

Troubleshooting

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Slides prepared by Kelly M. Elkins, Ph.D., Towson University, 2025

Background

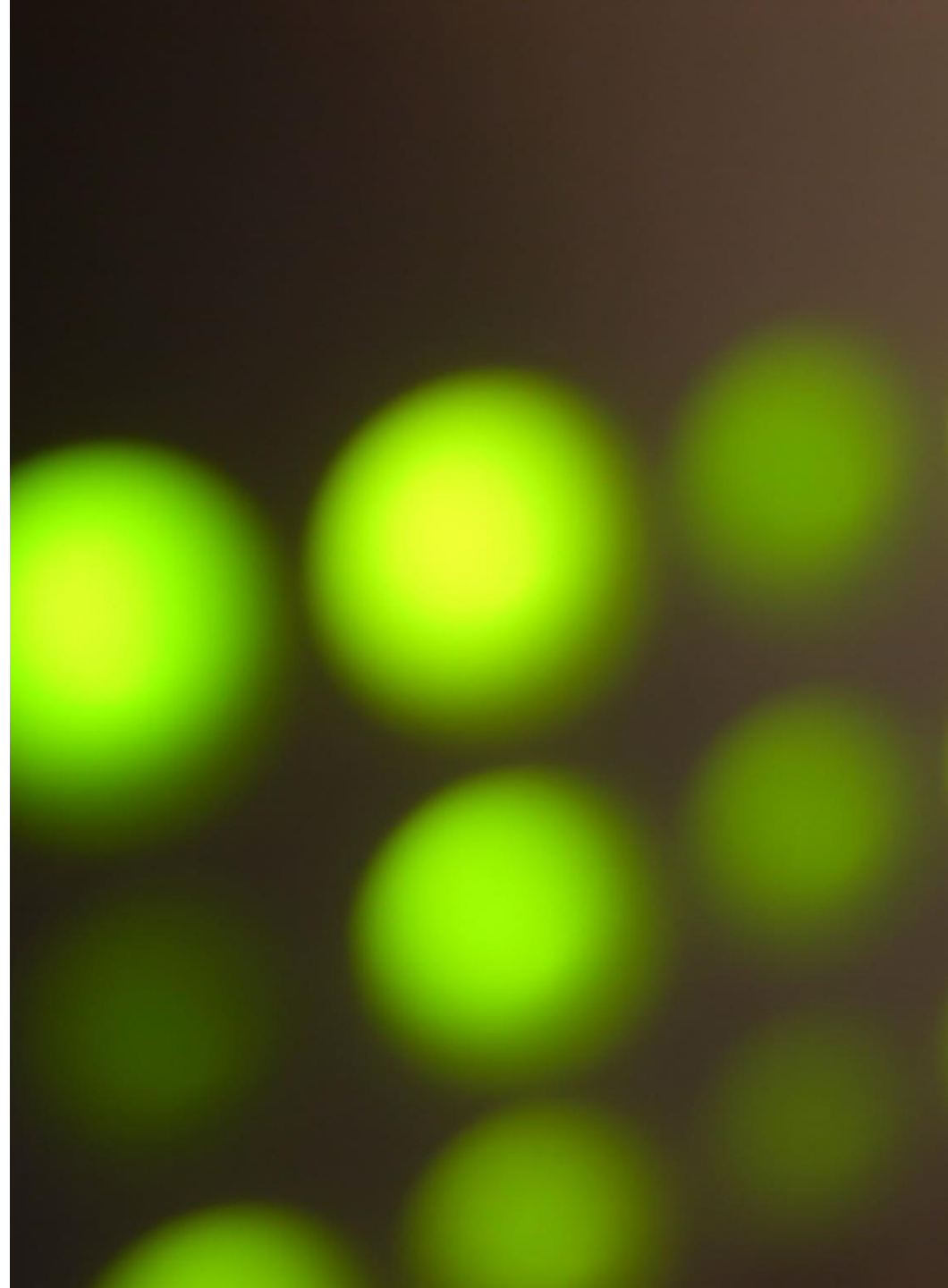
In October 2023, Towson University was awarded a cooperative agreement from NIST to develop a standardized DNA training curriculum for the United States that address the components in ANSI/ASB Standard 115, *Standards for Training in Forensic Short Tandem Repeat Typing Methods Using Amplification, DNA Separation, and Allele Detection*. 2020. 1st Ed.

This presentation addresses the knowledge-based portion of the training program and covers the topic outlined in 4.2.3i in ANSI/ASB Standard 115.

Learning Objectives

This material will provide trainees with an understanding of troubleshooting components, including:

1. thermal cycling errors (e.g., ramping, temperature control);
2. DNA detection errors (e.g., spectral calibration failure, resolution failure);
3. general equipment failure.



Terms & Definitions (ANSI/ASB 115)

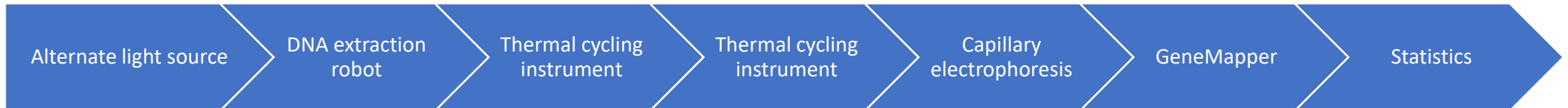
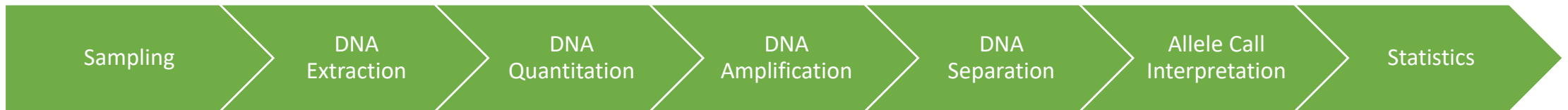
- **Artifact.** A non-allelic product of the amplification process (e.g., stutter, non-templated nucleotide addition, or other non-specific product), an anomaly of the detection process (e.g., pull-up or spike), or a byproduct of primer synthesis (e.g., “dye blob”) that may be observed on an electropherogram; some artifacts may complicate the interpretation of DNA profiles when they cannot be distinguished from the actual allele(s) from a particular sample.
- **Spectral calibration.** An examination of the contribution of overlap in the emission spectrum of fluorescent dyes used for a specific DNA test on a capillary electrophoresis instrument; permits the color deconvolution necessary for multi-color STR typing or sequencing to be performed; a poor spectral calibration may cause artifact peaks or inaccurate peak height determinations.
- **Stochastic.** 1) Chance, or random variation 2) in DNA testing, refers to random sampling error from extracts containing low levels of DNA and/or random variation in selection of alleles amplified at a particular locus.

Control Samples and Troubleshooting

Always include **positive** and **negative** controls in sample testing.

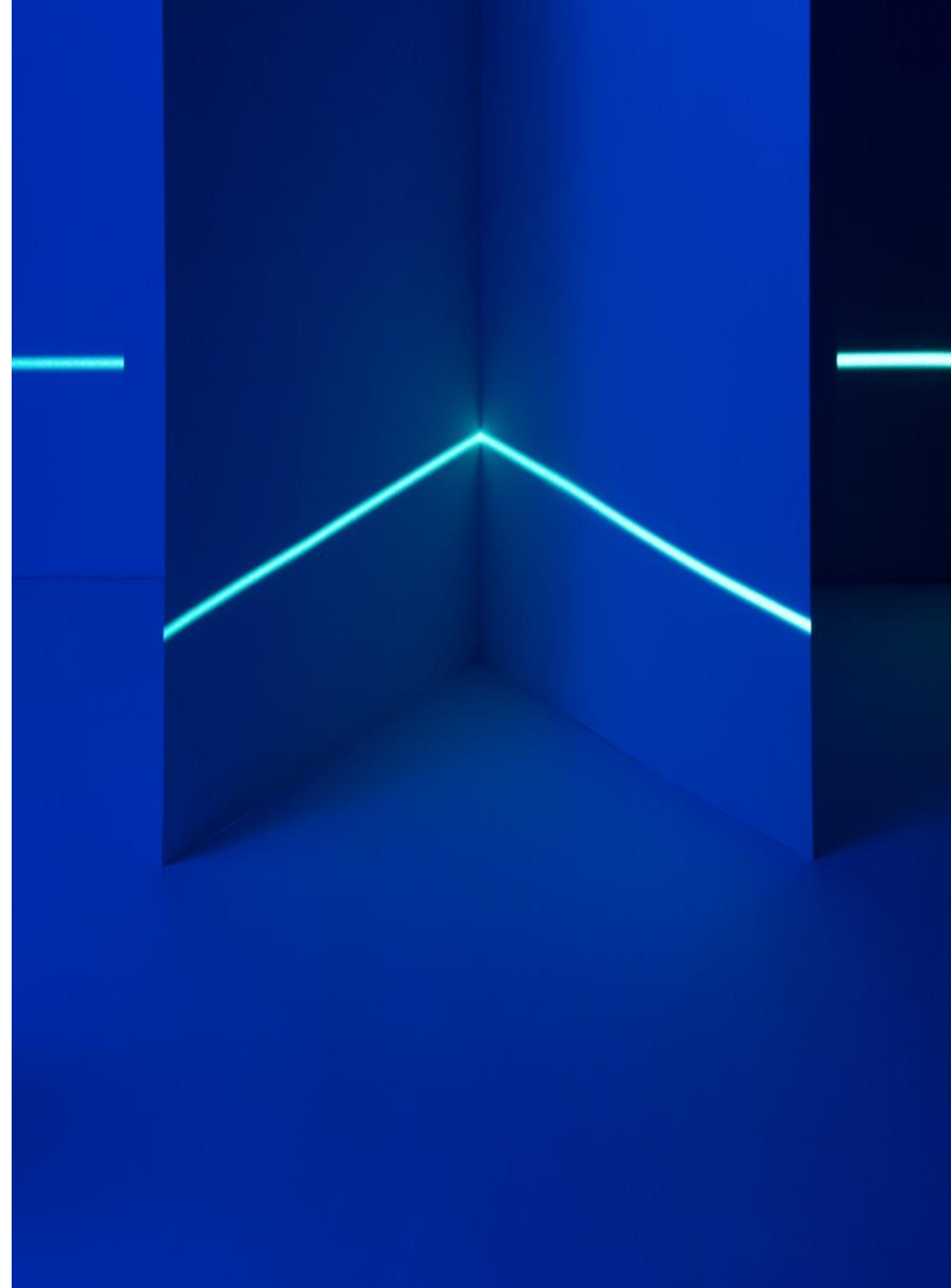
Controls will be used to determine if the method or instrument
performed properly.

DNA Typing Process & Instrumentation



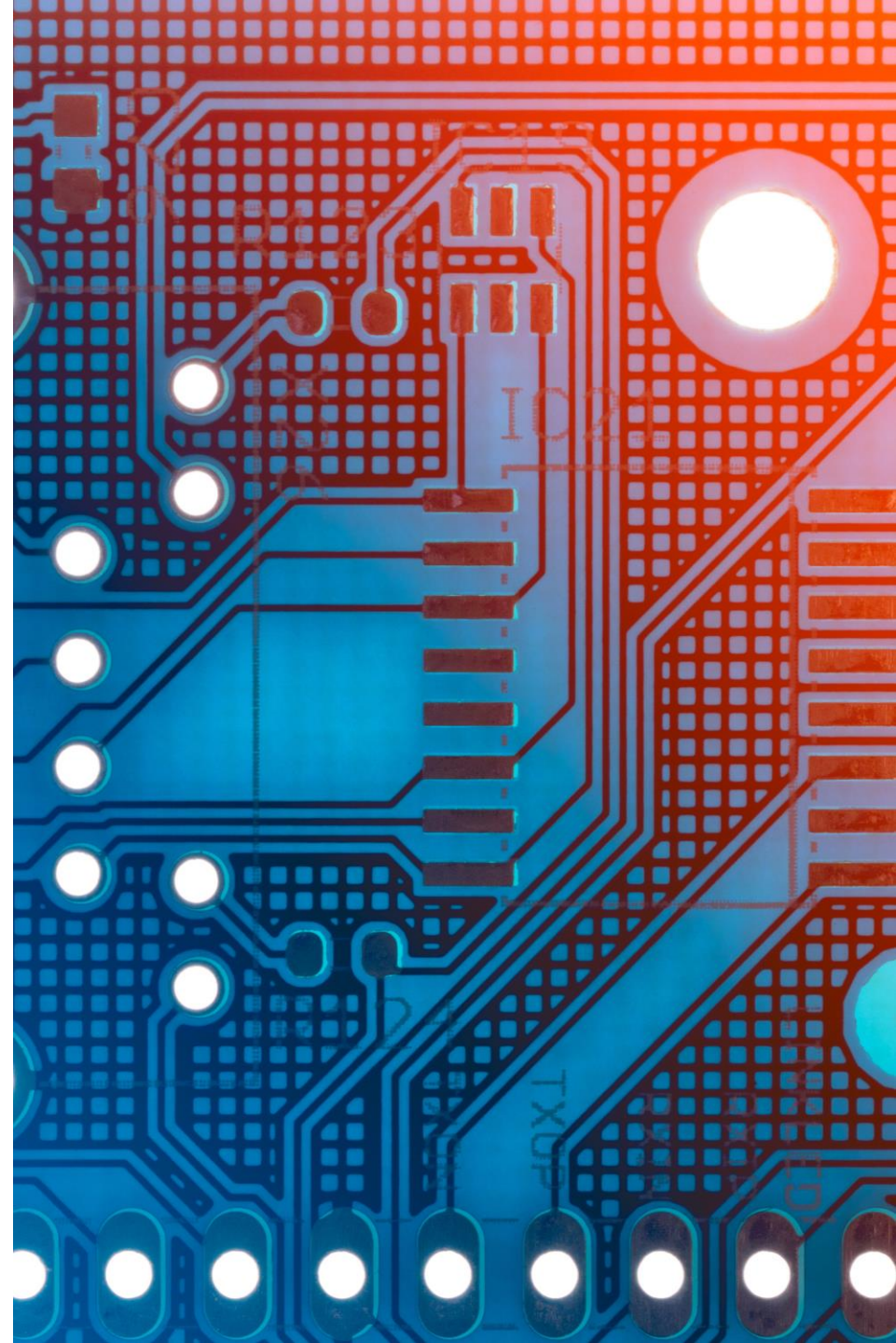
Interpretation: Detection of Thermal Cycling and CE Errors

- Control or sample issues
 - Positive controls and samples fail to amplify or lead to an unexpected result
 - Amplified DNA is not detected by the real-time detection system
 - Incomplete cycling leads to low DNA yield
 - Positive controls and sample STRs will fail to separate or be detected



Thermal Cycler Failure

- Power / battery / surge
- Electronics failure from hot – cold cycling
 - Circuit control board solder
 - Heater lid short
- Reduced DNA amplification / yield
 - Heating / cooling rate too low
 - Faulty heating element (Peltier thermoelectric thermal control assembly)
- No screen display
 - Blown fuse

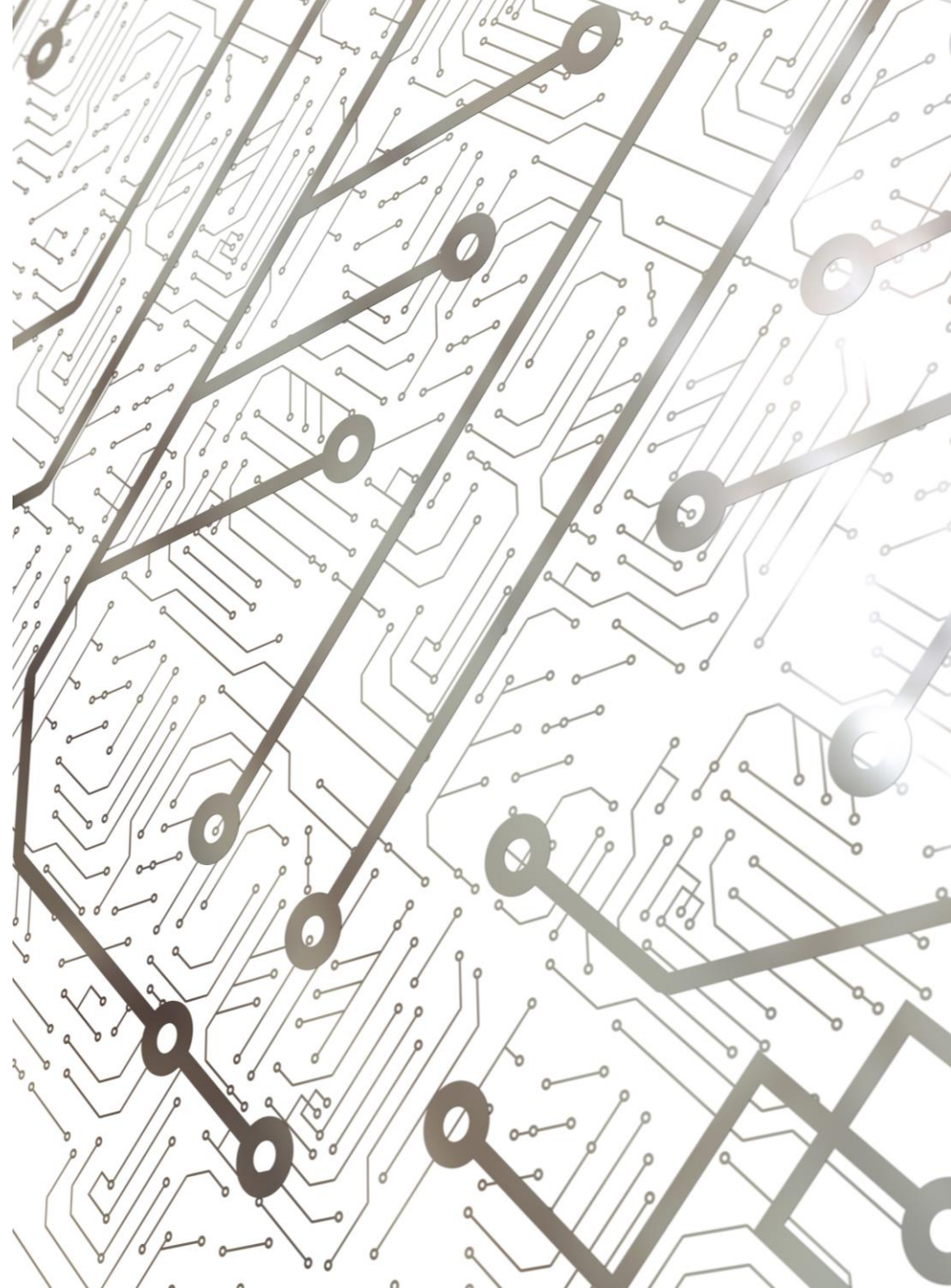


Thermal Cycling Error Response

- Check calibration and settings for temperature cycles and attainment
- Run instrument verification to check performance
- Check sensor error codes in manual and response
- Check lid seal: ajar or open lid can lead to an open circuit for the lid heater
- Decontaminate if contamination detected
- Use cotton swab with ethanol or isopropanol to clean lenses to improve detection sensitivity

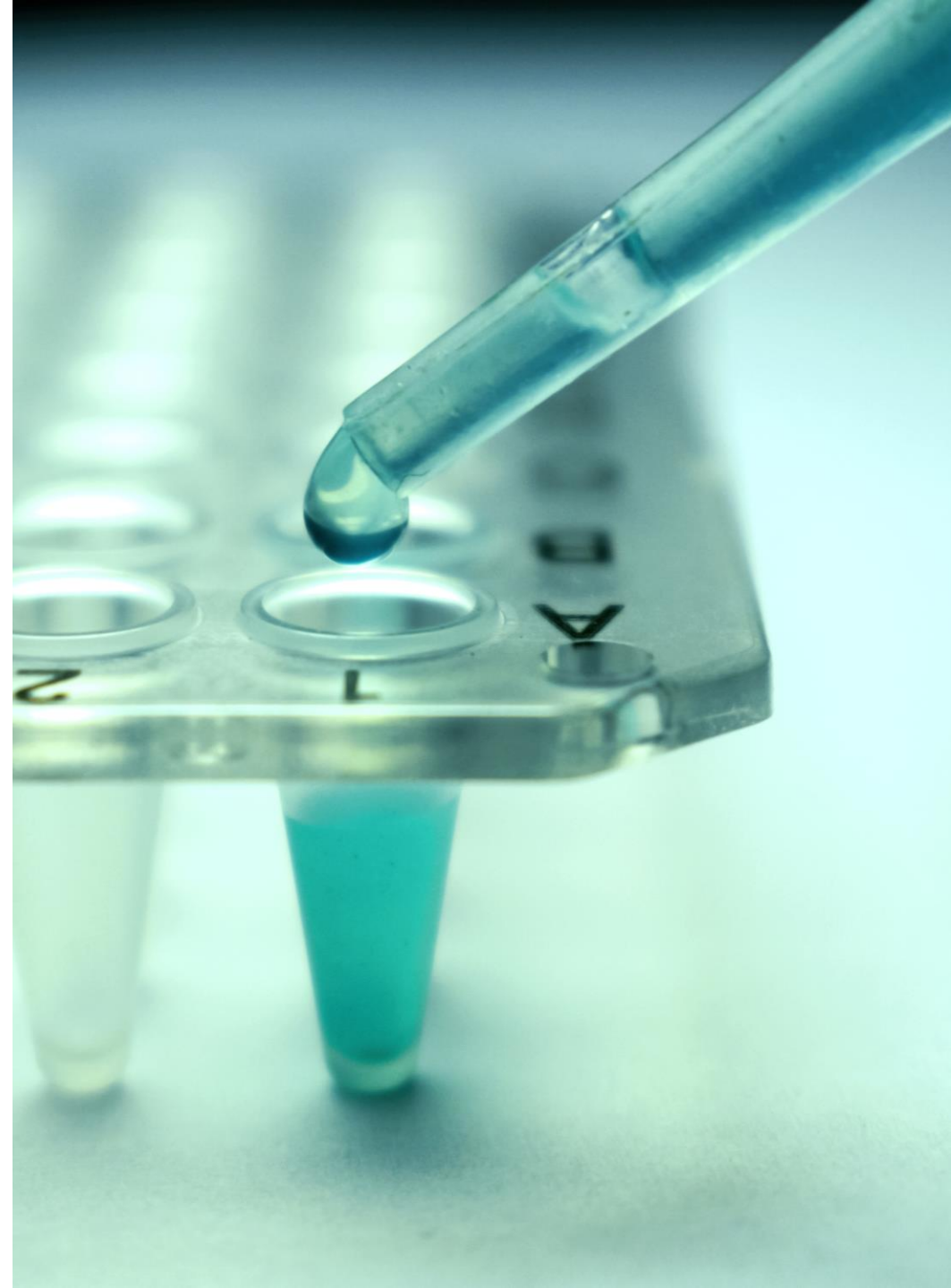
Veriti Thermal Cycler User Guide, Applied Biosystems, June 2010, https://assets.thermofisher.com/TFS-Assets/LSG/manuals/cms_042832.pdf

Rotor-Gene® Q MDx User Manual, v. 1, Qiagen, February 2018 ,
<https://www.qiagen.com/us/resources/download.aspx?id=d0f79b96-f0b1-400e-8a0a-def2a6d19e42&lang=en>



Thermal Cycling Errors: Temperature Control

- Symptoms
 - Failure to amplify
 - Low product yield or detection
 - Non-specific target amplification
- Solutions
 - Check tube fit in wells
 - Use thin-walled and low-profile plastics
 - Use clear plastics for fluorescence detection
 - Do not exceed fill volume
 - Increase annealing temperature to increase specificity
 - Mix primers and master mix well and rerun



Thermal Cycling Errors: Ramping (Ramp Rate)

- The higher the ramp rate, the shorter the run time.
- **Symptoms** of ramping errors
 - Non-specific priming or spurious annealing
 - Overshooting denaturation temperature
- **Troubleshooting** ramping errors
 - Set ramp rate to highest speed to avoid non-specific priming
 - Slow down ramp rate to avoid overshooting temperature

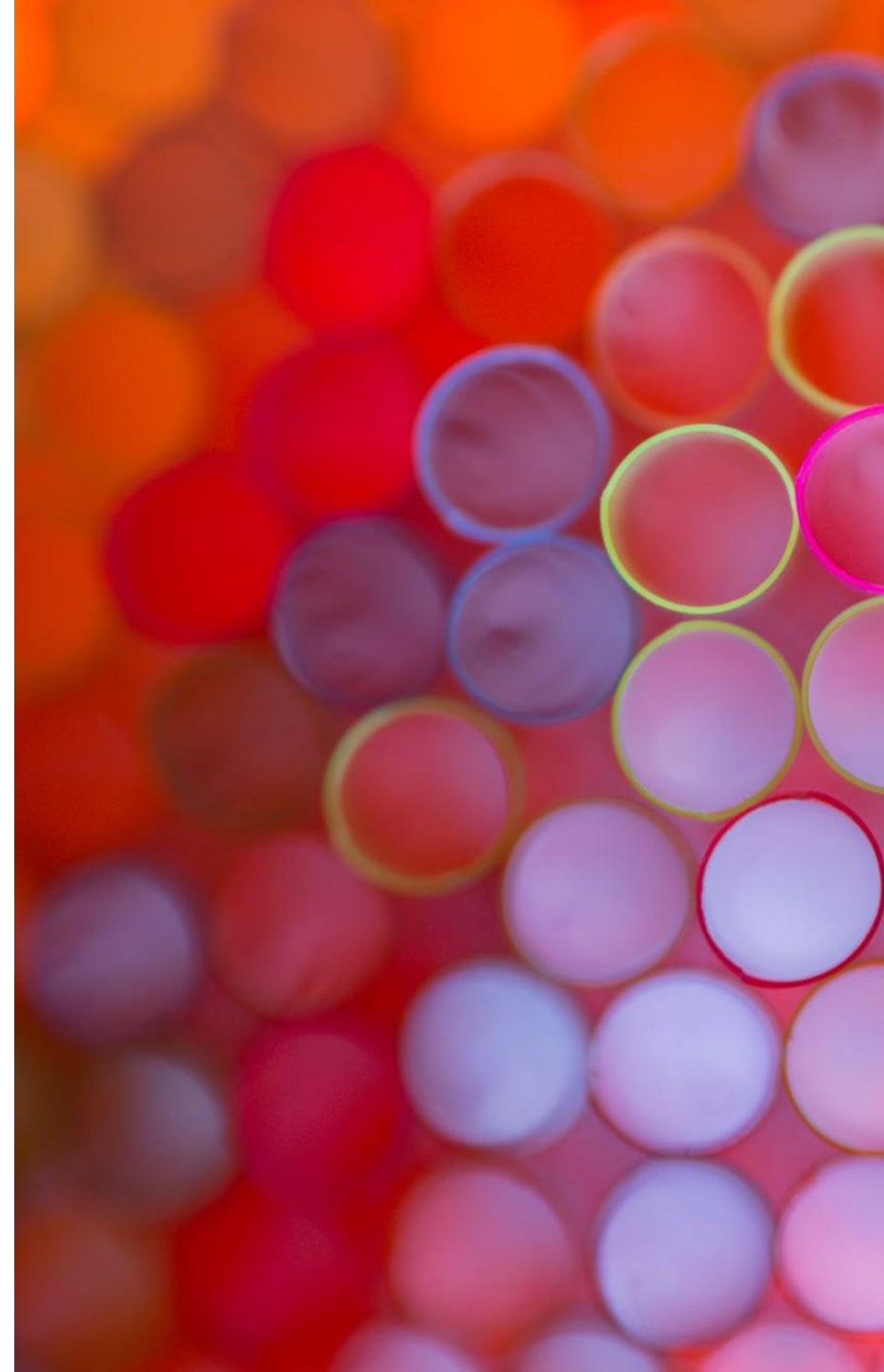


Thermal Cycling Issues

- Melted plate or tubes
 - Incompatible consumables
- Fluorescent detection issues
 - Improper sample position may lead to a reduction in signal
 - Cloudy or dusty windows or lenses lead to low detection and underestimating quantitation values
 - Low laser intensity leads to low detection
 - Gain set too high leads to oversaturation while too low will lead to the signal being undetectable from the background noise

Veriti Thermal Cycler User Guide, Applied Biosystems, June 2010, https://assets.thermofisher.com/TFS-Assets/LSG/manuals/cms_042832.pdf

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DNA Detection Errors: Spectral Calibration Failure

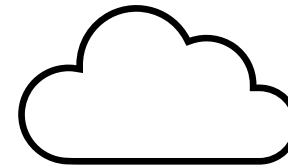
Leading to pull-up

- Rerun samples or matrix standards



Low intensity

- Cloudy detector window, clean off with cotton swab
- Replace laser or LED



DNA Detection Errors: Resolution Failure

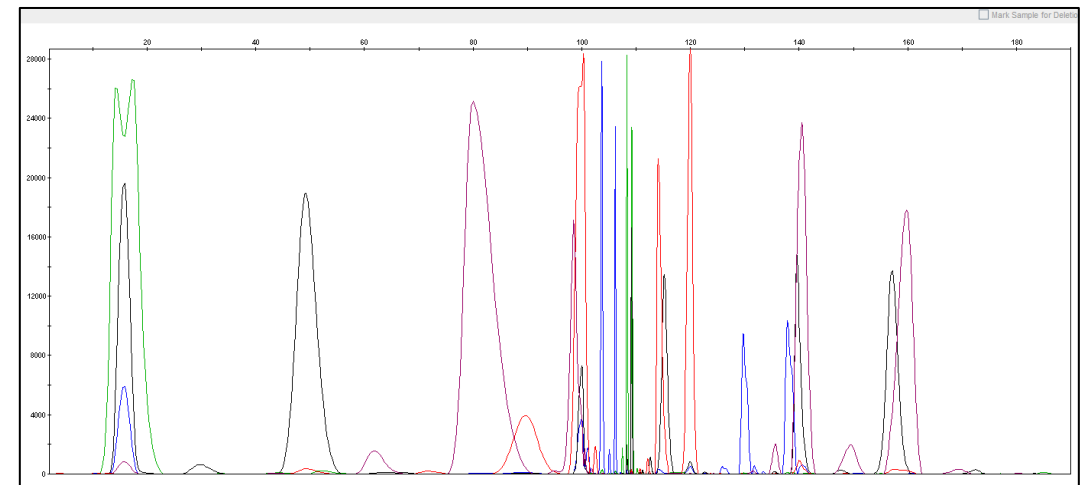
Poorly resolved peaks

- Change polymer and rerun



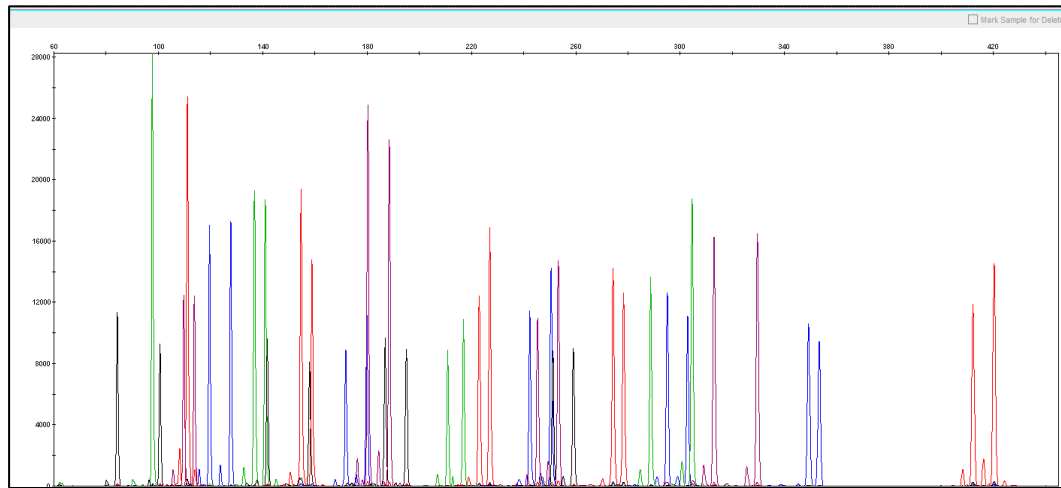
Secondary structure is forming, and migration is anomalous

- Run in Hi Di Formamide and heat and snap cool samples

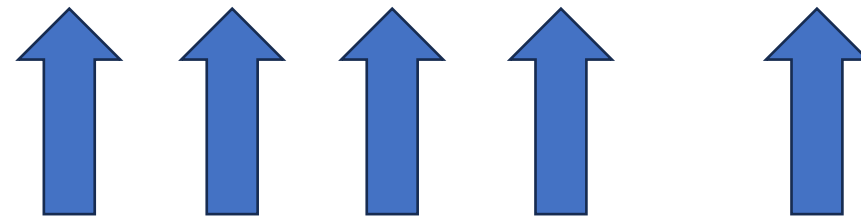
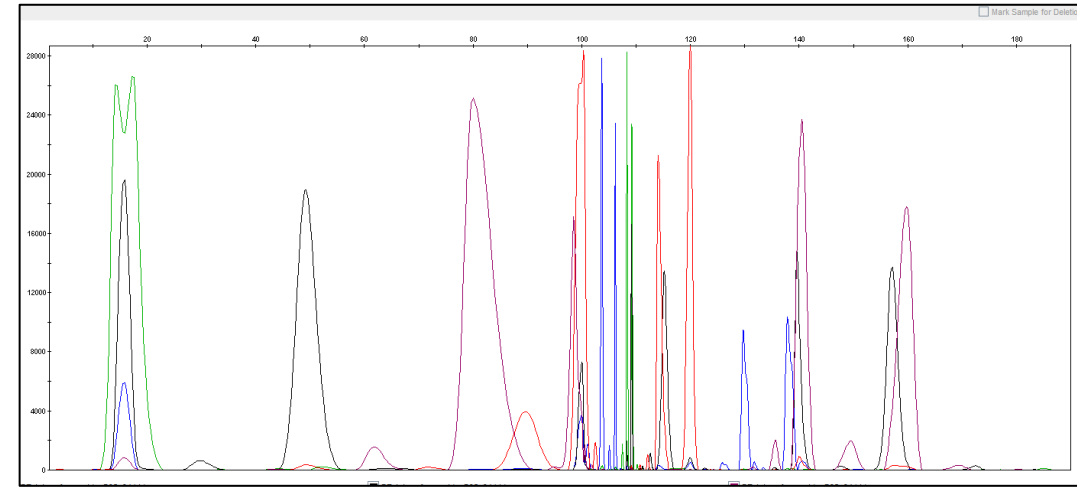


Example of Secondary Structure Formation

With HiDi Formamide



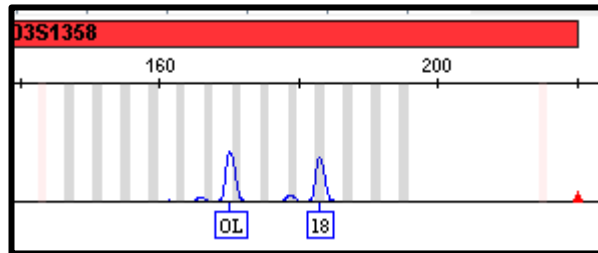
Without HiDi Formamide



DNA Interpretation: CE Run Issues

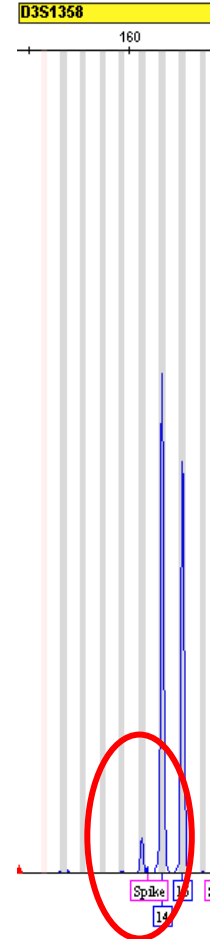
Off Ladder (OL)

- Ensure the correct size standard was used and mark the sizes in GeneMapper software
- Low CE room temperature or low buffer
- Rerun allelic ladder



Spikes

- Check for and remove bubbles and rerun
- Check that polymer remains and capillary has not run dry
- May be electrical voltage spike; rerun sample



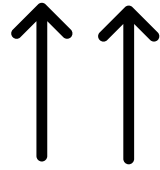
DNA Interpretation: CE Run Issues

Peak broadening

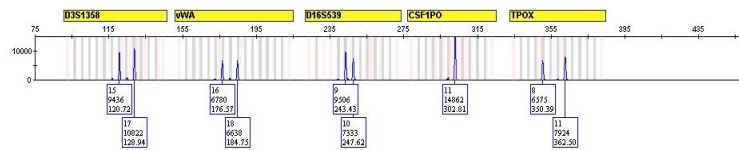
- Capillary is failing
- Replace capillary

DNA Interpretation: Signal Intensities

Too High



- Reduce DNA quantity inputted in PCR
- Reduce the quantity of DNA inputted for CE to 1 ng
- Reinject from 25 s down to 15 s and 5 s injection time

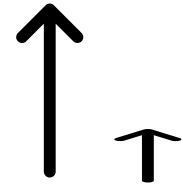


Too Low

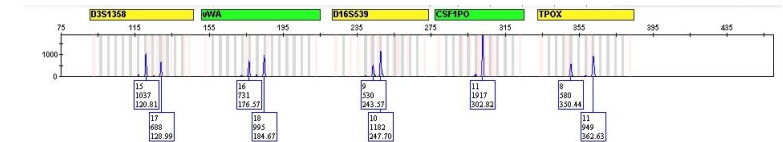


- Purify the PCR products (e.g., EZ1 cartridge)
- Concentrate the PCR products (e.g., ethanol precipitation)
- Perform WGA to enrich template prior to PCR or reamplify with a higher input of DNA
- Decrease the quantity of size standard to peak heights of approx. 500 RFU
- Increase the injection time
- Input more ladder if peak is below detection value

Imbalanced

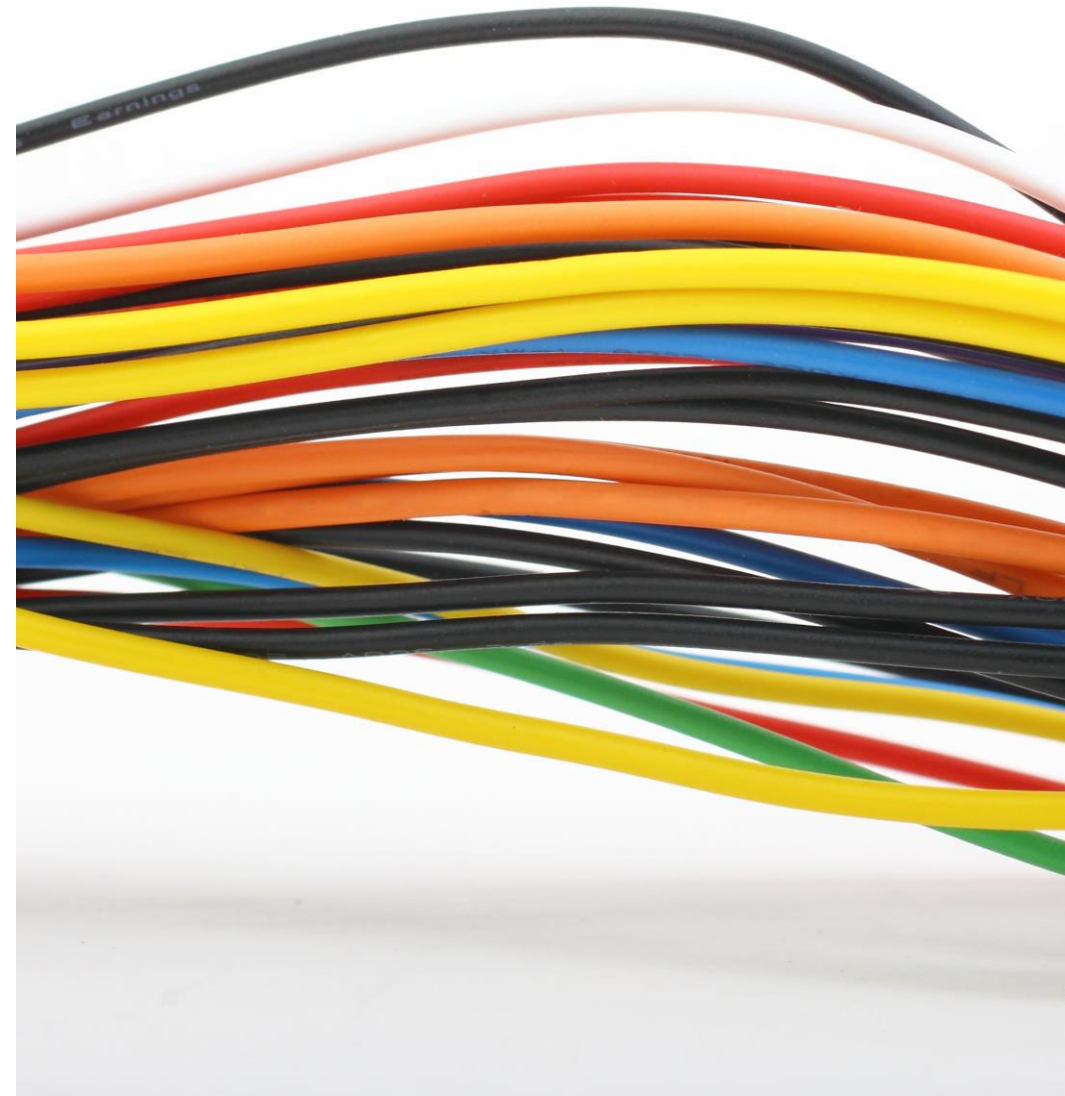


- Incorrect volume of primer or master mix or primer not well vortexed; reamplify
- Input more DNA



General Equipment Failure

- **Electrical**
 - Wire connections
 - Power source or surge
- **Mechanical**
 - Metal fatigue (e.g., crack)
 - Lubricant starvation (e.g., stuck parts)
- **Optical**
 - Alignment
 - Realign and reduce vibration
 - Chromatic aberration (e.g., light source variations)



General Equipment Failure

- Call instrument technical support or repair service unless you are trained to service the instruments
 - Consult your technical leader or lab director for guidance
 - Your lab may have service plans that cover instrument repair and maintenance
 - In cases of instrument failure, laboratories may choose to replace rather than repair



Study Questions

- Why is it important to use controls and standards in DNA typing?
- List some issues that may lead to little to no amplification.
- What could happen if the stated ramp rate is not used?
- What are some symptoms of spectral failure?
- How can you overcome resolution failure?
- What can you do if the peaks are below the analytical threshold on an electropherogram?

Suggested Readings

- ANSI/ASB Standard 115, Standard for Training in Forensic Short Tandem Repeat Typing Methods using Amplification, DNA Separation, and Allele Detection. 2020. 1st Ed.
https://www.aafs.org/sites/default/files/media/documents/115_Std_e1.pdf
- Butler, J.M. Ch 11: Low-Level DNA Testing: Issues, Concerns, and Solutions, Advanced Topics in Forensic DNA Typing: Methodology, Elsevier, 2011.
- FBI, Quality Assurance Standards for DNA Databasing Laboratories, effective Sept. 1, 2011. <https://ucr.fbi.gov/lab/biometric-analysis/codis/FBI%20Director%20Databasing%20Standards%20Revisions%20APPROVED%20and%20final%20effective%209-1-11.pdf>
- FBI, Quality Assurance Standards for Forensic DNA Testing Laboratories, effective Sept. 1, 2011. <https://ucr.fbi.gov/lab/biometric-analysis/codis/quality-assurance-standards-for-forensic-dna-testing-laboratories>