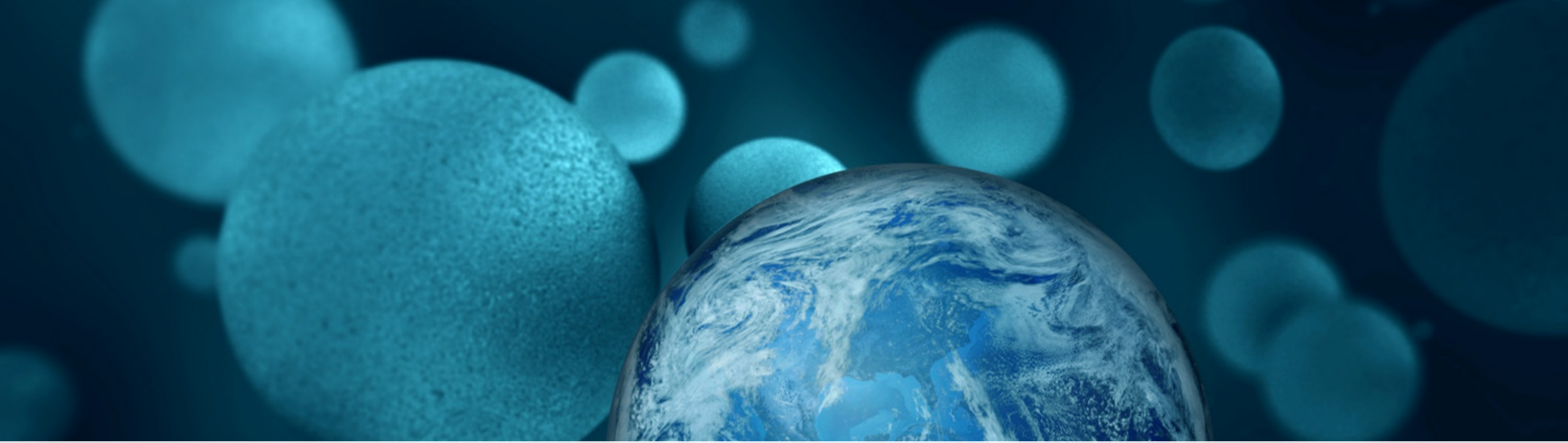

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Quant Studio 5 Instrument and Virtual Standard Curve

Megan Meyer
Field Application Scientist (FAS)

Innovation In DNA Quantification

Quantifiler™ Human and Y Kits
2004

Quantifiler™ Duo Kit
2008

Quantifiler Trio
2014

HID Real-time SW v1.1 | HID Real-time SW V1.2 | HID Real-time SW V1.3

2003
7000 Real-time PCR Instrument

2007
7500 Real-Time PCR instrument

2017
QuantStudio™ 5 Real-Time PCR instrument

Extending Your Real-Time PCR Instrumentation Options

- HID validated
- 0.2ml Standard 96-well format
- 6 Dye capability
- Smaller foot print
- 10 GB Onboard memory
- Bright light LED with 5 year lifespan
- Touch screen interface

Instrument Touch Screen

Open/Close door

Help

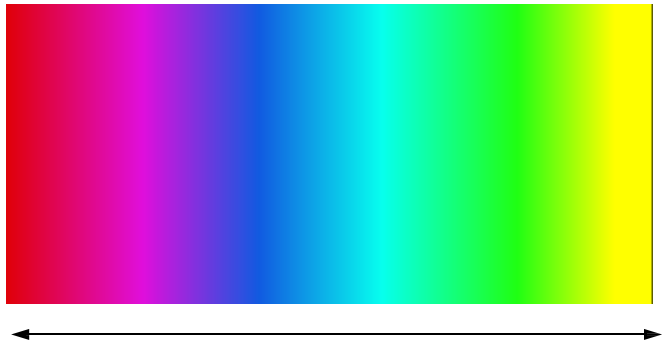
Sign In or Create a Local User Account

Settings:
- Calibration
- Runs
- Logs
- Ship Prep

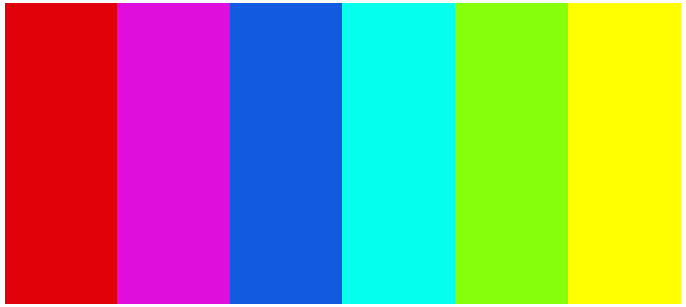
https://www.thermofisher.com/us/en/home/life-science/pcr/real-time-pcr/real-time-pcr- instruments/quantstudio-3-5-real-time-pcr-system/quantstudio-3-5-real-time-pcr-system- virtual-demo.html#touch

QuantStudio 5 Real-Time PCR Systems: VeriFlex Technology

- VeriFlex™ Blocks
- 6 programmable temperature zones
  - Independent temperature control in each zone (more precise than gradient)



Gradient



vs. VeriFlex™

- Can program at will, including multiple zones with same and different temperatures
- Great for optimization and also running multiple assays at the same time

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Optical System Comparison

	QuantStudio 5	7500
Excitation source	Bright white LED Excitation Source	Halogen Lamp
Lower energy use	✓	
Lower heat emission	✓	
Longer lifespan	✓	
Camera	CMOS Camera	CCD Camera
More efficient for shorter exposure times	✓	
Consumes less power	✓	
Detection	6 decoupled channels	5 decoupled channels

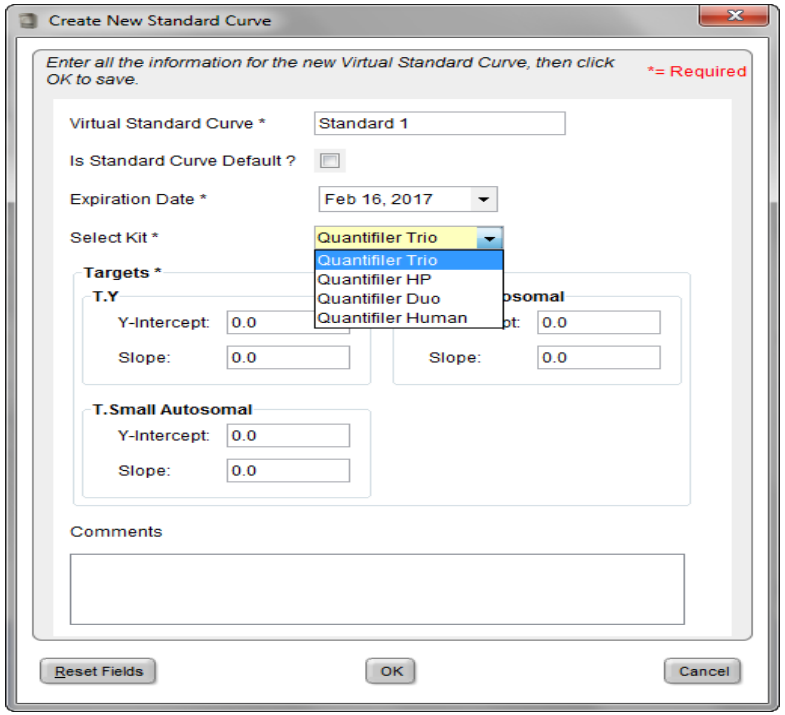
Recommended Maintenance and Calibration

Frequency	User-performed maintenance task
Weekly	Check disk space and power off the instrument for at least 30 seconds
	Clean the instrument surface with a lint-free cloth
Monthly	**Perform a background calibration (to check for thermal block contamination)**
	Run disk cleanup and defragmentation
	Perform instrument self-test
Every 2 years	**Perform ROI, uniformity, dye, and normalization calibrations**
As needed	Perform an RNase P instrument verification run After instrument installation, moving, or as needed
	Replace the instrument lamp

Broadening Your Software Capabilities: Virtual Standard Curve

The Virtual Standard Curve Feature as part of the HID Real Time SW enhancement to allow you to define your own standard curve values and use those values to calculate DNA quantitation:

- Reduction in Result Variation
- Streamlined and Time Saving
- Efficiency and Cost Savings
- Flexibility and Accuracy



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Number time… Can you figure out this sequence in 60 seconds?

8, 18, 11, 15, 5, 4, 14, 9, 19, 1, 7, 17, 6, 16, ?, ?, ?, ?, ?

10, 13, 3, 12, 2

What do these numbers mean to you?

10, 20, 40, 80, 160, 320, 640, 1280, 2560...

Looks like a sequence of doubling numbers, right?

PCR?? PCR efficiency?

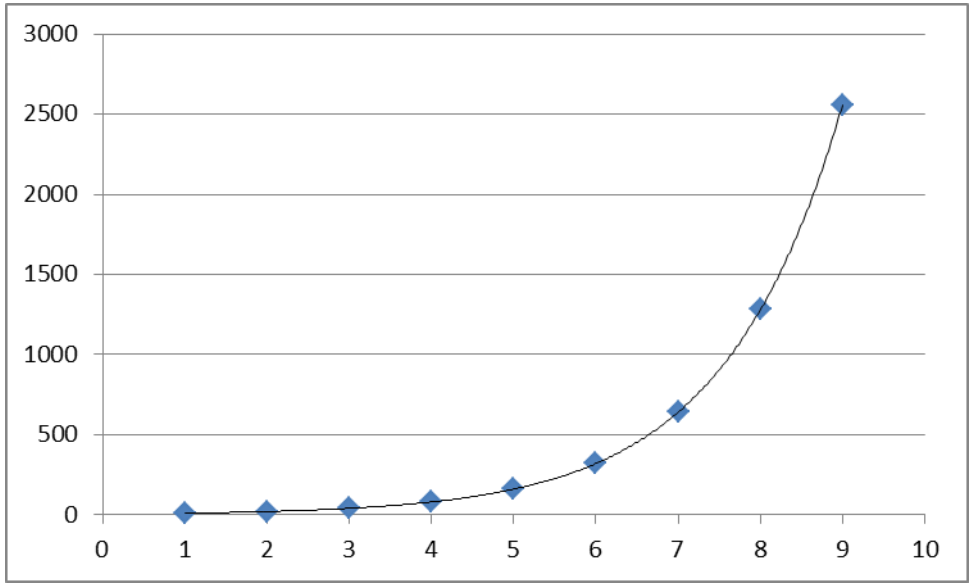
With qPCR doubling of product happens after every cycle with 100% PCR efficiency

Only need slope, Y-Intercept, R² to calculate PCR efficiency

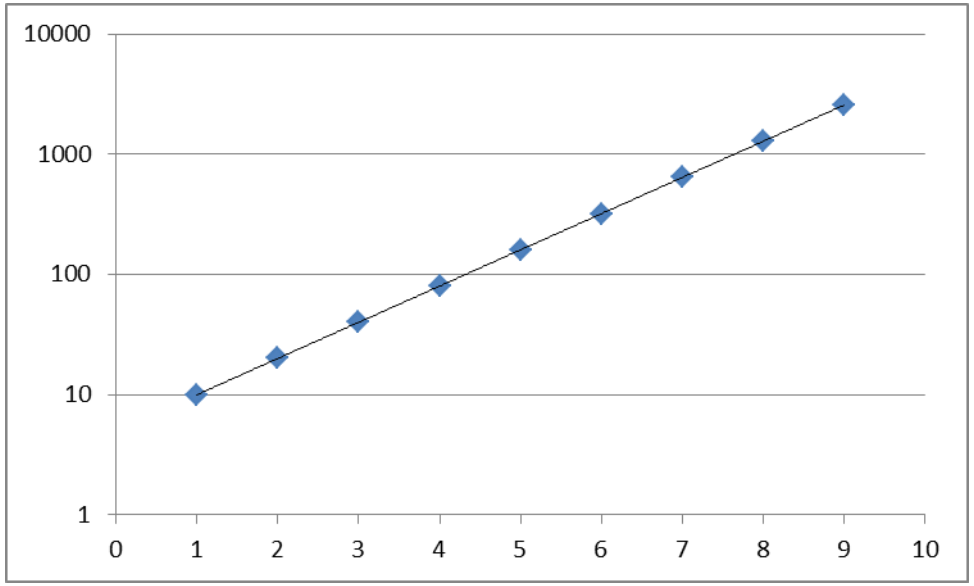
PCR Efficiency = $10^{(-1/slope)}-1$

Another look at the numbers…

10, 20, 40, 80, 160, 320, 640, 1280, 2560...



Linear graph / exponential curve



Logarithmic graph / linear curve

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Another look at the numbers…

10, 20, 40, 80, 160, 320, 640, 1280, 2560...

Starting at 10 copies of PCR product, after how many cycles do we see 100 copies of PCR product?

10, 20, 40, 80, 100, 160, 320, 640, 1280, 2560...

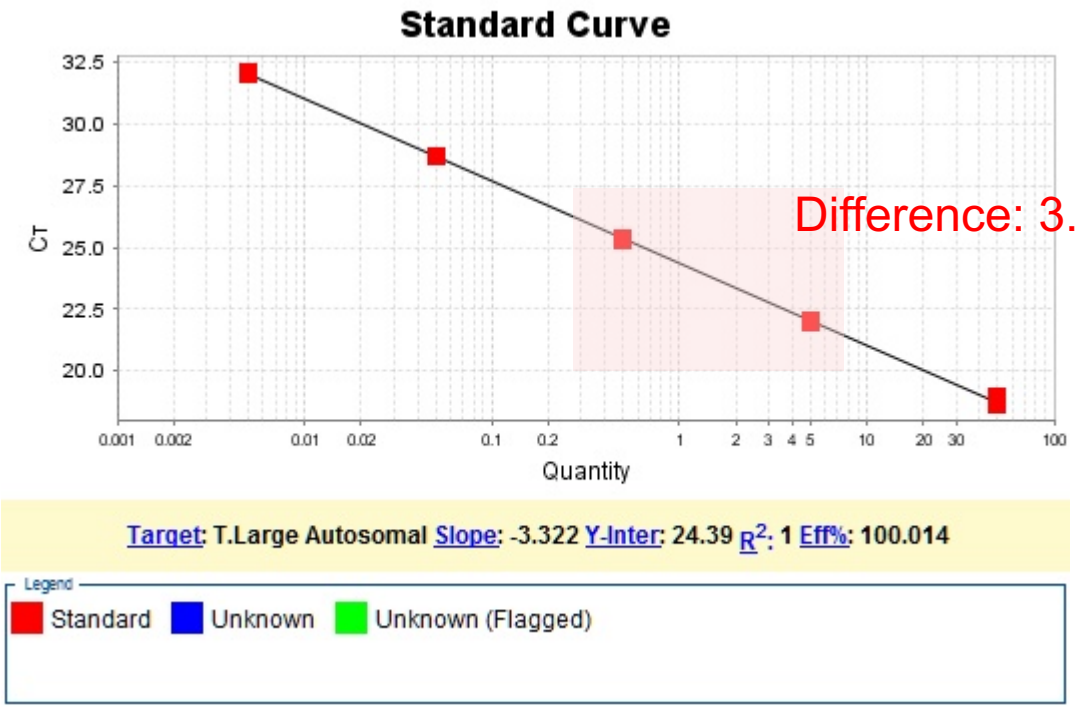
0, 1, 2, 3, 3.33 4, 5, 6, 7, 8

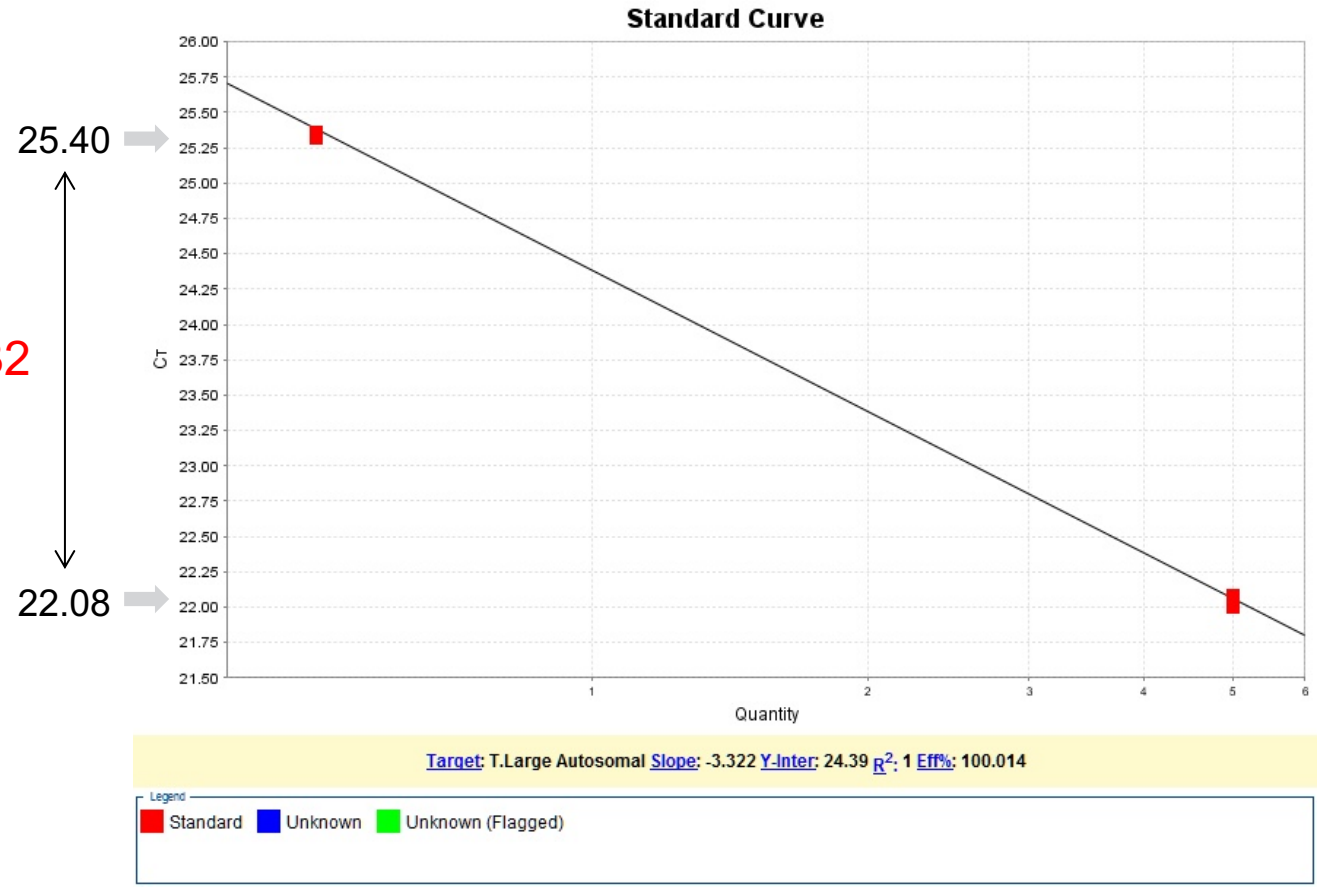
So, 100% PCR efficiency is also a 10-fold increase in product every 3.33 cycles

We can see this in action with Quantifiler Trio

Quantifiler Trio Standard Dilution Series

- 5 point, **10-fold** dilution series
- So the 5ng/ul and 0.5ng/ul standards should cross the cycle threshold (Ct) how far apart from each other?





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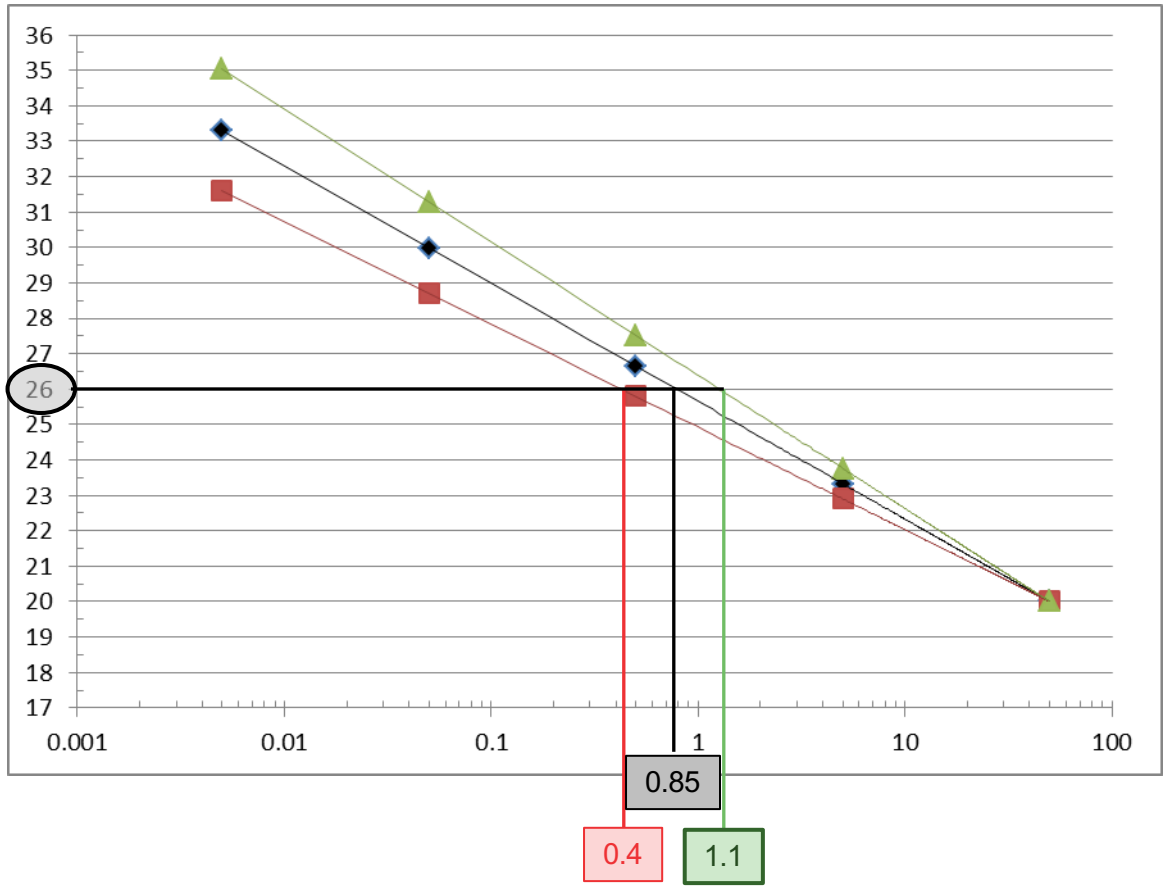
What if the slope is not -3.33?

Example 1: Pipette setting error



Only the slope is changing due to more/less standard DNA transferred to subsequent dilution points

More: 10.1ul versus 10.0ul for each transfer

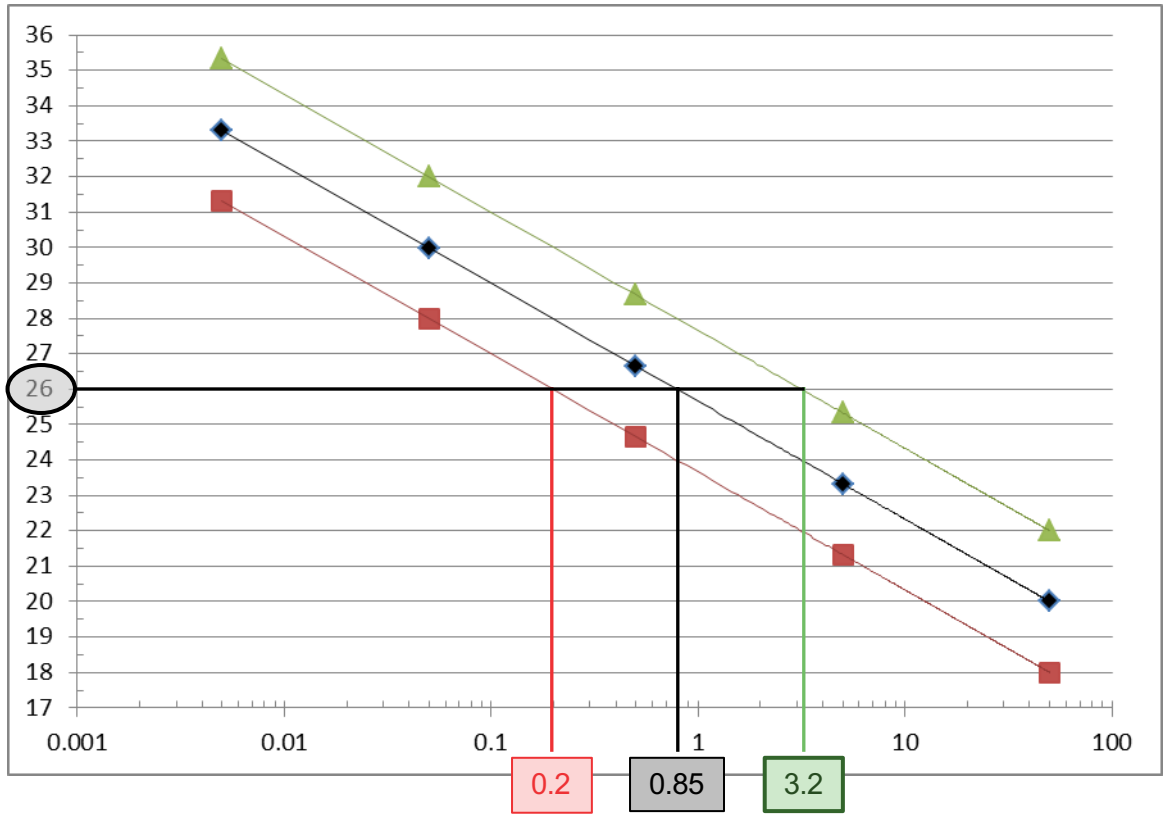
- Should be 50ng, 5ng, 0.5ng, 0.05ng…
- Actually 50ng, 6ng, 0.7ng, 0.08ng…
  - Ct values decrease due to more DNA than expected
  - Slope is less negative (-2.90)

Less: 9.9ul versus 10.0ul for each transfer

- Should be 50ng, 5ng, 0.5ng, 0.05ng…
- Actually 50ng,4ng, 0.3ng, 0.02ng…
  - Ct values increase due to less DNA than expected
  - Slope is more negative (-3.76)

What if the slope is not -3.33?

Example 2: Standard concentration error



Only the Y-intercept is changing due more/less standard DNA transferred first dilution point (non-pipette error)

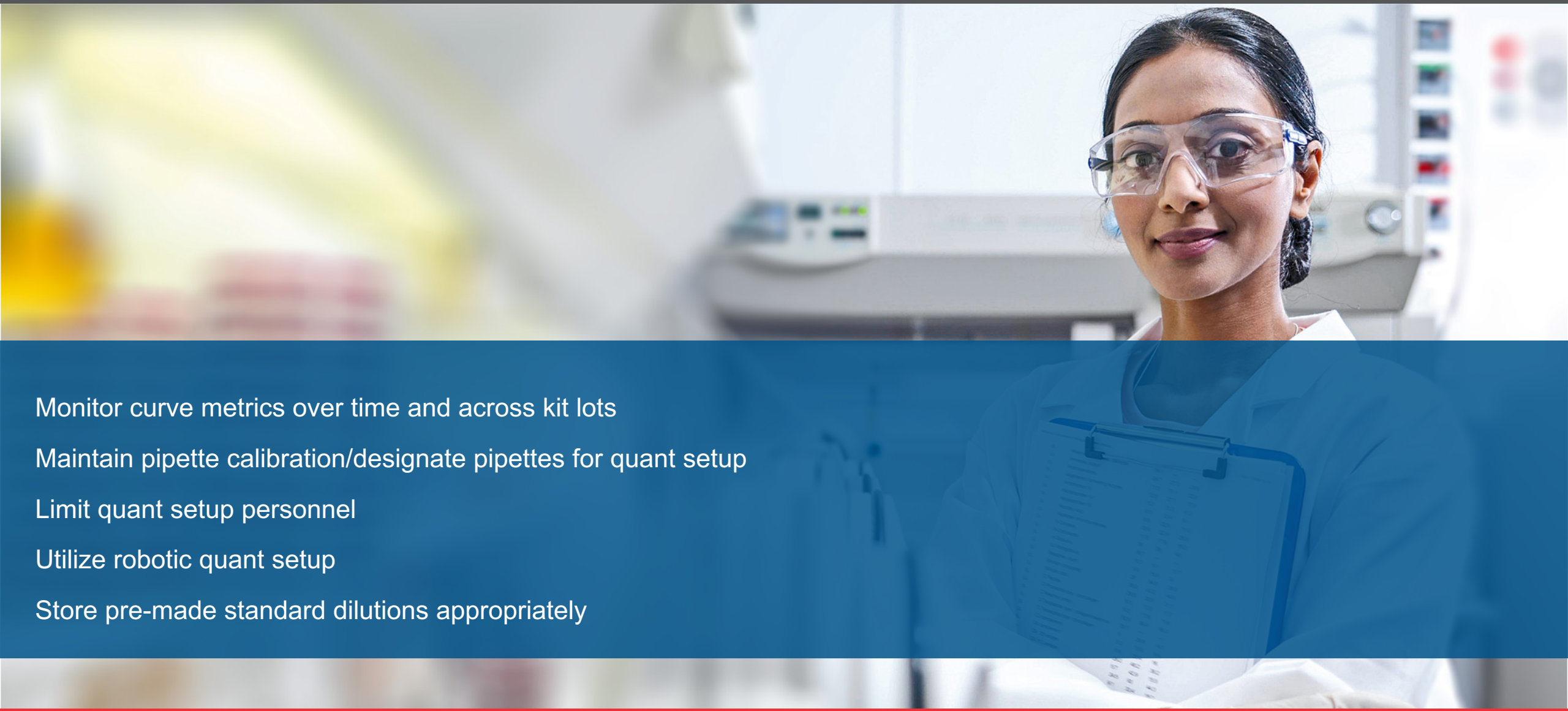
More: 52ng versus 50ng for initial transfer

- Should be 50ng, 5ng, 0.5ng, 0.05ng…
- Actually 52ng, 5.2ng, 0.52ng, 0.052ng…
  - Ct values decrease due to more DNA than expected
  - Y-intercept is lower
  - Unknown sample concentration falsely low

Less: 48ng versus 50ng for initial transfer

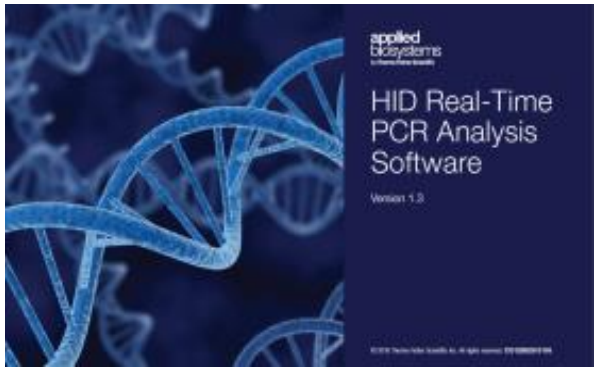
- Should be 50ng, 5ng, 0.5ng, 0.05ng…
- Actually 48ng,4.8ng, 0.48ng, 0.048ng…
  - Ct values increase due to less DNA than expected
  - Y-intercept is higher
  - Unknown sample concentration falsely high

What can you do to reduce Standard Curve variability?



- Monitor curve metrics over time and across kit lots
- Maintain pipette calibration/designate pipettes for quant setup
- Limit quant setup personnel
- Utilize robotic quant setup
- Store pre-made standard dilutions appropriately

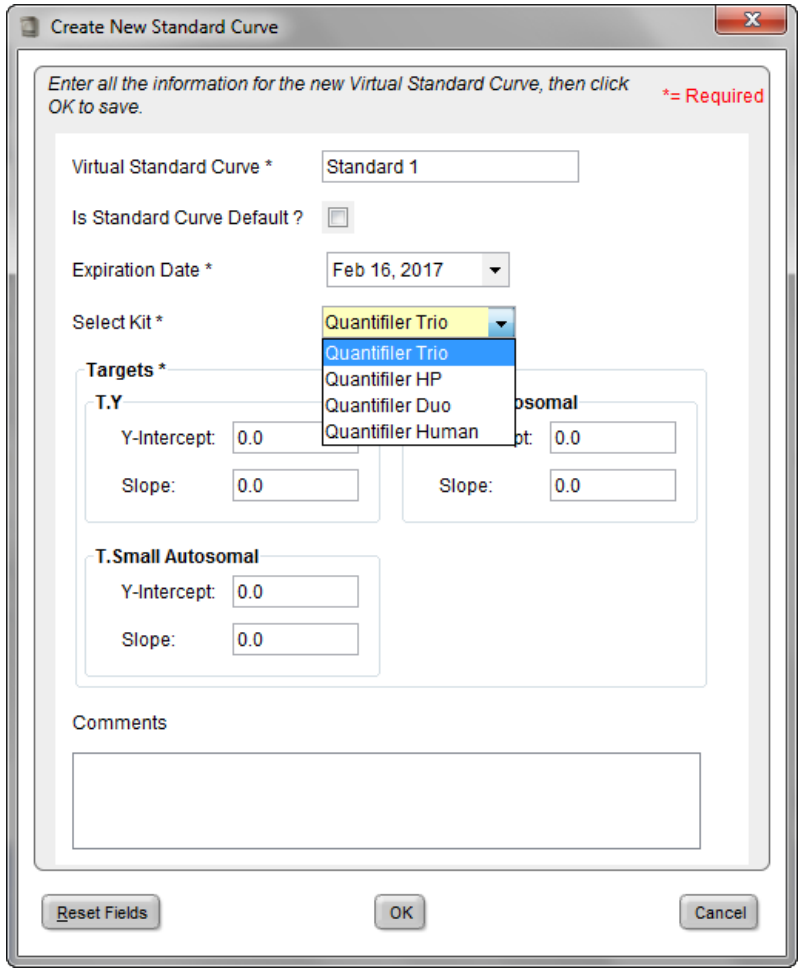
Virtual Standard Curve



HID Real-Time PCR Analysis Software v1.3

Developed to provide users access to:

- Quant Studio 5 Real-time PCR Quant Instrument
- Virtual Standard Curve analysis



Virtual Standard Curve

Manually define standard curve values to determine DNA quantity:

- Reduces variability
- Streamlines setup
- Increases efficiency and minimizes cost
- Flexible run options (pre and post application)

How to use Virtual Standard Curve for a run

- Option1:
  - Setup VSC (Using steps defined in previous steps)
  - Run a plate without standards
  - Analysis will pick the default file for the kit that's been run

- Option2:
  - Post run, add the VSC File
  - Re-Analyze
  - Analysis will use the VSC file added to the experiment

Virtual Standard Curve Benefits

- Streamlined and Time Saving
  - Simple User Interface allowing users to define their own standard curve values for all targets.
  - Analyst time saving as well as assist in workflow decision making.
- Efficiency and Cost Savings
  - The virtual standard curve feature allows for samples to be run in the place of standards, increasing throughput
- Flexibility
  - Adaptable analysis settings allowing for permutations of running and analyzing with traditional or virtual standard curves.
- Accuracy
  - Mitigates concerns of variability introduced due to operators on standard curve.

Thank you for your time & attention.