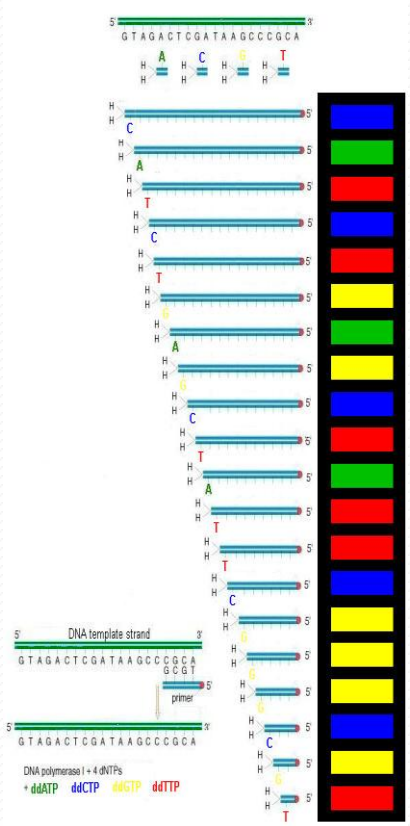
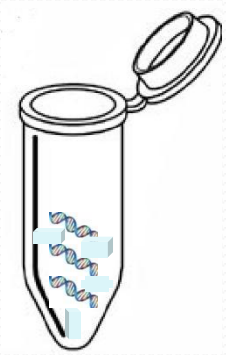
# DNA Sequencing
# Data Evaluation



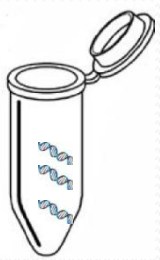

**Dr. Serageldeen A. A. Sultan**

PhD in Molecular virology
Yamaguchi University, Japan (2010)
Lecturer of virology
Dept. of Microbiology
SVU, Qena, Egypt
seaas@lycos.com

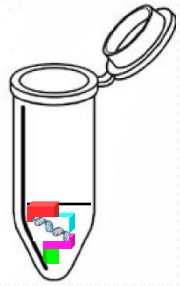
# Pathway of sequencing reaction



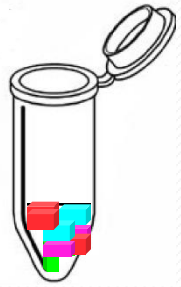
**1. Purify PCR product**



**2. Set up sequencing reaction**

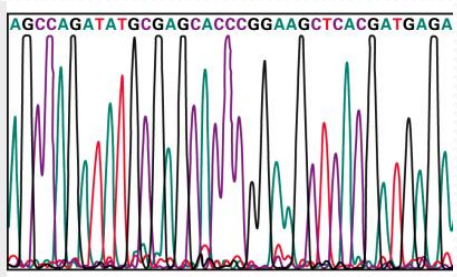


**3. Perform cycle sequencing**



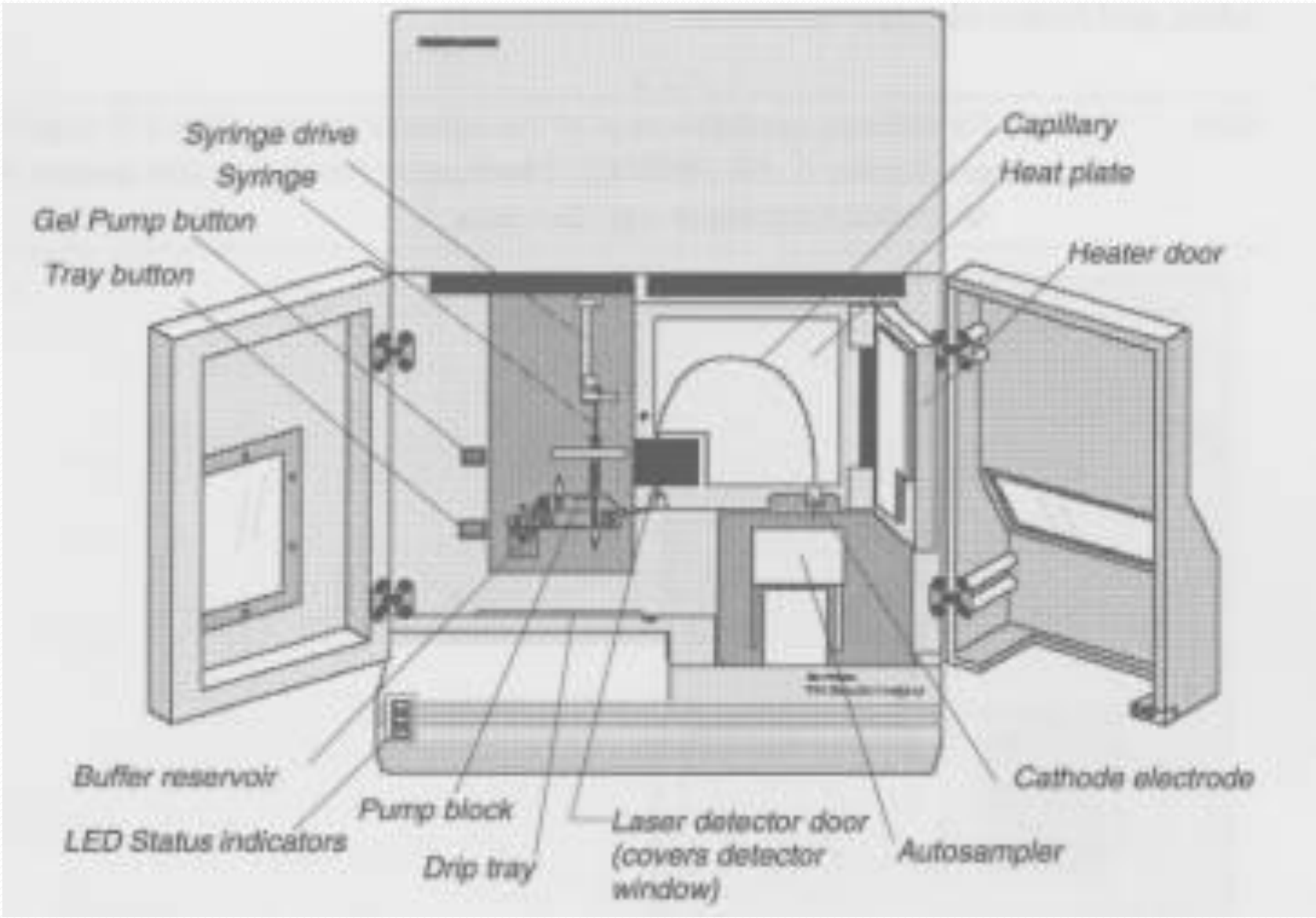
**4. Resolve sequence fragments**

**5. Read order of terminators (DNA sequence)**

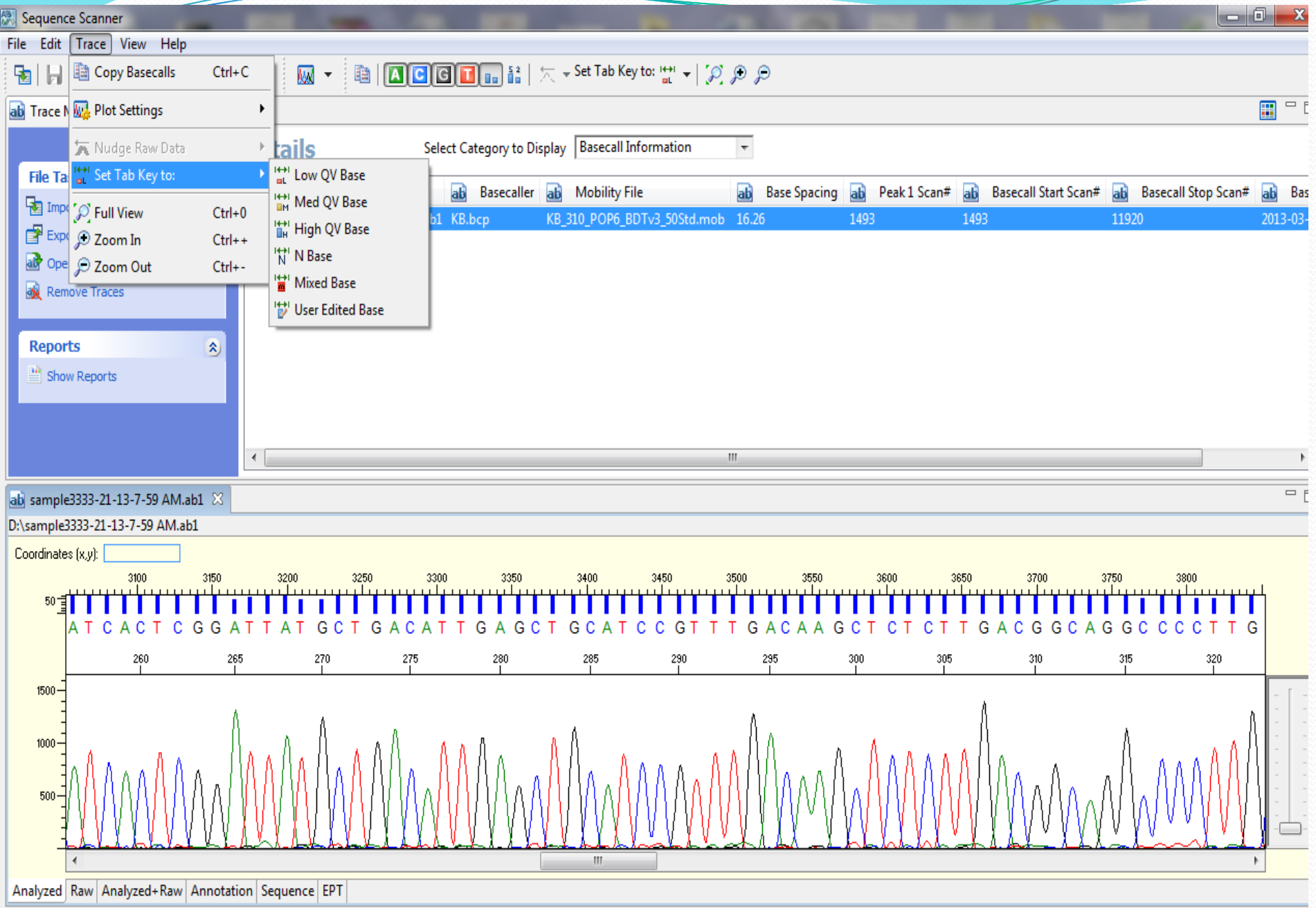


**ABI 310 Sequencer**

# Parts of ABI 310 Sequencer



# How can you evaluate sequencing results?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Plot Settings
Nudge Raw Data
Set Tab Key to:
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Zoom In Ctrl++
Zoom Out Ctrl+-
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Med QV Base
High QV Base
N Base
Mixed Base
User Edited Base
Select Category to Display
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Basecaller
Mobility File
Base Spacing
Peak 1 Scan#
Basecall Start Scan#
Basecall Stop Scan#
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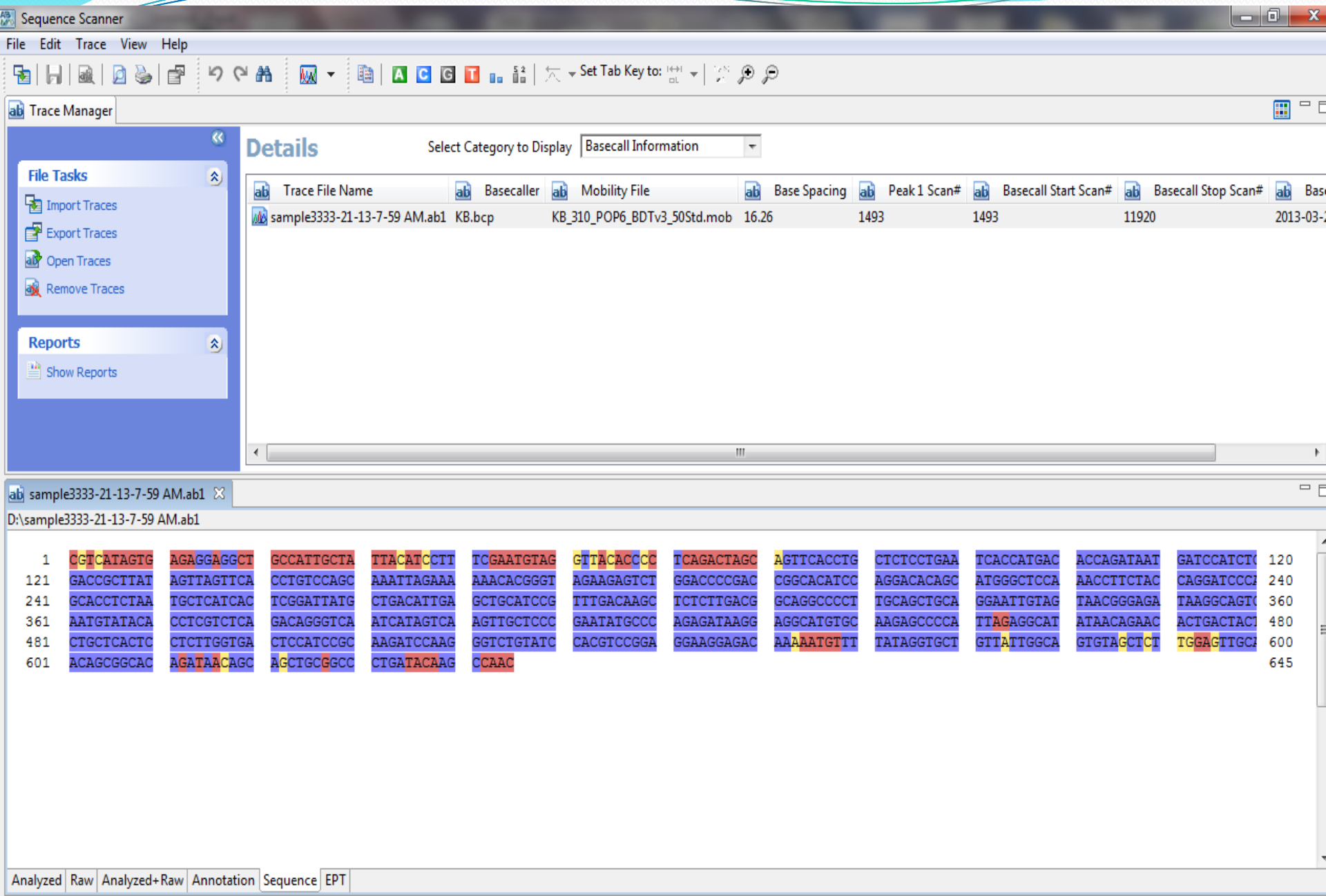
16.26
1493
1493
11920
2013-03-
Remove Traces
Reports
Show Reports
sample3333-21-13-7-59 AM.ab1
D:\sample3333-21-13-7-59 AM.ab1
Coordinates (x,y):
A T C A C T C G G A T T A T G C T G A C A T T G A G C T G C A T C C G T T T G A C A A G C T C T C T T G A C G G C A G G C C C C T T G
Analyzed
Raw
Analyzed+Raw
Annotation
Sequence
EPT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File Edit Trace View Help
Set Tab Key to:
Trace Manager
File Tasks
Import Traces
Export Traces
Open Traces
Remove Traces
Reports
Show Reports
Details
Select Category to Display
Basecall Information
Trace File Name
Basecaller
Mobility File
Base Spacing
Peak 1 Scan#
Basecall Start Scan#
Basecall Stop Scan#
sample3333-21-13-7-59 AM.ab1
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16.26
1493
1493
11920
2013-03-
sample3333-21-13-7-59 AM.ab1
D:\sample3333-21-13-7-59 AM.ab1
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121 GACCGCTTAT AGTTAGTTCA CCTGTCCAGC AAATTAGAAA AAACACGGGT AGAAGAGTCT GGACCCCGAC CGGCACATCC AGGACACAGC ATGGGCTCCA AACCTTCTAC CAGGATCCCA 240
241 GCACCTCTAA TGCTCATCAC TCGGATTATG CTGACATTGA GCTGCATCCG TTTGACAAGC TCTCTTGACG GCAGGCCCCT TGCAGCTGCA GGAATTGTAG TAACGGGAGA TAAGGCAGTC 360
361 AATGTATACA CCTCGTCTCA GACAGGGTCA ATCATAGTCA AGTTGCTCCC GAATATGCCC AGAGATAAGG AGGCATGTGC AAGAGCCCCA TTAGAGGCAT ATAACAGAAC ACTGACTACT 480
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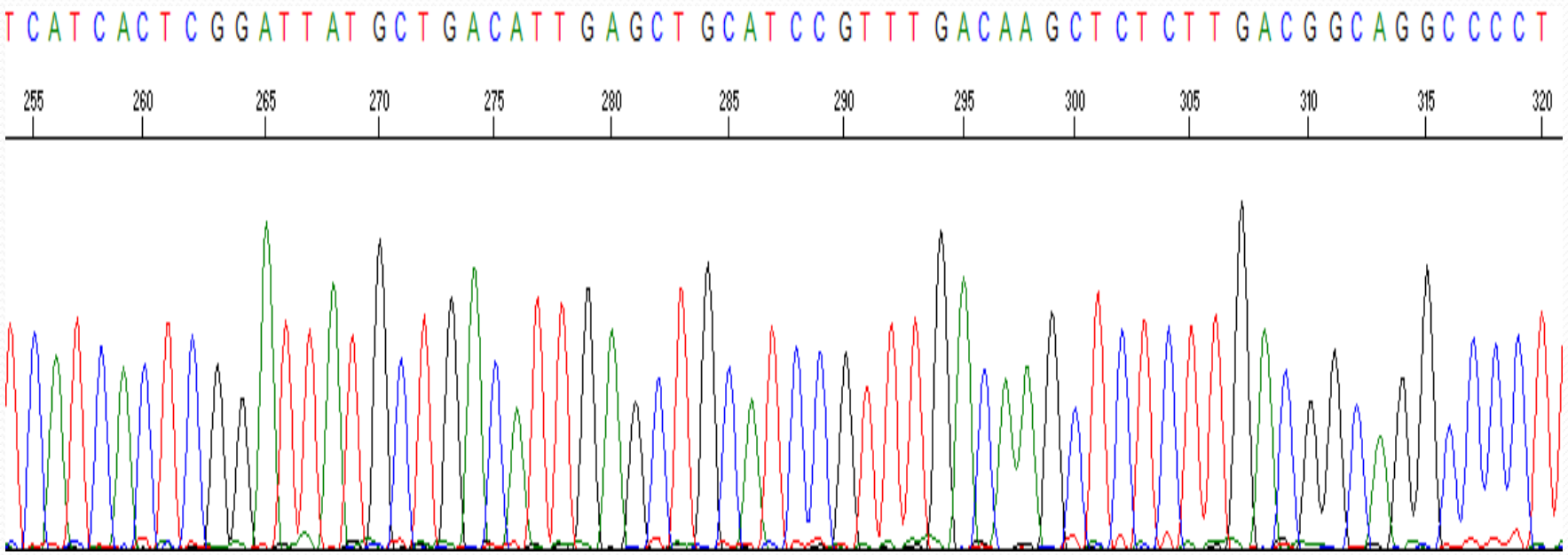
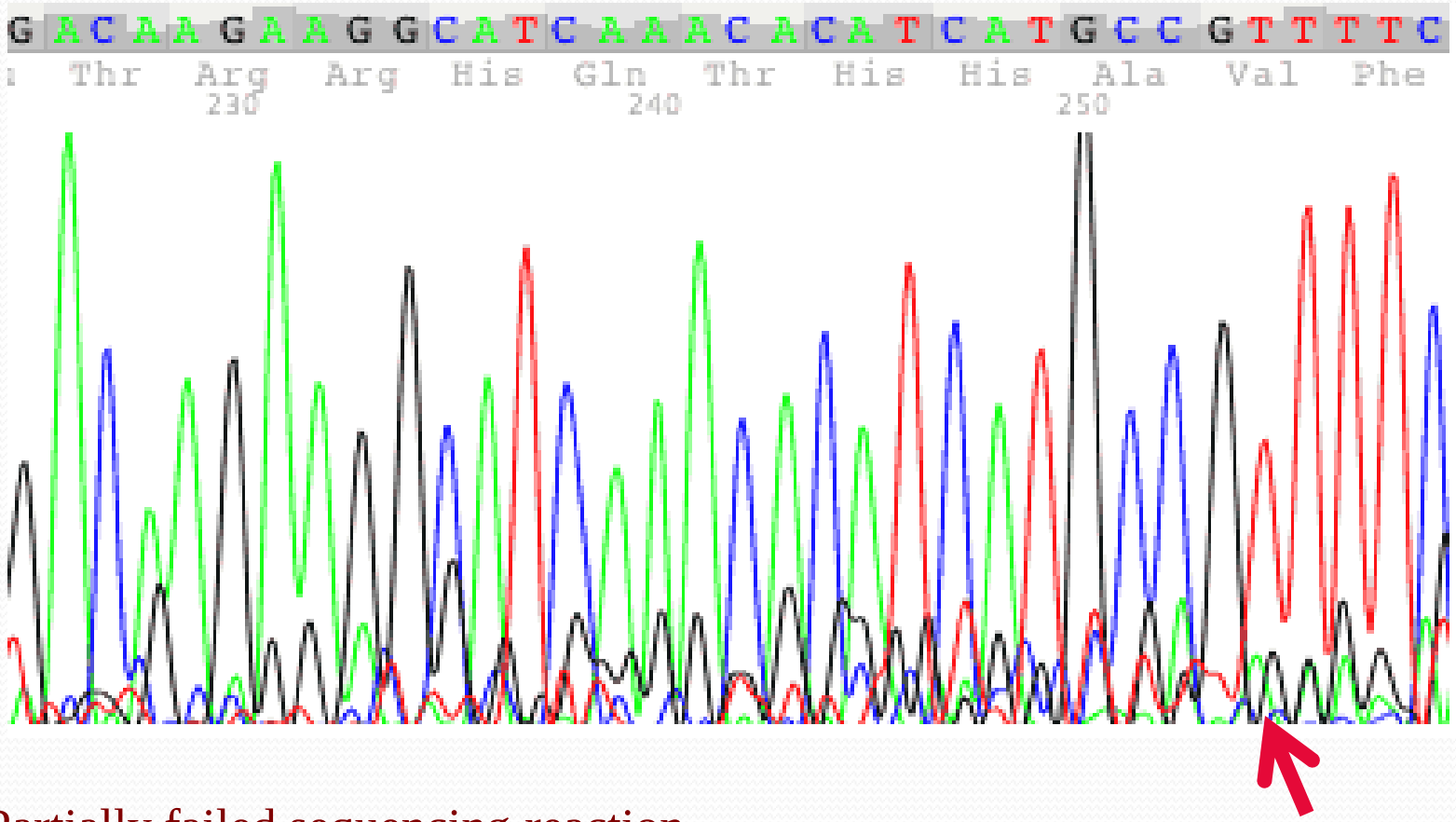
Analyzed
Raw
Analyzed+Raw
Annotation
Sequence
EPT



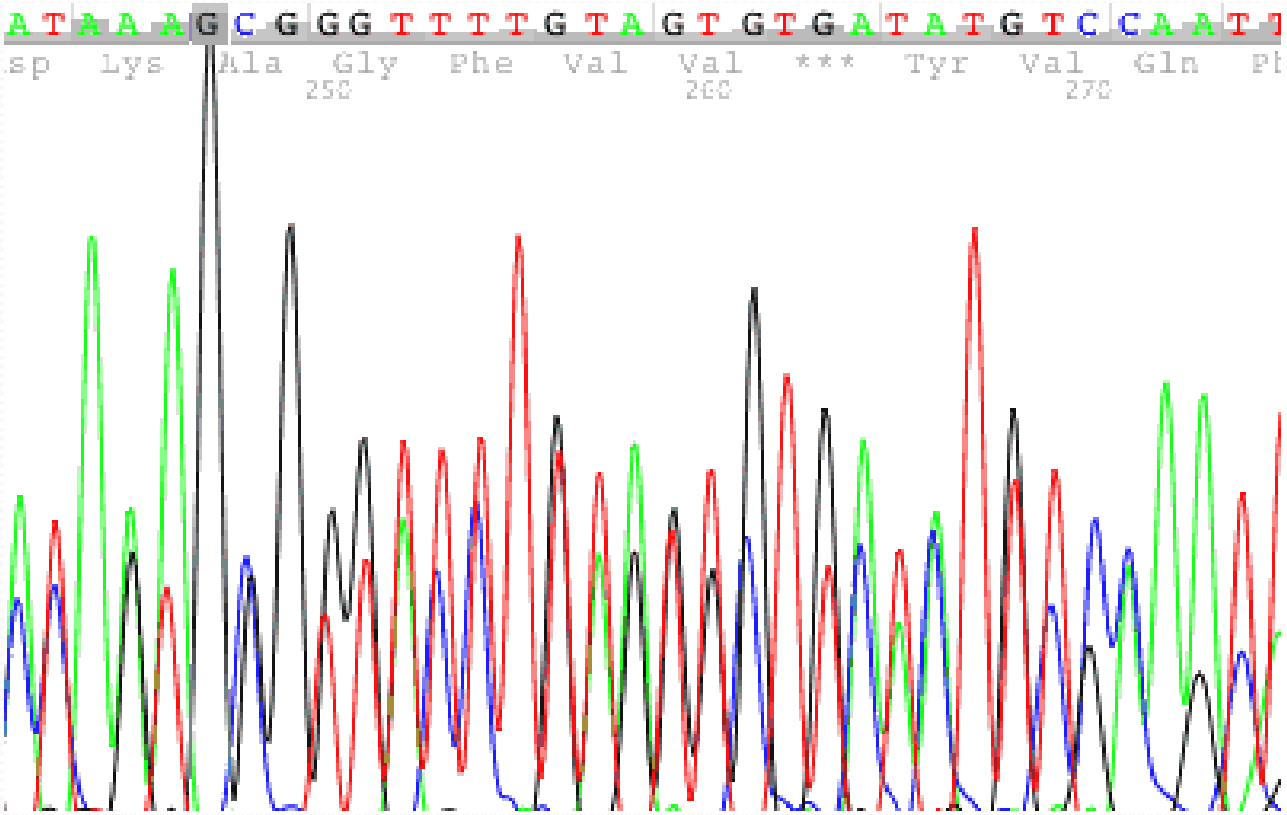

# DNA sequencing results

# Noisy or weak signal



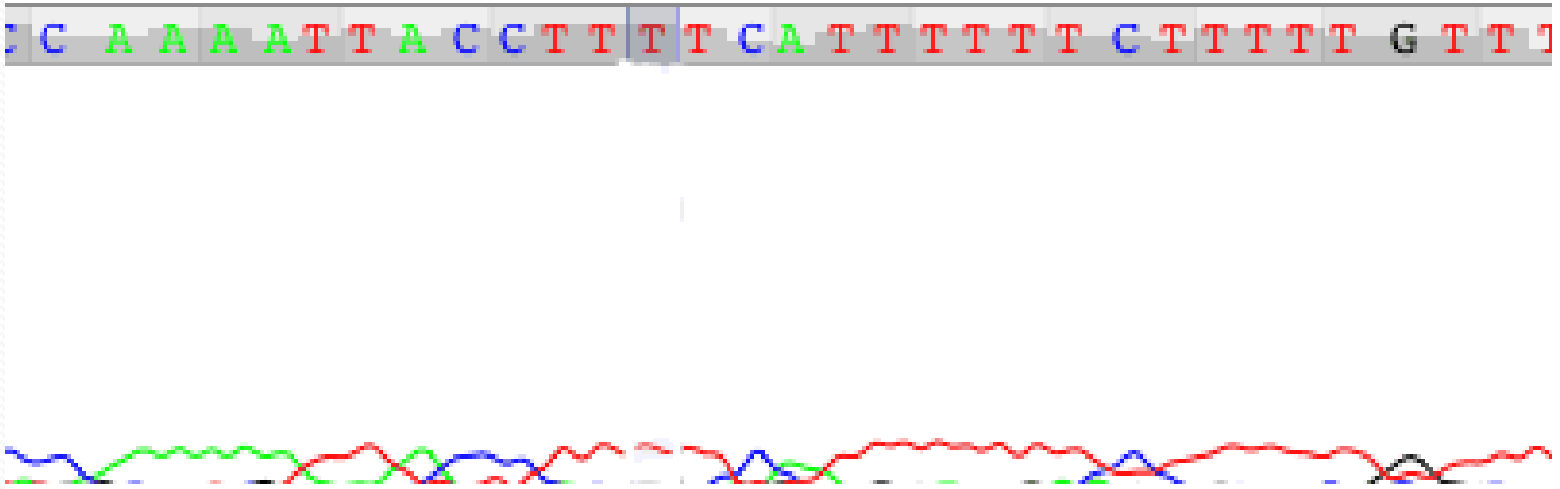

- Partially failed sequencing reaction.
- Too much or too little DNA.
- Partial loss of sample during clean-up

# Mixed DNA templates



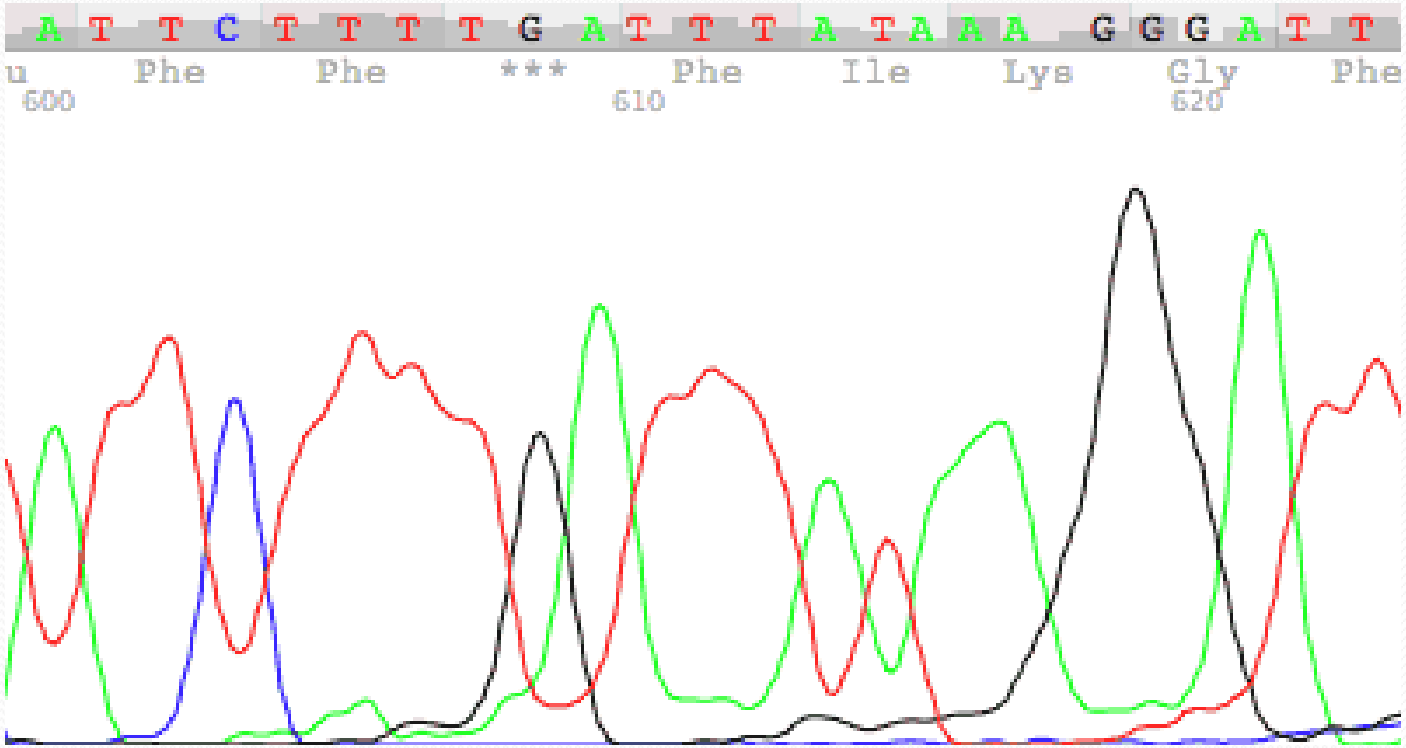

- Two or more templates were present in the reaction
- Two primers were present in the sequencing reactions
- Two priming sites are present in DNA template
- Different sequencing reactions were accidentally mixed at the clean up stage

# Short DNA sequencing read lengths



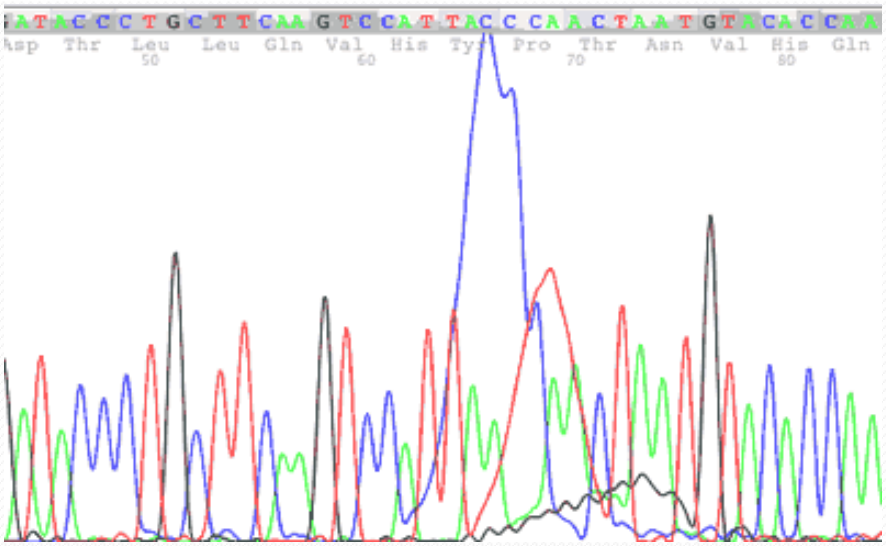

- Too much template DNA
- Excessive dilution of the BigDye reagent
- Too much primer

# Blurry trace chromatogram peaks

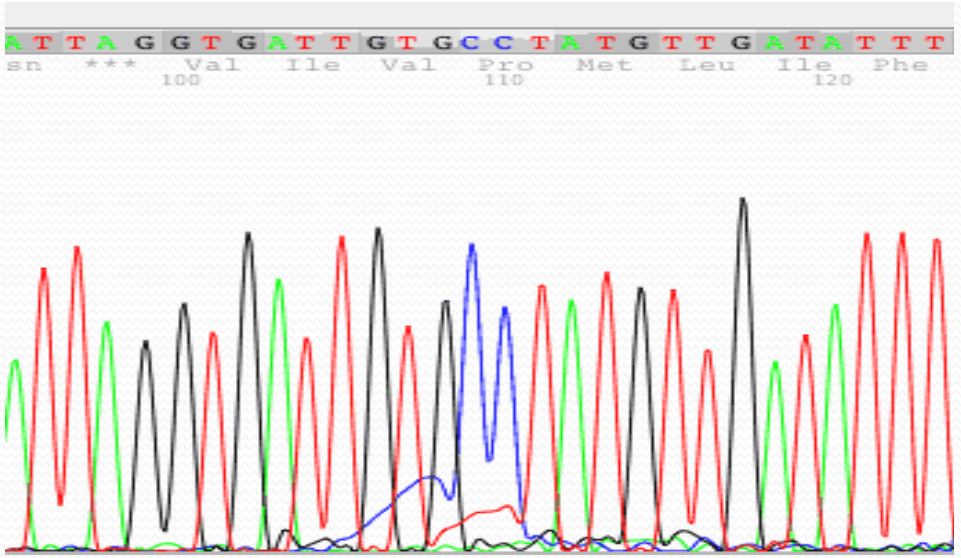



- **Capillary overload** (dirty samples with large amount of DNA, proteins or salts)
- **High sequencing run voltages**

# Dye Blobs (excess free dye terminators)



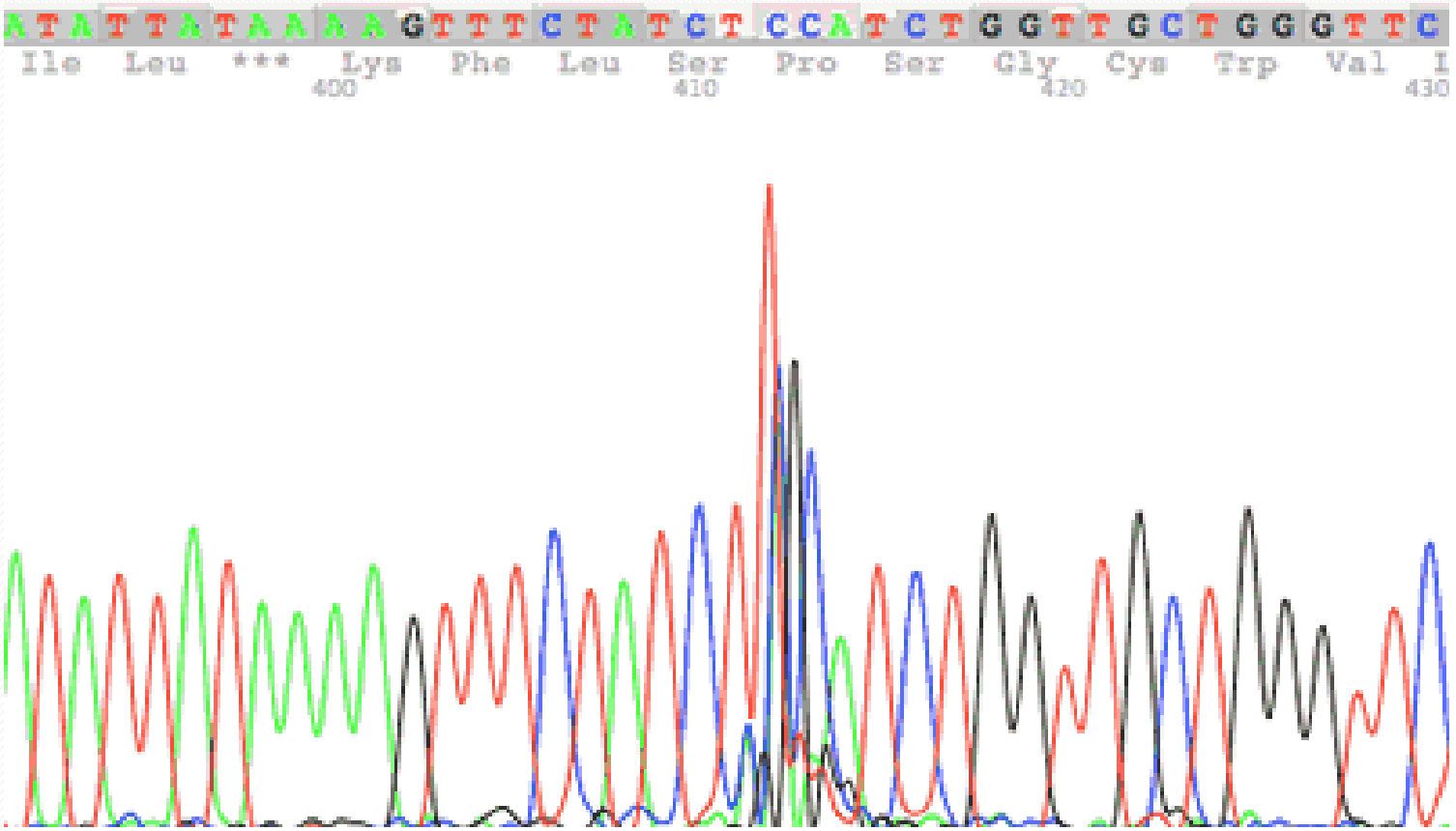
Moderate dye blob



Minor dye blob

-Poor post-sequencing clean up
-Lost pellet
-Failed reaction

# Sharp trace signal spikes

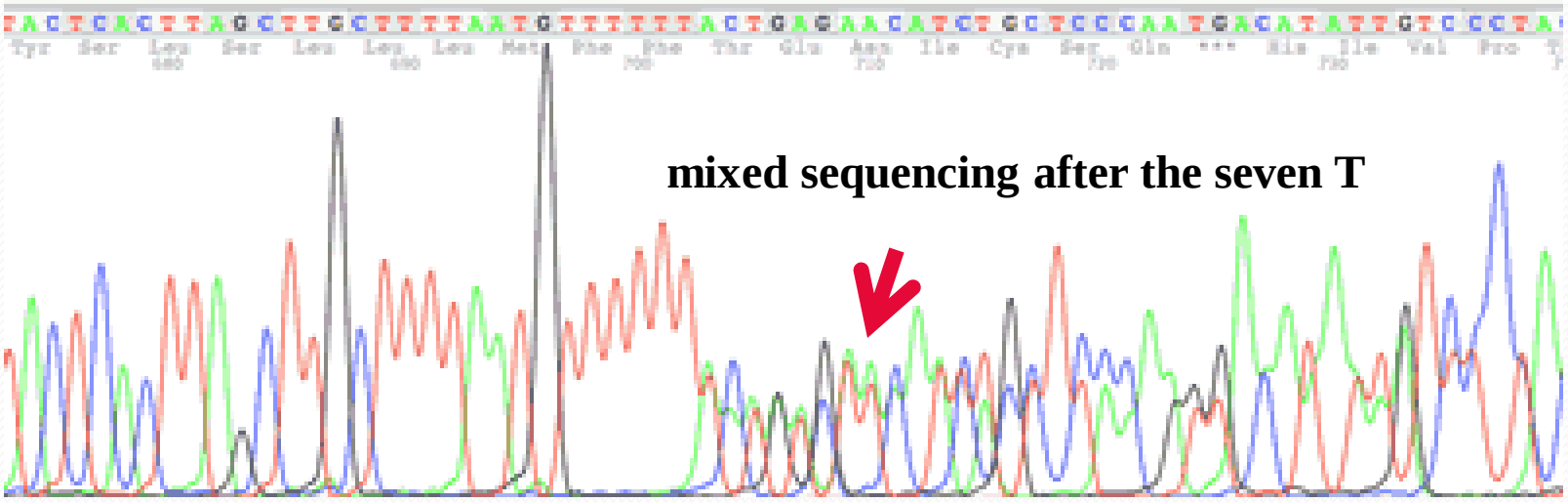


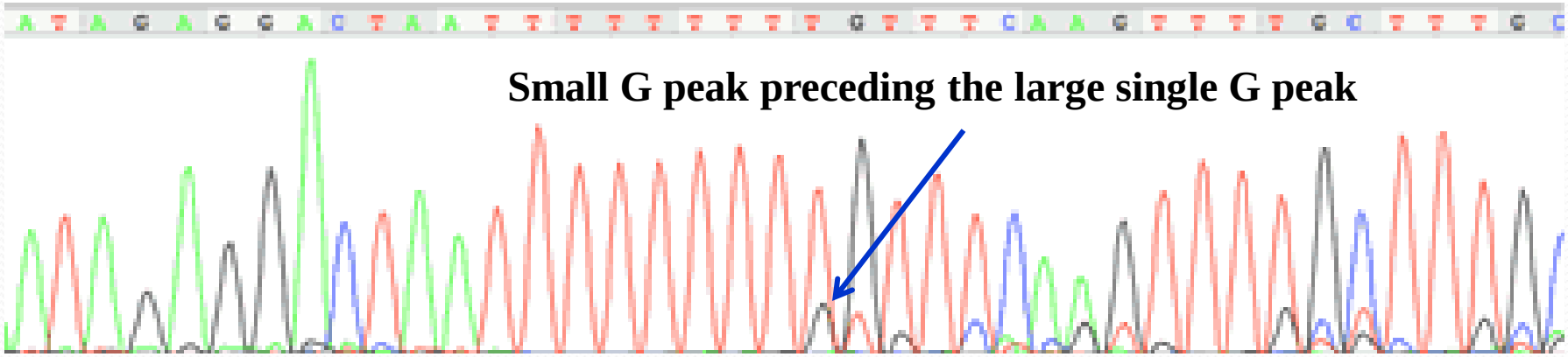

**Small gas bubbles forming in the capillaries during electrophoreses**

# Sequence Slippage

**Long runs of a mono nucleotide base**

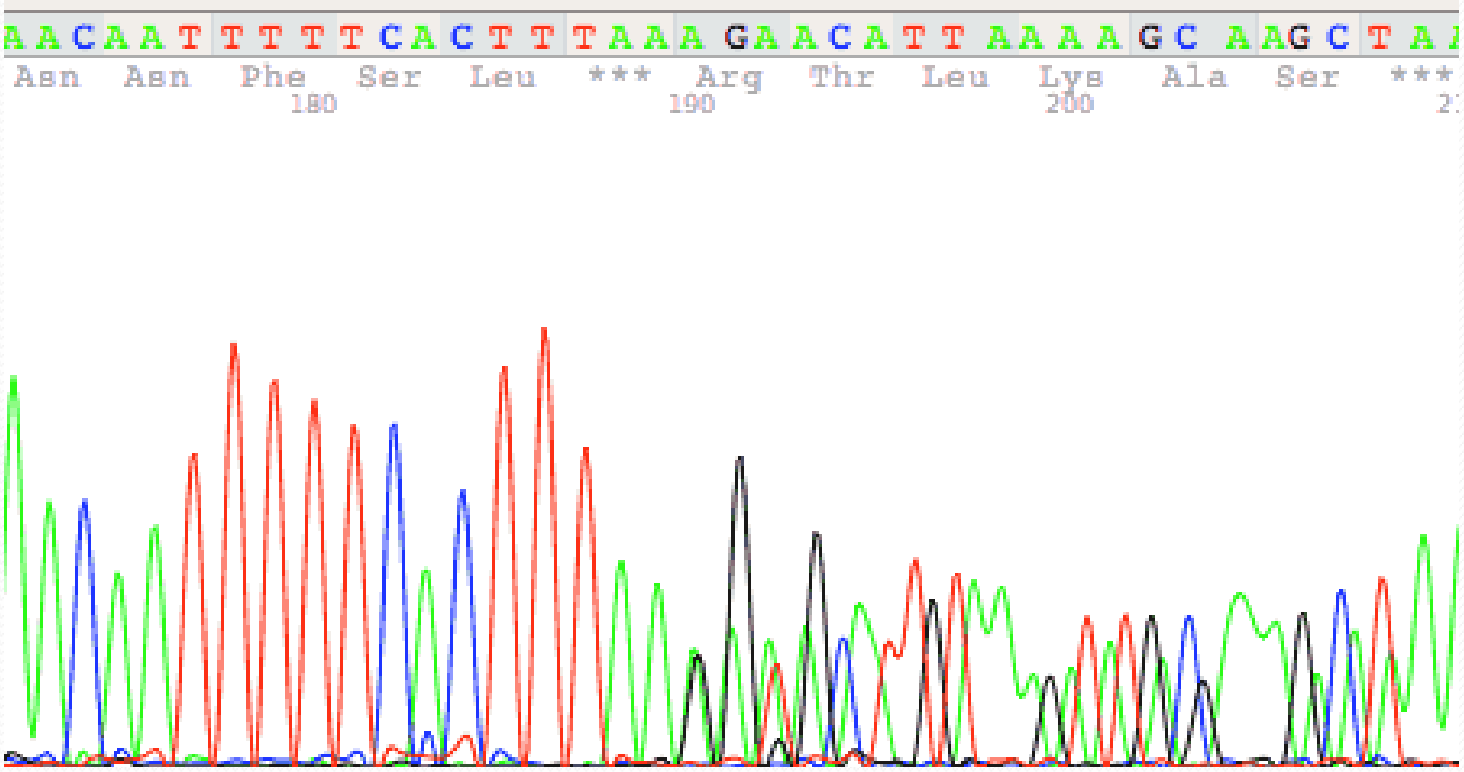
# Template insertion and deletions



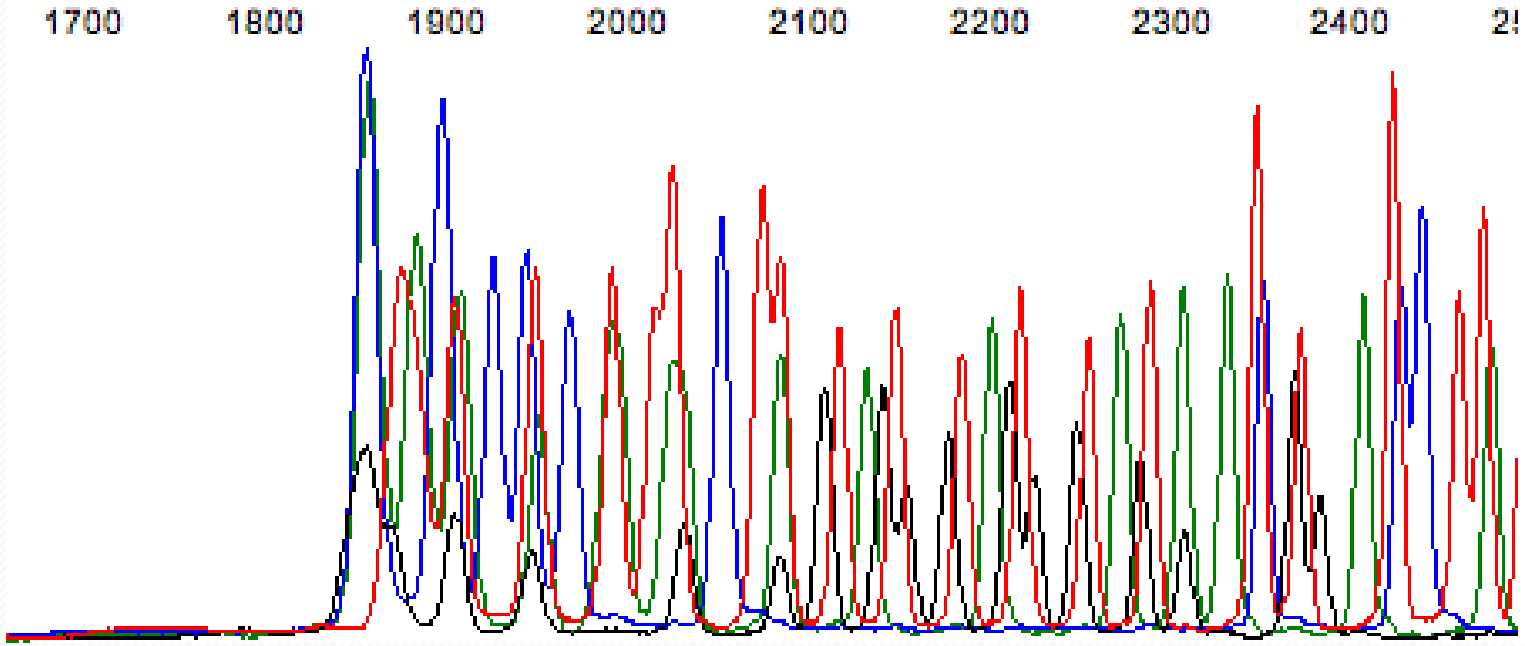

- Direct sequencing of PCR products derived from polymorphic templates
- Random mutation has occurred during the cloning process

# Chimeras and sequence rearrangements




- Cloning two or more unrelated DNA fragments at once in the same vector
- 'Unstable' sequences like repeats as long poly A tails

# Delayed sequencing signal start



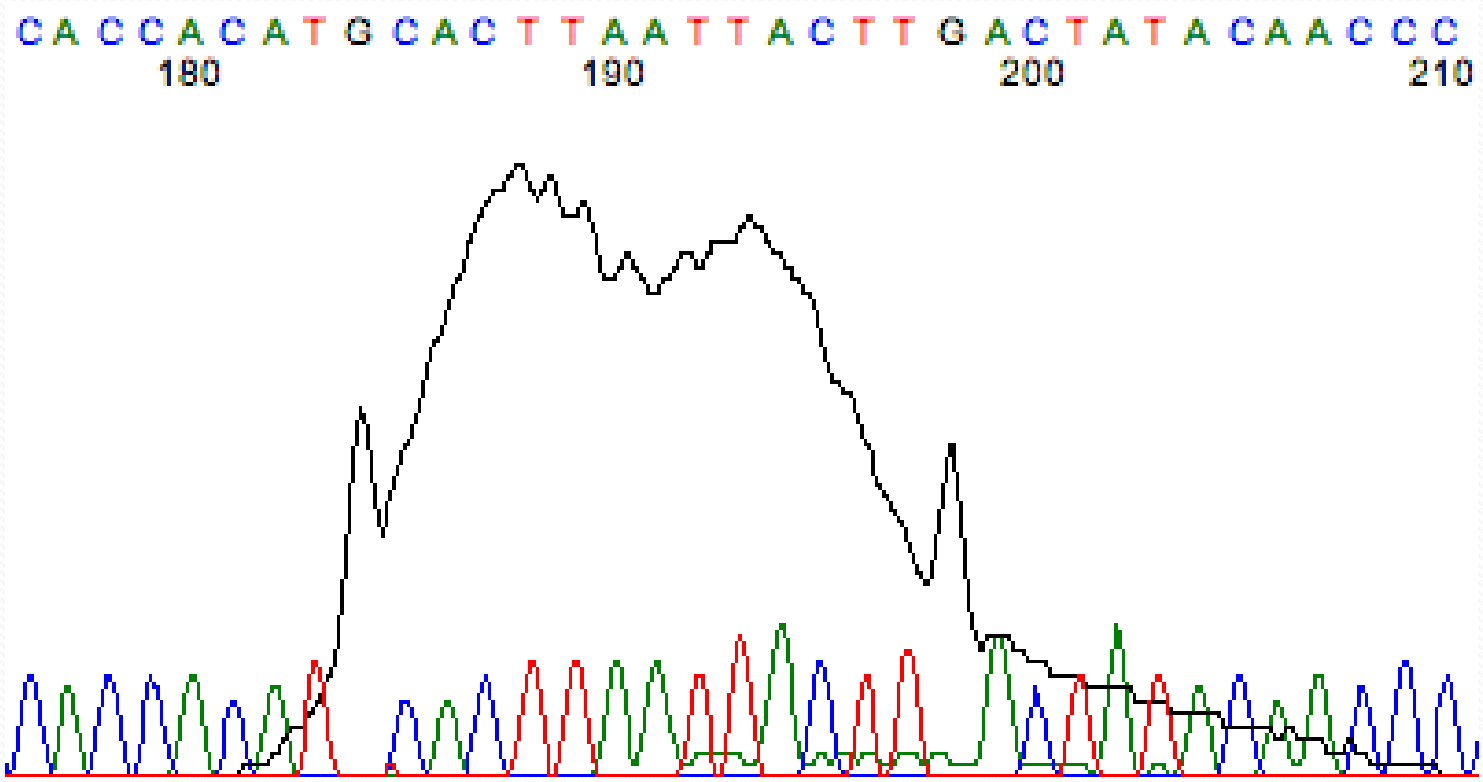

**- Capillary overload**

**The two most common causes of capillary overload are:-**

**too much template DNA**

**dirty template DNA contaminated with proteins and/or salt**

# Late "G" dye peaks




The breakdown of the BigDye sequencing chemistry before sample clean up (excess heating or high numbers of freeze/ thaw cycles)


THANK YOU