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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 $\frac{1}{2}$ of the Nobel Prize in 1980. Do you know who got the other half?



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DNA Sequencing

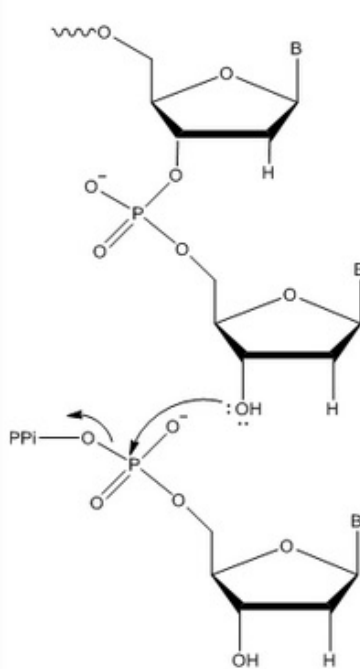
Factoid of the day: The person who this auditorium is named after. Paul Berg.



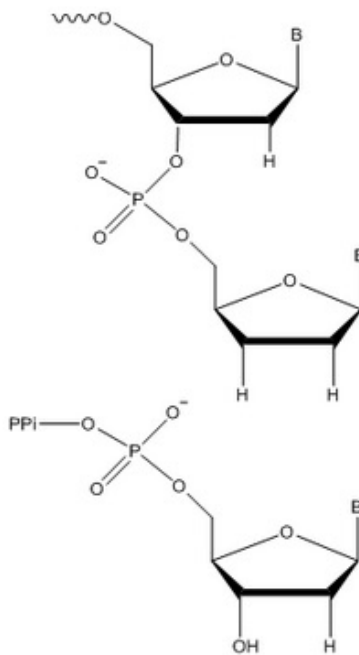
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Dideoxy Terminator Chemistry



chain extension



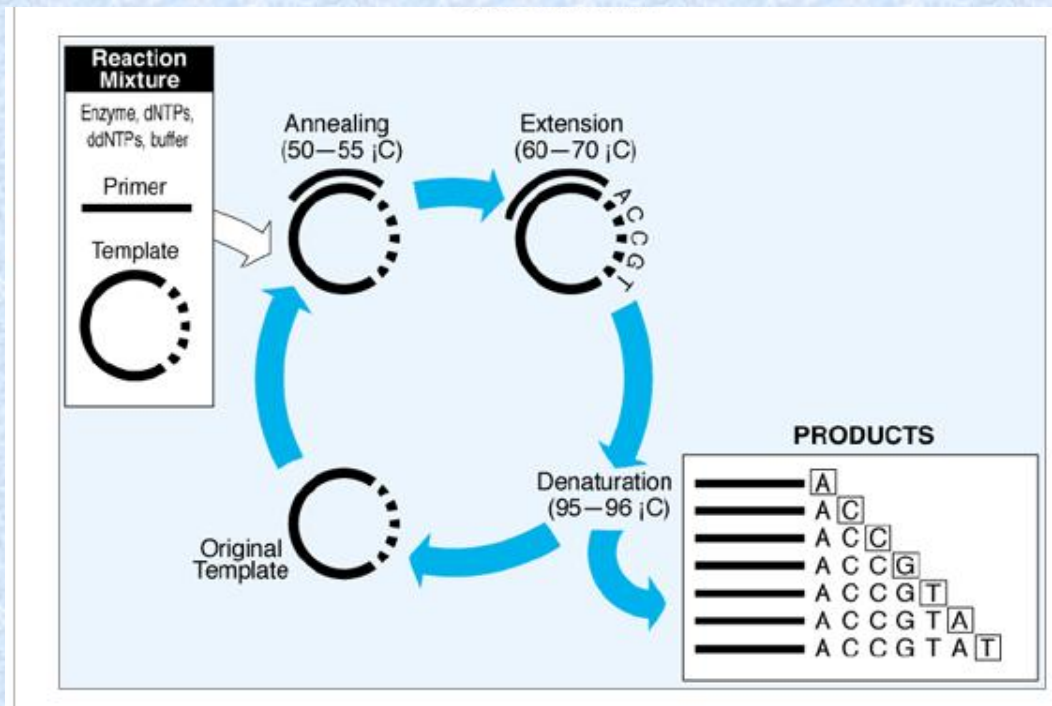
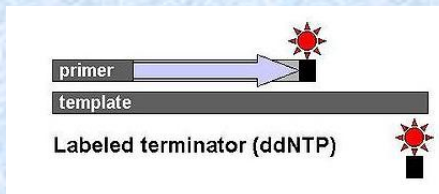
chain termination



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Cycle Sequencing Reaction



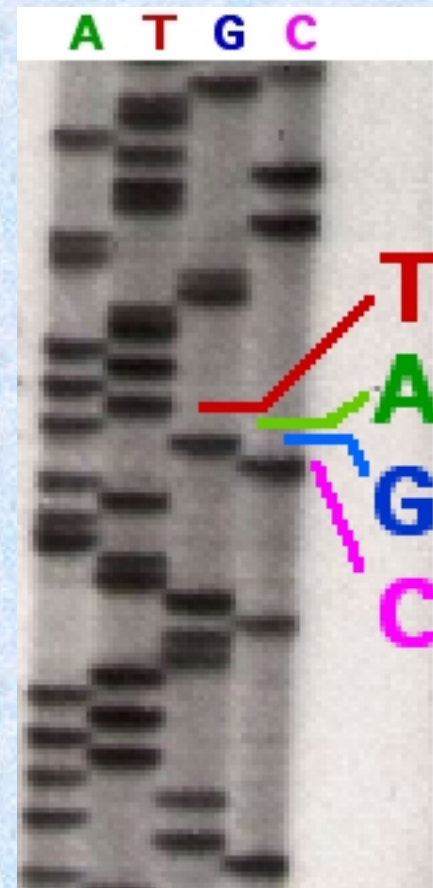
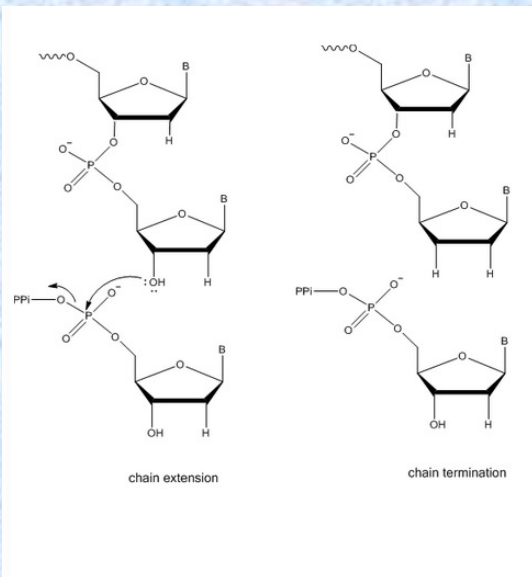


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Sequencing at PSU Over the Years

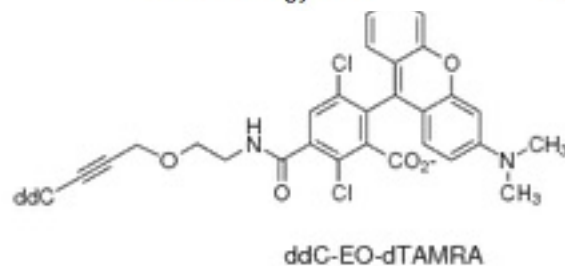
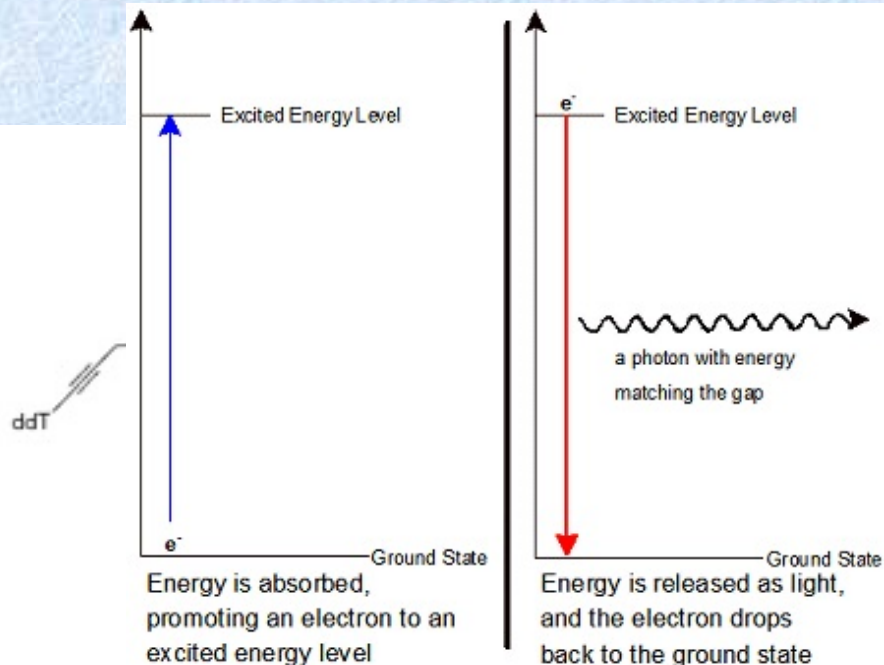
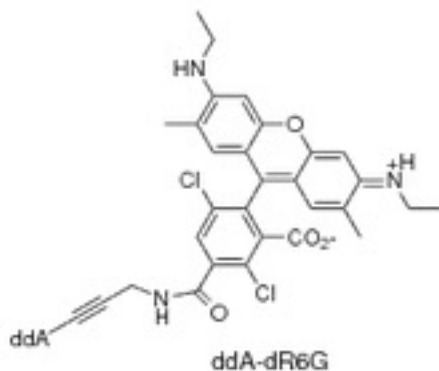
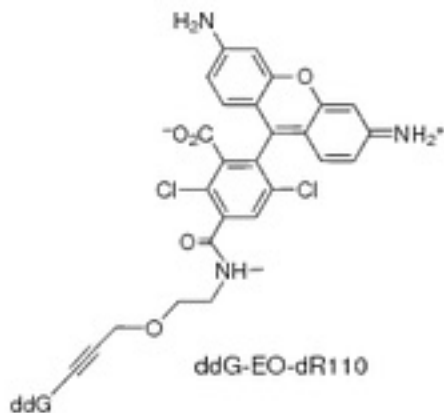
Method	Manual Gel
Bases per Day	1200?





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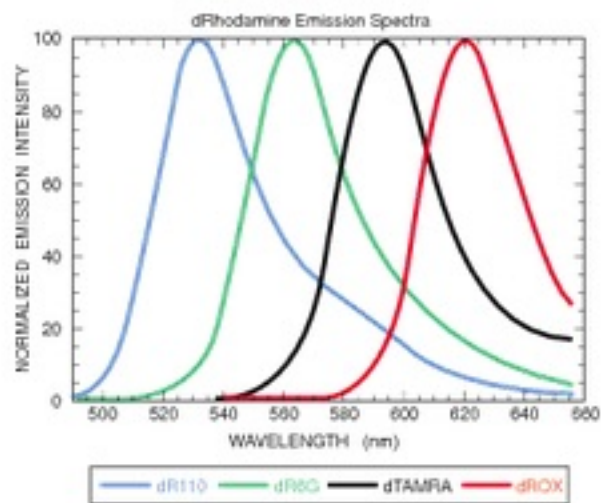
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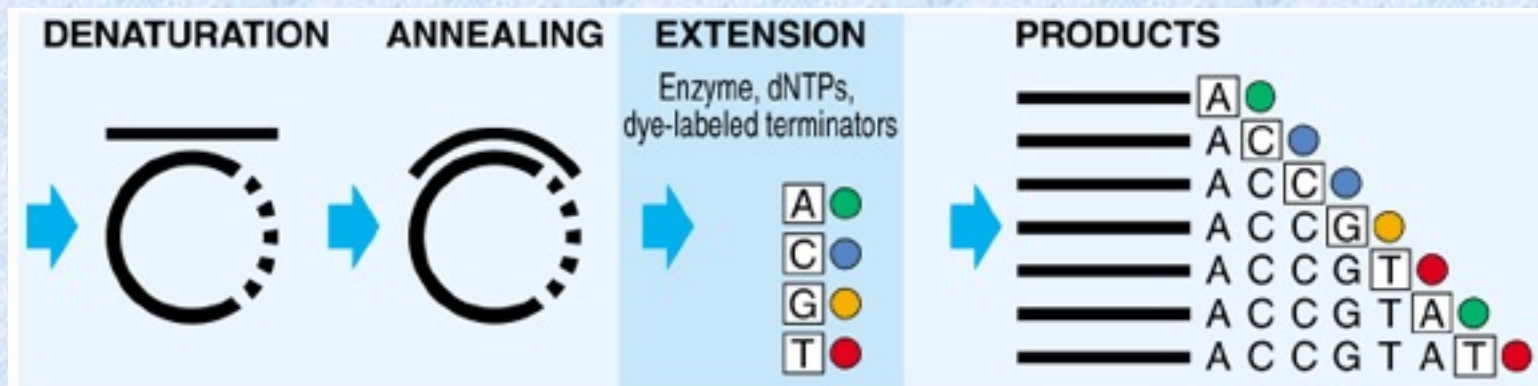
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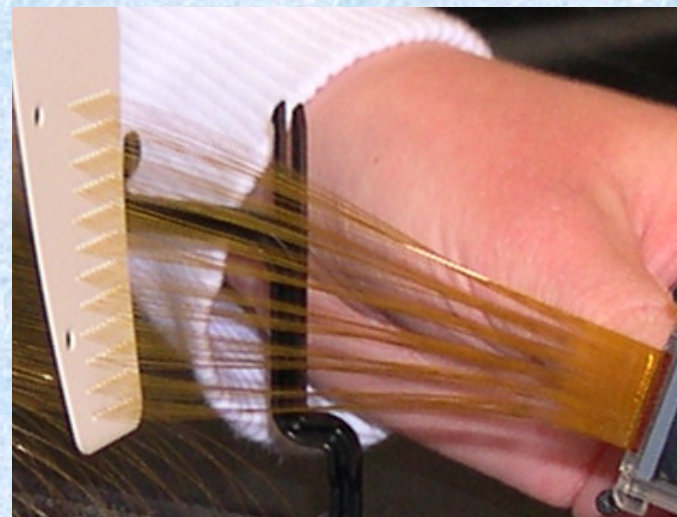
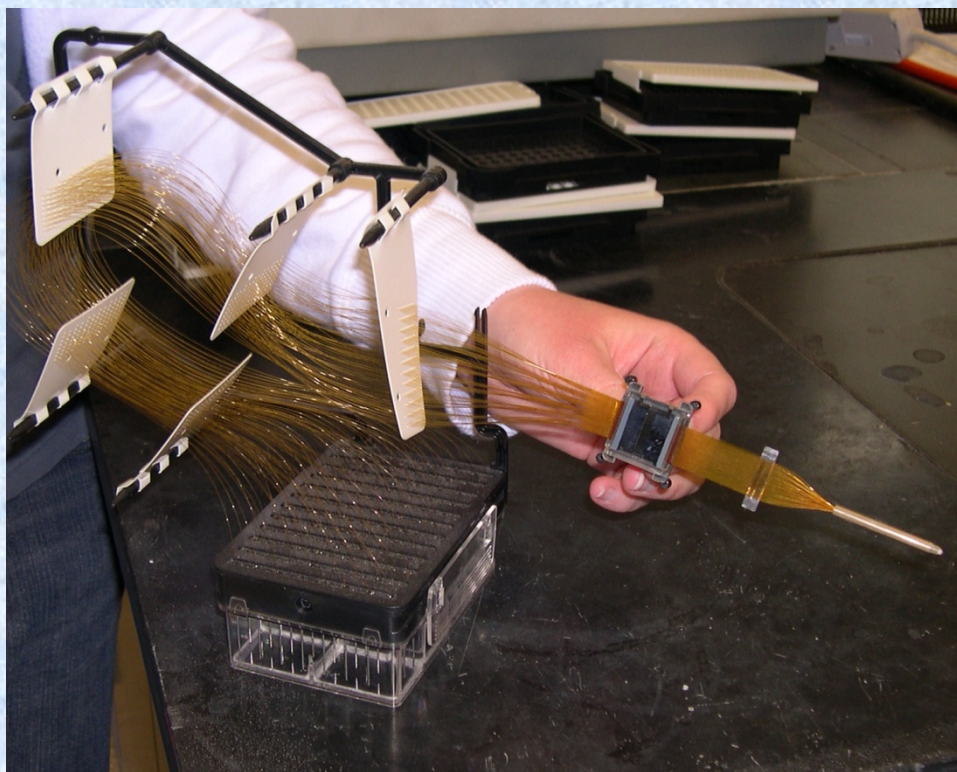
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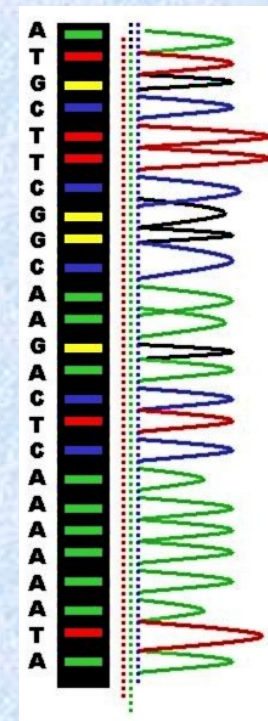
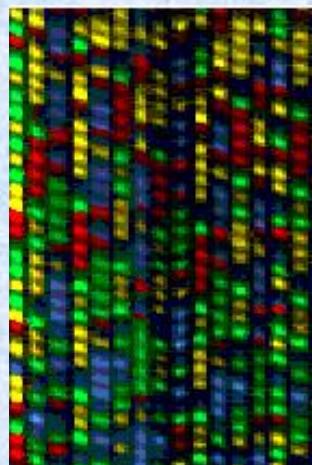


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Sequencing at PSU Over the Years

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

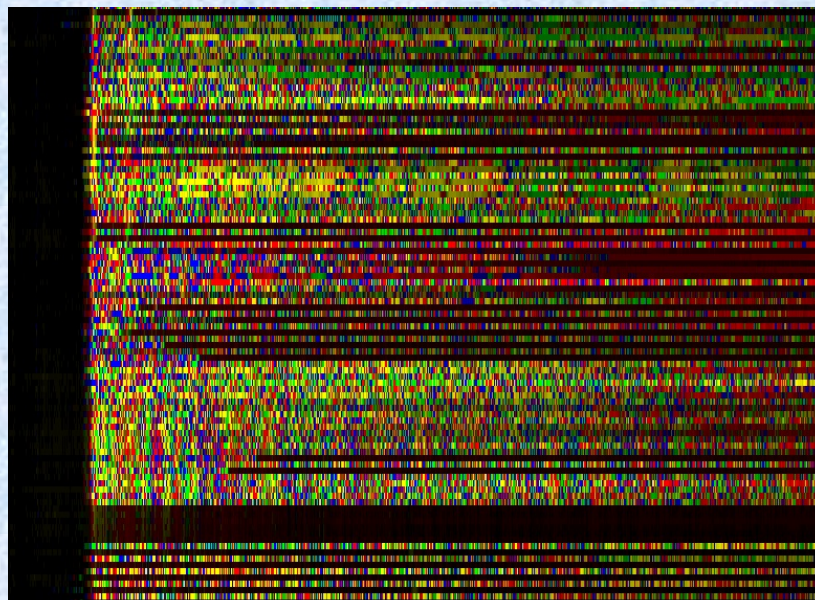




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Method	Manual Gel	377 Gel	3100 –16 Capillary	3730--96 Capillary
Bases per Day	1200?	20,000	100,000	0.5 to 1 million





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<http://dnalims.huck.psu.edu>

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For more information, please contact us.

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Voice: 814 867-4067

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Password *	<input type="password"/>
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Email Address *	<input type="text"/>

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Select the Affiliation Type before filling in Contact Info.

Affiliation

Name *	<input type="text"/>
Institution *	<input type="text"/>
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Bldg	<input type="text"/>
Room/Box No.	<input type="text"/>
Address 1 *	<input type="text"/>
Address 2	<input type="text"/>
City *	<input type="text"/>
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Phone *	<input type="text"/>
FAX	<input type="text"/>
User Email *	<input type="text"/>
PI *	<input type="text"/>
<small>Last, First (Do not add Ph.D., Dr., or Mr. etc)</small>	
PI Email *	<input type="text"/>

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PLEASE READ the instructions for primer and template requirements.
We MUST have 5 uls of template and 5 uls of primer for EACH reaction. If you do not supply the correct amount, you will have to wait longer for your results.

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PLEASE DROP OFF ALL SAMPLES IN ROOM 413 BETWEEN 8:00 AND 4:30PM. DO NOT BRING THEM TO ROOM 406. SAMPLES MUST BE HERE BY 9:00 TO BE RUN ON THE CUSTOM PLATE. IF WE HAVE TWO CUSTOM PLATES, RESULTS FOR THE SECOND PLATE WILL NOT BE AVAILABLE UNTIL THE NEXT MORNING. THANKS.

HAVE 35 OR MORE SAMPLES TO SUBMIT? ASK ABOUT PLATE SEQUENCING, IT COULD SAVE YOU MONEY.

Sequencing

Enter Individual DNA Sequencing Requests

[Upload and Import Excel File for Sequencing 96 Well Plates](#)

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Fragment Analysis

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
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Enter the Number of Reactions to Create Sequencing Requests For:

Select the Sequencing Type.

Service Requested:

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
<u>Services for use with Upload & Import</u>	
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



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DNA Sequencing Request Form - Mozilla Firefox

File Edit View History Bookmarks Tools Help

http://tanager.huck.psu.edu/cgi-bin/dna/seqTablepsu.cgi

Do you want Firefox to remember this password? Remember Never for This Site Not Now

Sequencing_short Request Form

Principal Investigator:

Comments:

TEMPLATES AND PRIMERS MUST BE IN WATER (NOT TRIS-EDTA) and each in separate tubes.	
Template Requirements per Reaction	5ul @ 200-300 ng/ul for plasmid DNA 5ul @ 20 ng/ul for PCR up to 400 bases; 40 ng/ul for 400 -> 1000 5ul @ 0.4 ug/ul for Large DNA
Primer Requirements per Reaction	5ul @ 1 uM for plasmid & PCR 5ul @ 10 uM for Large DNA
Primers Provided, N/C	T7, T7Term, T3, SP6, M13 Universal, M13-40, M13-47, BGHrevprim, M13 Reverse, M13 Reverse-48, Gal4AD, Gal4BD, Poly dT mix. Inquire about other available primers.

No Validation response means all required fields are filled in.

When entering Sample and Primer names, please ONLY use letters, numbers, and underscores.

Template Learn More			DNA Type		Primer Learn More	
Name	Conc. ng/ul	Volume	Type	Size bp	Name	Vol. 5ul@1uM
<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
1			-- Select --		-- Select --	5
2			-- Select --		-- Select --	5

Done

Microsoft PowerPoi... DNA Sequencing Re... 9:58 AM



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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: no file selected

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



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 μ l
- 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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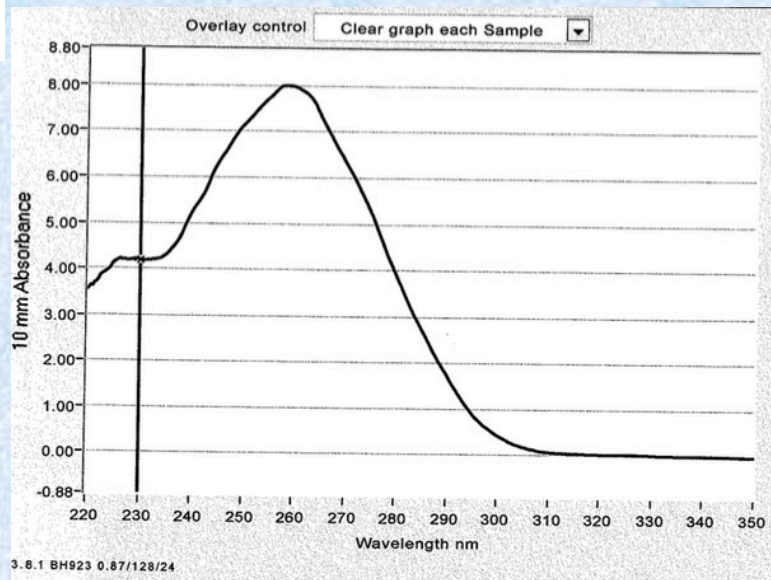
Keys to Success

- Sample Quality
- Sample Quantity

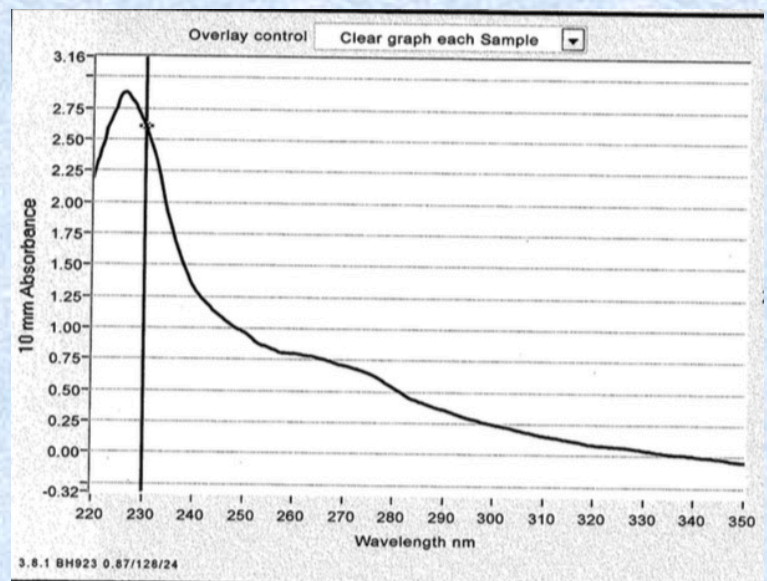


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$$260/280=2.07$$
$$260/230=1.90$$



$$260/280=1.52$$
$$260/230=0.31$$



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Extraction Tips

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



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.
- T_m 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 Geospiza.
- 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 fasta qual scf 1037	Results Availab
cs	1064804	Text Electropherogram	View	11854-36	D5	pGem	CP1	heintz,ginger	3023	Nov 20 2015	phd fasta qual scf 997	Results Availab
45467	1064790	Text Electropherogram	View	11854-37	E5	CTMx14	Mx14A	phillips,allen	1642	Nov 20 2015	phd fasta qual scf 700	Results Availab

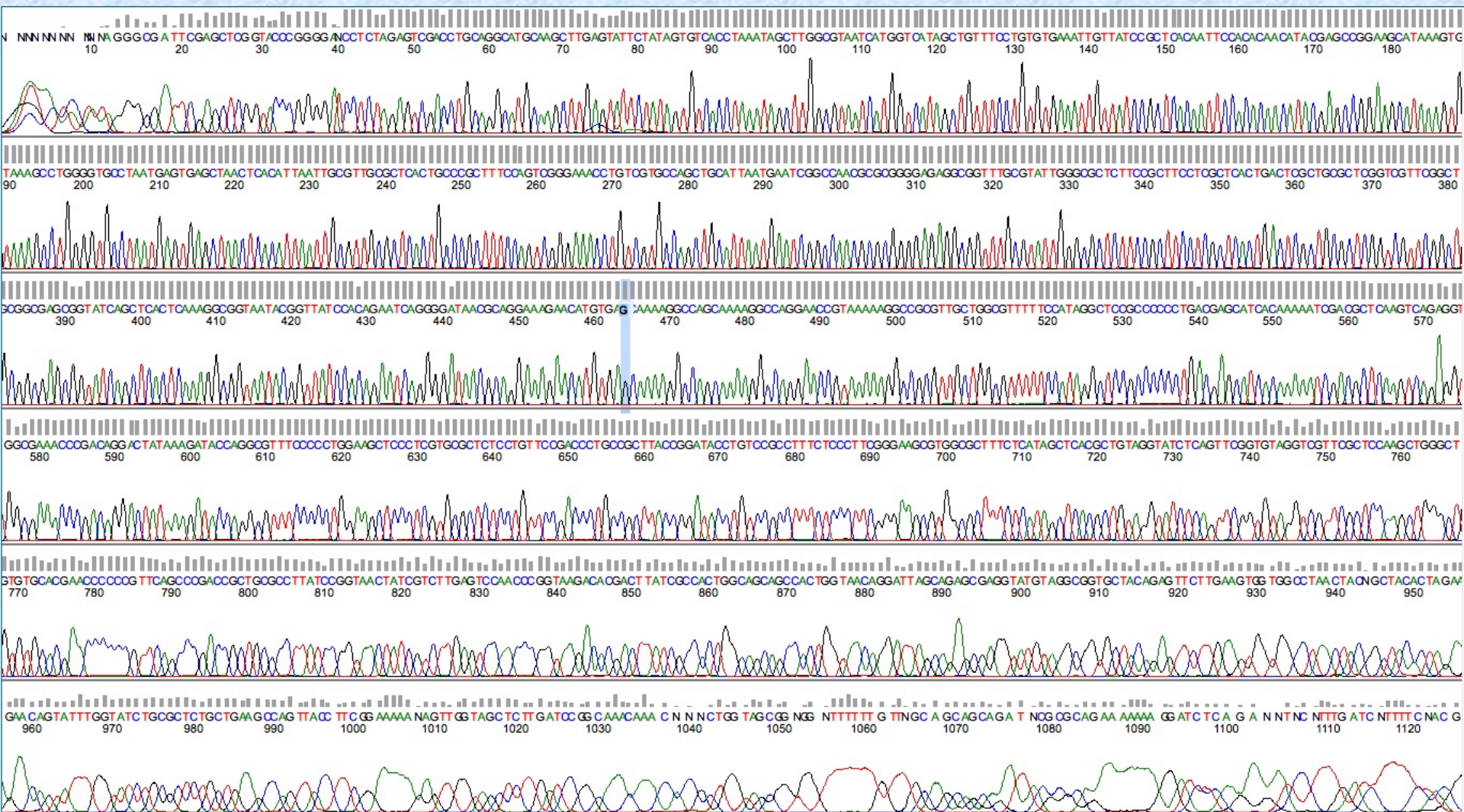




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Control PGEM Plasmid

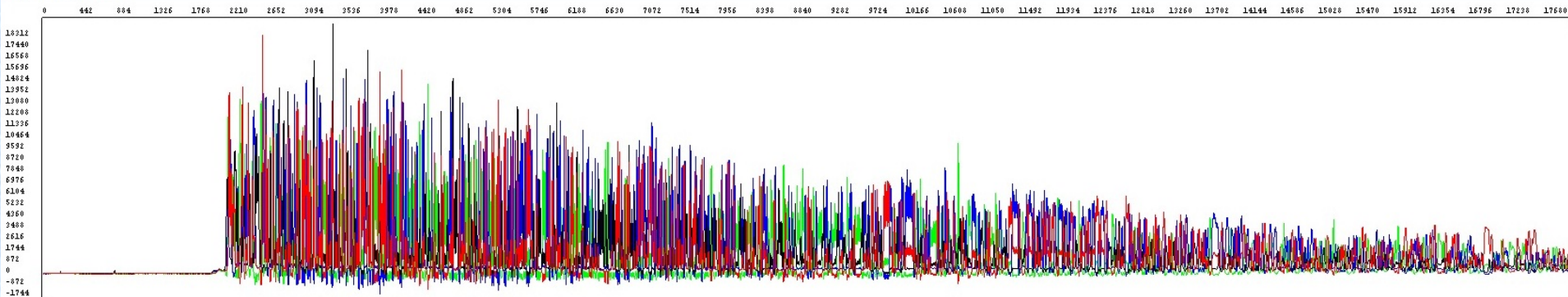




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CU4_pGem-CP1_2013-12-03

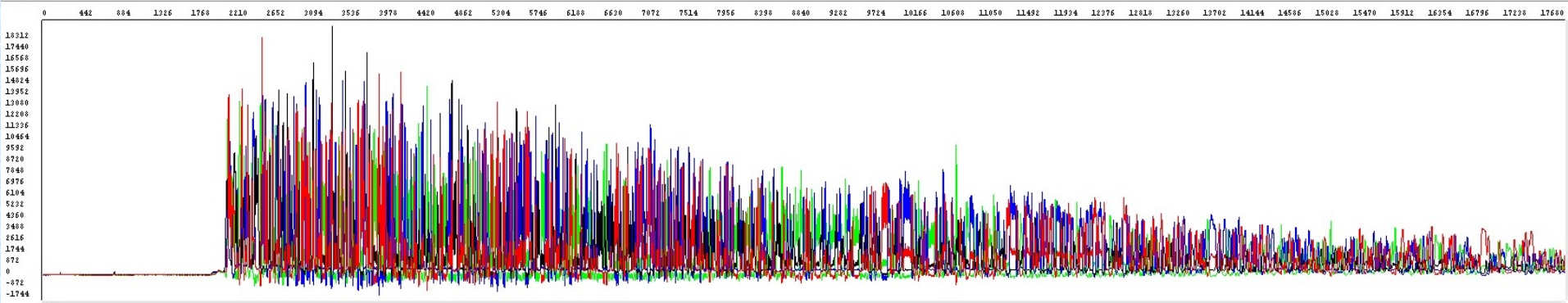




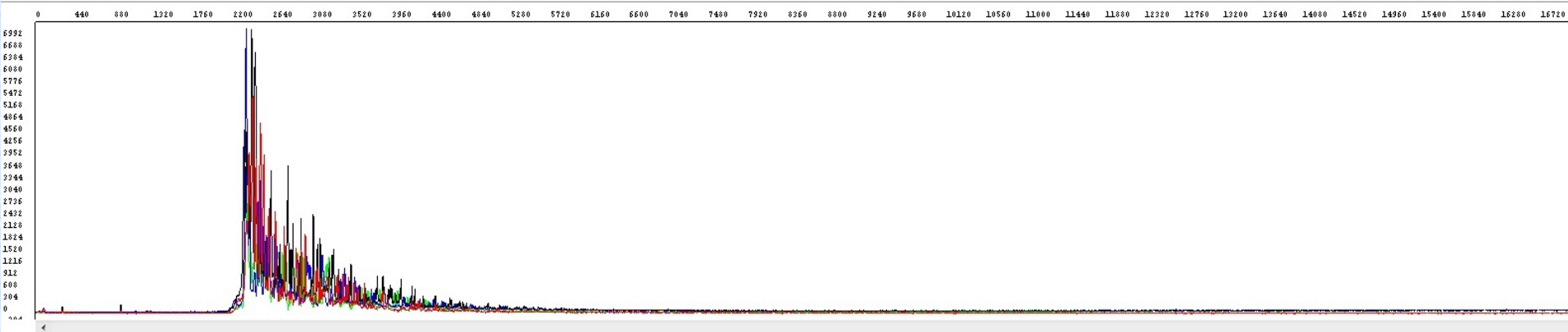
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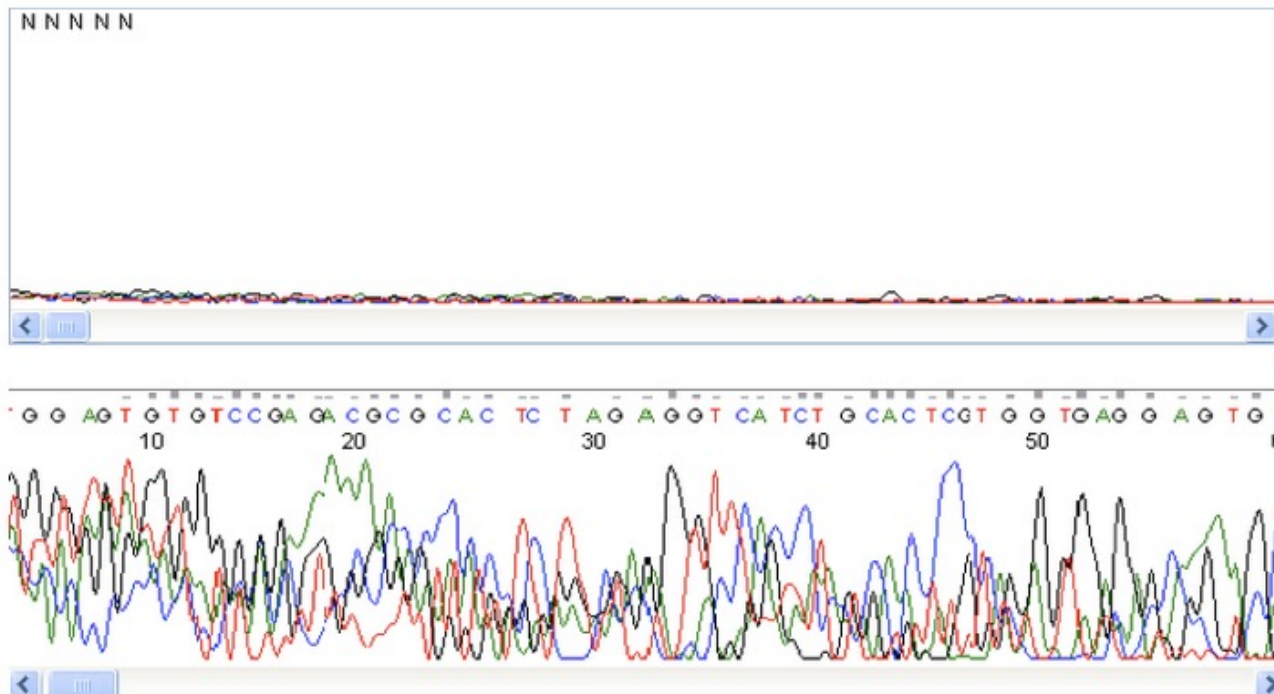
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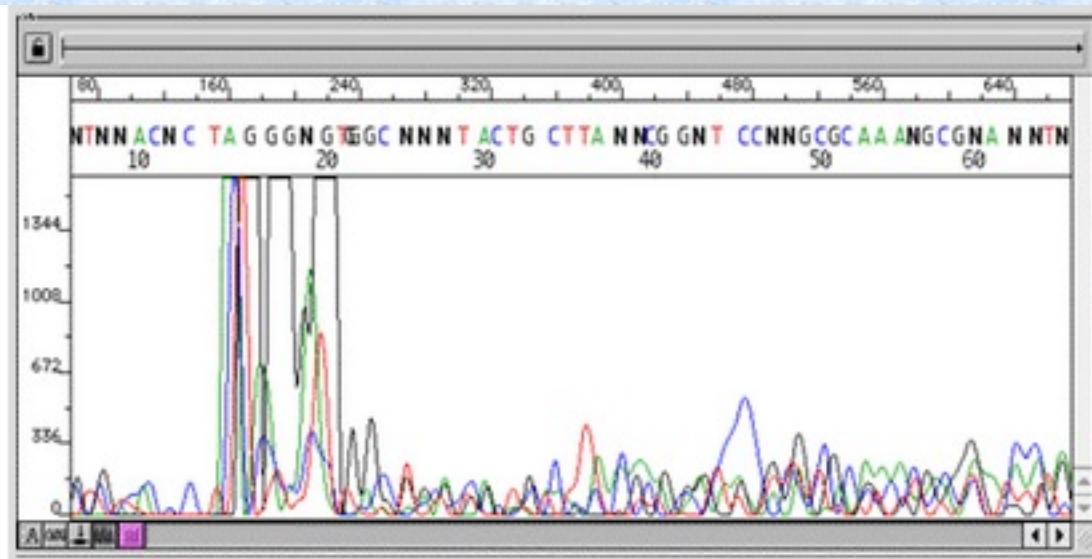


- Poor Quality or Wrong concentration
- Wrong Primer
- Degraded Primer
- Contamination
- Forgot to put template in the tube



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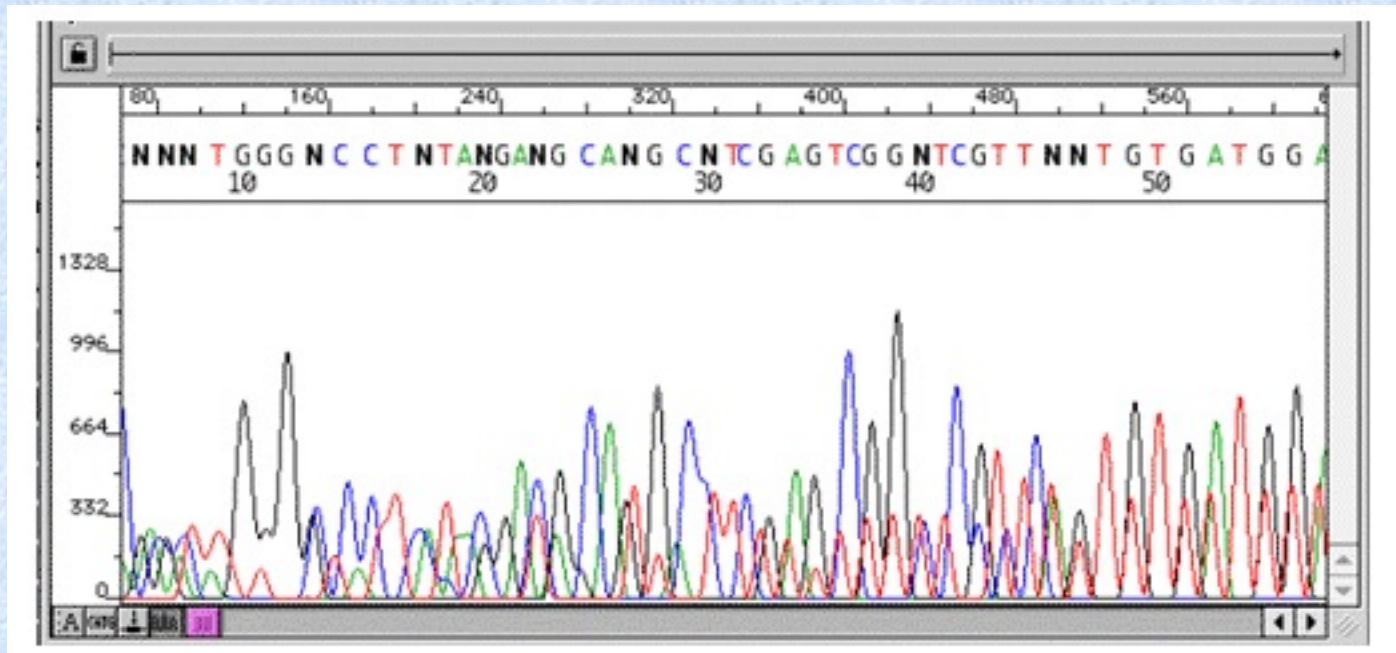


Cause: priming site not present



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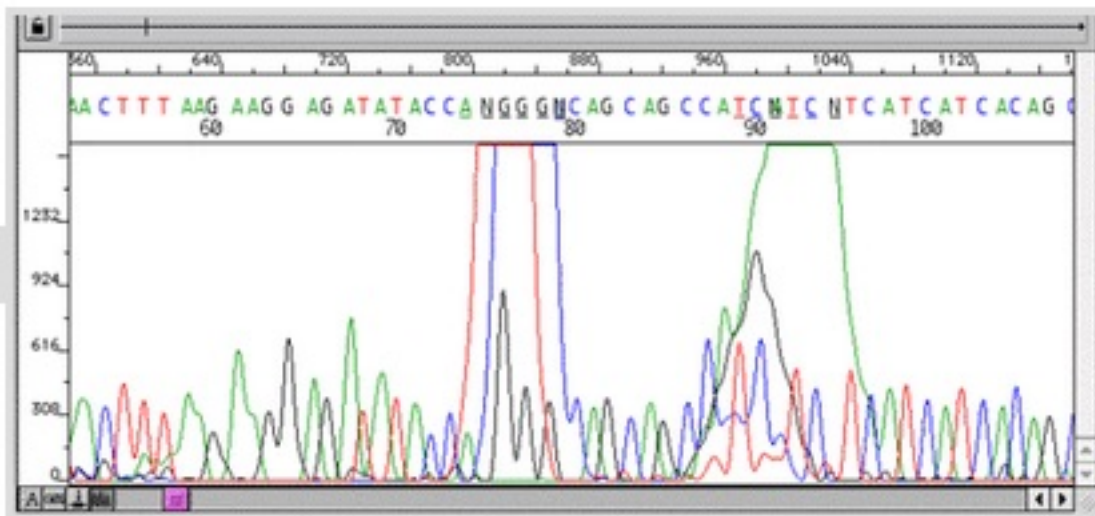
- Primer annealing to multiple sites
- Residual PCR Primers
- Mixed Plasmid or PCR products
- Degenerate Primers



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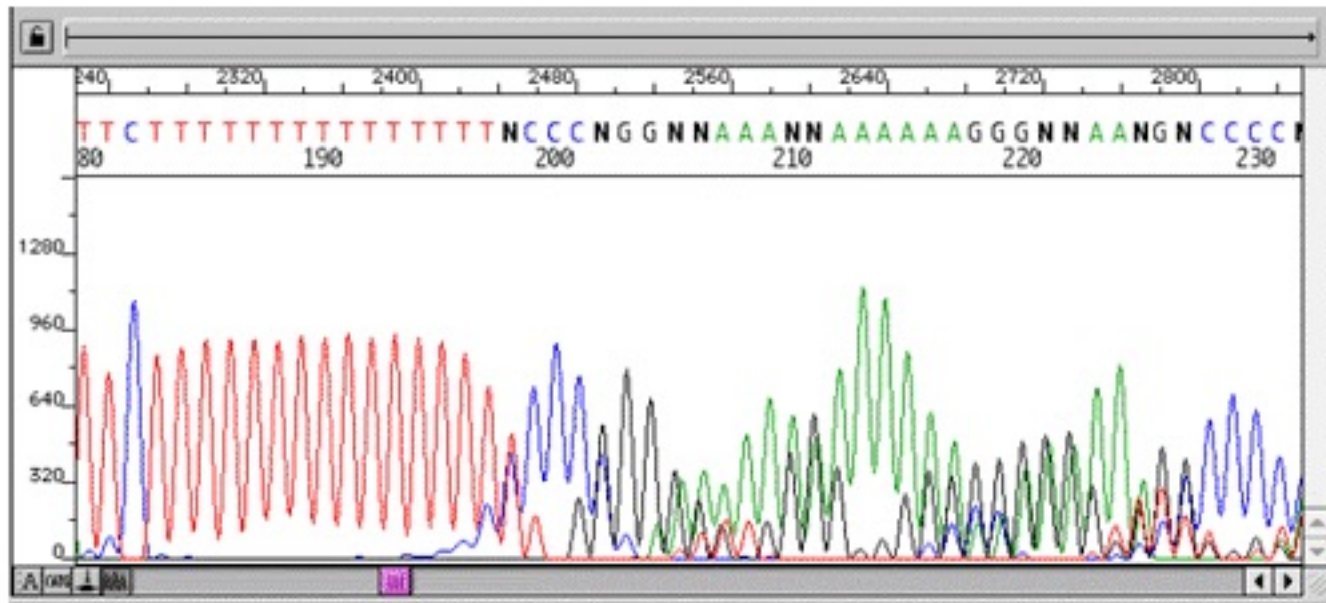
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Artifact: "dye blobs"





Cause: homopolymeric regions



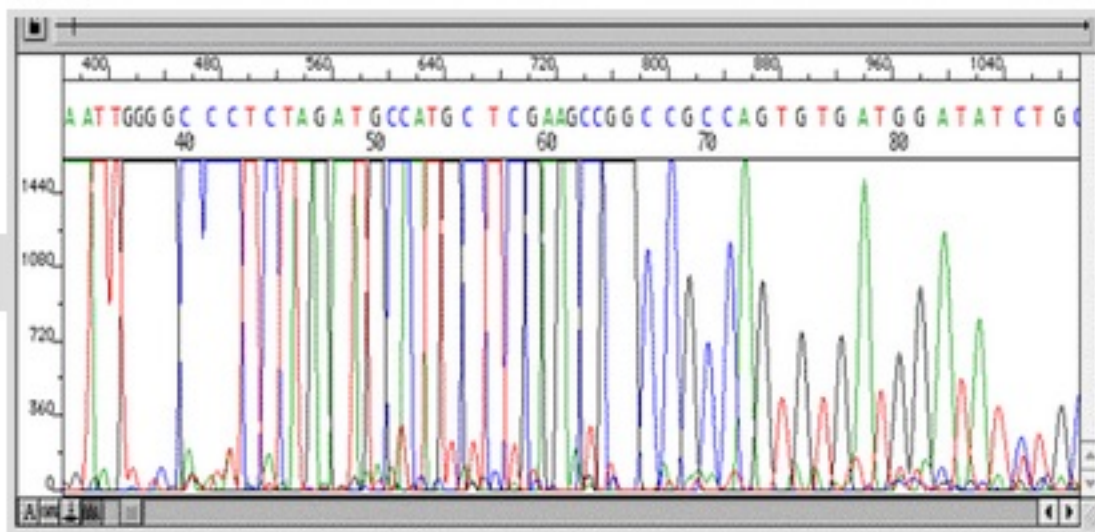
We have primers that can “anchor” and continue the sequence.



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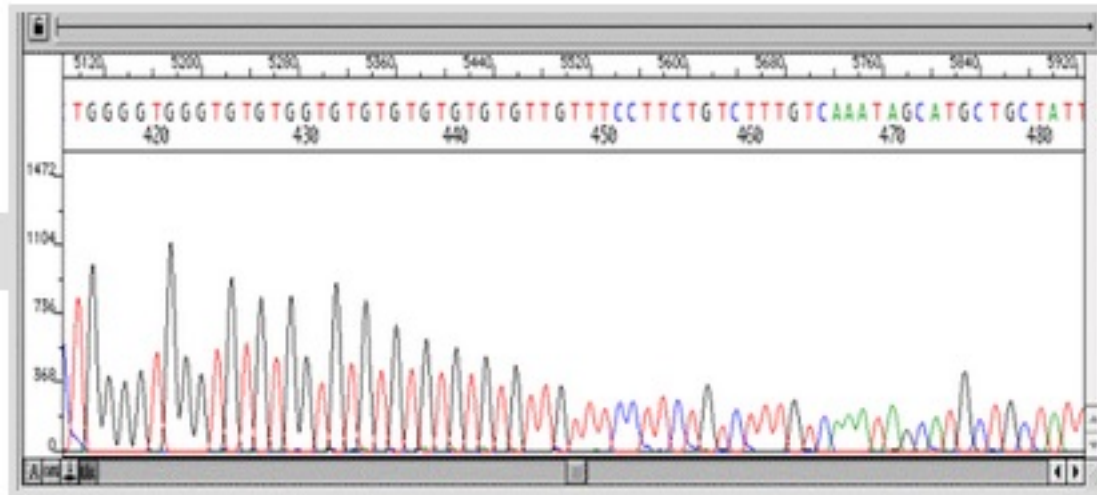
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Cause: too much DNA



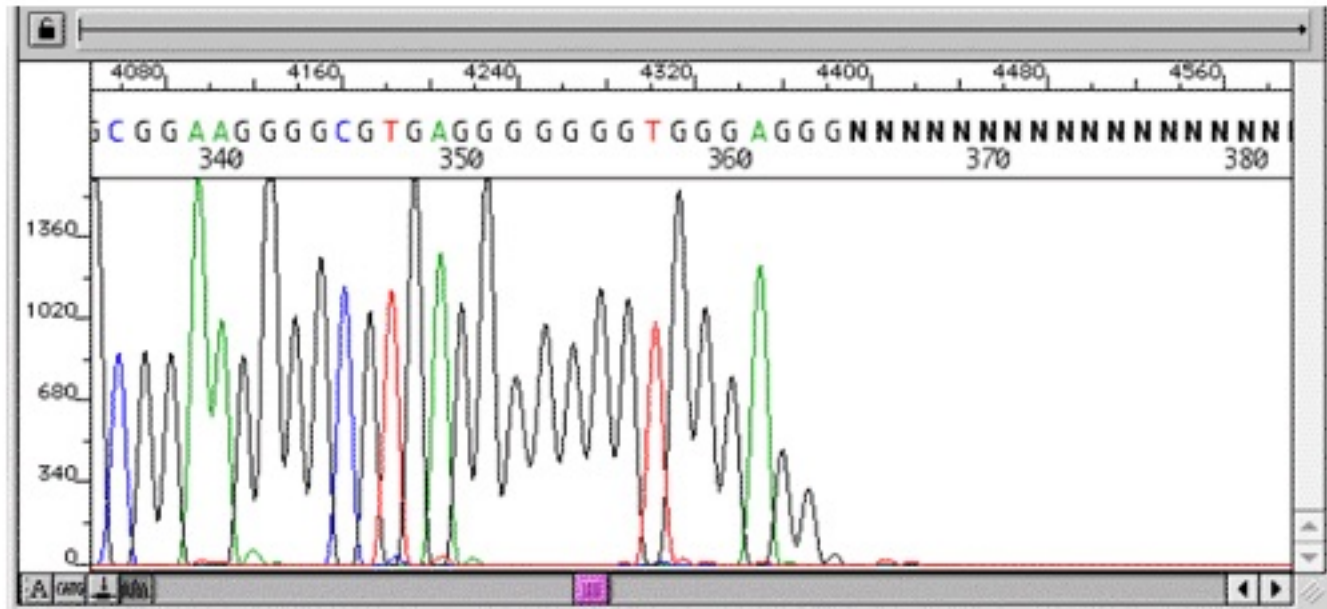
- Presence of salts
- Primer concentration too high

Cause: repetitive regions



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

Cause: secondary structure



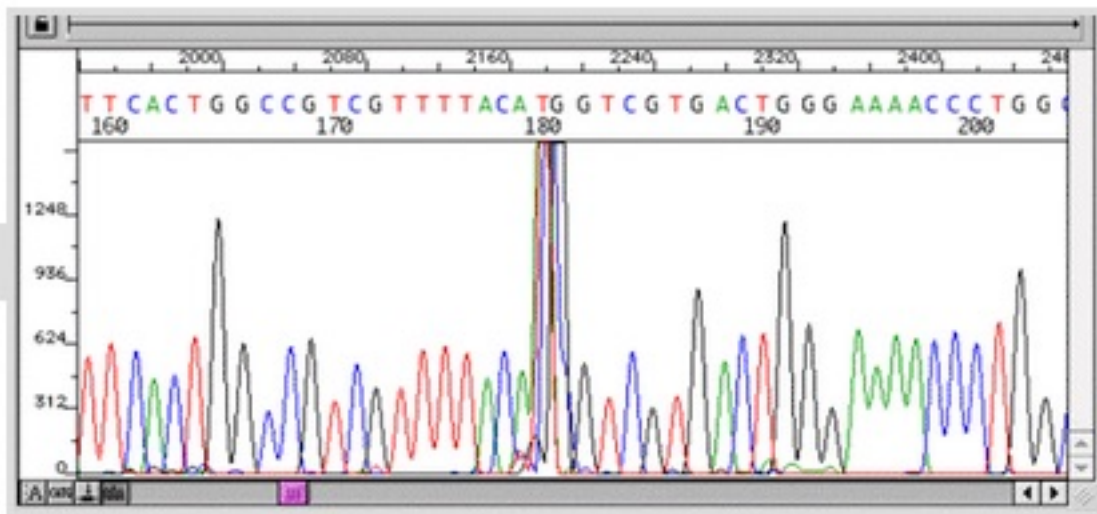
Request our special Stop Protocol.



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Artifact: "spikes"

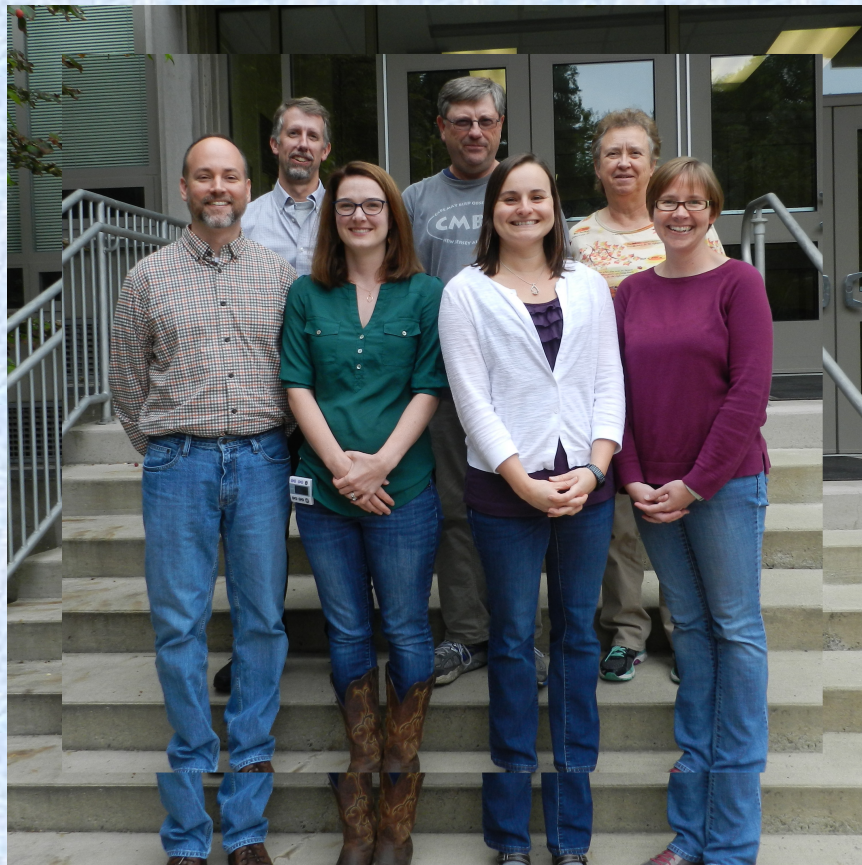


Request a Re-run. Possibilities are a bubble in the array or could be low template concentration and electrical spike.



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