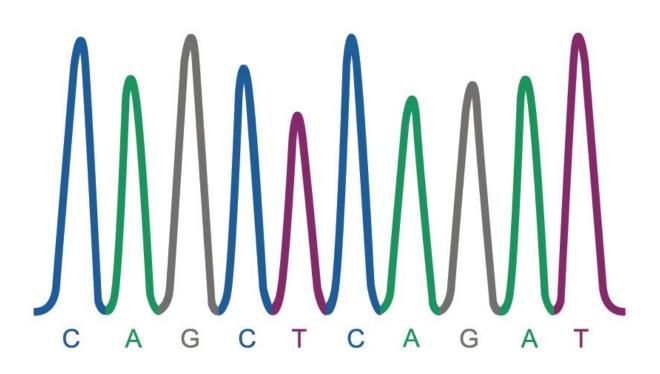


## **Quality Control**



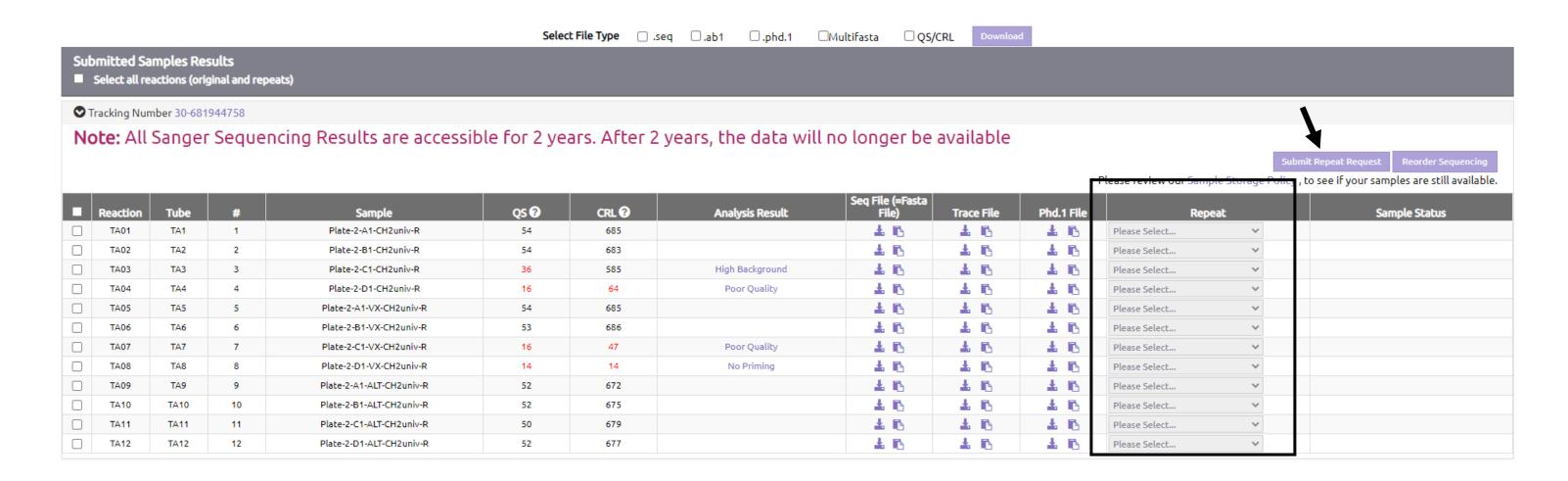
#### TO PASS OUR QUALITY CONTROL:

- 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
  - 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

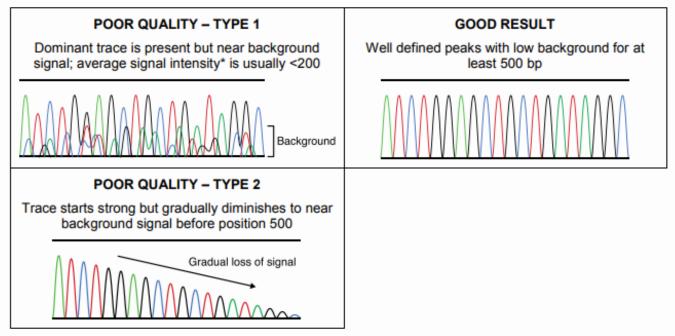


QS 🚱	CRL ?	Analysis Result
54	685	
54	683	
36	585	High Background
16	64	Poor Quality
54	685	
53	686	
16	47	Poor Quality
14	14	No Priming



#### 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.



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

#### Troubleshooting

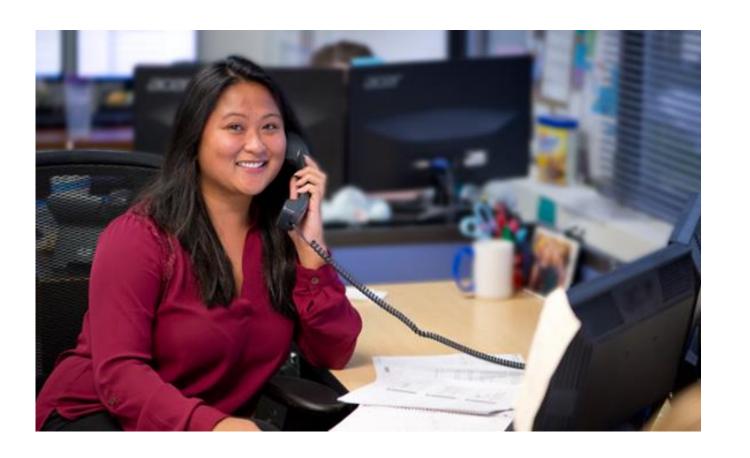
There are several possible causes for a poor quality result. Select your DNA type below to jump to the appropriate section in the guide. The list of potential causes is not exhaustive but represents the most common issues. For more assistance, please contact Technical Support at 877-436-3949 ext. 2 or dnaseq@genewiz.com.

DN	IA Type	Page	
1.	Plasmids	2	
2.	Purified PCR Products	4	
3.	Unpurified PCR Products	7	

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



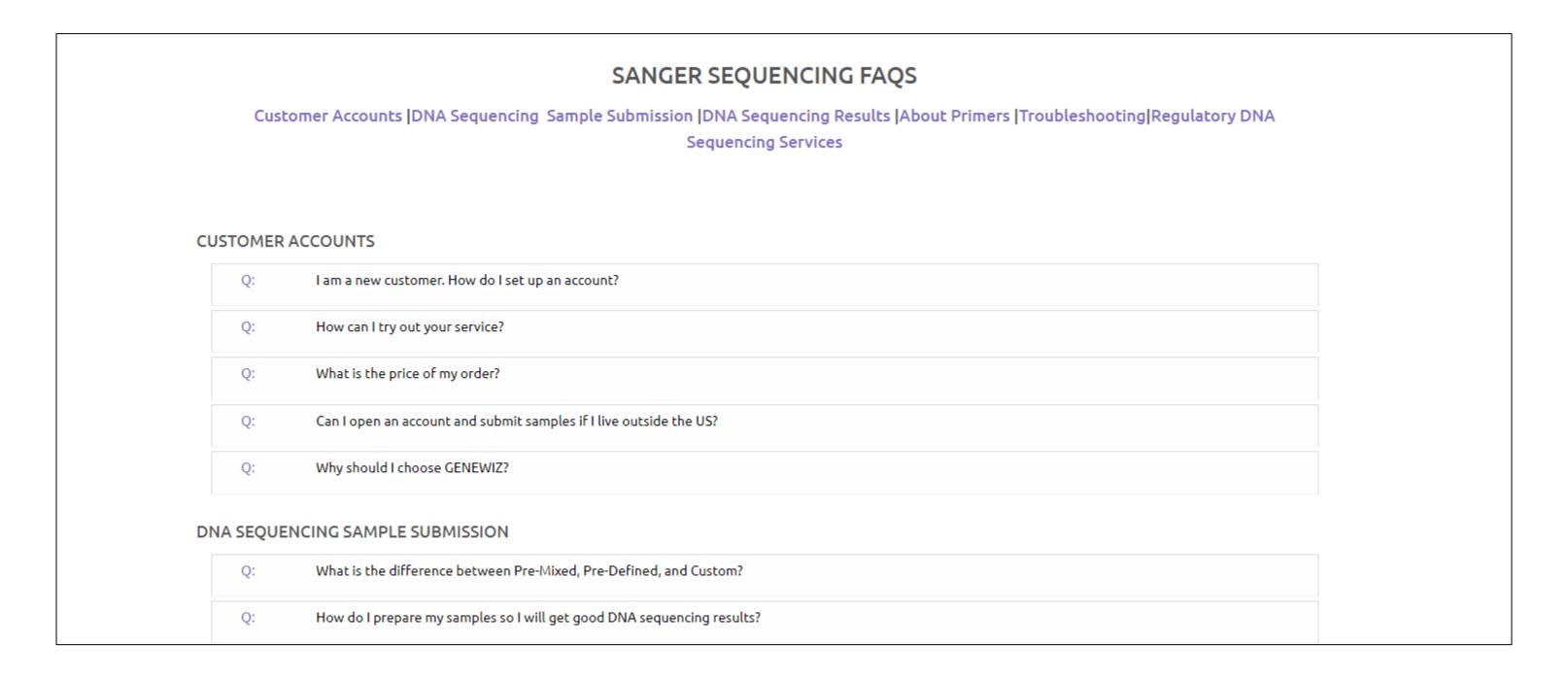
- Personalized Ph.D. level customer support
  - +1-877-GENEWIZ (436-3949) ext. 2
  - Email: DNAseq@azenta.com
  - Live Chat
- In-depth troubleshooting at no extra cost
- Free & ½ price repeats
- 5 day sample storage or longer on request



## Sanger FAQ on Website







## Azenta Oligo Services



#### **OLIGO RETAIN FOR SANGER**

Bundled with Sanger and Prep Services

#### **OLIGO-RAPID SERVICES**

- Standard, unmodified oligos for 15 60mer lengths, 2<sup>nd</sup> 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

