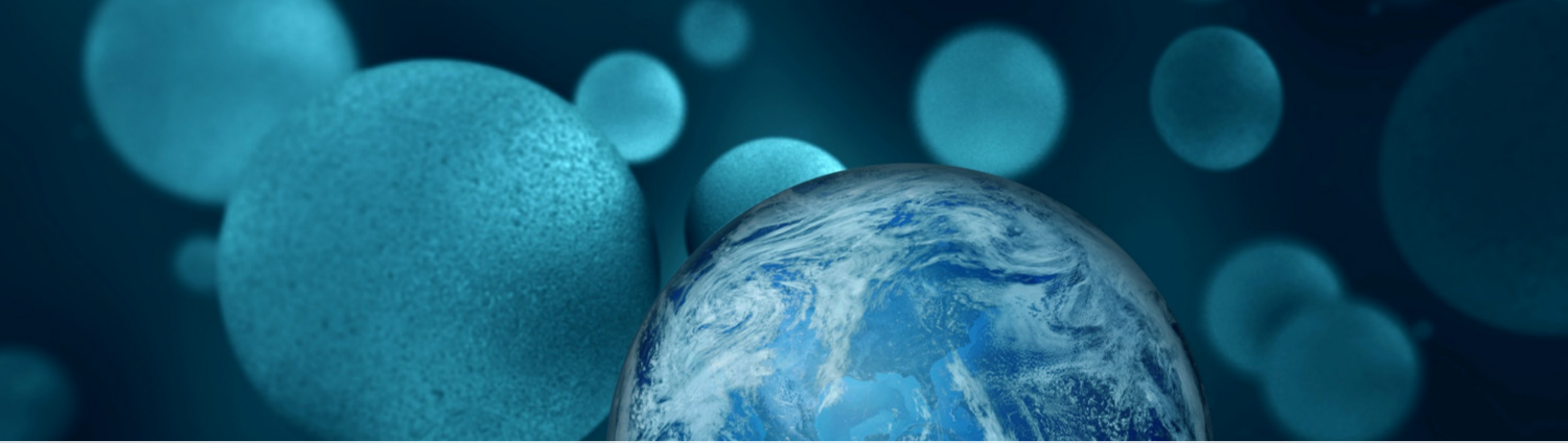

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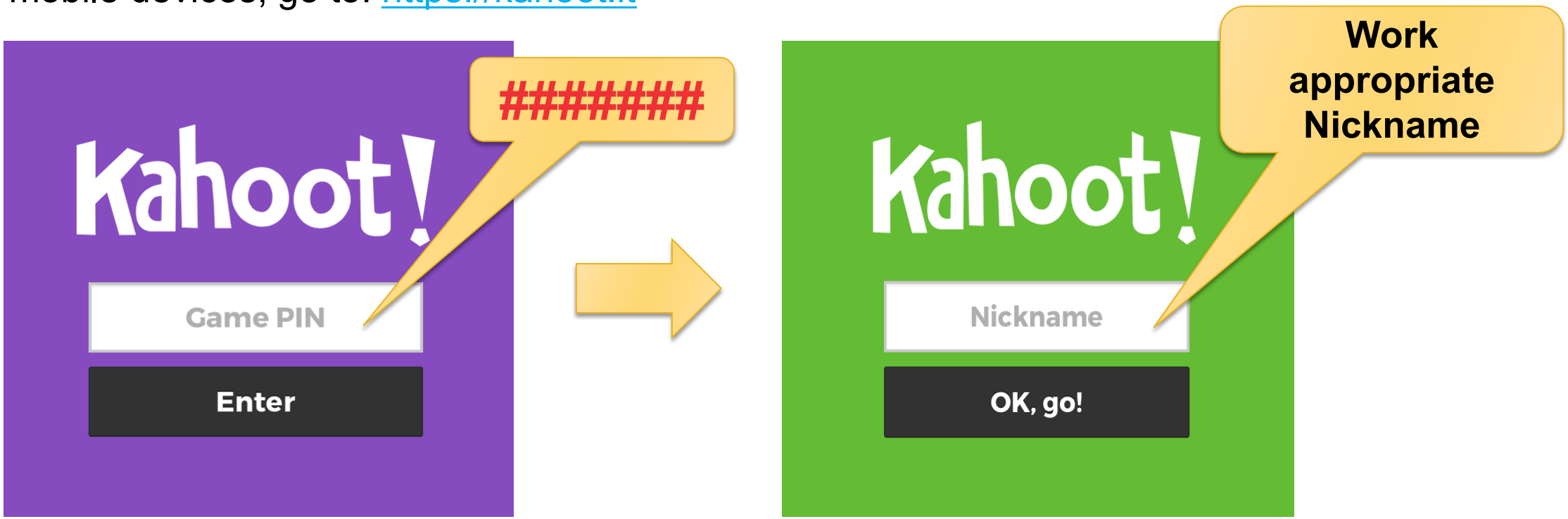
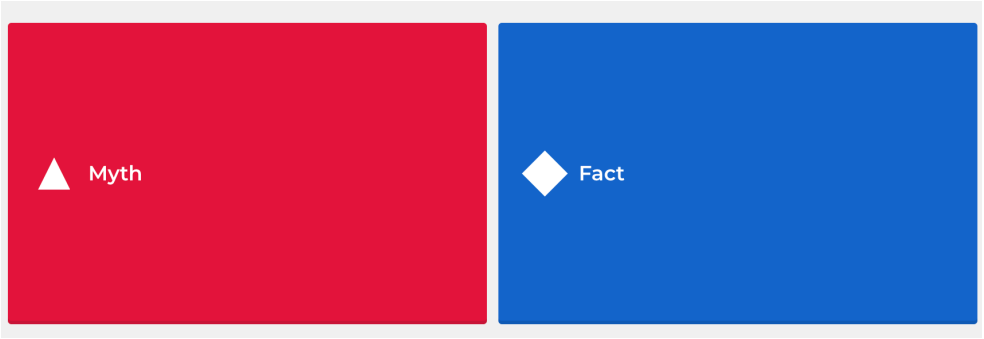
MythBusters – Review of lessons learned from extraction to data analysis

Megan Meyer, Field Application Scientist
John Brill, Field Service Engineer

Let's Play A Game…Yes, You Have To!

On your mobile devices, go to: https://kahoot.it





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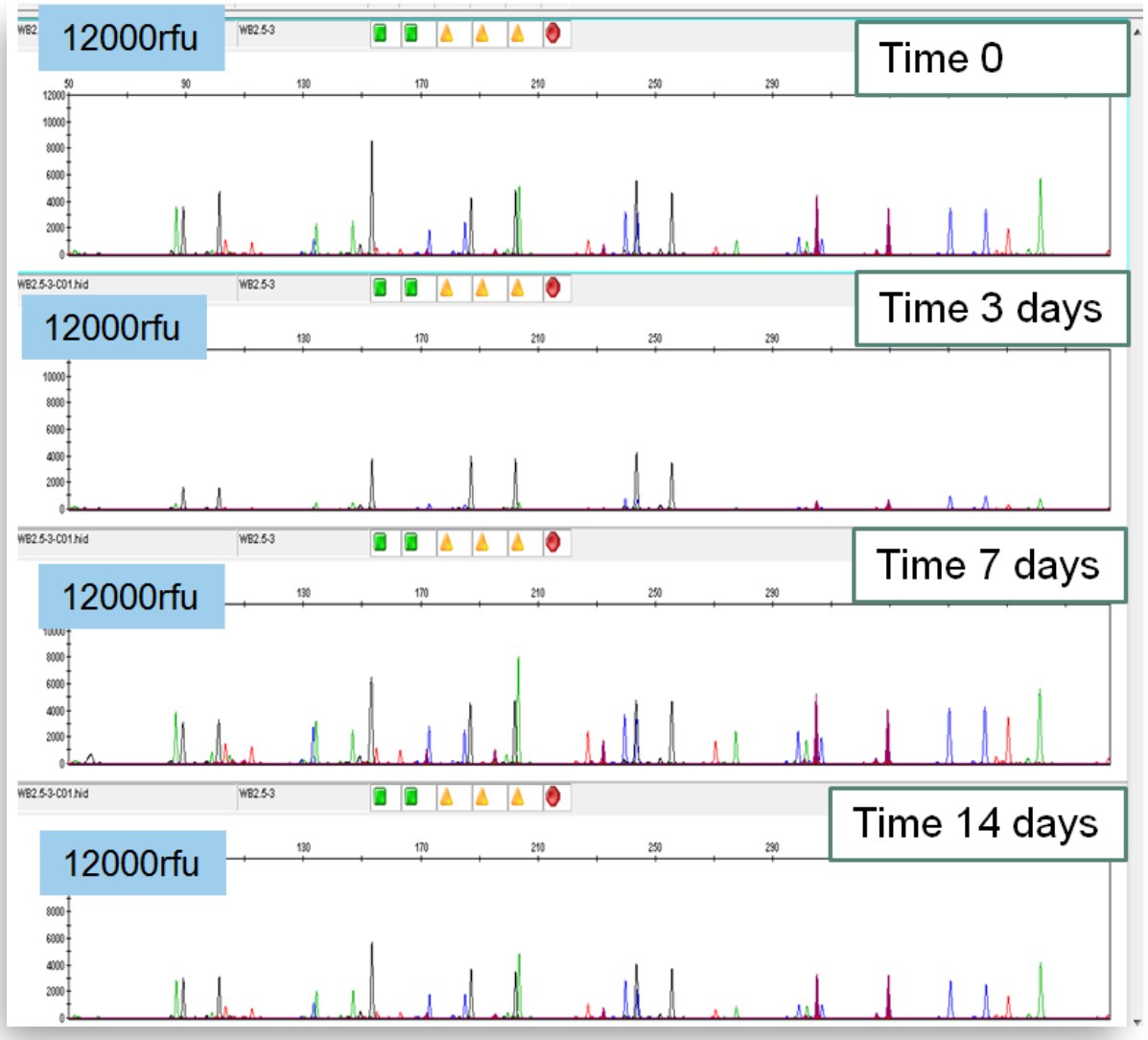
Extraction

Question 1

- MYTH or FACT:

Ethanol is a component of most magnetic bead based extraction kits and can cause downstream sample inhibition if not completely removed?

FACT: Ethanol – Effect of Time



Ethanol spiked sample amplified best at day 7.

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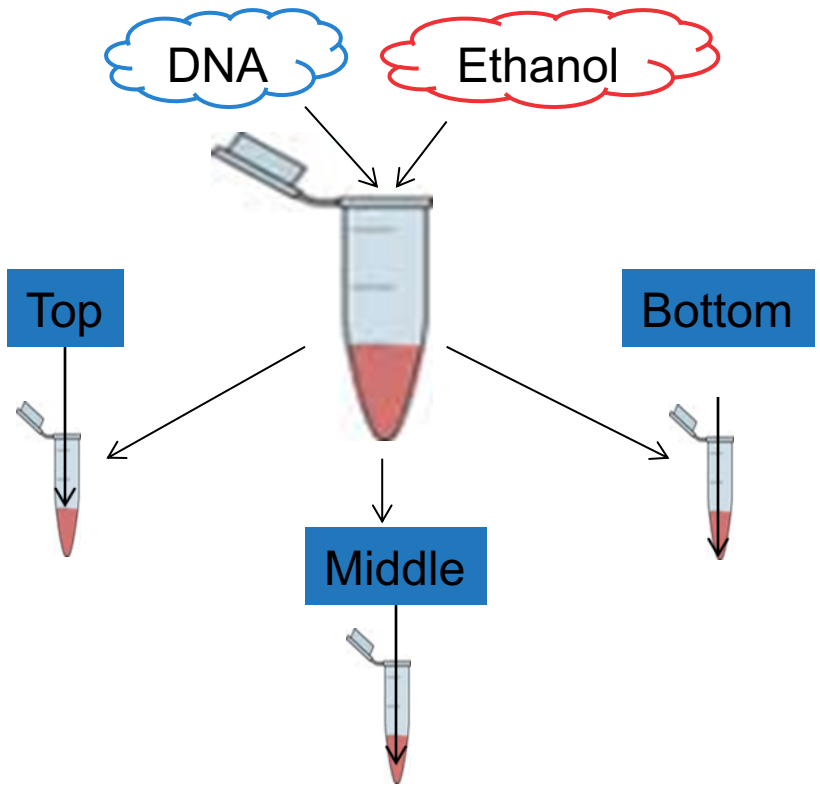
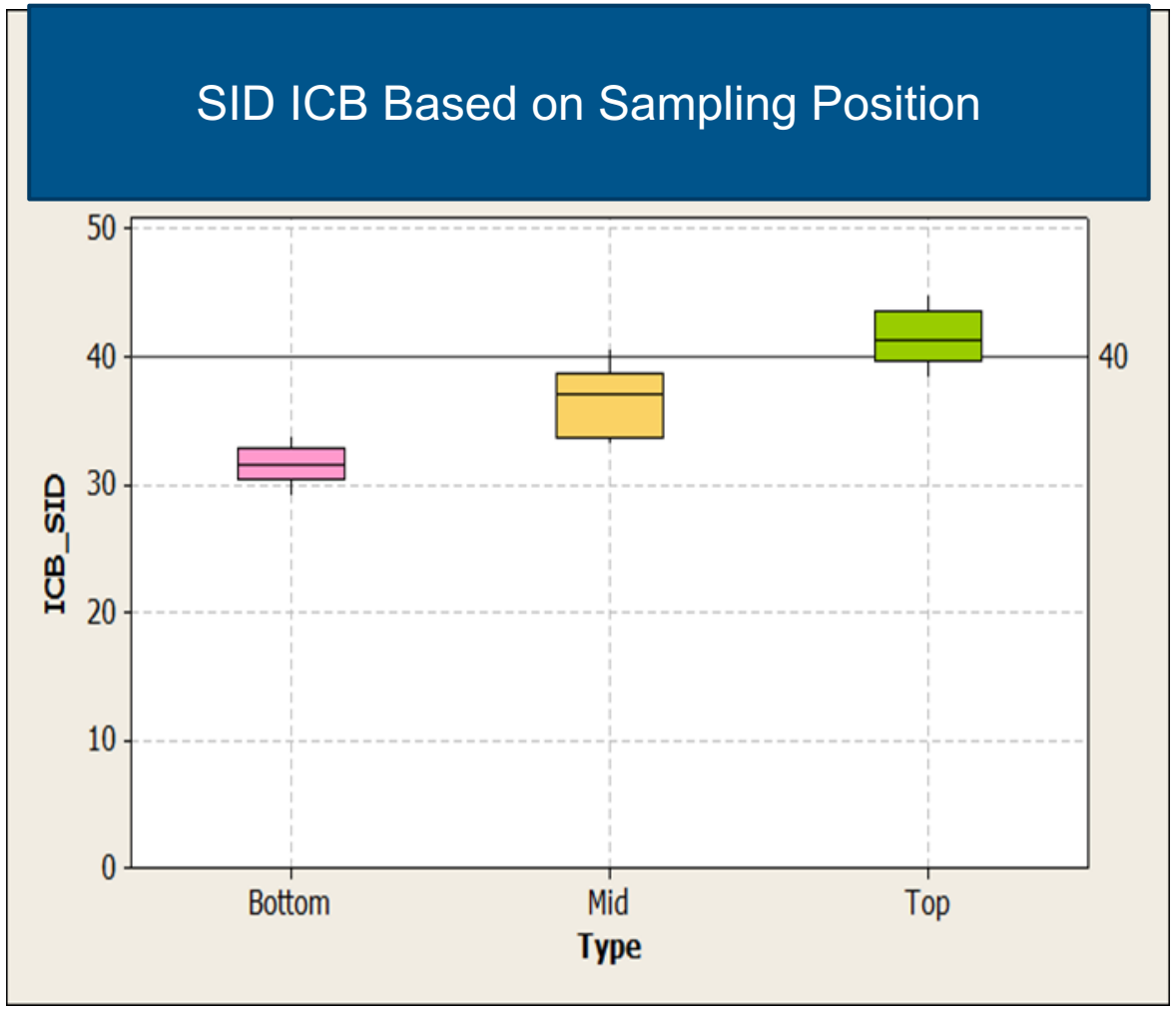
FACT: Ethanol: Effect of Sampling





Sampling position and time could be explanations for different degrees of inhibition among repeat amplifications of the same sample.

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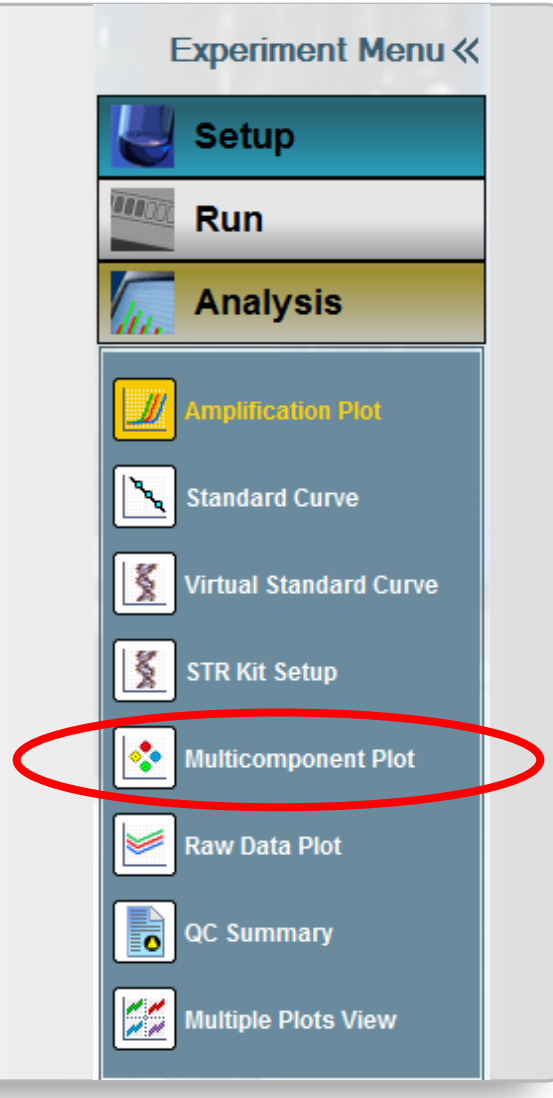
Quantitation

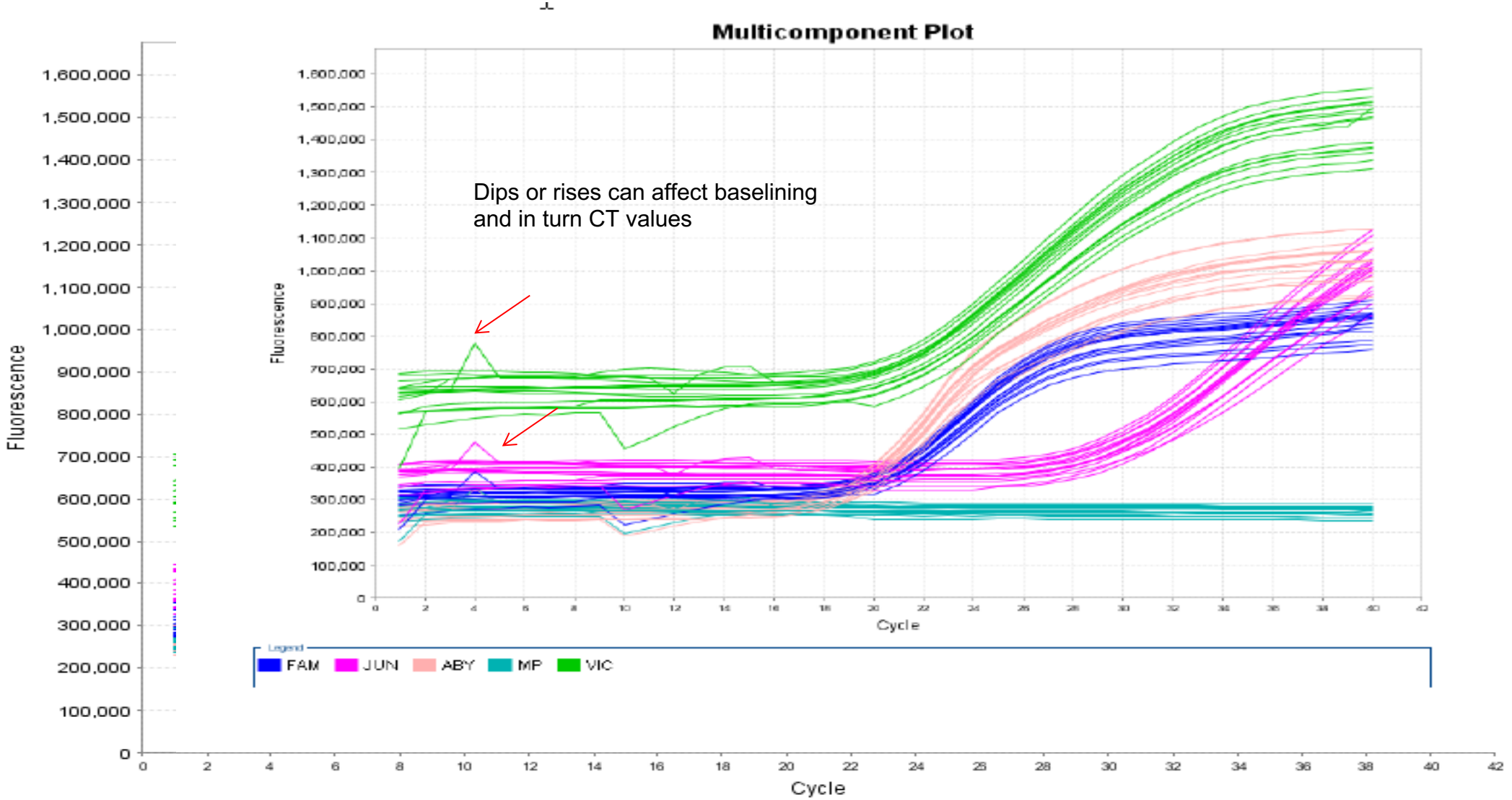
Question 2

- MYTH or FACT:

When using Quantifiler™ Trio, it is important to look for signs of bubbles using the Multicomponent Plot?



FACT: Closer Look at the Data: Multicomponent Plot



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Question 3

- MYTH or FACT:
  - Applied Biosystems DOES NOT offer a product to help minimize bubbles for Quantifiler™ Trio setup?

https://pixel.nymag.com/imgs/daily/selectall/2016/11/30/30-poltical-bubbles.w710.h473.jpg

MYTH: Automation Enhancer: Loves to Burst your Bubble

- R&D from Thermo Fisher qPCR business and Product Assurance group in HID business provided a solution by adding Quantifiler Automation Enhancer to the PCR Reaction Mix.
- Addition prevents bubbles from being introduced during robotic mixing and pipetting
- Validation to assure that the performance of the Quantifiler Trio kit is not compromised by adding the Quantifiler Automation Enhancer
- Quantifiler Trio Reaction Mix with added Quantifiler Automation Enhancer at 1000:1 ratio is stable at 4C for at least six months.
- Can be ordered at no charge through your Account Manager/ Support Team

Quantifiler Trio and Bubbles

- Minimizing bubbles with pipetting techniques
  - Dispense the DNA onto the side of the well, above the surface of the master mix.
  - Dispense the DNA into the master mix, but only until the first stop of the pipette. Raise the tip above the surface of the liquid to touch the side of the well to perform the blowout.

- What to do if you see bubbles
  - Tap the rack with sealed plate on the benchtop to bring the bubbles to the liquid
  - Working with individual wells, either tap the well with a tool such as a marker or pen, or flick the well with your gloved fingertip.
  - Centrifuge the plate following this process to consolidate any reaction mix to the bottom of the wells

- User Guide Revision E: October 2015

Quant Troubleshooting from FSE Perspective

1. Wavy Data
   - Lamp, Lamp holder, Lamp power cable, over heating
2. Run will not start/abort in middle of run
   - Block or heated cover not heating or block is over heating due to blocked ventilation or communication issue
3. High background
   - Contaminated block due to debris on plates over time or contaminated heated cover due to optical seal not placed correctly or old. May be able to clean out but if heated cover is contaminated then it must be replaced.
4. High amount of outliers or quadrants of samples not amplifying properly
   - Block has 4 Peltier's, one for each quadrant. If one fails then amplification will be poor for those samples but rest of block will work fine.
5. Door will not open.
   - Reboot instrument and see if block/heated cover move to their home position. Could be due to broken tray latch/something preventing block/heated cover to move (i.e. sticky substance).

Amplification

Question 4

- MYTH or FACT:
- Applied Biosystems DOES NOT validate specific thermal cyclers for HID amplification kits?

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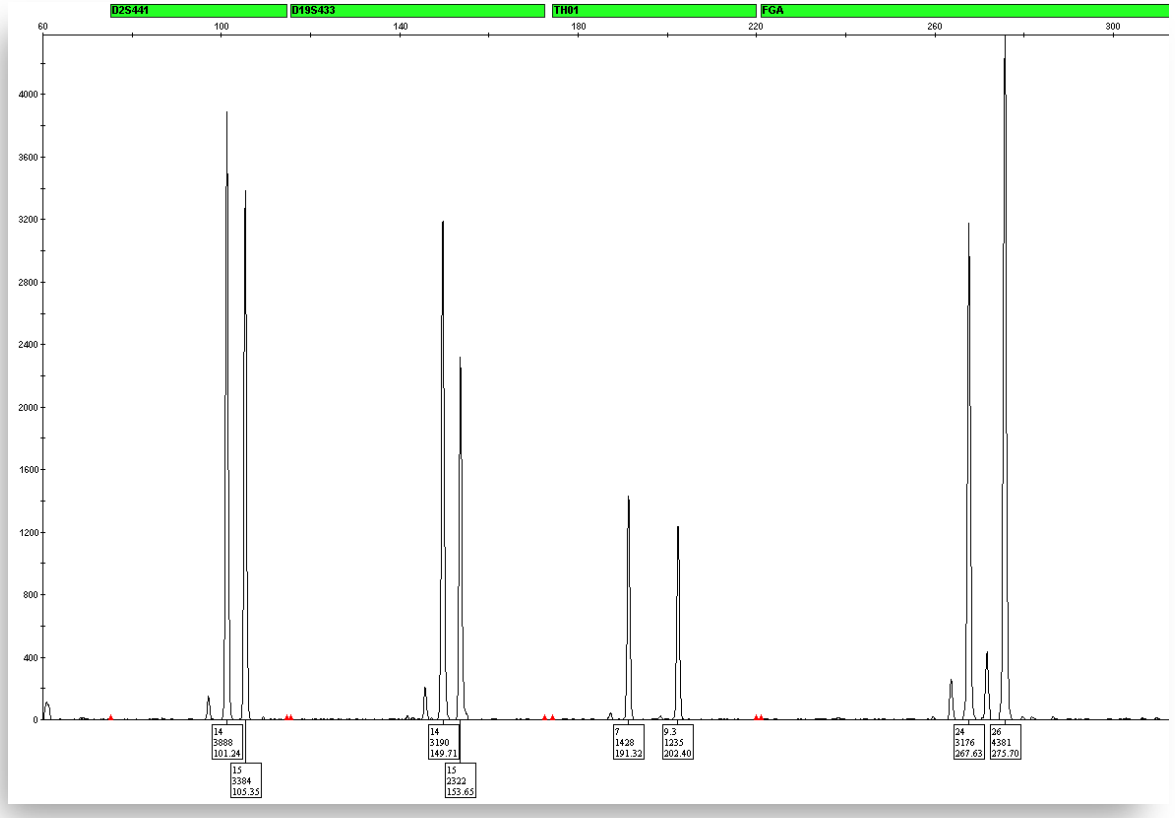
MYTH: Thermal Cycler Considerations

- Non-validated thermal cyclers have different thermal properties/ramp rates
- Edge vs Interior wells
- Loci such as TH01 have a GC-rich self-complementary region of the target sequence that can form several secondary structures (DNA hairpins), particularly with suboptimal temperatures
- Proper use of plate and tube accessories, as applicable
  - Compression pads
  - Tray & retainer sets



Remember, when designing megaplexes, small marker-level tradeoffs must be made when finalizing the overall optimal kit conditions. Slight deviations can have a large impact on the profile.

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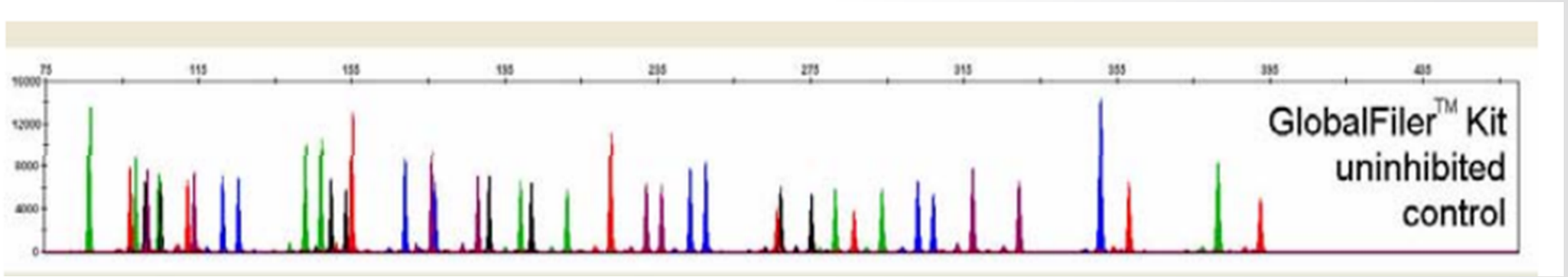
Question 5

- MYTH or FACT
  - A single dye channel can show patterns of inhibition while other dye channels appear unaffected?



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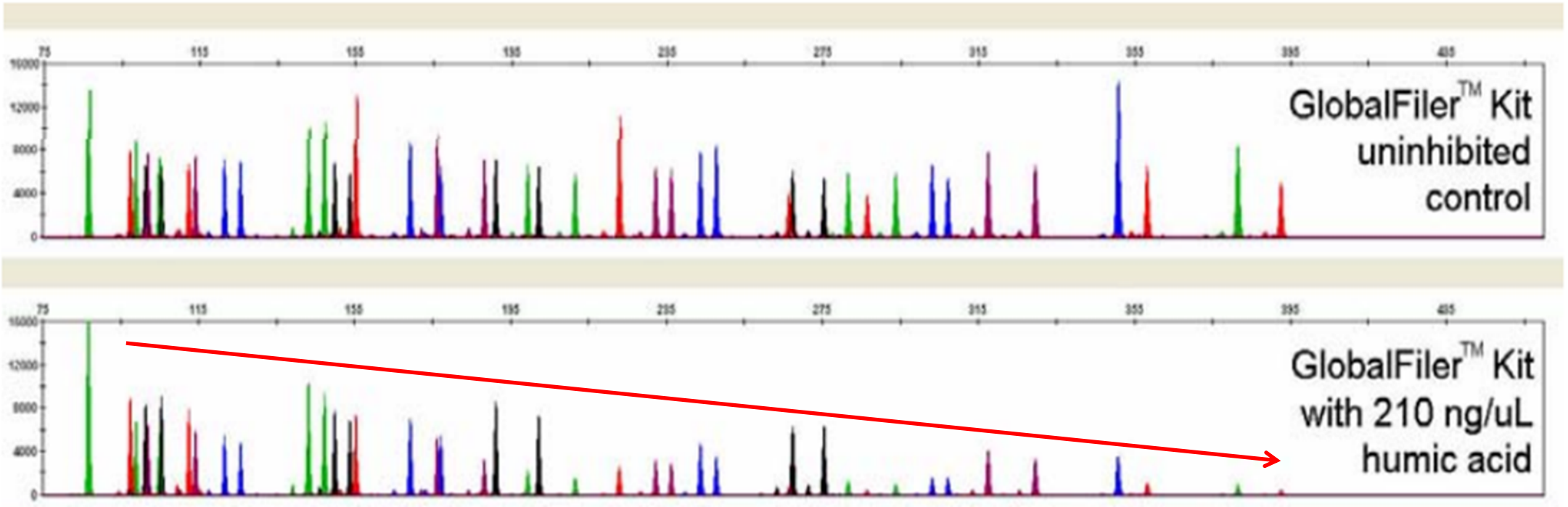
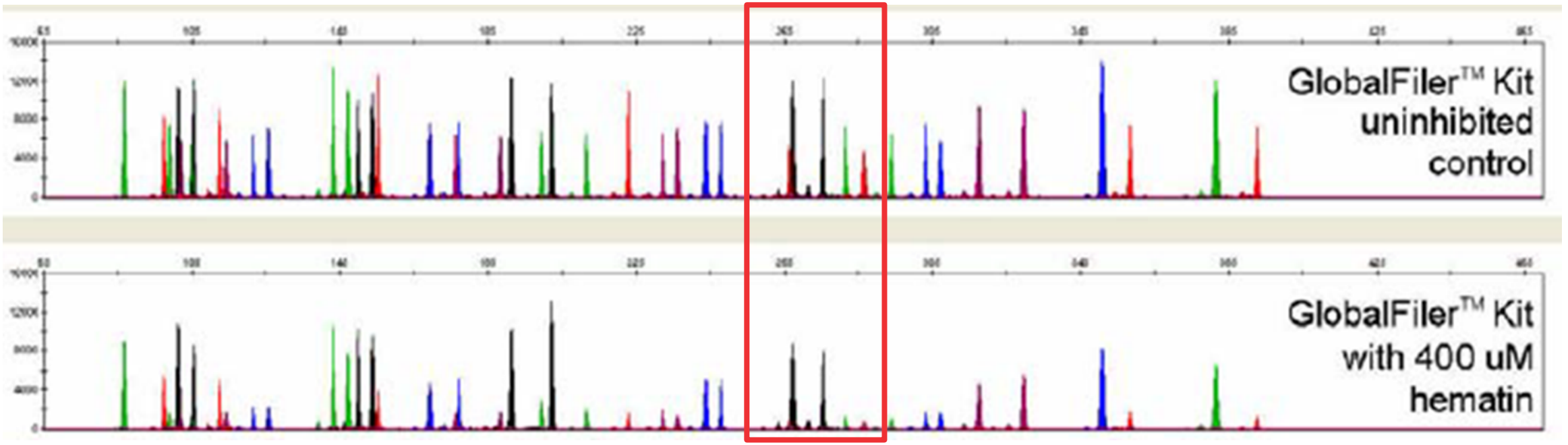
FACT: Inhibition Patterns: Traditional Ski Slope



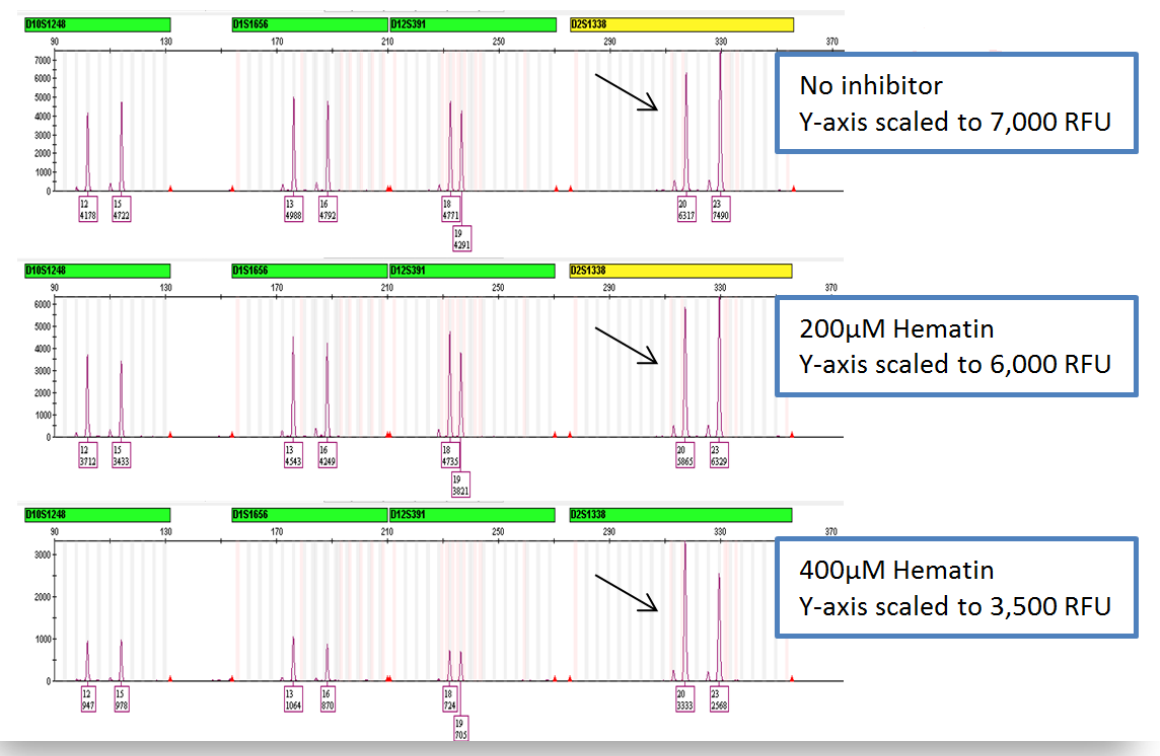
Most dye channels and loci demonstrate the traditional ski-slope pattern.

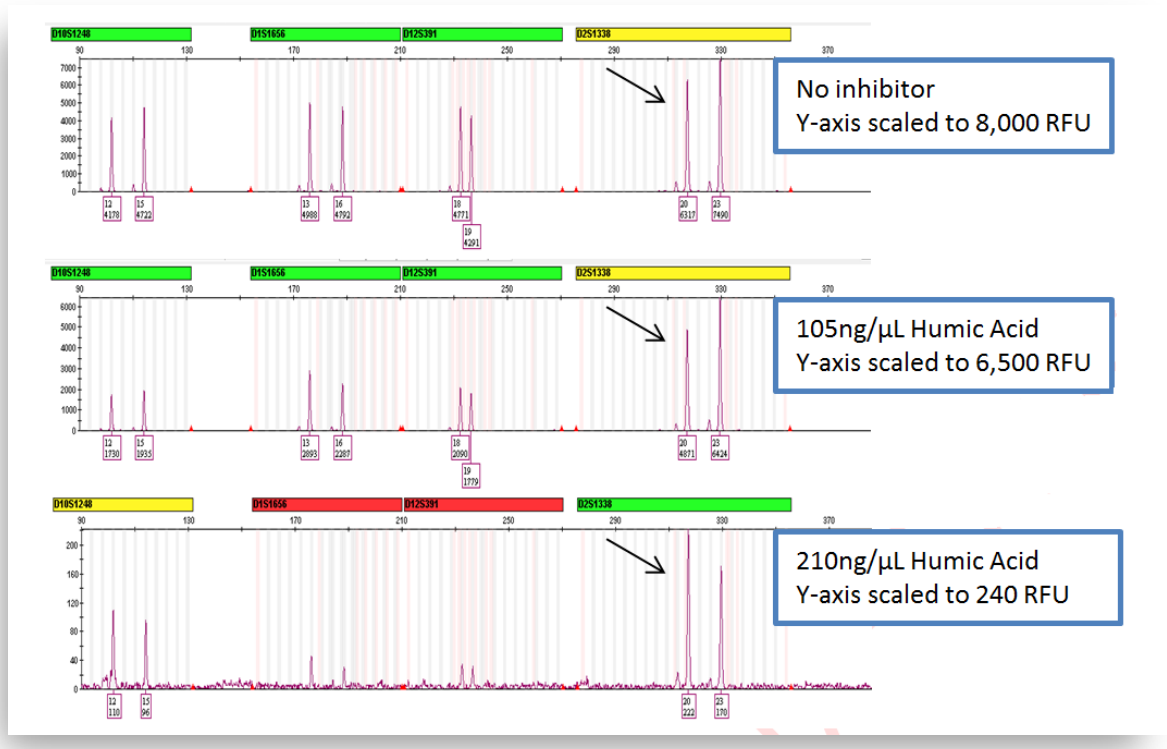
FACT: Inhibition Patterns: High NED



Due to kit design, the NED dye channel is generally least impacted by inhibitors. Therefore, common inhibition profiles show high NED peak heights relative to the other dye channels.

Inhibition Patterns: Reverse SID





Amplification bias of the D2S1338 locus can be observed in the SID dye channel.

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Question 6

- MYTH or FACT
- Improper cycling conditions and long term storage of amplicon are two reasons that (–A) could occur?

IMPORTANT! If you using are the GeneAmp™ PCR System 9700, select the Max ramping mode. If you are using the Veriti™ Thermal Cycler, select the 100% ramping rate. *Do not* use 9600 emulation mode.

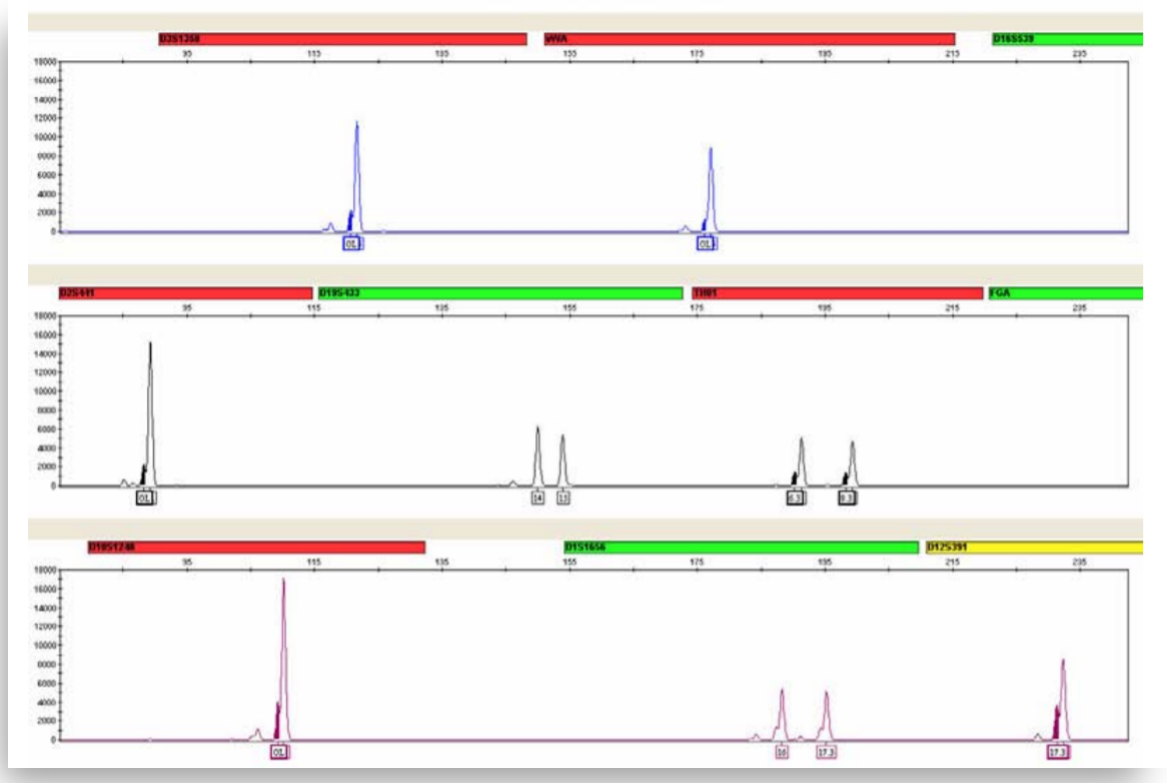
Initial incubation step	Cycle (29 or 30 cycles)		Final extension	Final hold
	Denature	Anneal/Extend		
HOLD	CYCLE		HOLD	HOLD
95°C, 1 minute	94°C, 10 seconds	59°C, 90 seconds	60°C, 10 minutes	4°C, Up to 24 hours[1]

[1] The infinity (∞) setting allows an unlimited hold time.

FACT: -A Considerations

- Incorrect thermal cycling parameters
  - GlobalFiler and GlobalFiler Express use different ramp speeds than prior kits
- Expired kits
- Aged/improperly stored amplicon
- Sample specific exonucleases
- Chelex* extraction
- Direct amplification w/ certain substrates
- Altered PCR conditions

*Chelex extraction has not been confirmed internally to hinder adenylation in GlobalFiler, but Chelex and other extraction methods should be considered when troubleshooting –A.



Tip: to reduce/eliminate –A in stored/aged amplicon, perform an additional 5-10 minute soak at 60°C just prior to CE prep.

Question 7

- MYTH or FACT

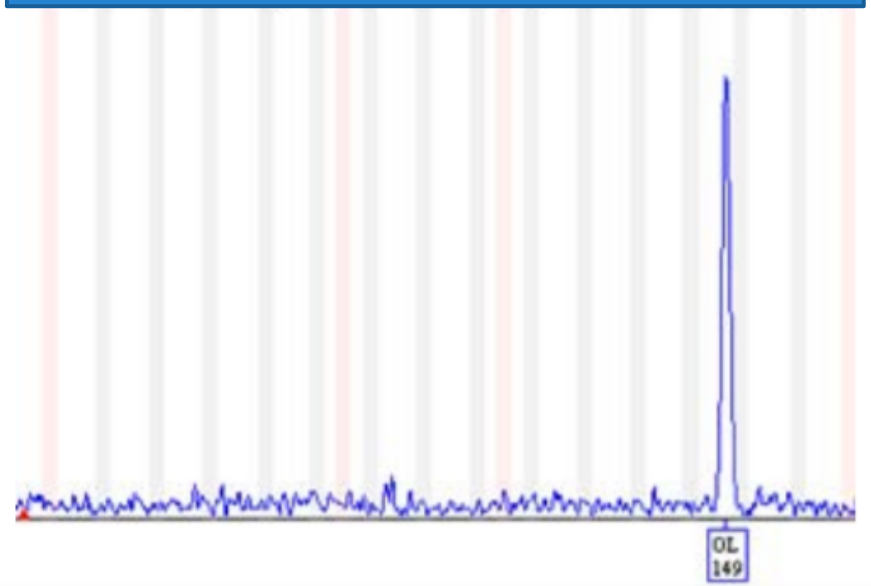
During development, R&D scientists DO NOT focus extensively on effective primer design and kit optimization to prevent artifacts.

http://science.phillipmartin.info/science_dna.htm

MYTH: Artifact Considerations

1. Greater sample/substrate variety than ever before
2. Greater chemistry and instrument sensitivity than ever before
3. 24 primer sets = more opportunity
4. Direct amplification

Artifact at vWA in GlobalFiler attributable to yeast or fungus – no detectable stutter



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November 29, 2017

TECHNICAL NOTE

Artifacts Identified Post-Developmental Validation: GlobalFiler™ PCR Amplification Kit

The purpose of this document is to assist with data interpretation by providing a repository of GlobalFiler artifacts, identified and characterized post-developmental validation of the GlobalFiler kit (PN 4476135 and 4482815) as a result of investigating customer reports. Many of the artifacts included in this document fall below the peak amplitude threshold (PAT) used during developmental validation, appear outside of the read region, or are attributable to specific sample types that were not encountered during developmental validation.

Tips for Identifying Artifacts:

- Peak morphology
- Sample specific? In controls?
- Is it reproducible?
- OL or OMR allele calls
- Height of suspect artifact relative to other peaks
- Lack of stutter

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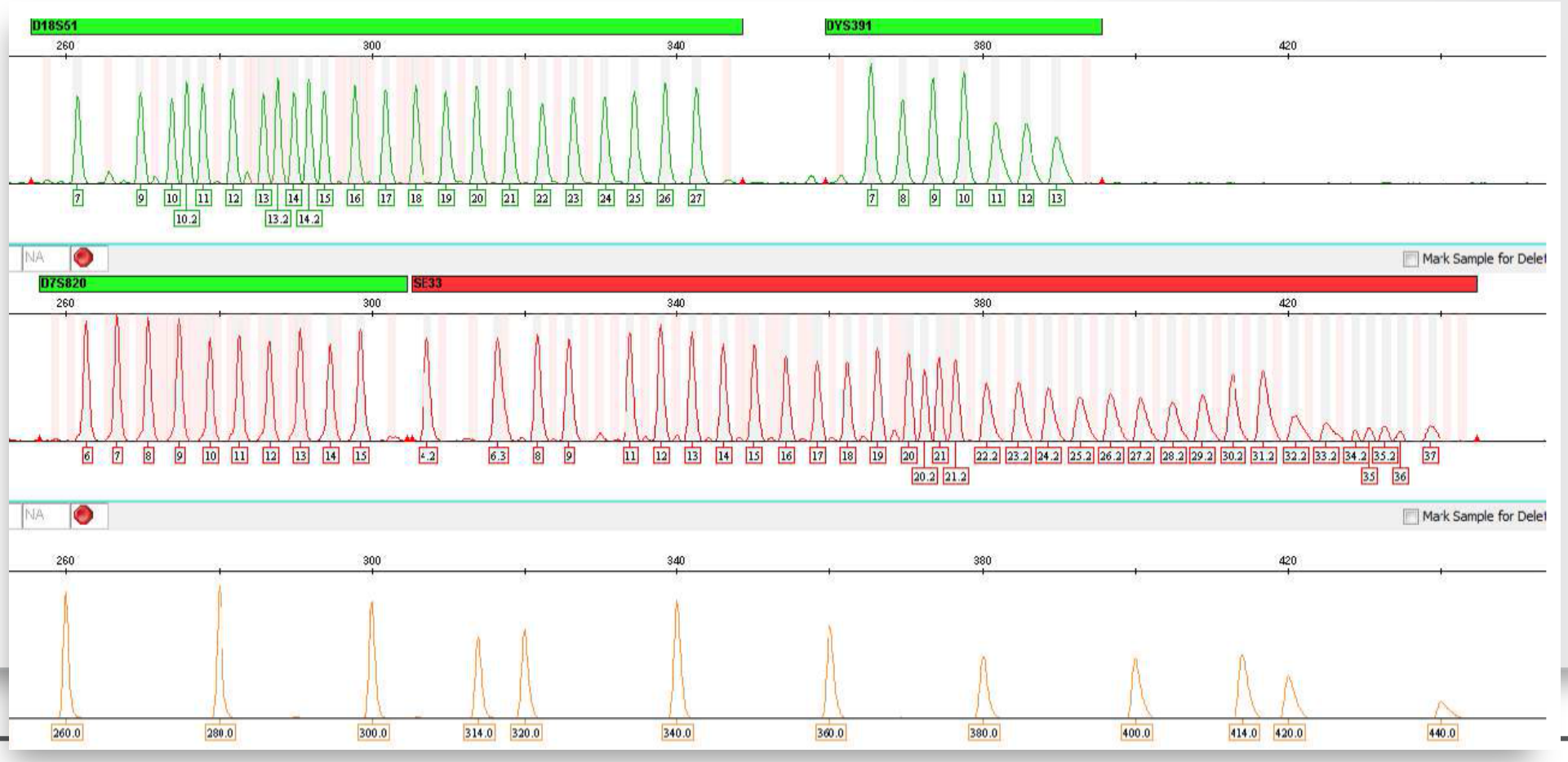
Capillary Electrophoresis

Question 8

- MYTH or FACT:
- A primary reason for Loss of Resolution (LOR) with a capillary is the accumulation of contaminants over time?



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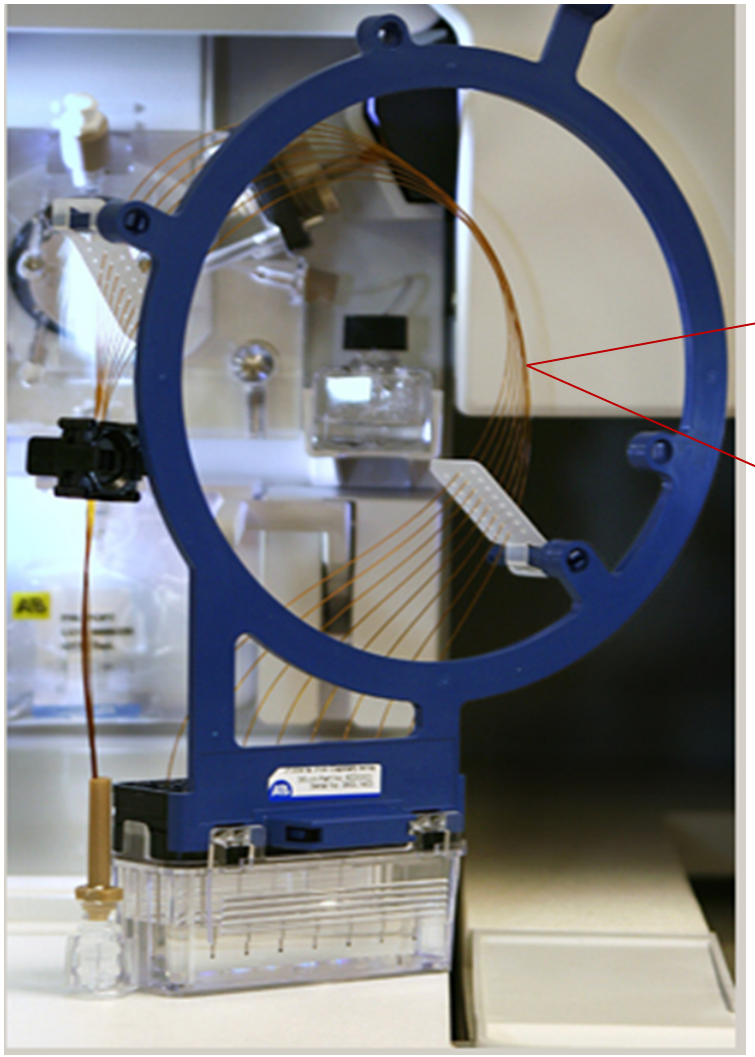
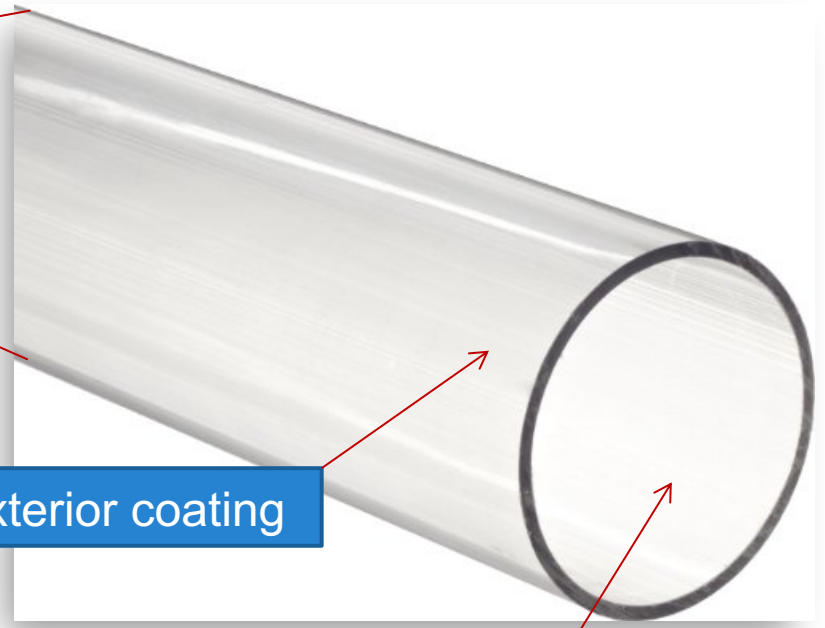
FACT: What is an array?

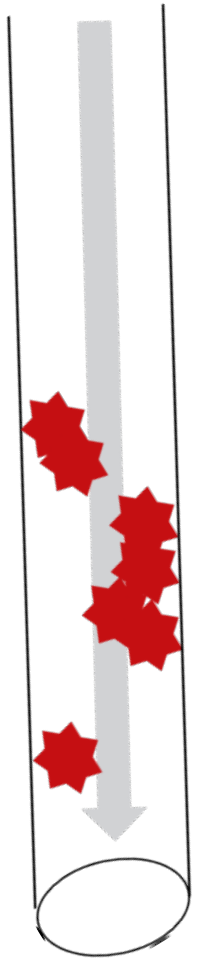


Hollow glass tube

Flexible exterior coating

Internally uncoated

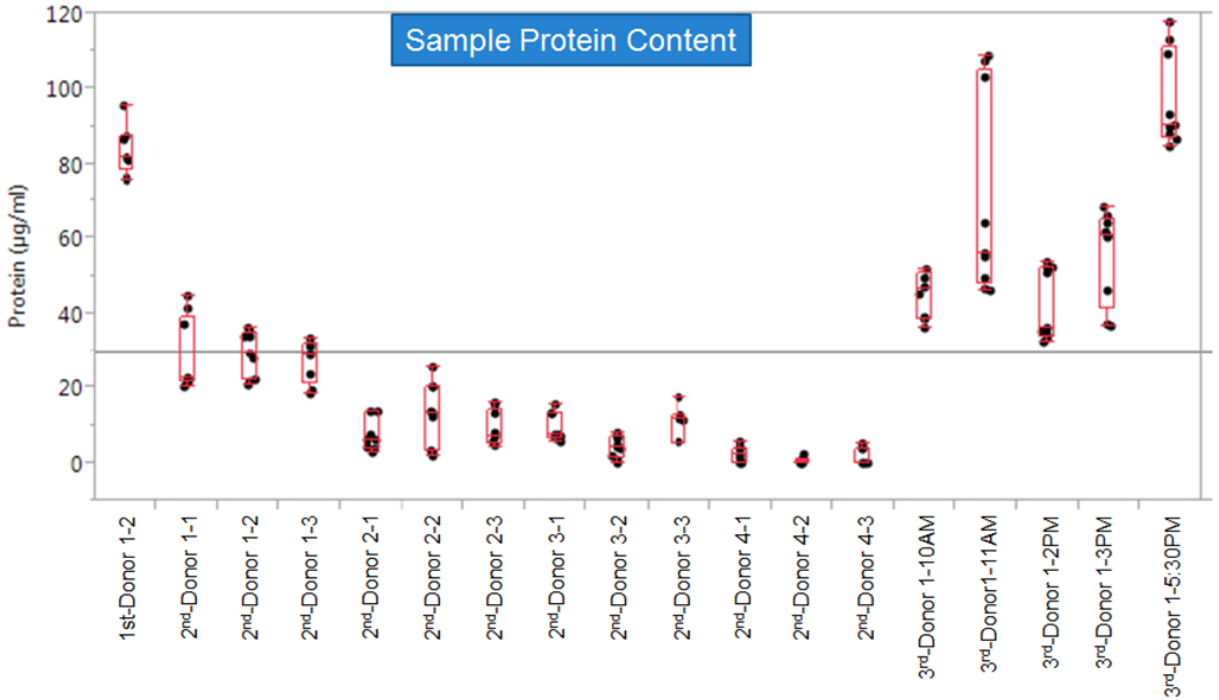




Progressive array deterioration is caused by an accumulation of foreign contaminants.

LOR: Direct Amp Workflow

- Direct amp workflows can cause early onset of LOR
- High protein samples cause LOR
- Sample protein content is variable
- Changing the array is only a temporary solution



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Tips for Extending Array Life

1. Perform wash wizard weekly
2. Install a fresh polymer pouch at least once every 14 days
3. Run a single dummy injection of HiDi blanks or Negative Template Controls on idle days
4. Coordinate casework so instruments are run as often as possible

WASH the pump chamber and channels

Fresh polymer + Weekly washes + High frequency = Longer array life

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Question 9

- MYTH or FACT:

3500 series arrays DO NOT have an expiration date?

https://www.google.com/search?q=expiration+date+clipart&rls=com.microsoft:en-US&tbm=isch&source=iu&ictx=1&fir=3-OWUhVsOmlntM%253A%252CZjdIqt7T16om5M%252C_&usg=__x3aoxNqt8JLg100LA3yFDIw---k%3D&sa=X&ved=0ahUKEwjv69C-u4DaAhXvYN8KHWuzBiEQ9QEIOTAI#imgrc=3-OWUhVsOmlntM:&spf=1521740123554

MYTH: 3500 series Arrays

- 3500/xL arrays are able to produce robust data through the listed expiration date or **160** injections, whichever comes first.
- Arrays have a 1 year from manufacture expiration date can be used beyond the expiration date or minimum injection specification. If the array is still producing robust data, use it!
- Higher throughput labs who consistently run their instruments with little idle time obtain longer average array lifespans than lower throughput labs

Question 10

- MYTH or FACT:

3500 polymer pouches can be stored anywhere in a refrigerator?

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MYTH: 3500 series polymer pouches

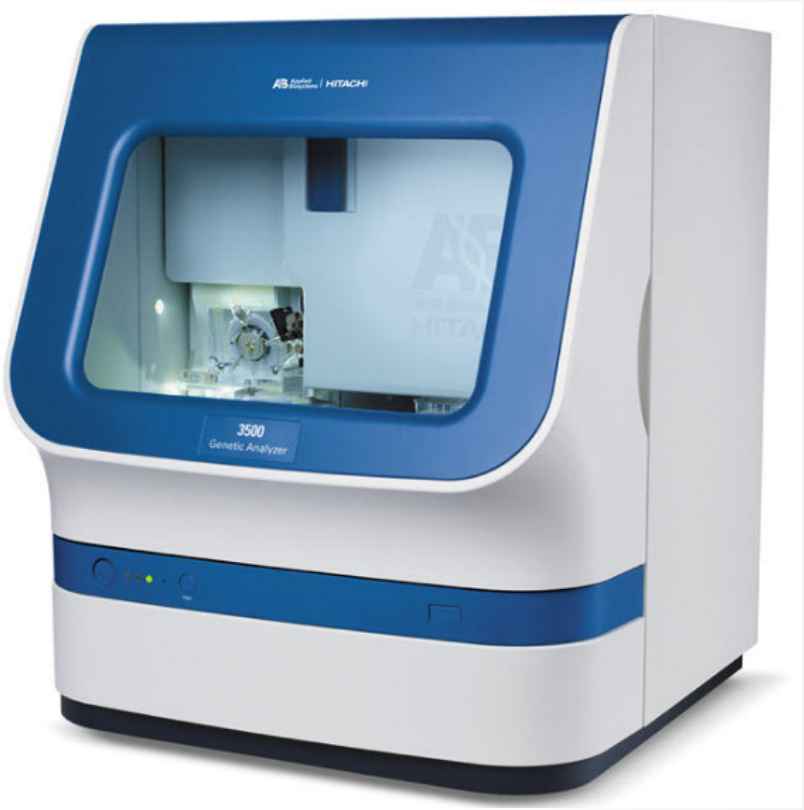
- Polymer pouches should be kept in a tightly controlled temperature area of the fridge
- Consider where the pouches are stored and ensure it is not the coldest part of the fridge
- Pouches that are near the cooling unit of the fridge or drop below 2° C are likely to crystallize
- Pouches should never be frozen

href="http://www.clker.com/cliparts/4/U/3/T/I/d/open-refrigerator-th.png"><img

Question 11

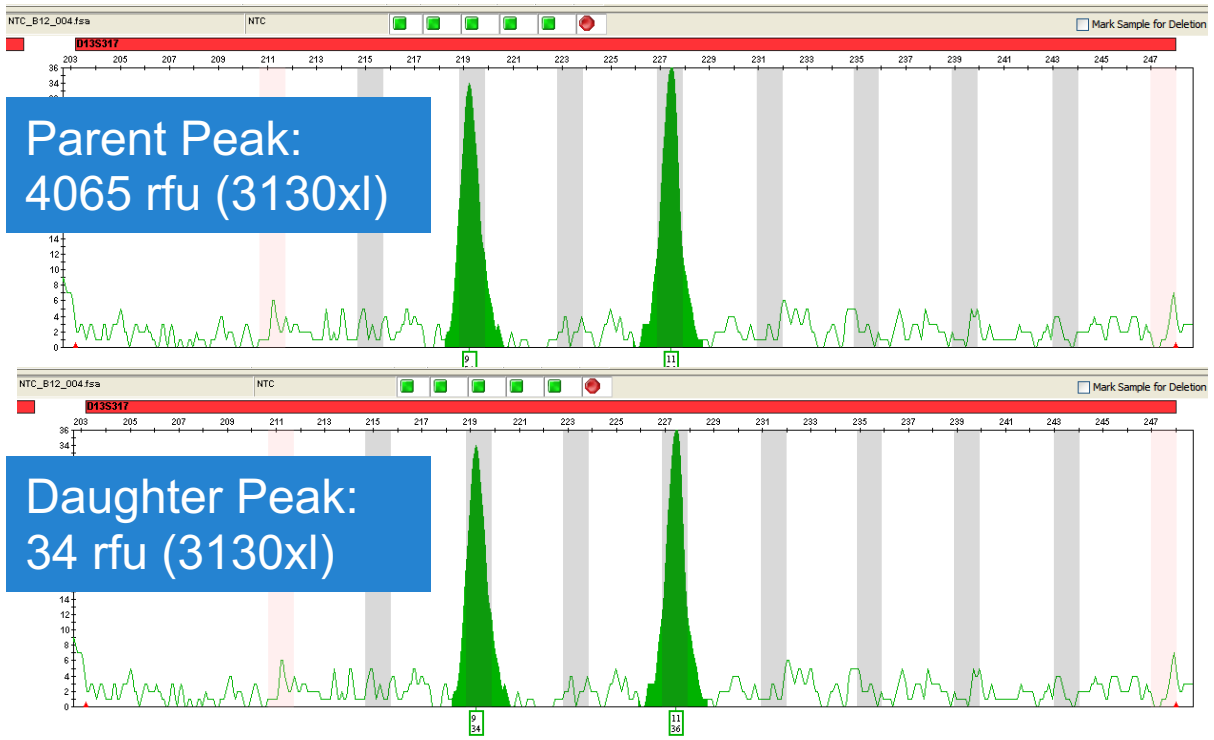
- MYTH or FACT:

Carryover is the physical transfer of DNA from one injection to the next and can be completely prevented?



MYTHS: Carryover

- Carryover can be minimized but can never be completely eliminated with the current technology.
- The primary mechanisms for minimizing carryover are the polymer fill and rinse steps of the run module, and the duck bill design of the septa to physically wipe the ends of the capillary.



34/4065 = 0.83%

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Question 12

- MYTH or FACT:

HiDi™ Formamide Blanks should be used to assess carryover?

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MYTHS: Carryover

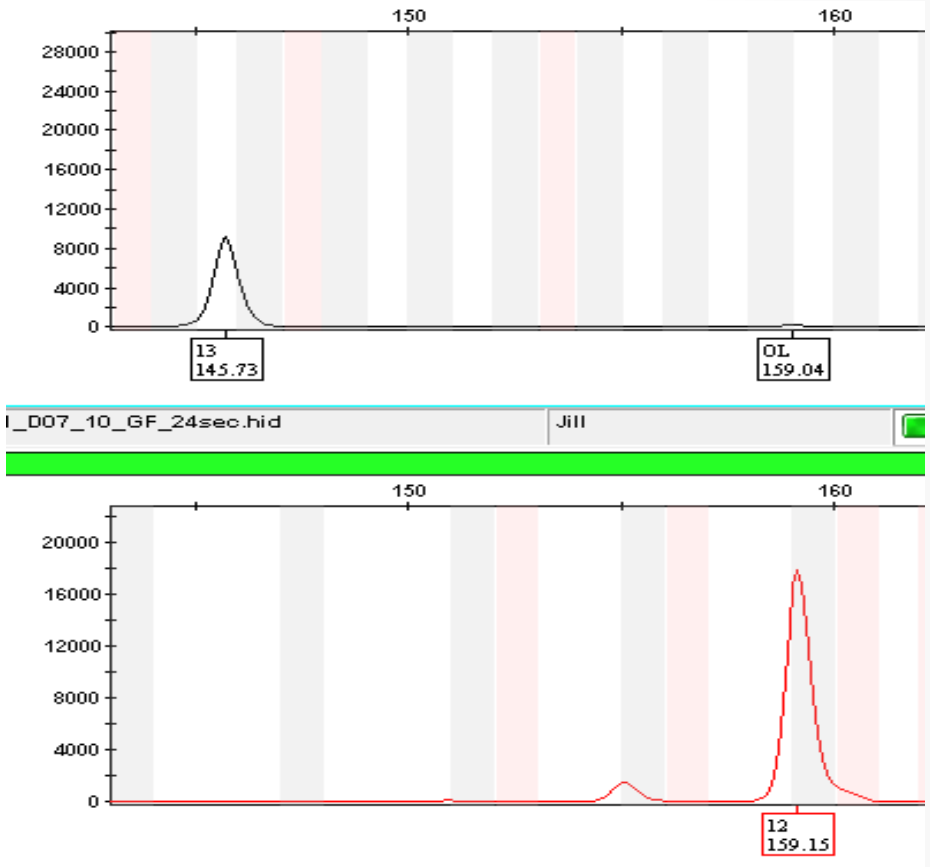
- HiDi blanks have little to no salt content and actually promote carryover. Negative Template Controls should be used instead.
- We have completed extensive carryover studies and are available to share our findings and to provide guidance. For a more thorough carryover discussion, contact your HID FAS.

Data Analysis

Question 13

- MYTH or FACT:

GeneMapper™ ID-X can calculate the % pullup for you?



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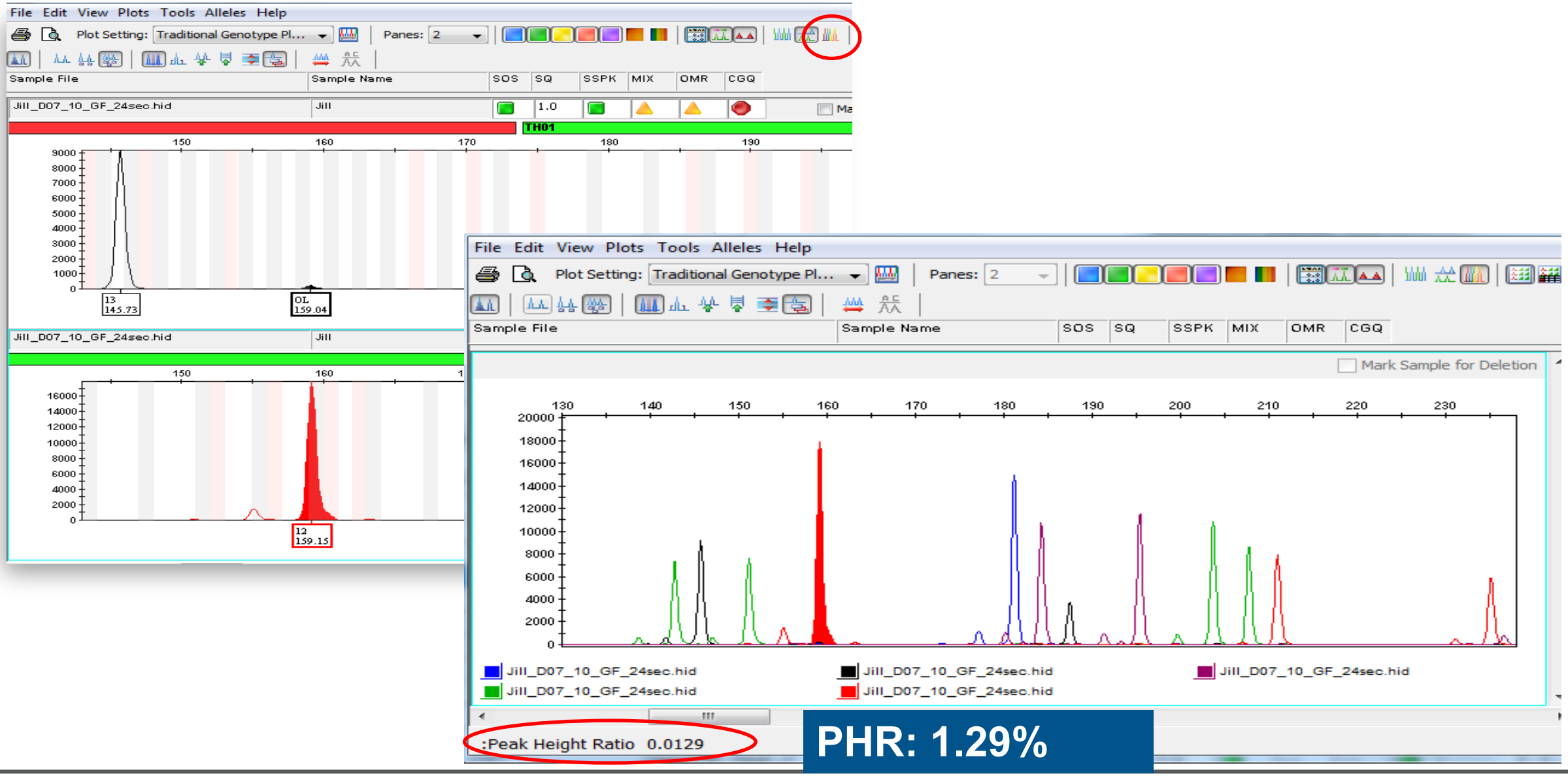
FACT: Calculating Peak Height Ratios



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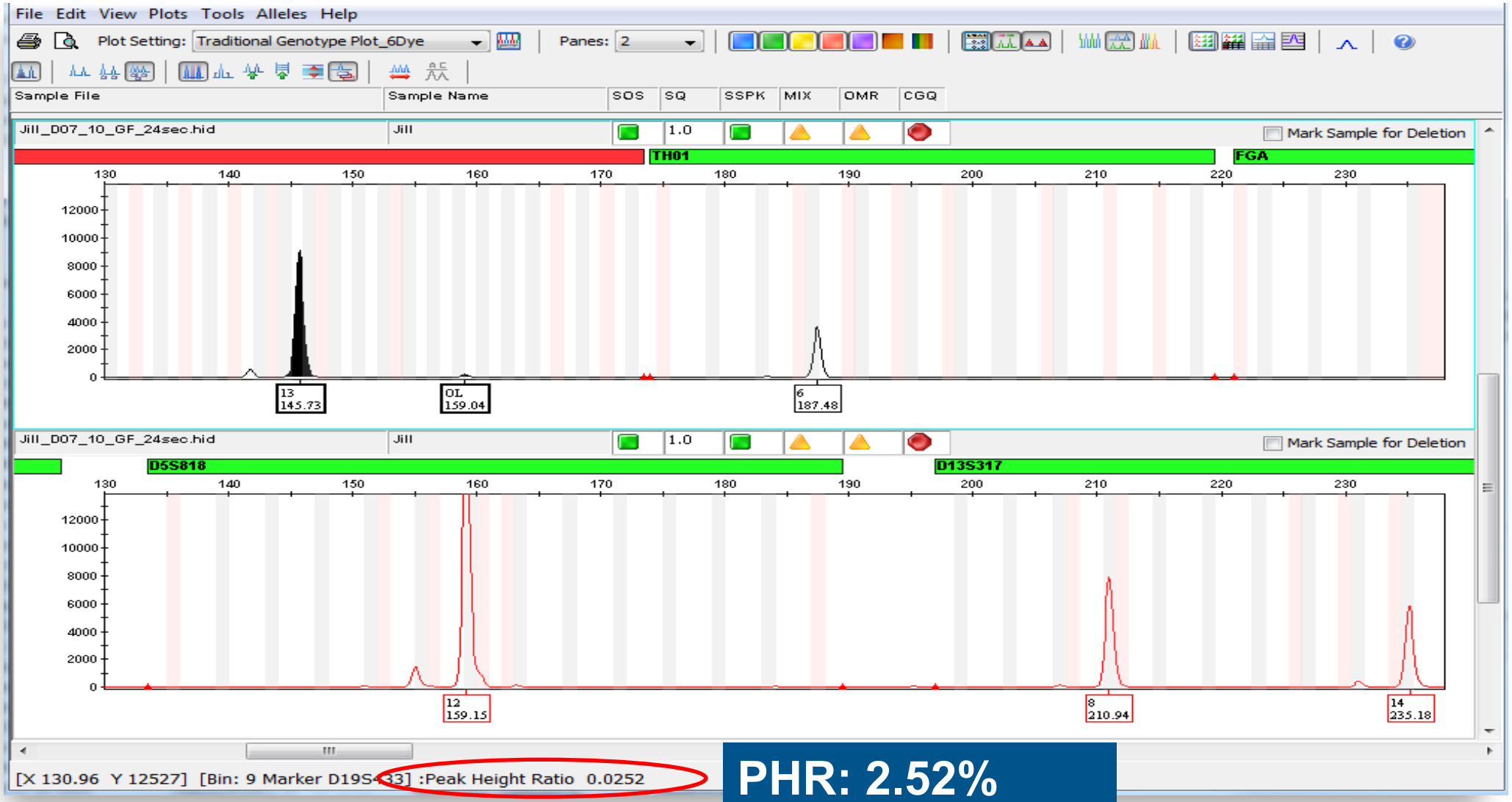
FACT: Calculating Peak Height Ratios



PHR: 2.52%

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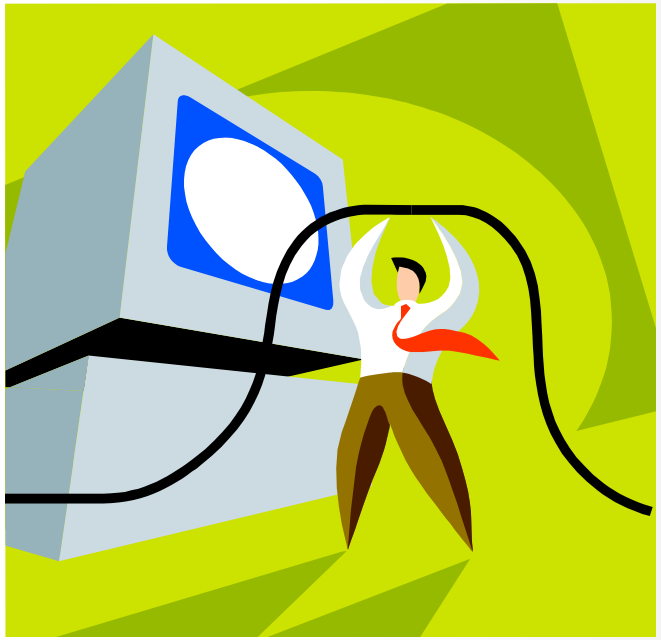
Instrument and Computer Communication

- -Communication issues(31XX and 37XX) / services will not start (3500) – 31XX and 37XX use a windows user to log in to PC and grab calibration file. If instrument cannot log in then it will not boot up and stay at a solid yellow light. Password cannot change and password cannot expire. Depending on customer network policy, 3500 services need admin rights in order to start properly. The calibration file for the 3500 is on board so no matter what the 3500 will go to green light even without a customer PC connected.



Additional CE Troubleshooting from FSE Perspective

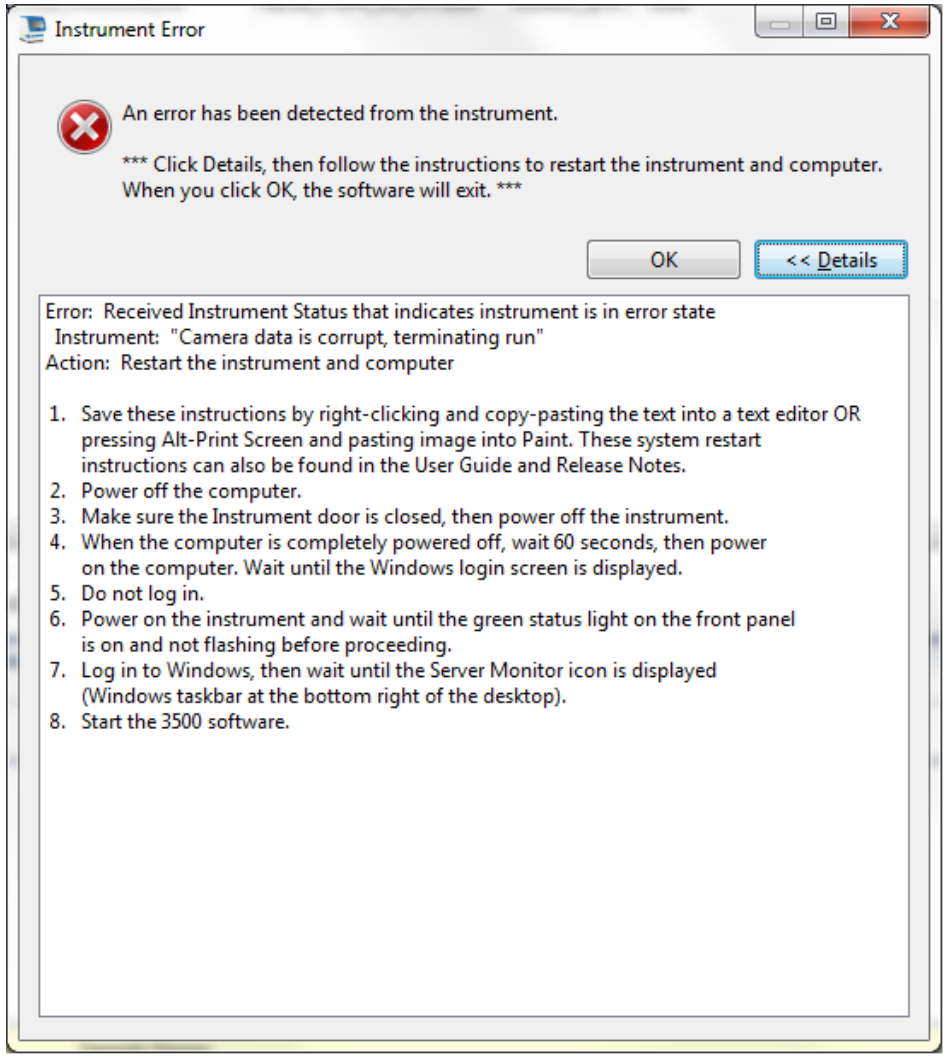
1. Samples work on part of the tray/low level samples
   - Possible autosampler alignment or laser alignment.

2. Machine red light on boot up
   - Instrument runs a self-test during boot up and applies all calibration values. If parts are damaged or missing then machine will red light.

3. Flat baseline in raw data but machine green lights when turned on.
   - No EP current. Could be arching in system due to bubbles. Pay attention to EPT data!
   - CCD camera has failed and needs to be replaced.
   - Laser has failed and needs to be replaced.

3500/xL Camera Corrupt Error – Sporadic Occurrences

- Often caused by system/network updates, incorrect or rushed system startup sequence or other sources of communication barriers
- Resolution = FULL system restart
- Prevention:
  - Perform FULL system restart after every network update
  - Daily full system restarts can help if done properly but are not always necessary – restarts should be done on a weekly basis at minimum
  - Attempting to start a Spatial can be used as a diagnostic tool
    - If Spatial fails to start, perform full system restart
    - If Spatial starts, proper communication has been established & errors should not occur

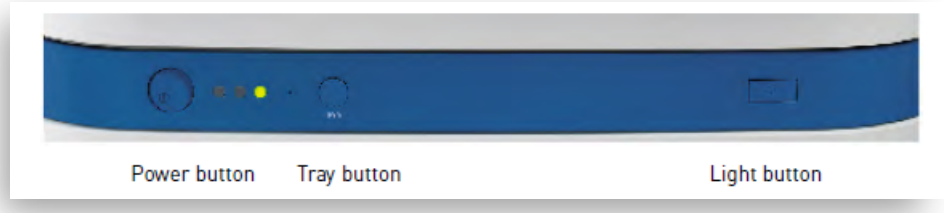


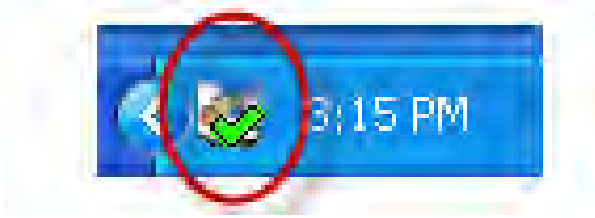
3500/xL Communication Issues

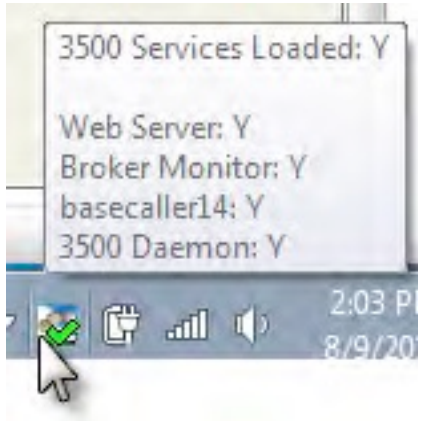
Formal Startup Sequence

1. Power on computer but do not log in
2. Power on instrument – wait for green light
3. Log in to Windows
4. Wait ~1-2 minutes for green checkmark to appear in task bar
5. Launch the Data Collection software and log in

- If the task bar status icon remains a red X, start the services manually by Right-clicking the status icon and selecting any services that are not started.







Most instrument/computer communication issues can be prevented or resolved by following the formal startup sequence.

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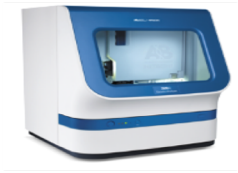
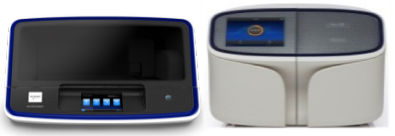
Conclusion and Workflow Considerations

Collection	Extraction	Quantification	Amplification	Detection & Analysis	Next Gen Sequencing
 Copan NUCLEIC-CARD™ and 4N6FLOQSwabs™	 AutoMate *Express*™ DNA Extraction System	 7500 Real-Time PCR Instrument	 ProFlex™ Thermal Cycler	 3500/3500xL Genetic Analyzer 3130/3130*xl* Genetic Analyzer	 Ion Chef™ System and Ion S5™ Systems
	 PrepFiler™ Forensic DNA Extraction kit	 QuantStudio 5 Real-Time PCR Instrument + HID Real-Time PCR Software v1.3	 GlobalFiler™ STR Kits	 GeneMapper™ *ID*-X Expert System Software	 Precision ID Panels
		 Quantifiler™ HP and Trio Kits	 Y-Filer™ Plus Kit		

Not only is a kit itself complex, but it must be able to work well with other parts of the workflow, of which there are many variants. If there is a workflow issue, the GlobalFiler profile may not look optimal due to an upstream or downstream complication.

To download resources

Thermofisher.com/forensics-resources

Or

TEXT: 313131
MSG: HIDinfo

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Forensics Resources

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Forensic Toxicology

- Sample Prep Forensic Toxicology
- Targeted Drug Screening & Quantitation
- Unknown Compound Identification
- Volatile Blood Alcohol Analysis

applied biosystems

Forensic DNA Analysis

Casework/Database Workflow: Product & Poster Highlights

- Extraction
- Quantification
- Amplification
- Analysis
- Validation

NGS for Forensics: Product & Poster Highlights

- Construct Library

HID Product Communications Registration

http://resource.thermofisher.com/pcs/index.php

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Would you like to receive regular updates on all technical communications relating to HID products that you are currently using?
To register, please complete the form below.

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Submit

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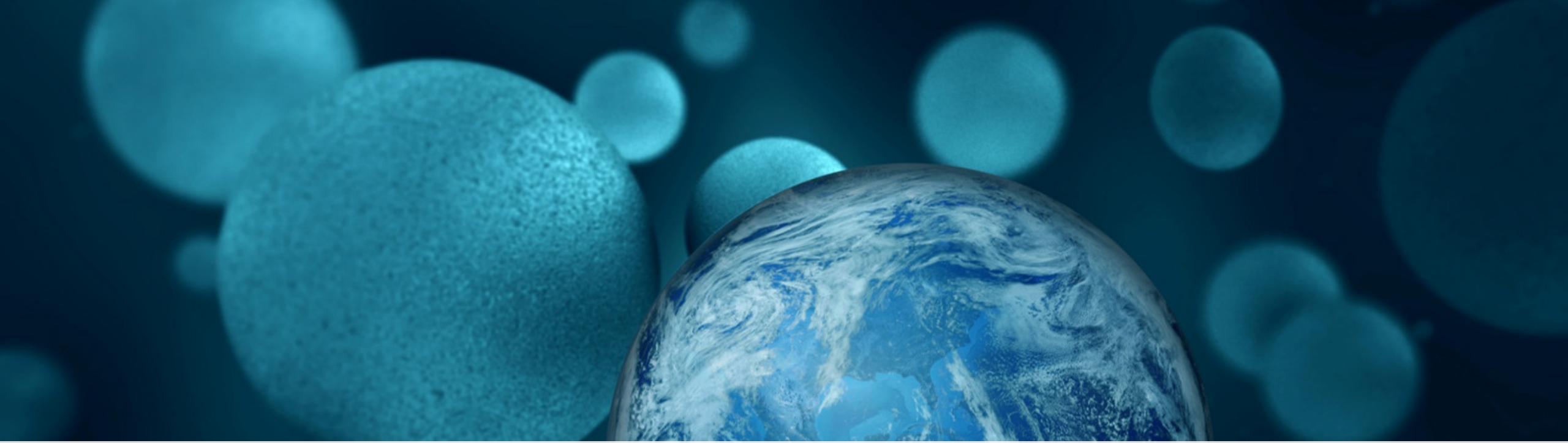

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Thank You!

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