ACGT, INC.

ACGT, Inc. DNA Sequencing

Northwestern University

May 15, 2019

ACGT, INC.

Meet your Presenters

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  Assistant Manager, DNA Sequencing
- Manan Chandra, M.S.
  Accounts Manager

ACGT, INC.

Who Are We?

ACGT, Inc. is a Contract Research Organization that provides a broad range of DNA sequencing and molecular biology services to the pharmaceutical, biotechnology, academic, clinical, and government sectors.

ACGT, INC.

ACGT, Inc. History & Milestones

- **1993** - Started DNA Sequencing Service
- **1999** - Launched Contract Genomic Services
- **2004** - Moved to Present Facility
- **2005** - Launched GLP-level Services
- **2010** - Launched Next Generation Sequencing Services
- **2011** - Launched ID Services
- **2012** - CLIA Registration
- **2012** - Opened Branch Facility in Germantown, MD to serve NIH Research Community
- **2013** - CAP and CLIA Accreditation
- **2013** - Molecular Diagnostic Services
- **2019** - Working toward being cGMP-compliant

ACGT, INC.

Credentials

- GLP Compliant (21 CFR 58) since 2004
- US EPA GLP Compliant (40 CFR 160)
- BSL-2 Compliant
- Compliant with HIPAA Regulations
- Controlled Substance DEA License
- CLIA Certified
- CAP Accredited
- Women/Small Minority Owned Business

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Partnering with Sequencing Core

COLLABORATING WITH SEQUENCING CORES

ACGT is now a dedicated provider to the Northwestern University Sequencing Core at the Feinberg School of Medicine.

ACGT, Inc. ▪ 35 Waltz Drive, Wheeling, Illinois 60090 ▪ Toll Free 800.557.ACGT (2248) ▪ Fax 847.520.9163 ▪ www.acgtinc.com

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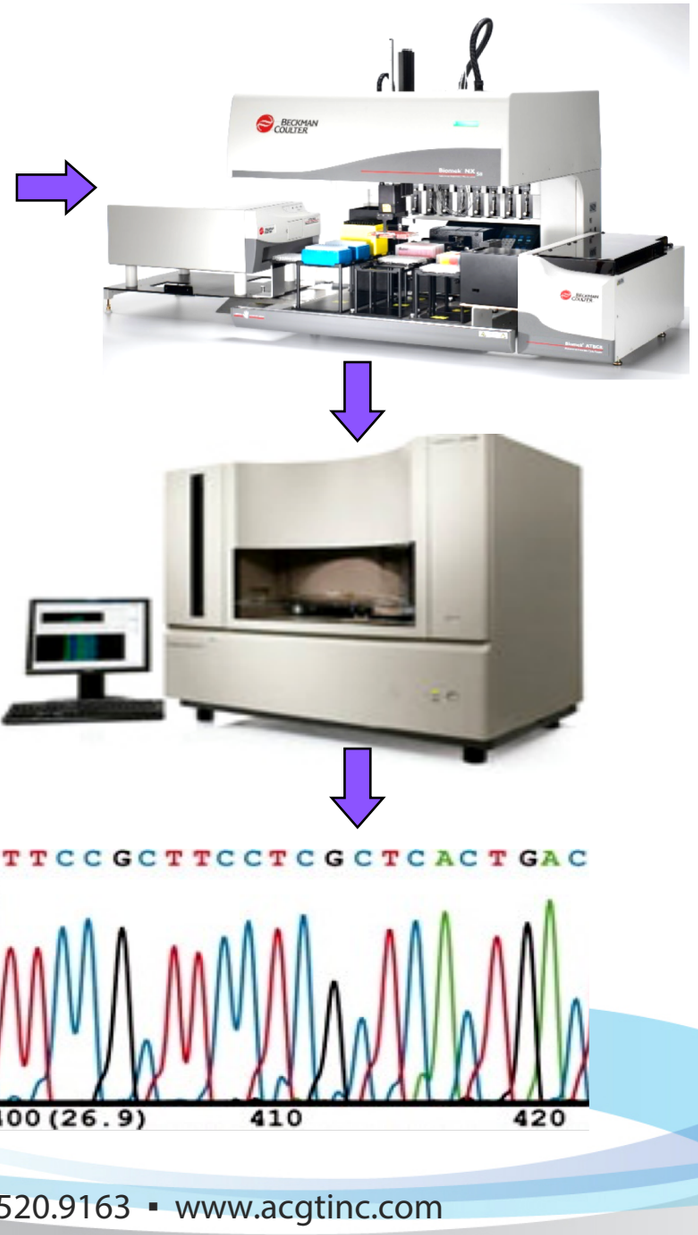
Sanger DNA Sequencing

- Two 3730XL DNA Analyzers
- The most advanced BigDye Terminator V3.1 chemistry
- Proprietary CleanSEQ sample clean-up for superior qual
- All critical Sanger DNA Sequencing equipment is certified for GLP projects and clinical analysis
- Data review of each sample
- Extensive troubleshooting for problematic samples
- A significant portion of our workflow is automated using the biomek NXP automation station.
- Capacity of 3450 reactions/ day.



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ACGT, Inc. Services Based On Sanger Sequencing

- Quick Single Pass in a Single Tube or 96-well Plate Format
- Full Sequence Analysis
- PCR and Sequencing
- Direct Colony Sequencing
- Load-and-go Sequencing

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Highlights

- Generate 750 – 1000 bp of clean data
- 92-96% success rate on first run
- Free primer banking
- Free universal primer
- Free shipping via ACGT drop-box
- Dedicated technical support staff
- All types of samples/ tubes accepted

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Levels of Service

- LCO (Low Cost Option) – Sample and primer are pre-mixed in a single tube.
- Standard Service – Ideal for samples with known concentration. We accept Plasmids and purified PCR products for this service. Samples and primers are submitted in separate tubes.
- Premium Service – Comprehensive service for samples of unknown concentration. Un-purified PCR products may be column purified at an extra charge.

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Common Reasons for failure of Sequencing Reactions

- DNA quality and quantity
- Primer Binding
- Salt contamination
- Excessive Signal Intensity
- Mixed Sequences
- Difficult (Hard to sequence) Templates

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Sample Submission Guideline

	Template DNA type	Size of DNA	Template Concentration (*ng /ul*)	Volume of Template per reaction	Primer concentration per ul	Volume of Primer per reaction	Total Reaction volume
LCO service	Plasmid	up to 10kb	20	10ul	10pMol/ulOr33-66ng/uL	2ul	12ul
	PCR DNA	100-200bps	0.2-0.6				
		200-500bps	0.6-1				
		500bps-1kb	1-2				
		1kb-2kb	2-4				
		Over 2kb	4-10				

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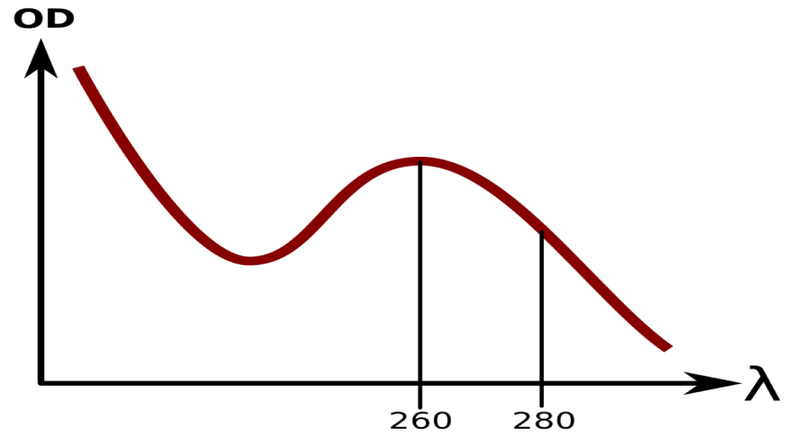
Sample Submission Guideline

	Template DNA type	Size of DNA	Template Concentration (ng/ul)	Volume of Template to send per reaction	Primer concentration (pmol/uL) or in (ng/uL)	Volume of primer to send per reaction
Premium/Standard service	Plasmid	up to 10kb	25-50	10ul	10 pmol/ulOr33-66 ng/uL	7ul
	PCR DNA	100-200bps	1-3			
		200-500bps	3-5			
		500bps-1kb	5-10			
		1kb-2kb	10-20			
		Over 2kb	20-50			
	Large DNA *	Over 10kb	100-200			

*(e.g, BACs, PACs, YACs, Cosmids, and fosmids)

Sample Submission Guideline

- Nanodrop 260/280 value should be 1.8
- Nanodrop 260/230 value should be 2.0
- After purification, the DNA should be eluted in de-ionized water instead of elution buffer. Buffer components have a tendency to inhibit the sequencing reaction and result in failed runs
- If a template must be analyzed in both directions, pre-mixed samples of the template with forward and reverse primers in separate tubes must be submitted



Unusable data/ No signals

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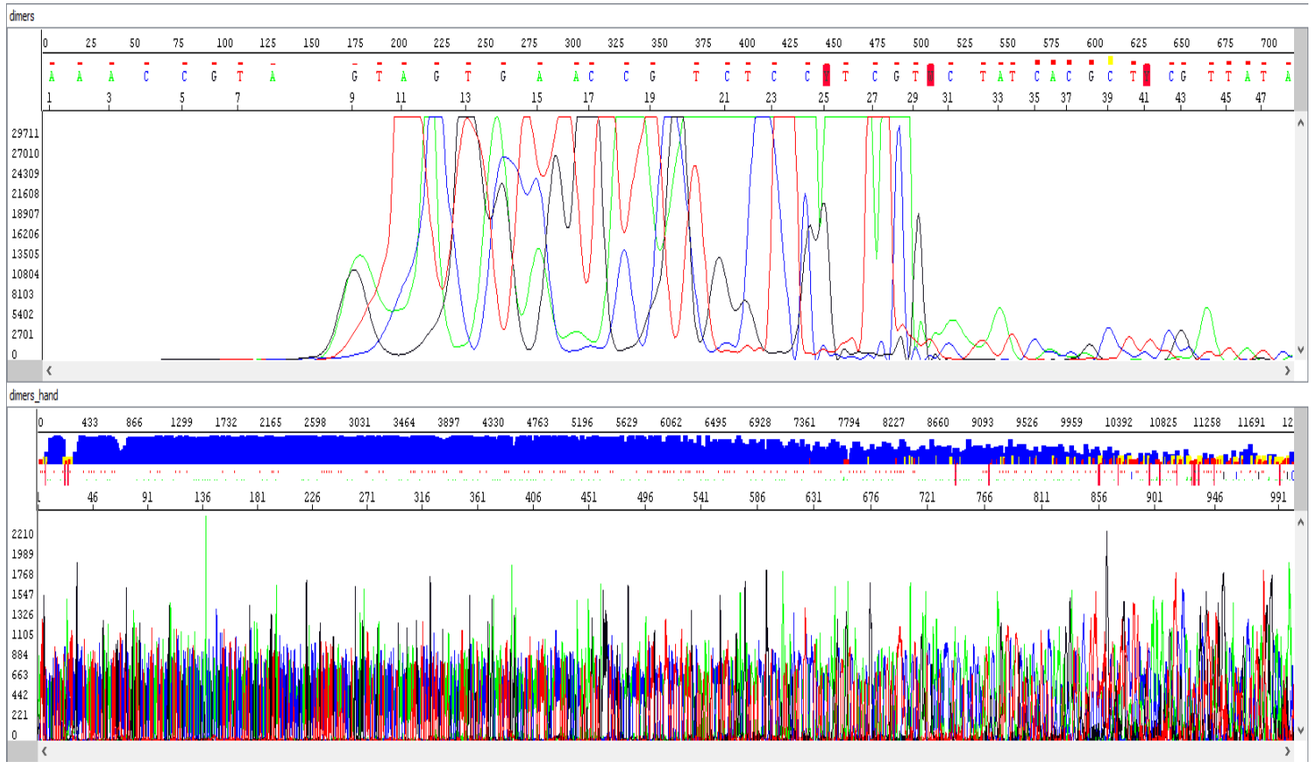
- Cause 1: Problems in primer binding. Sometimes due to PCR amplification or cloning, there may be an induced mutation, deletion or insertion in the primer binding site.
- Solution: Please check if the theoretical sequence of the construct does include the binding site for the primer being used. If not, repeat the sequence with a different primer.



- Cause 2: DNA quantity may be too low for sequencing. Products may be lost during clean-up.
- Solution: Check DNA concentration on a nanodrop or spectrophotometer. If the quantity is too low, re-prep DNA

- Cause 3: Thermal cycler conditions.
- Solution: Use correct thermal cycler parameters and regularly calibrate the thermal cycler.

Noisy data/ Weak signals

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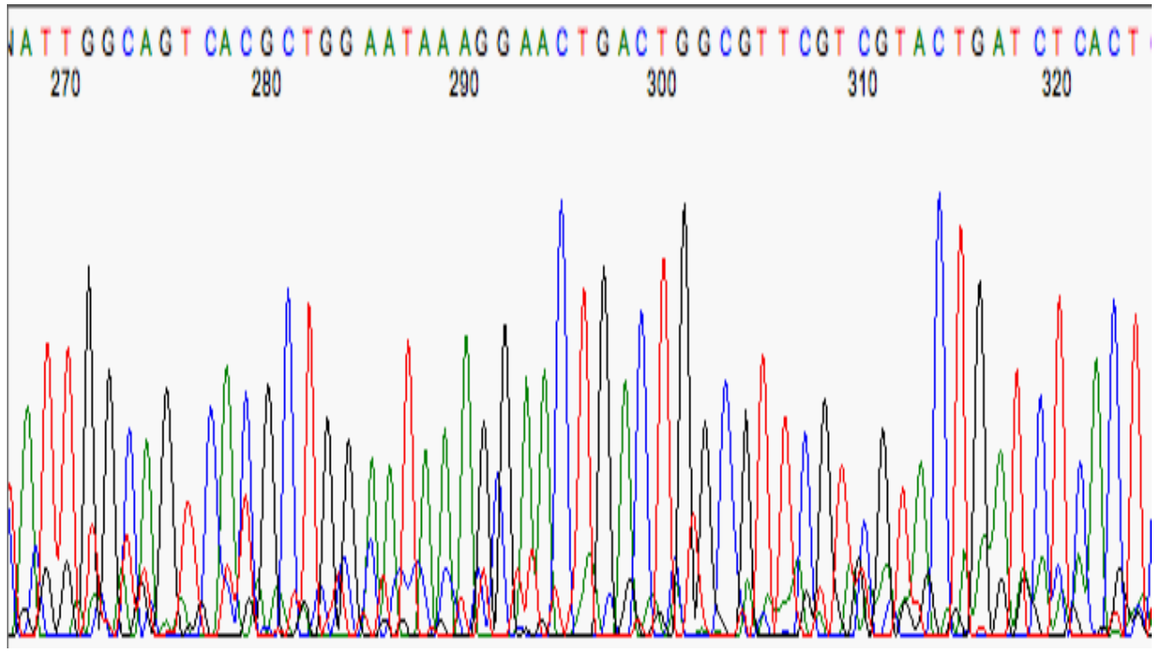
- Cause 1: Salt contamination of DNA results in poor read length with signals diminishing very early.
- Solution: Check for salt and/or protein contamination via a nanodrop or spectrophotometer. Avoid using an elution buffer for DNA clean-up.

- Cause 2: A low Primer Melting Temperature may also result in sub-optimal signals.
- Solution: Ensure that the Tm of your primer is not out of range.

- Cause 3: Nested PCR, i.e. same primer used for amplification and sequencing.
- Solution: Gel purification may result in cleaner reads.



Mixed Sequence

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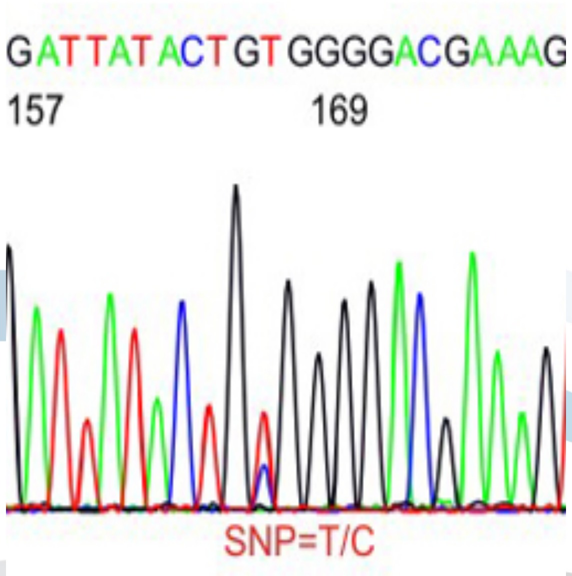
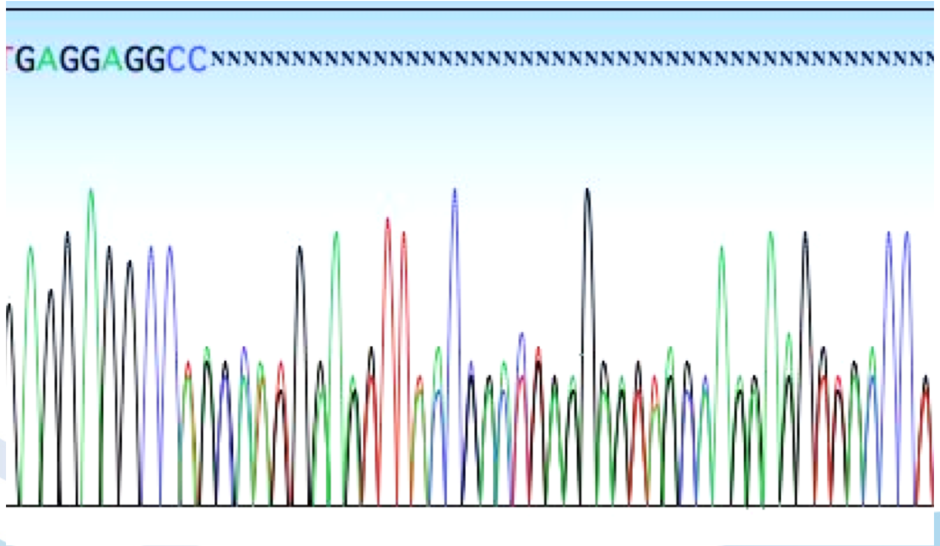
Sequencing is initiated from a specific primer/target interaction, therefore:

1. Only one target per sample, must be present in sufficient quantity in the sample
2. Only a single target sequence in the sample should result from primer binding





Radomized (unreadable) sequence caused by several different templates present in one reaction

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- Whole Mixed Sequence: Usually due to an unspecific primer binding site.
- Solution: The sequencing reaction should be repeated with an alternative primer. Please check the specificity of the primer (i.e. binding site is present only once.

- Mixed Sequence up to a certain region: Usually occurs when more than one PCR product is present in the sample. The mixed sequence is seen up to the end of the shorter PCR product. This may also be the result of the presence of multiple clones in case of plasmids.
- Solution: Check the PCR amplification conditions to be sure to receive only one single amplification product. Verify via agarose gel purification.

High Intensity Peaks

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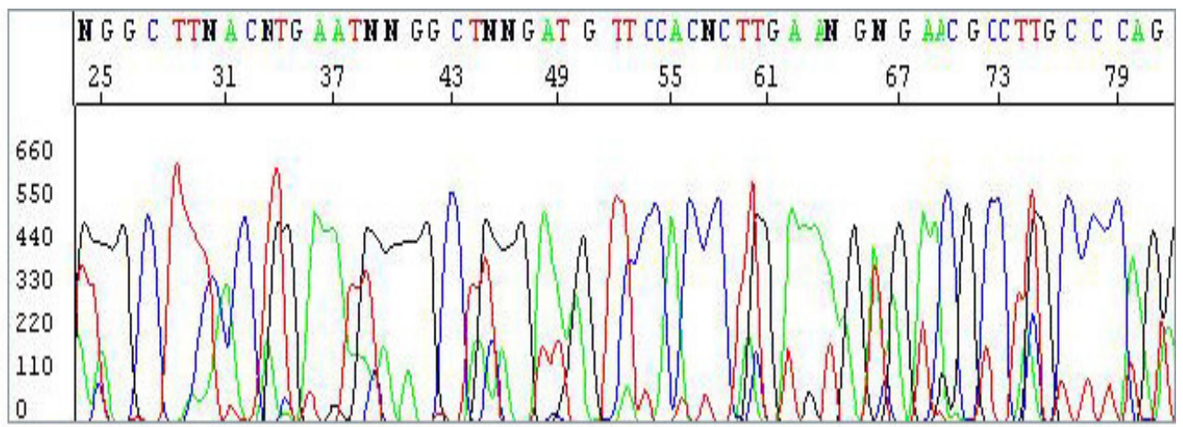
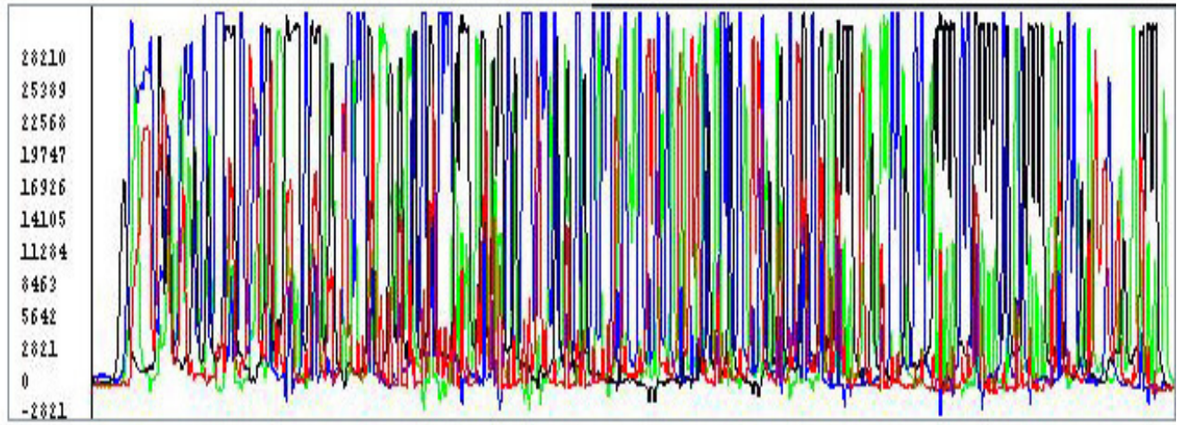
- High intensity peaks are typically the result of too much DNA template present in the sample.
- More common in PCR products of short length.



- Solution: Check DNA concentration of your samples. The optimal way to quantify the DNA is via agarose gel electrophoresis with quantitatively well-defined bands to be able to compare with the target. If possible, try to generate PCR products >200 bp.



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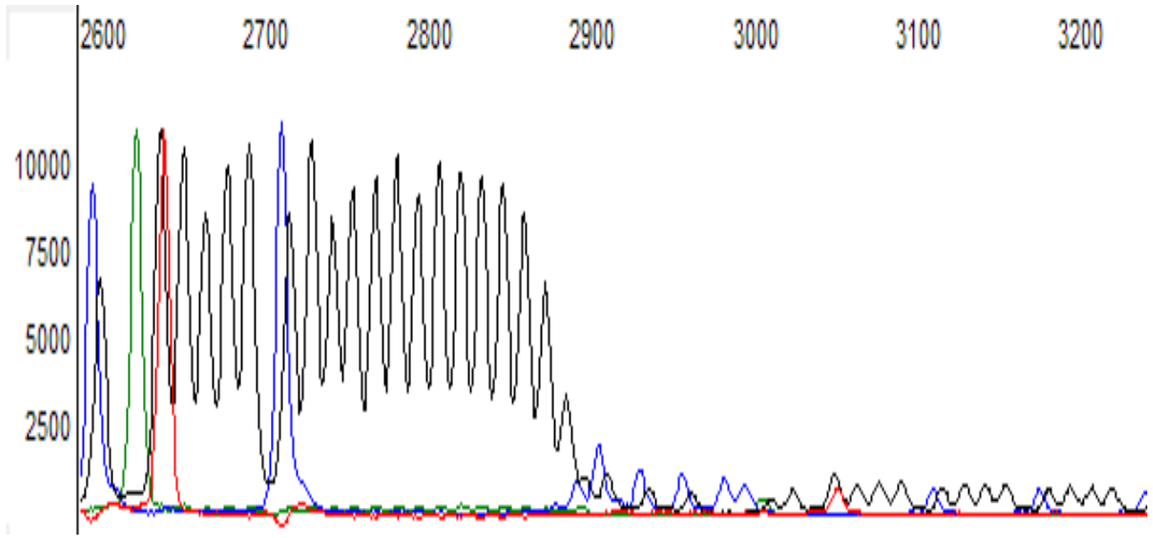
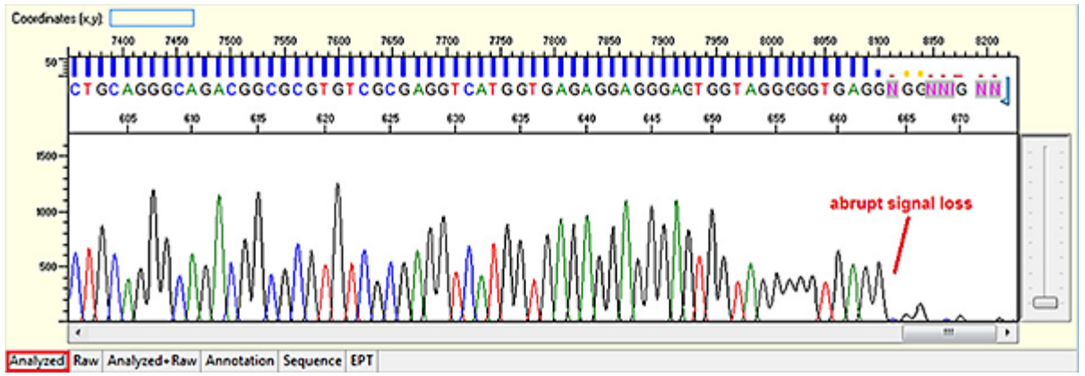
Hard to Sequence Samples

- High GC Content
- DNA with repetitive sequences
- Templates with a tendency to form secondary structures
- Plasmid cloned in a gateway vector
- Solution: Sequencing the template from the opposite direction or designing a primer to bind downstream of the secondary structure





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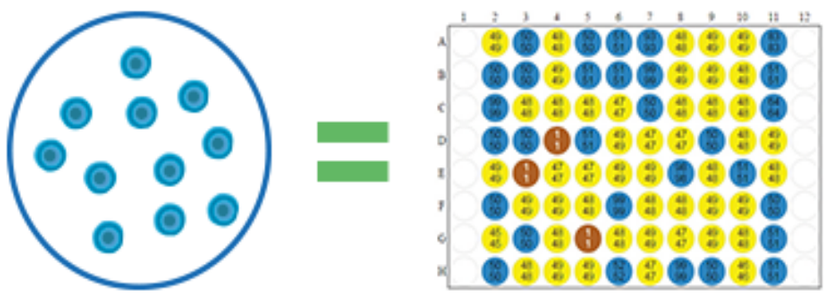
Direct Colony Sequencing

- We accept bacterial colonies re-suspended in buffer with the primer sent separately.
- DNA is extracted and amplified directly from the colony to generate the sequence data
- Works on plasmids, BACs, cosmids and phages.
- We will retain original bacterial sample tubes for two weeks

SAMPLE TO ANSWER



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Load-and-go Service

- We accept pre-performed Big Dye sequencing reactions (clean or un-clean) for loading onto the 3730 machine.
- Samples may be submitted in a liquid or dried format.

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Primer Synthesis, Design and Banking

- Primers or templates can be banked at our facility for subsequent orders at no extra charge.
- Select the option on the online order form.
- Can be viewed on your ACGT account.
- We also offer a primer design and synthesis service. For primer design, we would require a reference (target) sequence.

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PCR & Sequencing

- This service is for PCR and sequencing of Genomic DNA targets.
- Designed to detect sequence variants in short genomic DNA regions (from 300 bp to 2 kb).
- Publication quality sequence data and variant analysis.
- Pricing is project specific.
- Results will be available in 2-3 business days from sample receipt (excluding time needed for primer synthesis).

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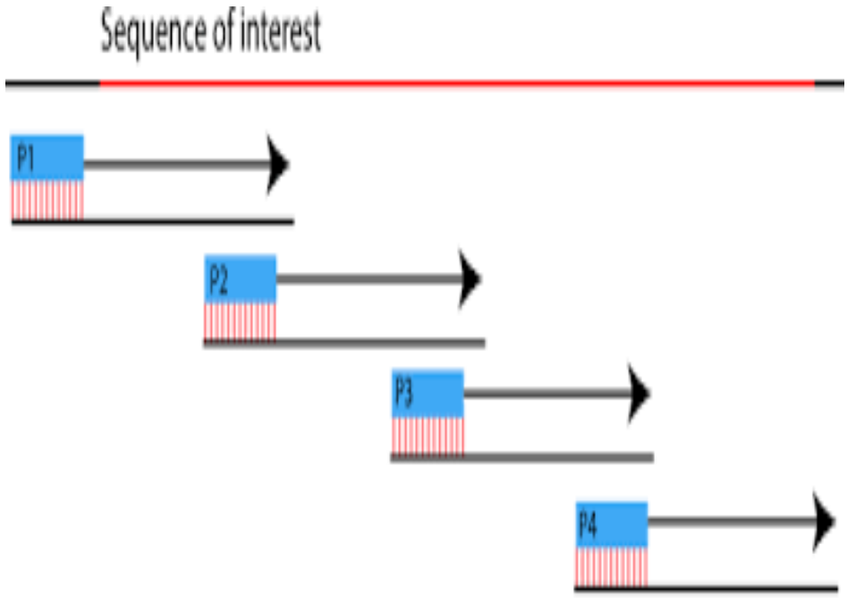
Full Sequence Analysis (FSA)

- We offer both ss-FSA as well as ds-FSA.
- Design and synthesis of internal primers
- Consensus sequence determination
- Variant analysis report in comparison with reference sequence



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