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Sanger Sequencing

Deborah Grove, Ph.D.
Director for Genetic Analysis
Genomics Core Facility
Huck Institutes of the Life Sciences

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DNA Sequencing

- Chemical Sequencing by Maxam and Gilbert in the early 1970s-laborious, 24 bases
- Frederick Sanger in 1975 –Dideoxyterminator Chemistry
- Sanger and Gilbert received ½ of the Nobel Prize in 1980. Do you know who got the other half?

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DNA Sequencing

Factoid of the day: The person who this auditorium is named after. Paul Berg.

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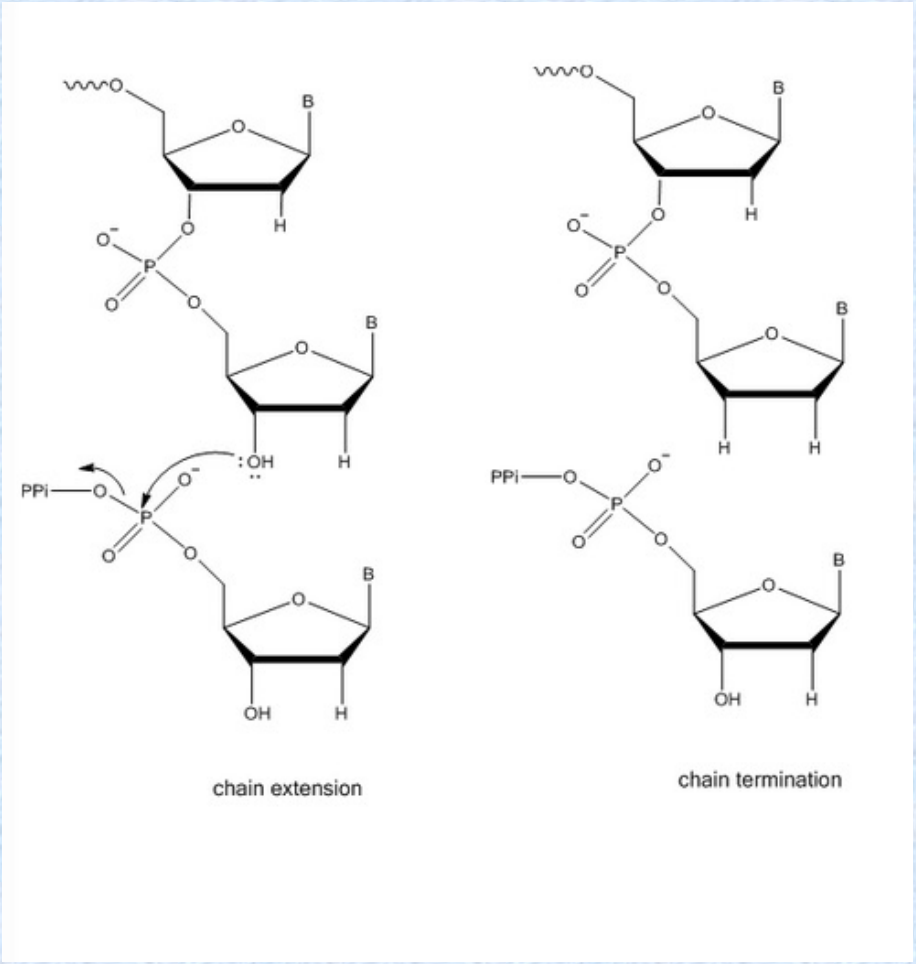
DideoxyTerminator Chemistry



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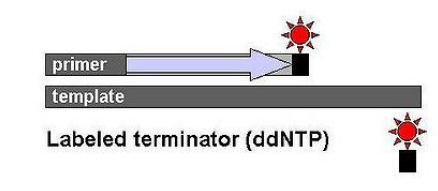
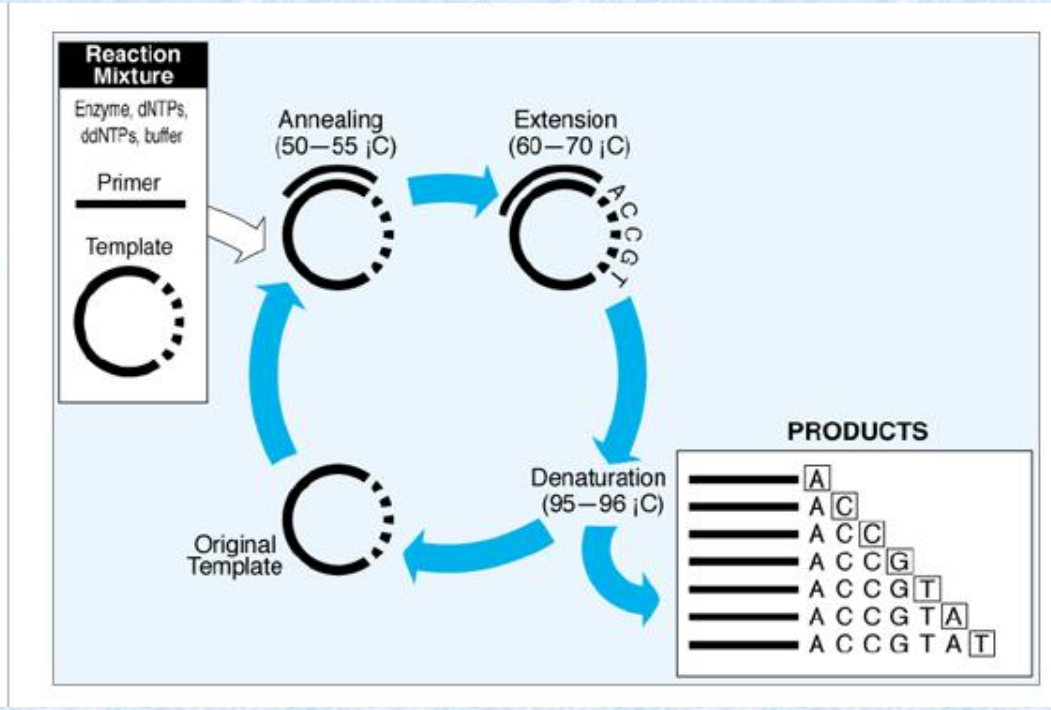
Cycle Sequencing Reaction





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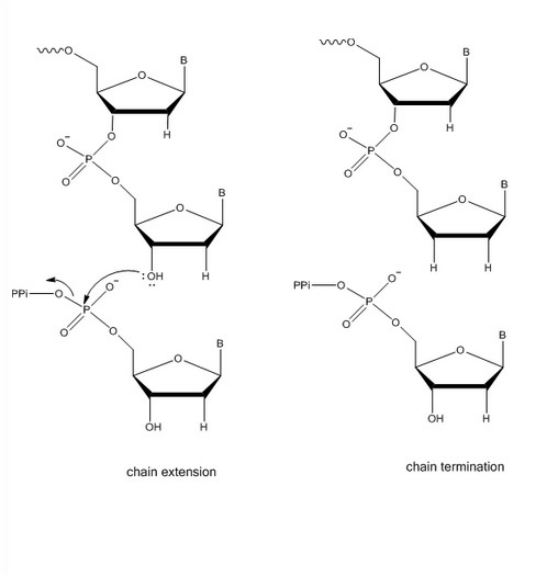
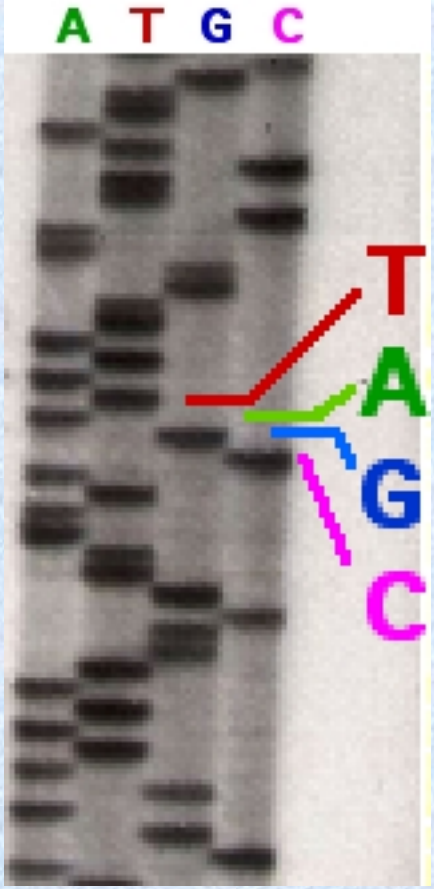
Sequencing at PSU Over the Years

Method	Manual Gel
Bases per Day	1200?





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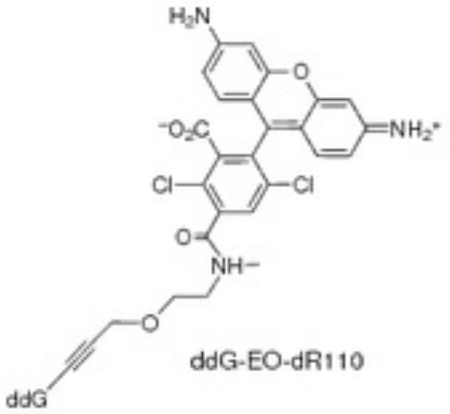
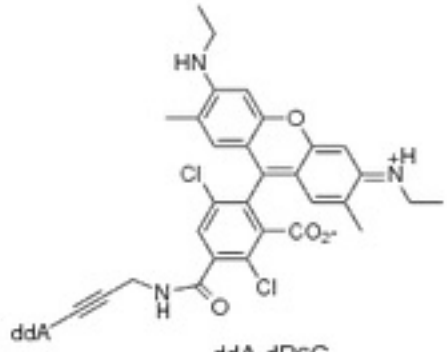
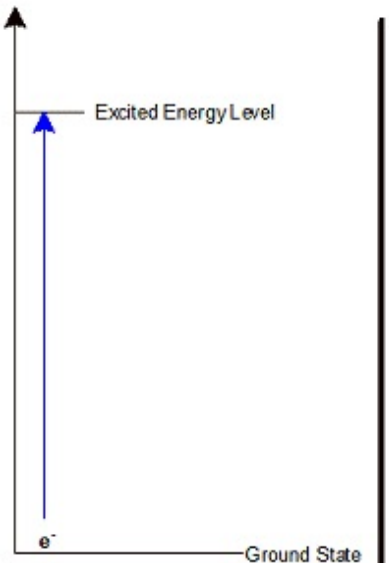


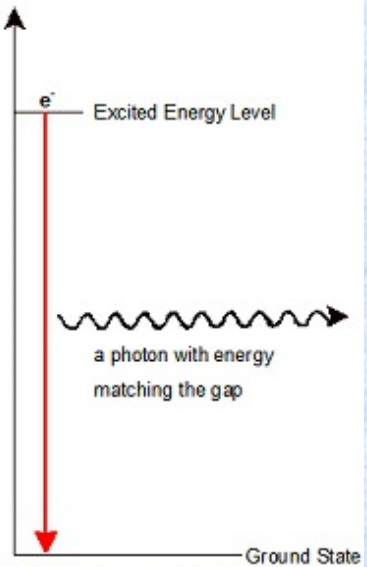
ddG-EO-dR110



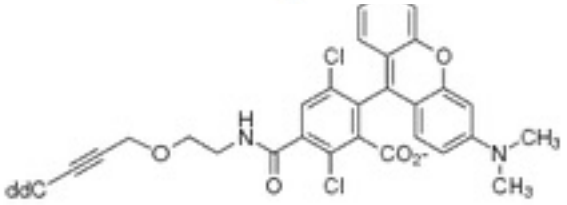
ddA-dR6G



Energy is absorbed, promoting an electron to an excited energy level



Energy is released as light, and the electron drops back to the ground state



ddC-EO-dTAMRA

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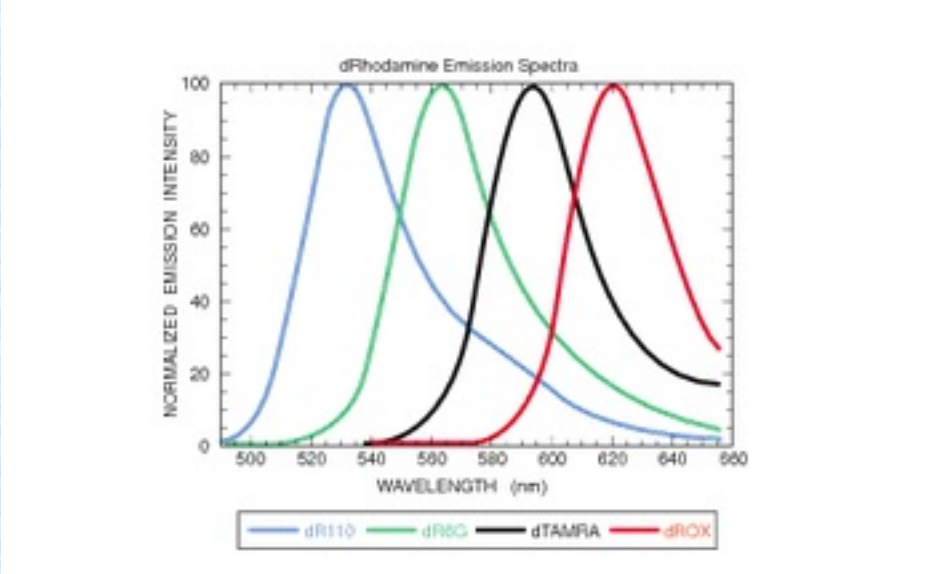

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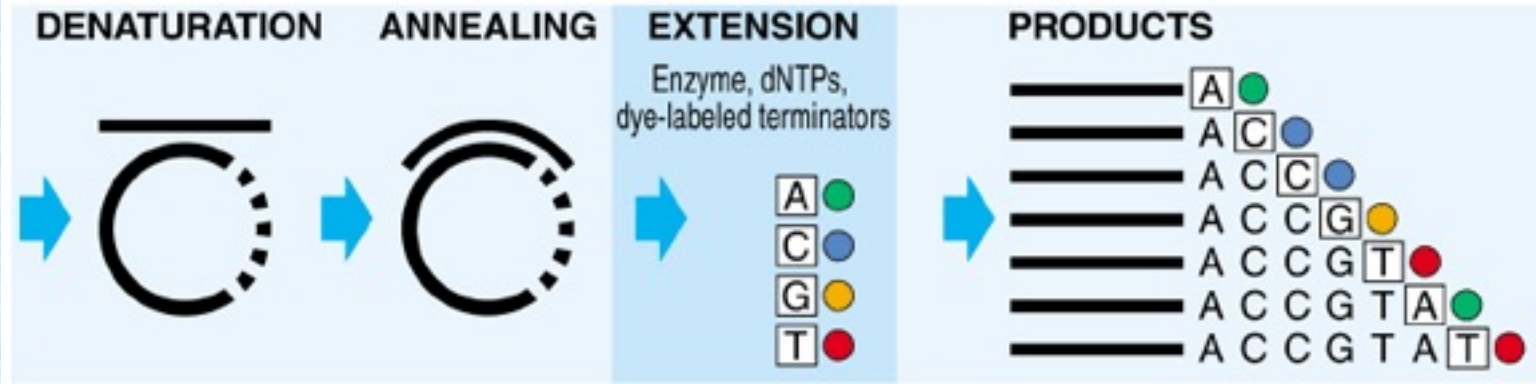

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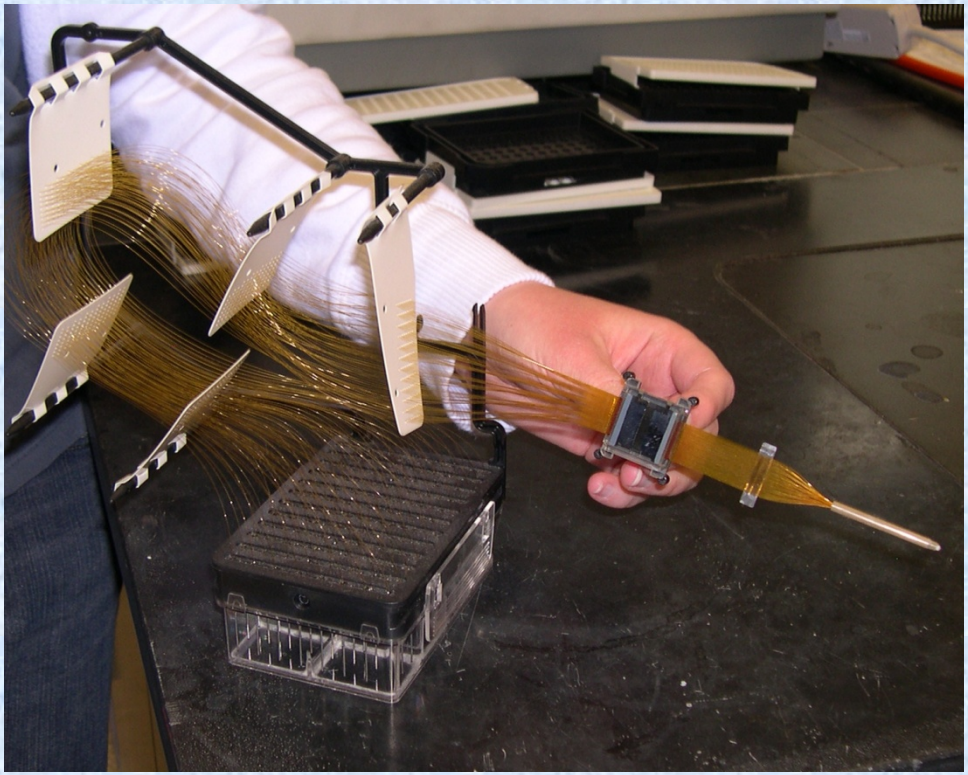

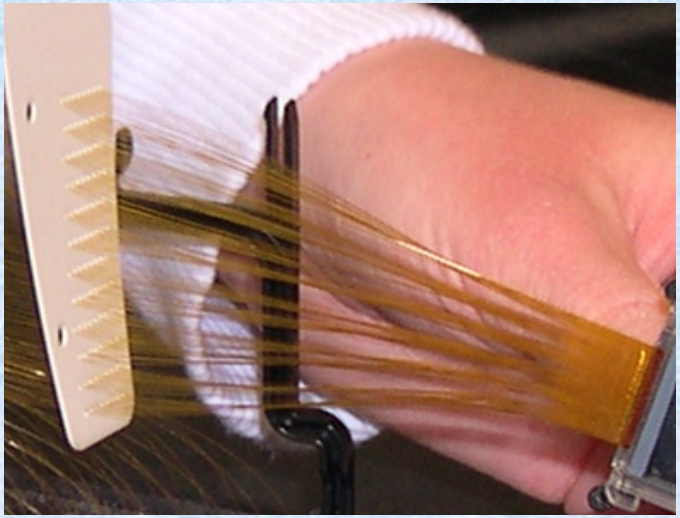

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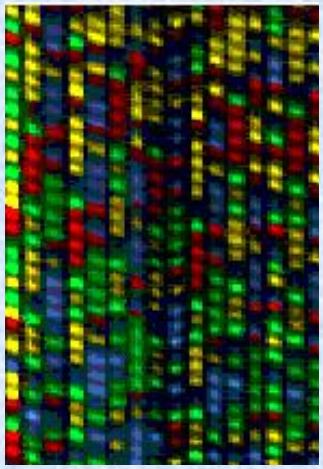
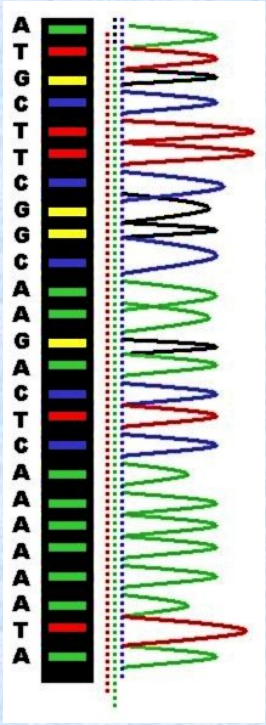
Sequencing at PSU Over the Years

Method	Manual Gel	377 Gel
Bases per Day	1200?	20,000





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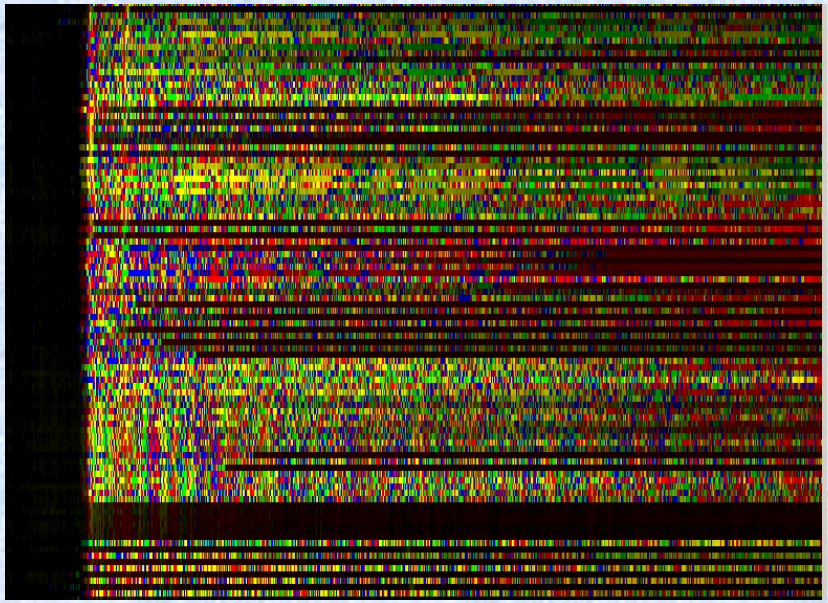
Method	Manual Gel	377 Gel	3100 –16 Capillary	3730--96 Capillary
Bases per Day	1200?	20,000	100,000	0.5 to 1 million



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http://dnalims.huck.psu.edu

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University Park, PA 16802

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PO Box 272531
Ft. Collins, CO 80527
970 290-9222
www.dnatools.com

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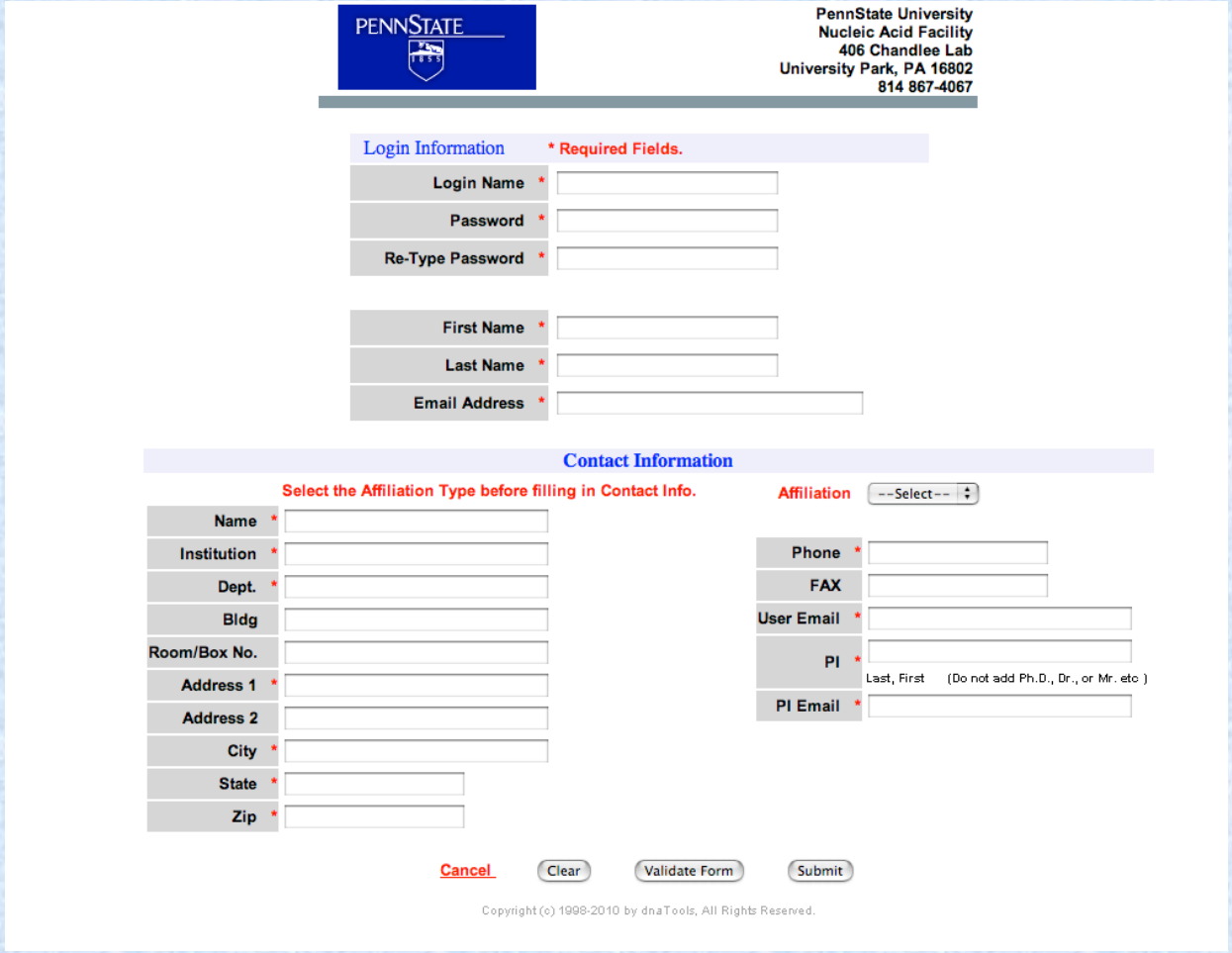

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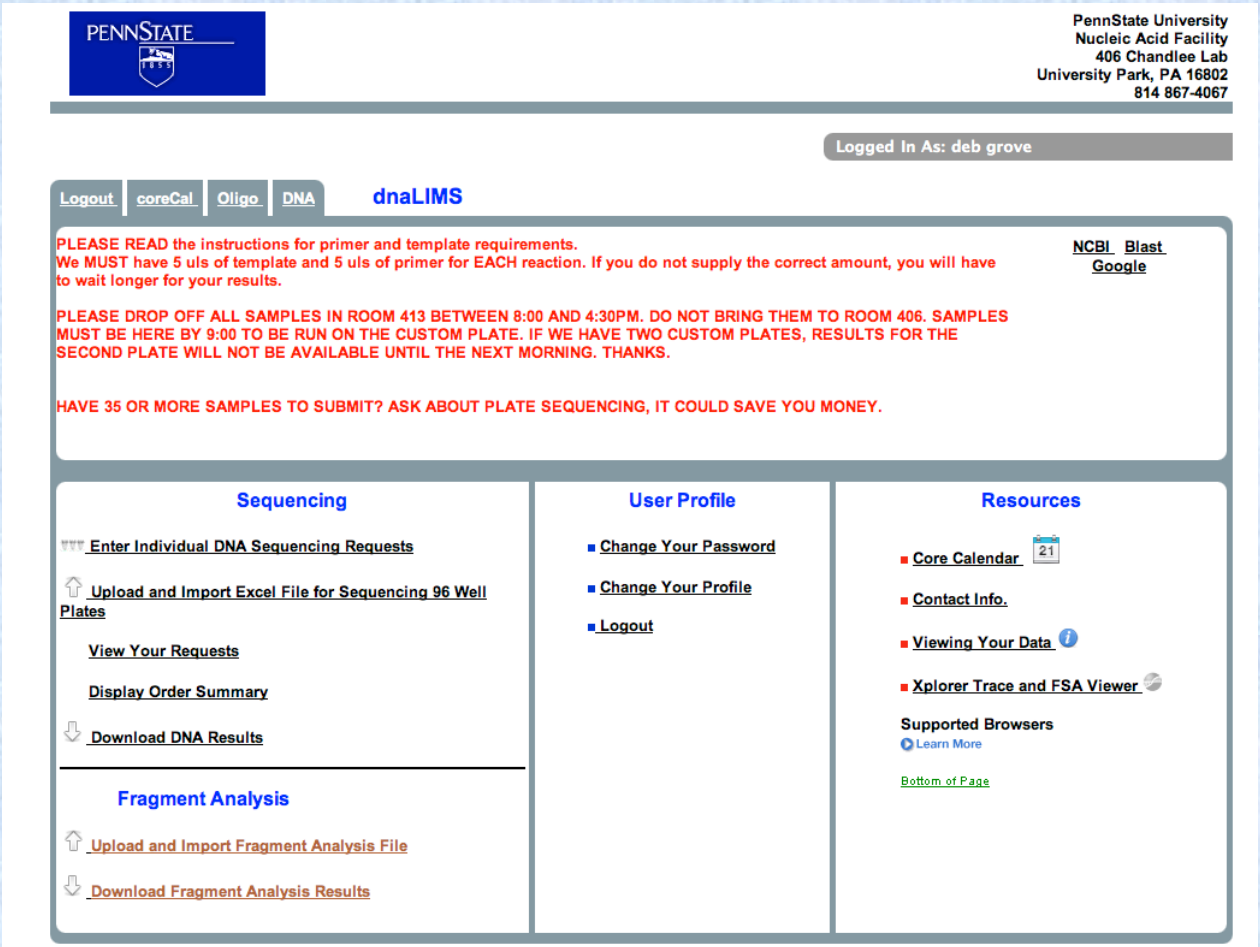

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Enter the Number of Reactions to Create Sequencing Requests For:

Select the Sequencing Type.

Service Requested: Sequencing_short

Clear Submit

Service Type	Service Description
Sequencing_short	Cycle Sequencing + Electrophoresis, 700-800 bases. PCR Products and Plasmids up to 10K. This is TEMPLATE size, NOT Read Length. Cost per Reaction $ 6.81
Sequencing_long	Cycle Sequencing + Electrophoresis. PCR Products and Plasmids OVER 10K. This is TEMPLATE size, NOT Read Length. Cost per Reaction $ 8.7
Sequencing_Genomic	Cycle Sequencing + Electrophoresis, 700-800 bases. Genomic DNA over 200K and up to 3MB. Inquire for details of template and primer concentration. Cost per Reaction $ 12.62
Electrophoresis_Only	Electrophoresis Only, Individual Tubes. Cost per Reaction $ 5
Services for use with Upload & Import	
User_Prep_96	96 Well Plate. Cost per Plate $ 229.47
User_Prep_96_Electrophoresis_Only	96 Well Plate. Cost per Plate $ 70.24

Standard Primer Information		
Primer	Sequence	Tm
T7	TAA TAC GAC TCA CTA TAG GG	60
Poly-dT	A	60
T3	ATT AAC CCT CAC TAA AGG GA	na
T7-term	GCT AGT TAT TGC TCA GCG G	60
SP6	CGA TTT AGG TGA CAC TAT AG	60
Gal4AD	TAC CAC TAC AAT GGA TG	60
Gal4BD	TCA TCG GAA GAG AGT AG	60
M13	TGT AAA ACG ACG GCC AGT	60
M13-40	GTT TTC CCA GTC ACG AC	60
M13-47	CGC CAG GGT TTT CCC AGT C	60
M13R	CAG GAA ACA GCT ATG ACC	60
M13R-48	AGC GGA TAA CAA TTT CAC A	60
BGHrevprim	TAGAAGGCACAGTCGAGG	na

Clear Submit

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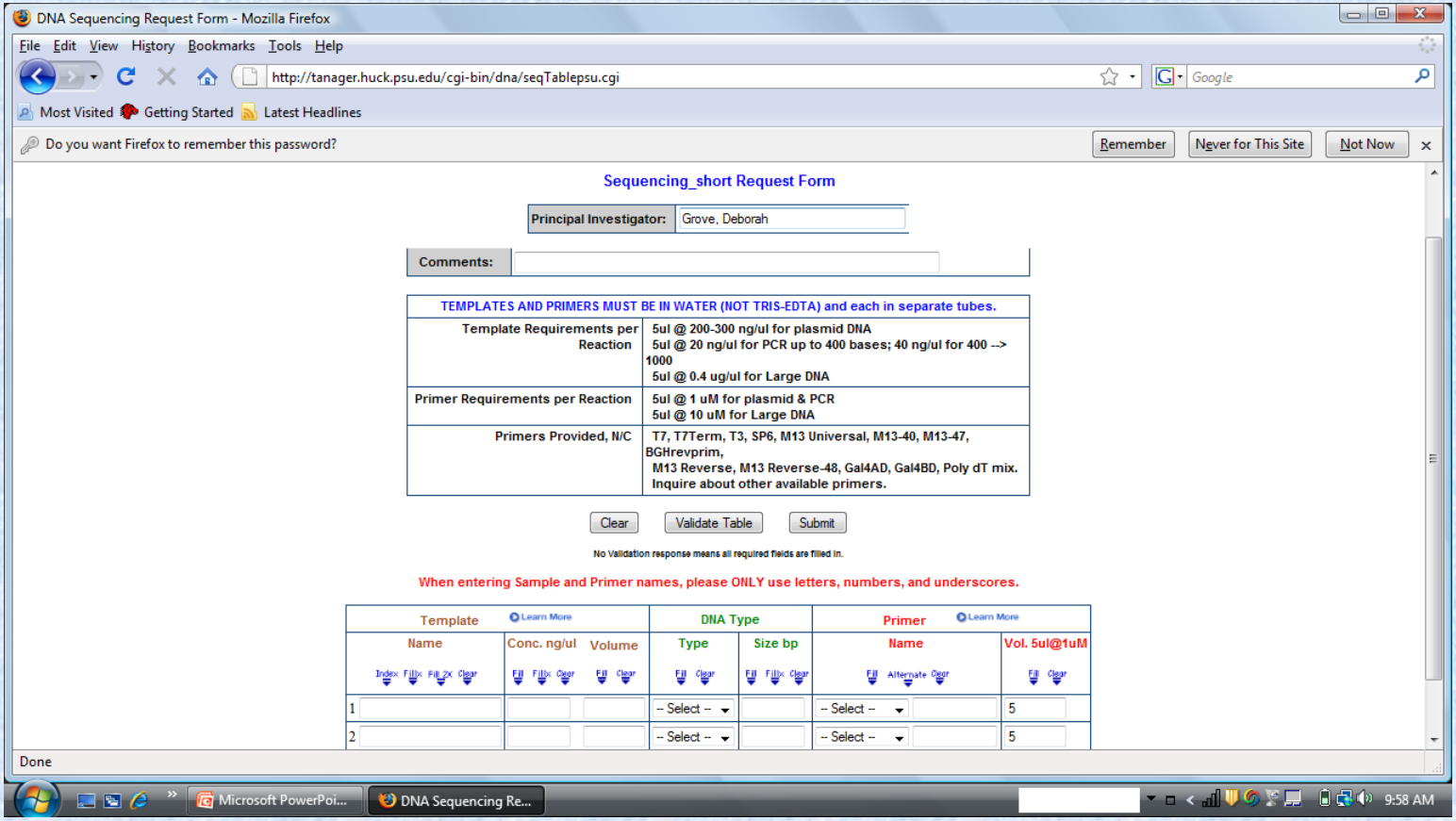

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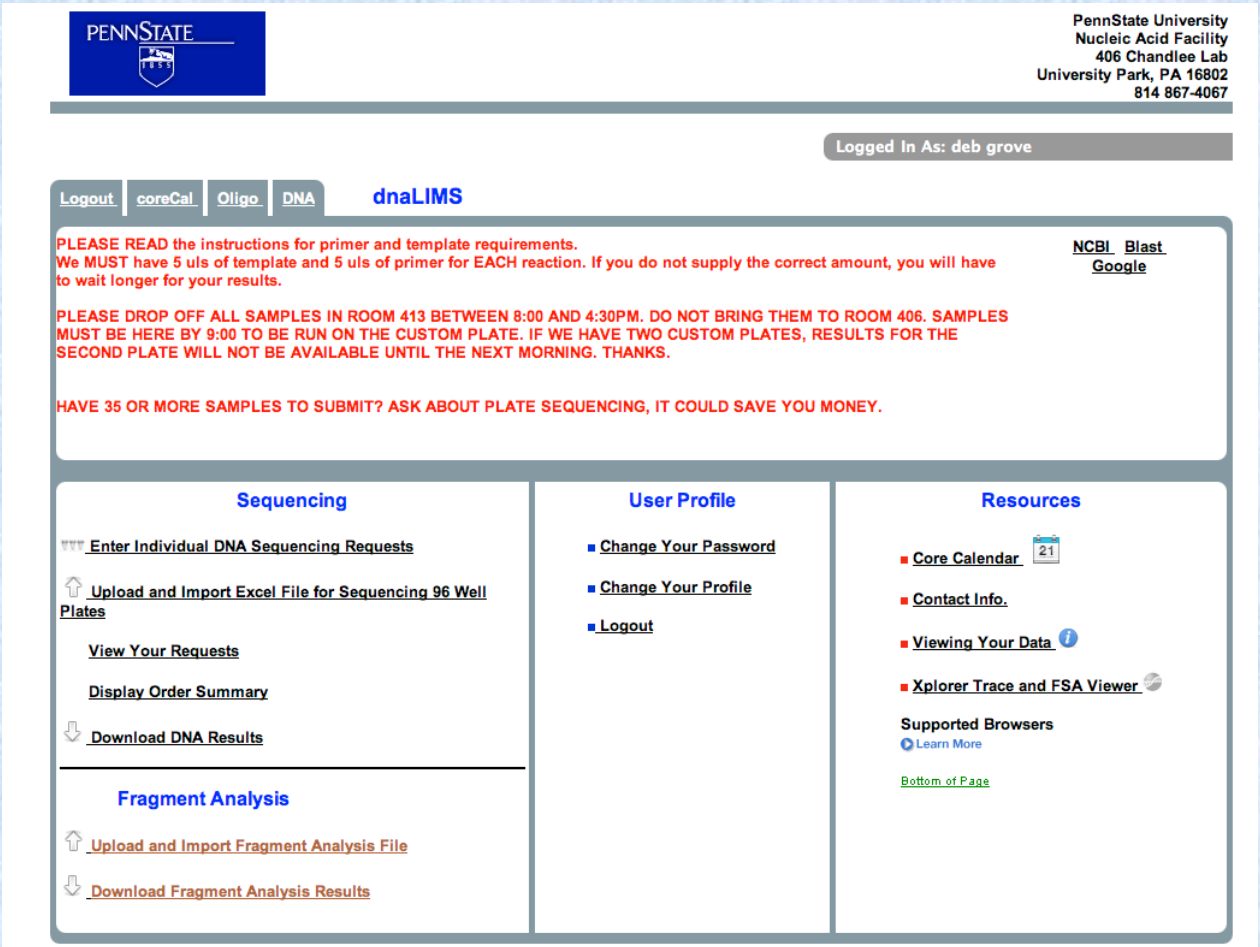

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Import requests from electronic spreadsheets.

Right click to Download this Sequencing Template to your PC.
Edit the template file with the program Excel, i.e. Open Excel first then the file from within Excel.
No spaces or illegal characters allowed. Allowable characters include: a - z, A - Z, 0 - 9, and hyphens.
Save the edits to a Tab delimited file with a .txt extension on your PC.
Use the Choose File button to select the modified template for uploading and importing.
Do Not have Duplicate Sample and Primer name pairs on multiple lines in the import file.

Principal Investigator:	**Grove, Deborah**
Service Request:	-- Select --

Click here for Information on How to Prepare Plates for Sequencing

Comments:

Select the Browse or Choose File button to locate the upload file.

Upload File: Choose File no file selected

After selecting the file, depress the Submit button to upload the file.

Clear Submit

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CSR Preparation

- Samples are all thawed and spun down
- Primers are added to the 96 well plate first followed by template using calibrated pipettors
- PGEM control is added to each plate
- Samples in plate are denatured at 98 degrees for 5 minutes
- CSR mix is made and added to all wells
- CSR is run in Thermal Cycler

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Cleanup

- Water is added to CSRs to a final volume of 20 uls
- Plates with Sephadex are spun to remove water
- CSR samples are pipetted on top of Sephadex
- Plates are again spun and fragments from the primer extension reaction are eluted from Sephadex
- Unused dNTPs, ddNTPs and other small molecules are retained in Sephadex beads

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Keys to Success

- Sample Quality
- Sample Quantity

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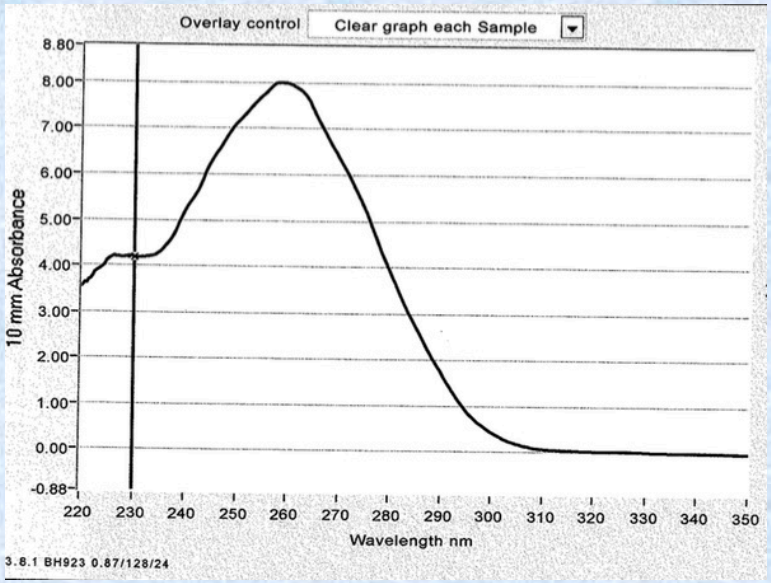
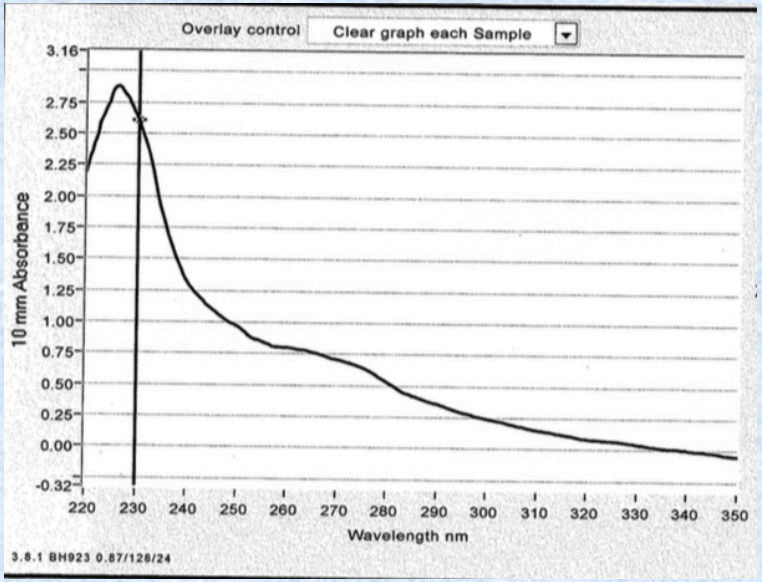


260/280=2.07
260/230=1.90



260/280=1.52
260/230=0.31

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Extraction Tips

Protocols from labs getting 800 to 1200 bases are available in room 413 Chandlee

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Extraction Tips

- Don’t overgrow your cultures.
- Use less of your culture than the maximum for the kit.
- Some solutions can go bad such as the NaOH/SDS. Make fresh.
- Some find second washes are necessary.
- Use the Tris elution buffer – never Tris EDTA! Some say water is OK.
- Some heat the elution buffer to 65 and leave it on the column for 5 min.

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Extraction Tips

- Isopropanol should be ice-cold and Ethanol should be at room temperature.
- Be sure you get rid of Ethanol. Let it dry longer than suggested. Up to an hour if you are not in a hurry.
- Be sure your spin columns are at room temperature.
- Elute PCR products in a minimal volume to reach an optimal concentration.

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Extraction Tips

- For valuable samples retain your supernatants until recovery is verified.
- Centrifuge tubes in the same orientation in order to recover DNA in a compact pellet.
- Calibrate your pipettors.

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Primer Selection

- Use accurate data. Look at electropherogram.
- Primers should be 22 bases or less.
- Eliminate primer dimers and secondary structure.
- Tm greater than 58 degrees by Nearest Neighbor Analysis.
- GC content of 50 to 55% and GC lock at 3’ end.
- Genomics Core has Oligo 7.
- Avoid false priming sites with a good design program.

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View your Electropherograms

- Click on View.
- Use FinchTV from Geopspiza.
- Use Sequence Scanner free from Life Technologies.

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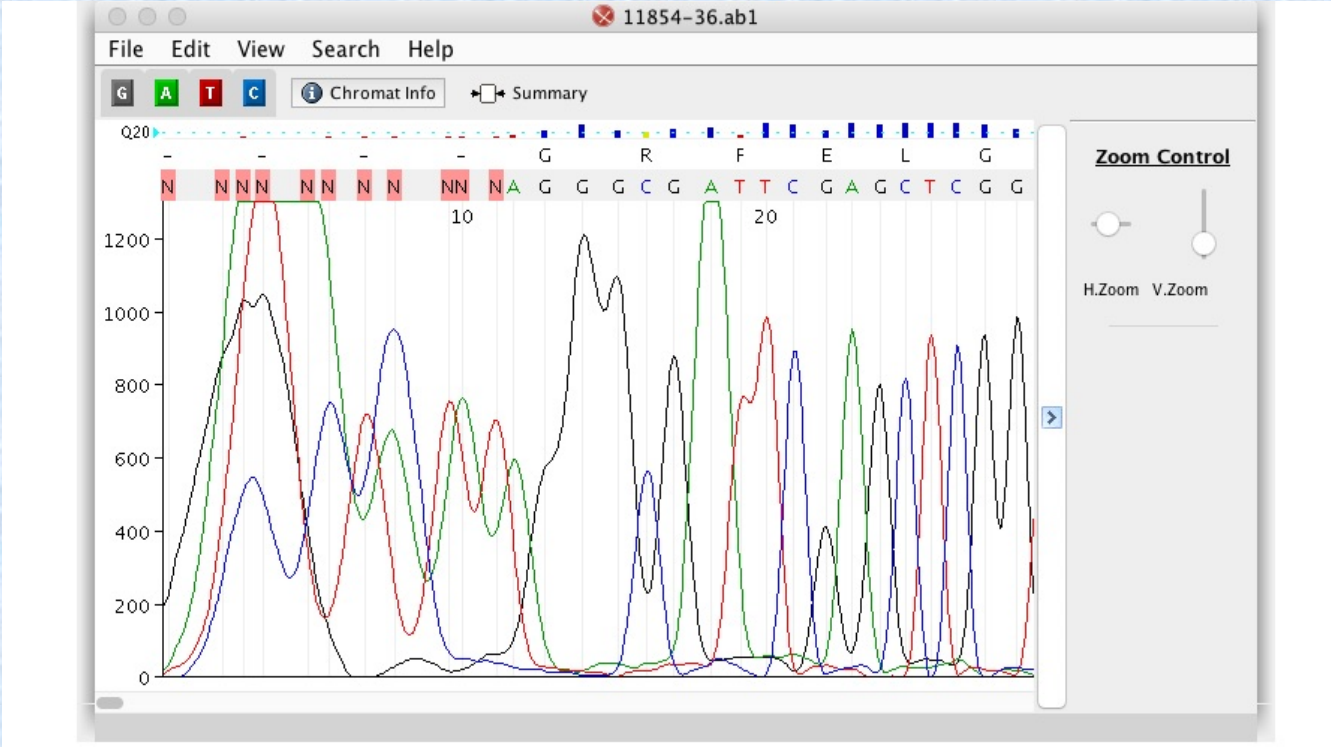
45466	1064789	Text Electropherogram	View	11854-35	C5	iraDPEP2	lacbsu	mcgibbon,louise	2995	Nov 20 2015	phd qual 1037 fasta scf	Results Availab
cs	1064804	Text Electropherogram	View	11854-36	D5	pGem	CP1	heintz,ginger	3023	Nov 20 2015	phd qual 997 fasta scf	Results Availab
45467	1064790	Text Electropherogram	View	11854-37	E5	CTMx14	Mx14A	phillips,allen	1642	Nov 20 2015	phd qual 700 fasta scf	Results Availab



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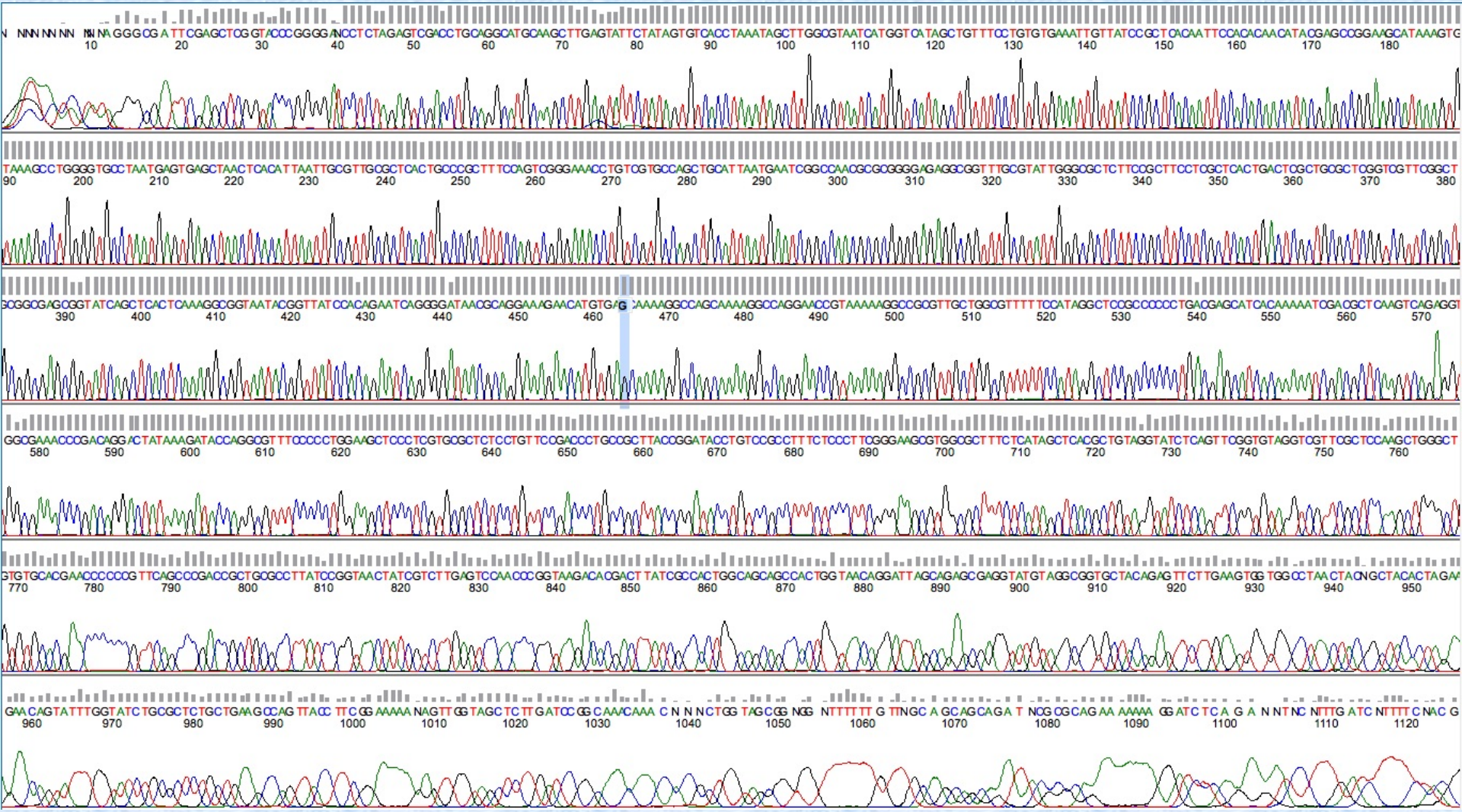
Control PGEM Plasmid



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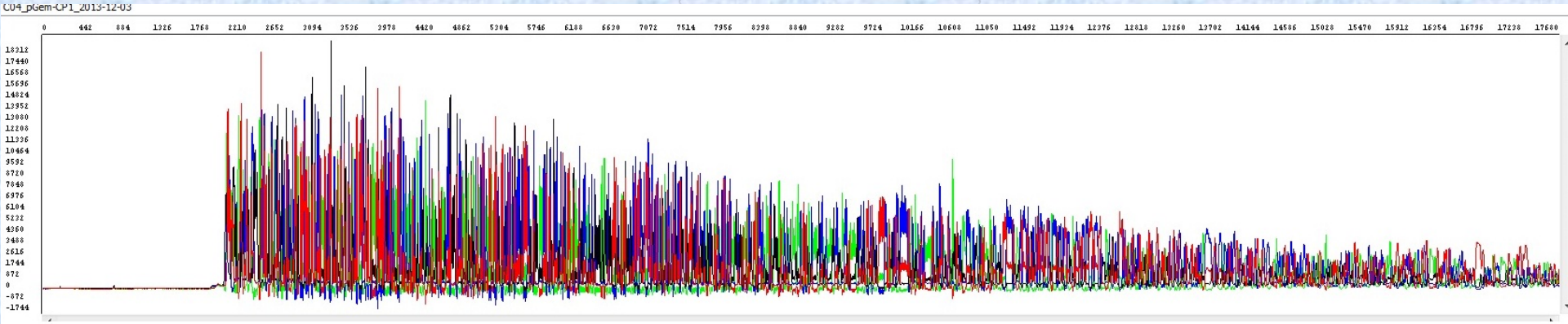

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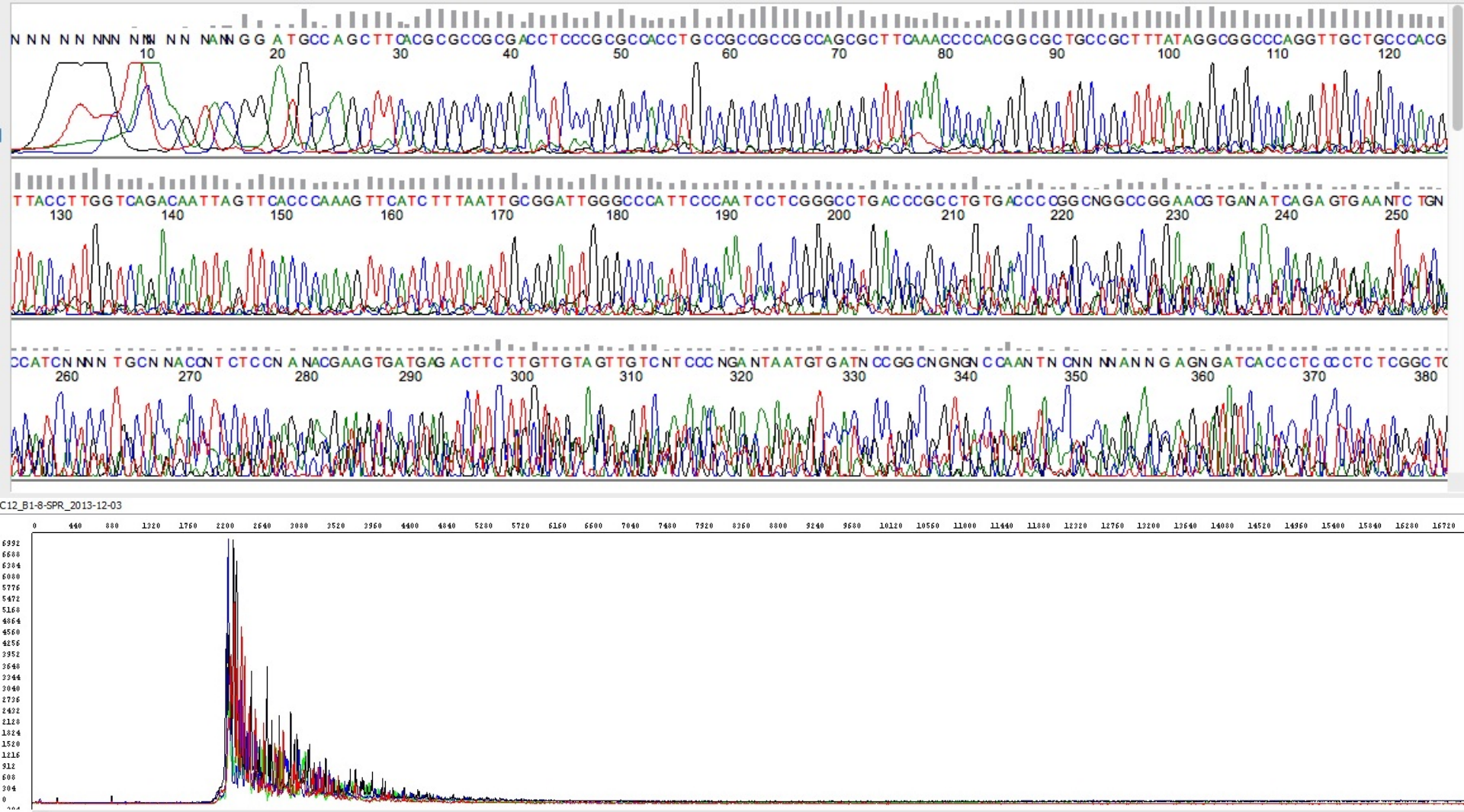

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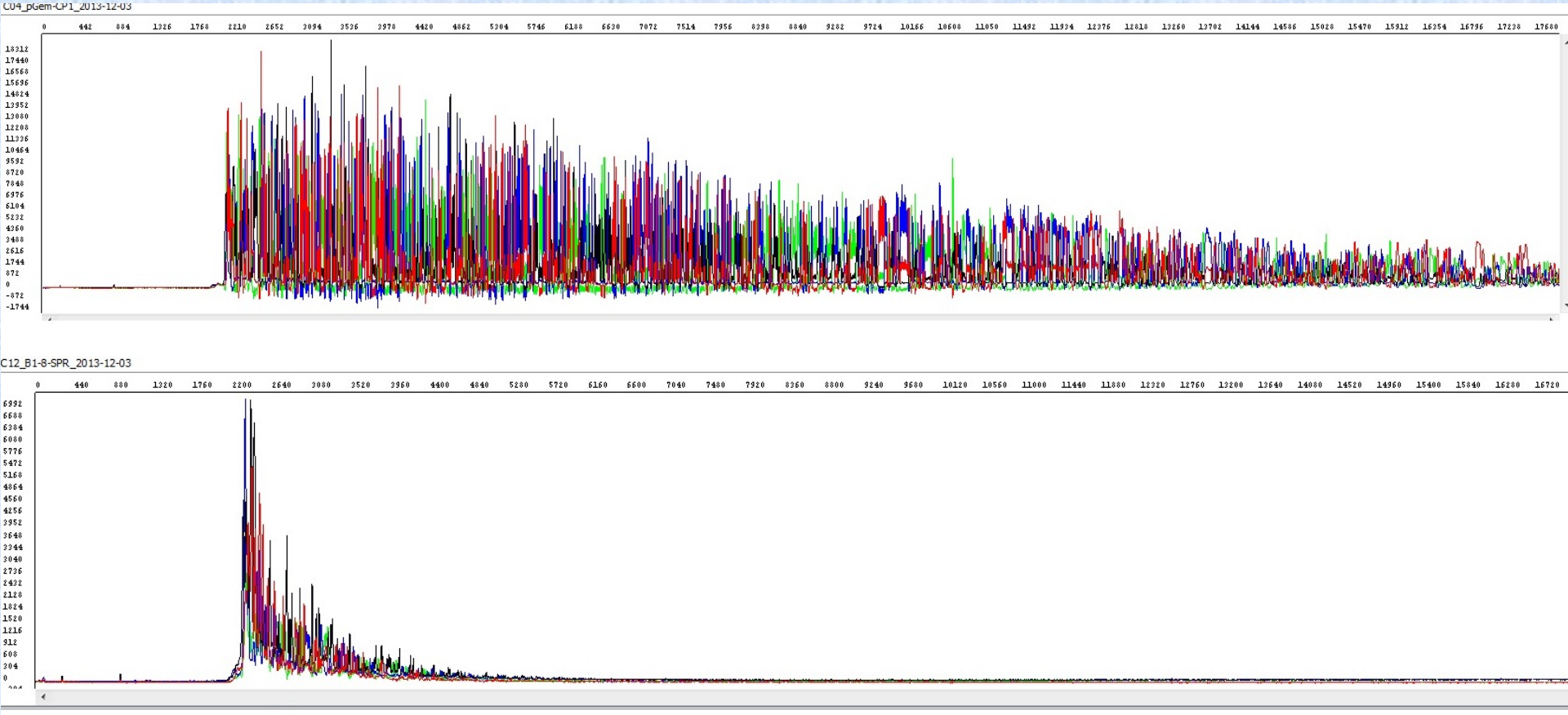

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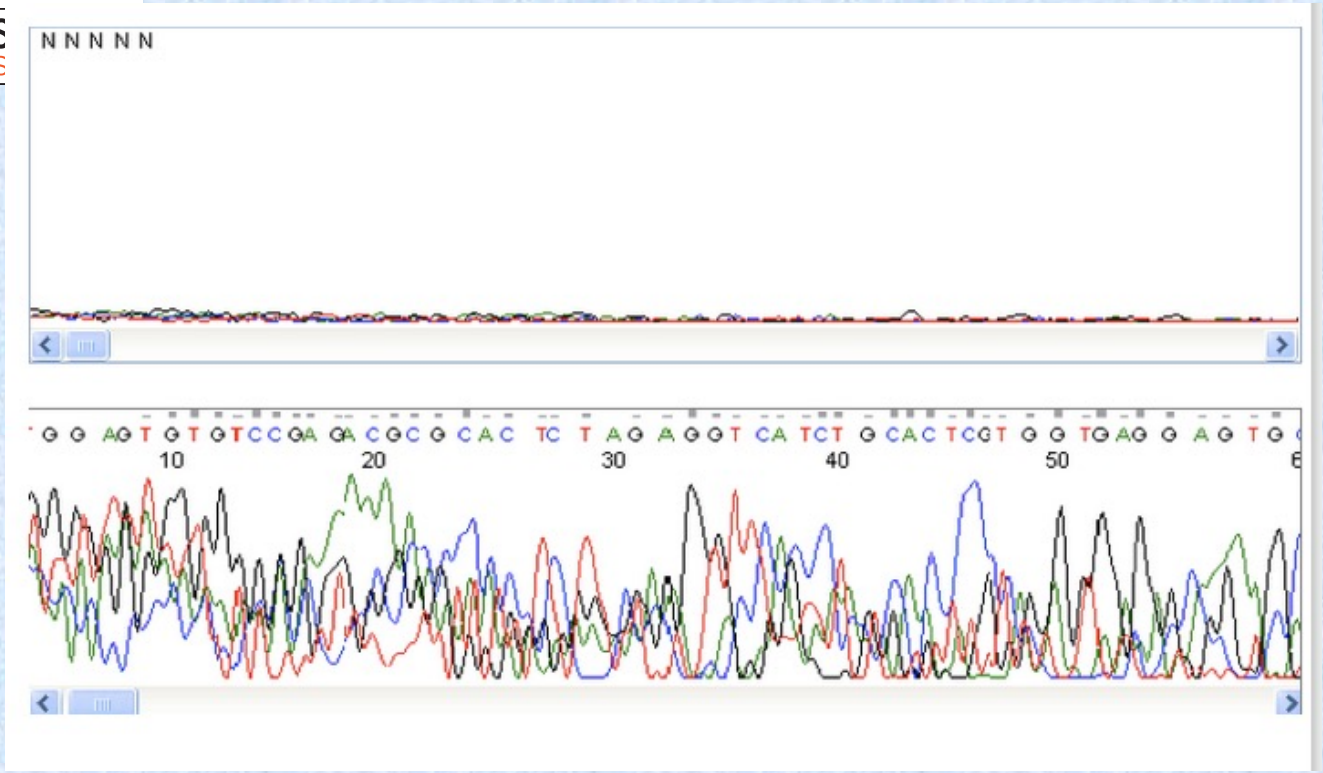

- Poor Quality or Wrong concentration
- Wrong Primer
- Degraded Primer
- Contamination
- Forgot to put template in the tube

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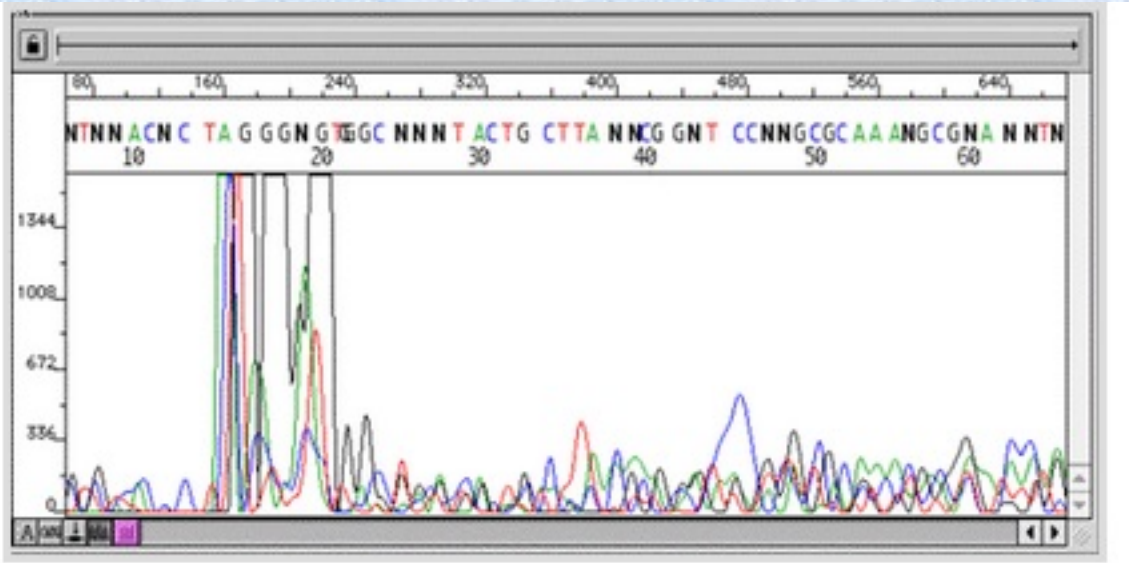

Cause: priming site not present

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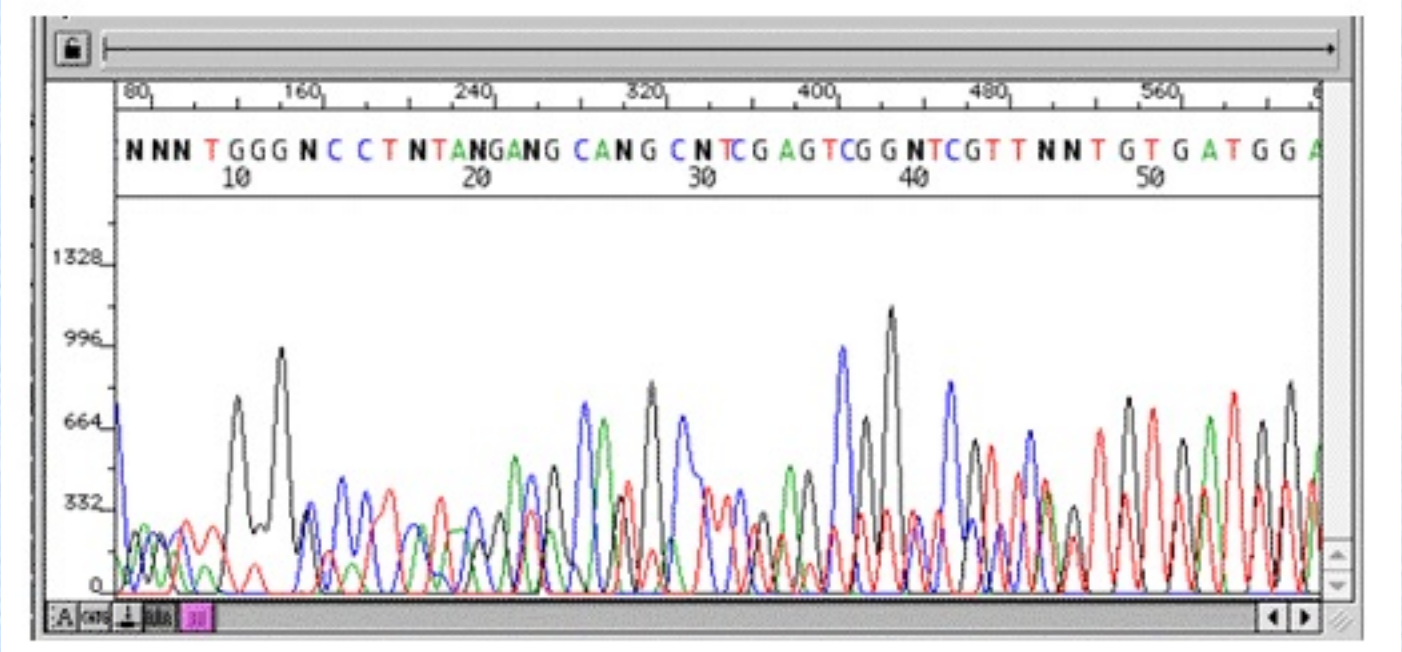

- Primer annealing to multiple sites
- Residual PCR Primers
- Mixed Plasmid or PCR products
- Degenerate Primers

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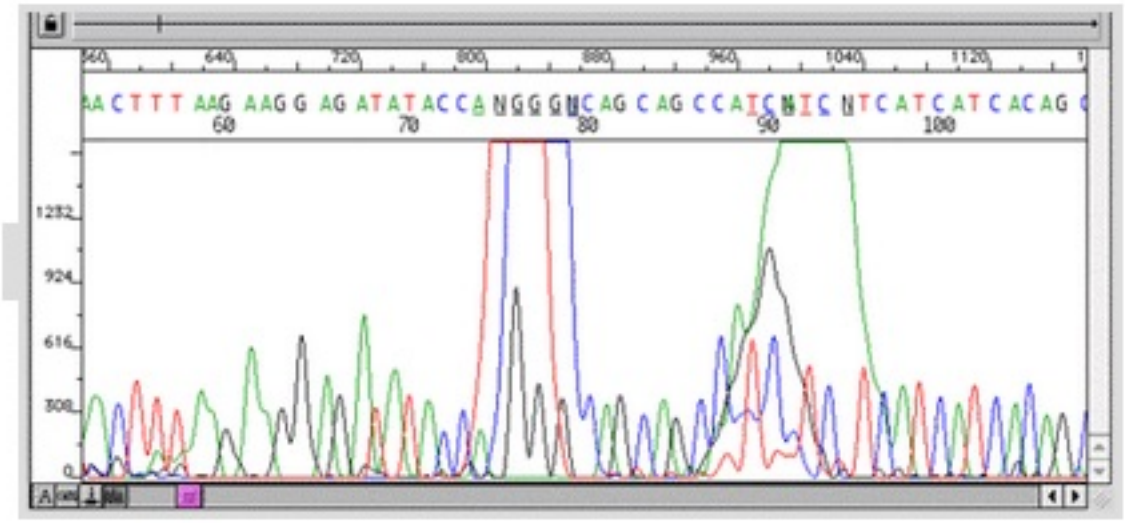
Artifact: "dye blobs"



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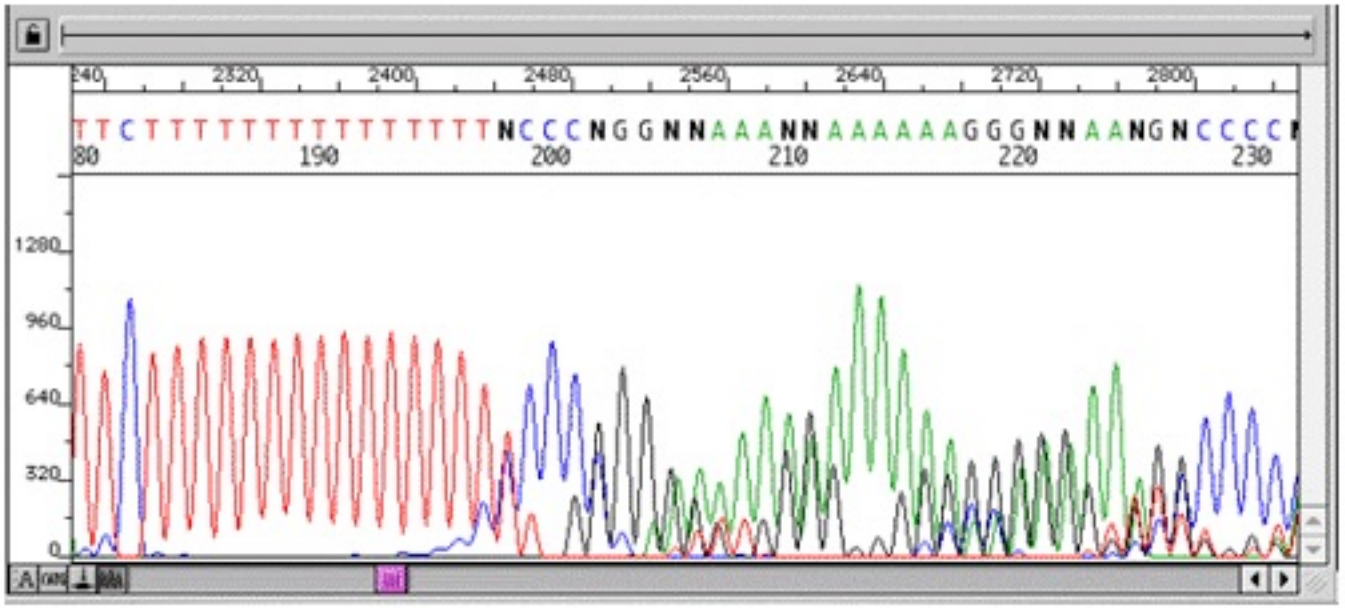
Cause: homopolymeric regions



We have primers that can “anchor” and continue the sequence.

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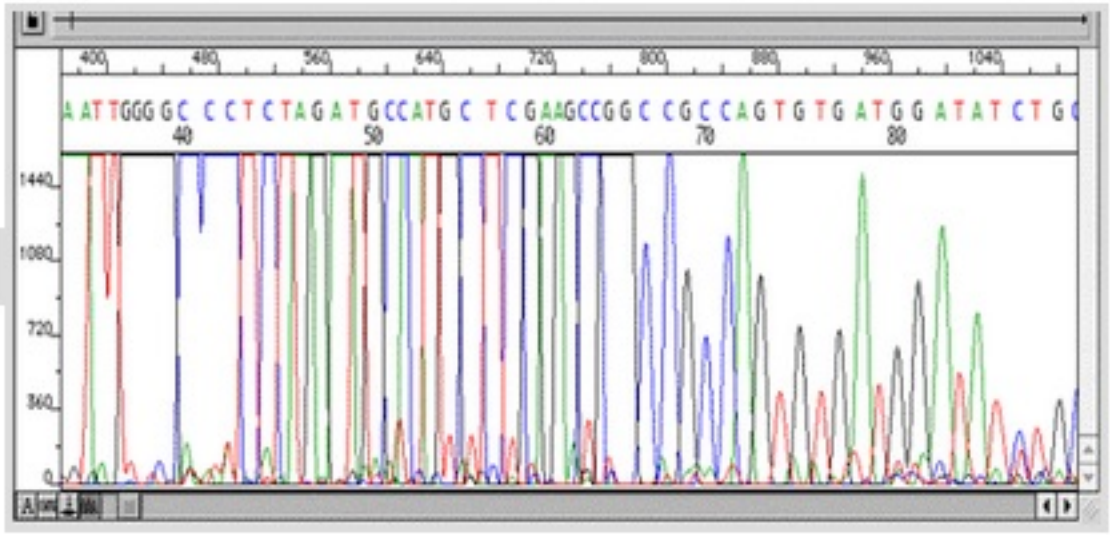
Cause: too much DNA



- Presence of salts
- Primer concentration too high

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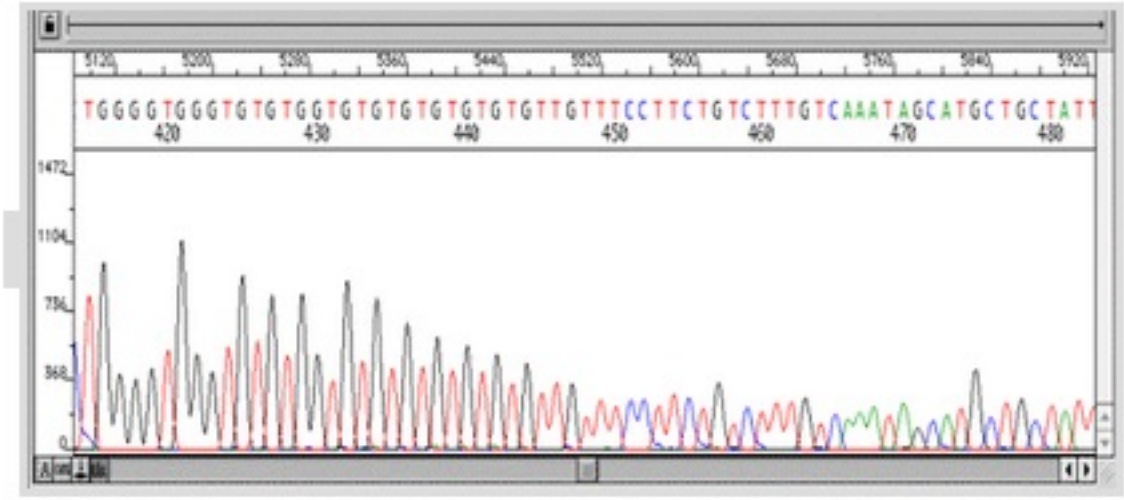
Cause: repetitive regions



I have designed primers for this before to continue the sequence.

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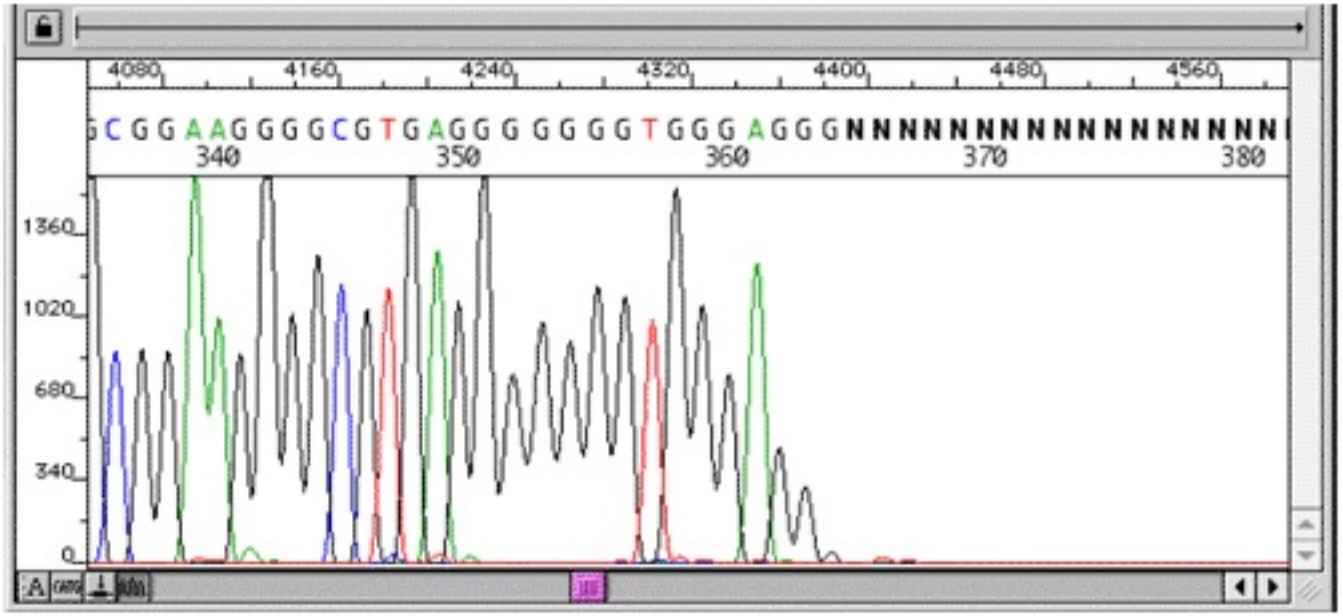
Cause: secondary structure



Request our special Stop Protocol.

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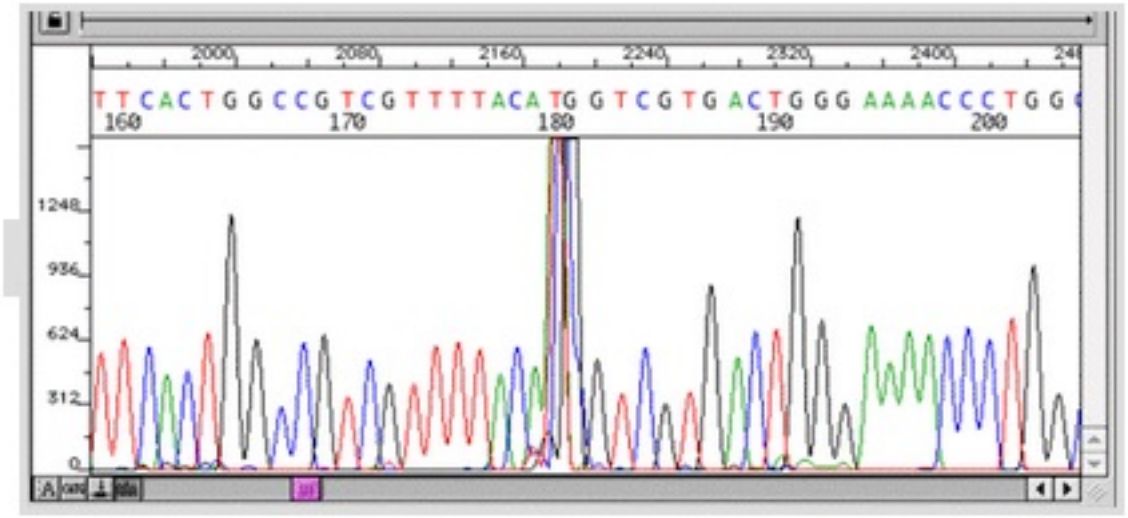
Artifact: "spikes"



Request a Re-run. Possibilities are a bubble in the array or could be low template concentration and electrical spike.

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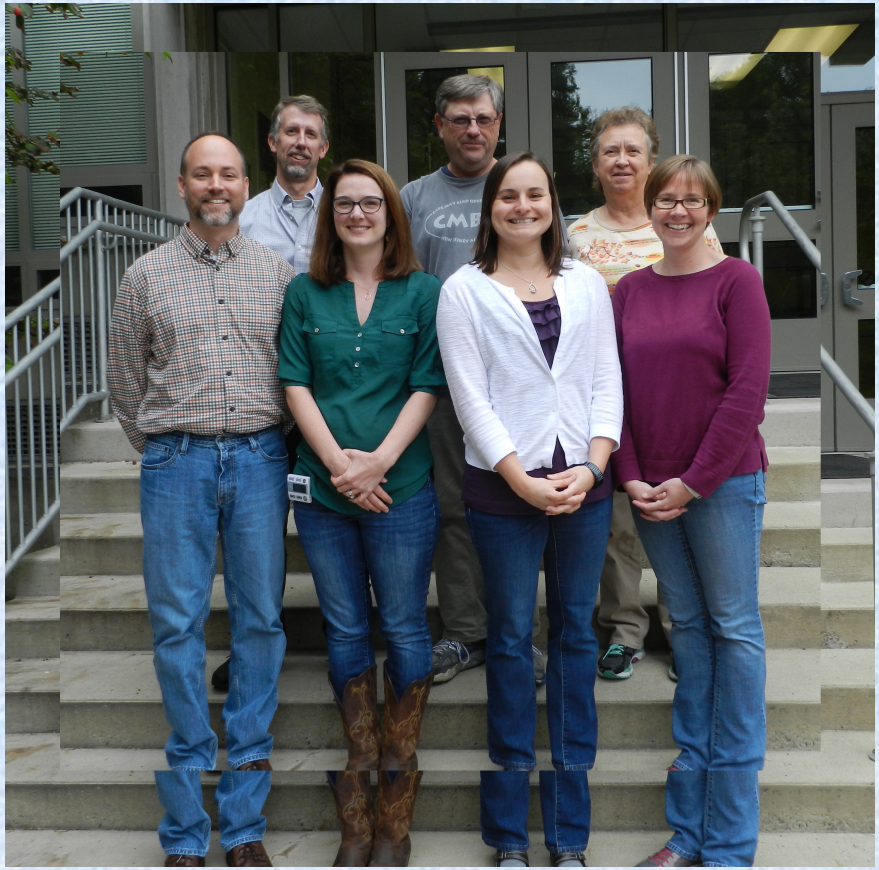

Front: Dan Hannon, Kerry Hair, Ginger Heintz, Ashley Price
Back: Dr. Craig Praul, Dr. Greg Grove, Dr. Deborah Grove