



Quant Studio 5 Instrument and Virtual Standard Curve

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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



Sign In or
Create a
Local User
Account

Open/Close
door

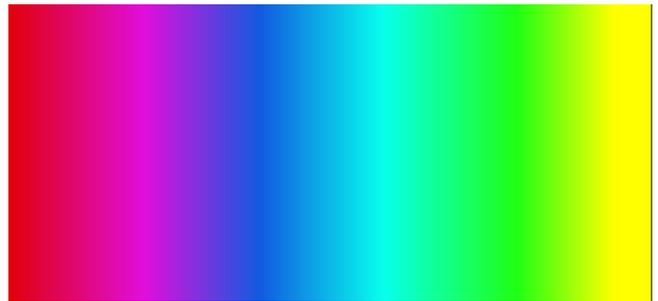
Help

- 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>

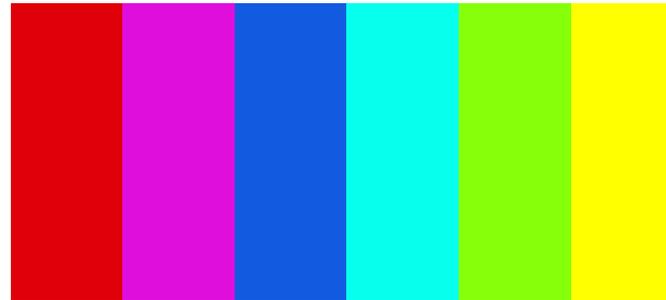
QuantStudio 5 Real-Time PCR Systems: VeriFlex Technology

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



← 60°F 65°F →

Gradient



60°F 61°F 62°F 63°F 64°F 65°F

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

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

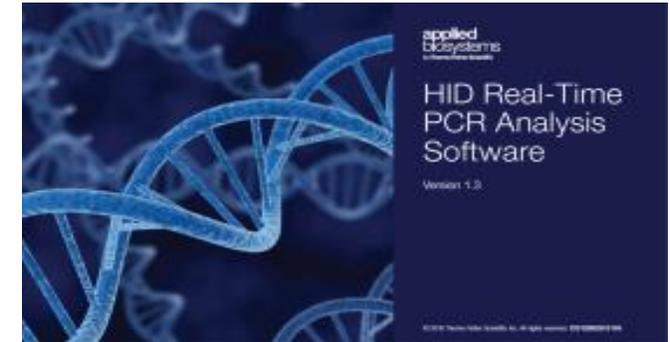
5 decoupled channels

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

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

The screenshot shows a dialog box titled 'Create New Standard Curve'. It contains the following fields and options:

- Virtual Standard Curve ***: Text box containing 'Standard 1'.
- Is Standard Curve Default?**: Unchecked checkbox.
- Expiration Date ***: Dropdown menu showing 'Feb 16, 2017'.
- Select Kit ***: Dropdown menu with a list: 'Quantifier Trio' (highlighted), 'Quantifier HP', 'Quantifier Duo', and 'Quantifier Human'.
- Targets ***:
 - T.Y**: Y-Intercept: 0.0, Slope: 0.0.
 - T.Small Autosomal**: Y-Intercept: 0.0, Slope: 0.0.
 - T.Mitochondrial**: Y-Intercept: 0.0, Slope: 0.0.
- Comments**: Empty text area.

At the bottom are buttons for 'Reset Fields', 'OK', and 'Cancel'. A note at the top right says '* = Required'.

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?

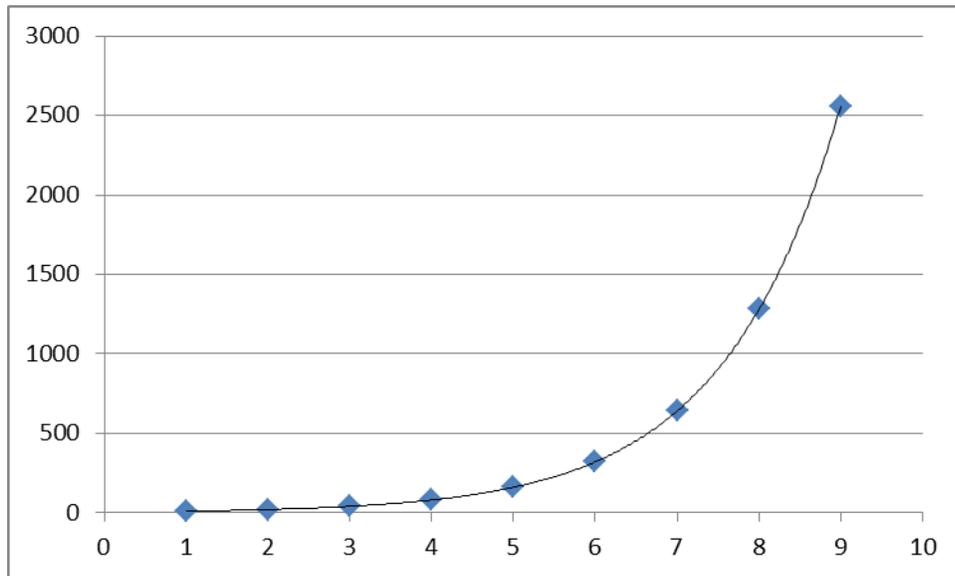
With qPCR doubling of product happens after every cycle with 100% PCR efficiency

Only need slope, Y-Intercept, ~~F~~ to calculate PCR efficiency

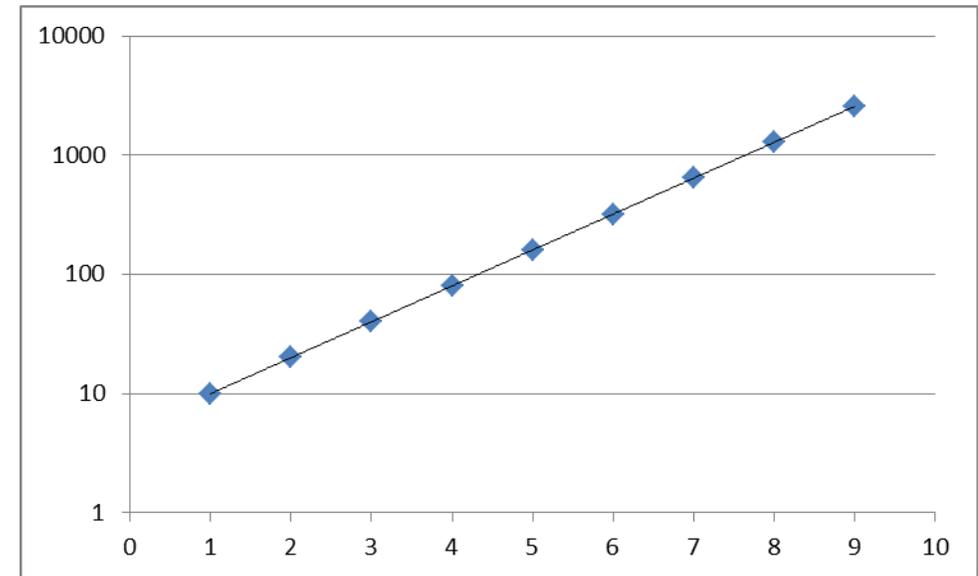
$$\text{PCR Efficiency} = 10^{(-1/\text{slope})} - 1$$

Another look at the numbers...

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



Linear graph / exponential curve



Logarithmic graph / linear curve

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...

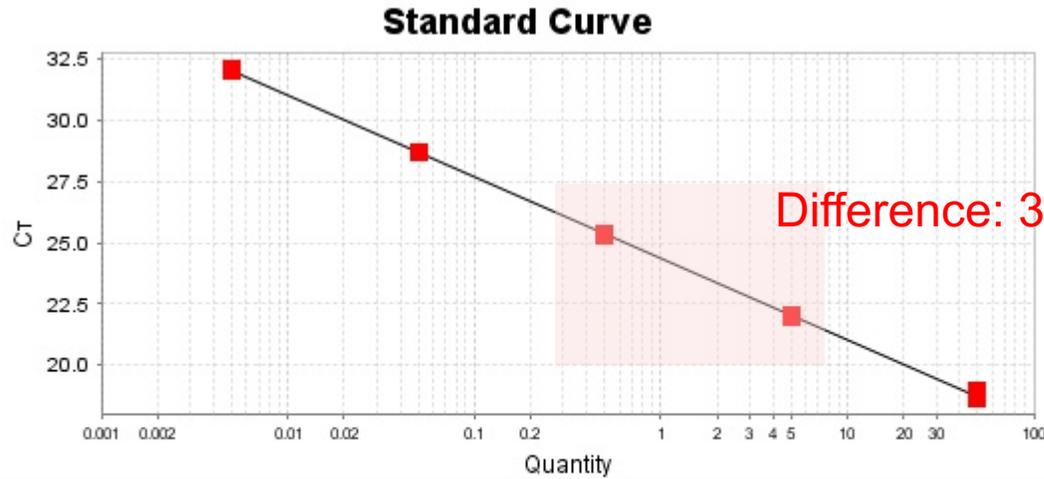
0, 1, 2, 3, 3.33, 4, 5, 6, 7, 8

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?



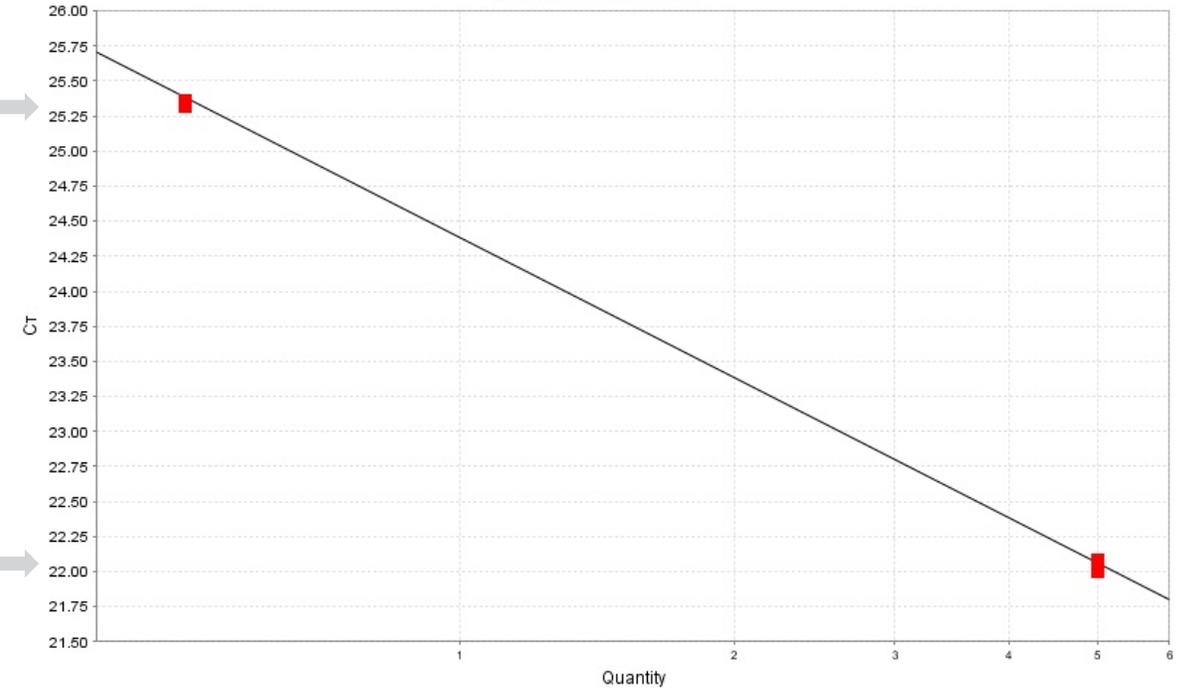
Target: T.Large Autosomal Slope: -3.322 Y-Inter: 24.39 $R^2: 1$ Eff%: 100.014

Legend
■ Standard ■ Unknown ■ Unknown (Flagged)

25.40

22.08

Standard Curve

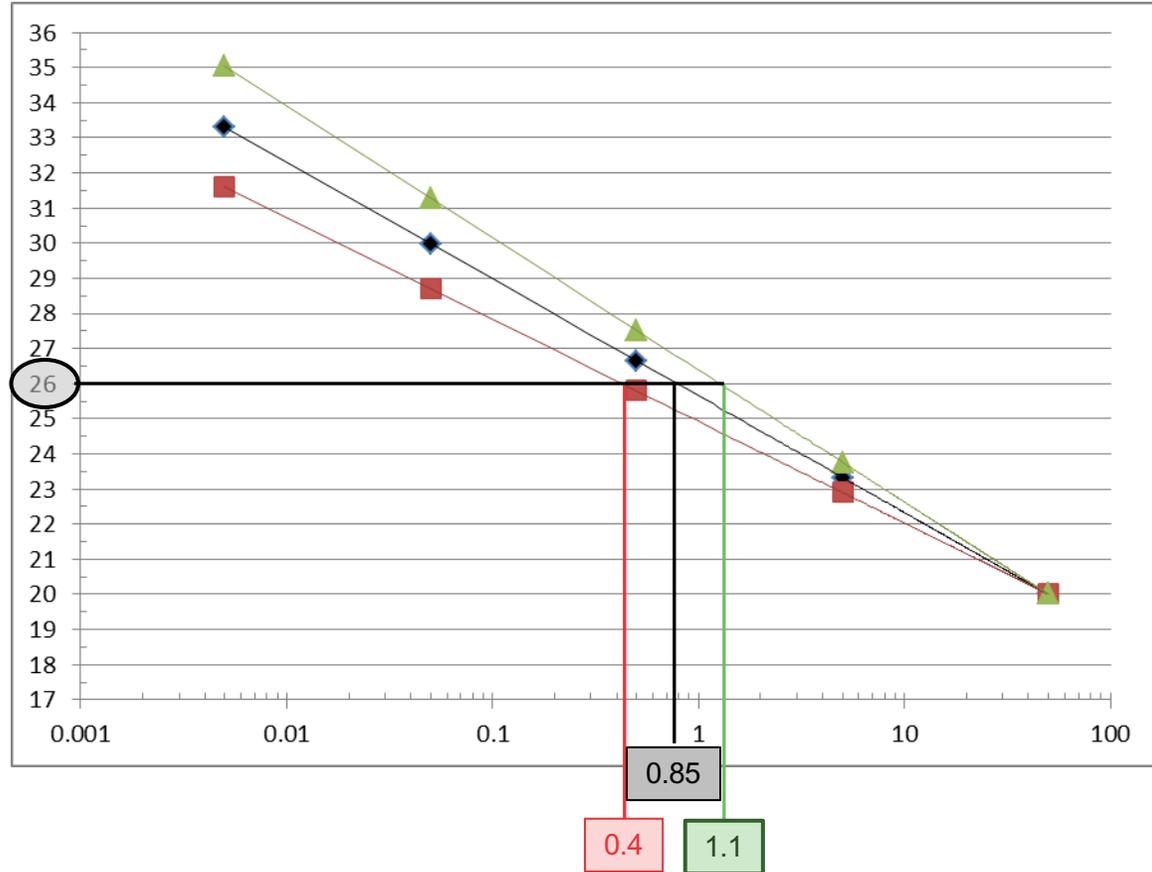


Target: T.Large Autosomal Slope: -3.322 Y-Inter: 24.39 $R^2: 1$ Eff%: 100.014

Legend
■ Standard ■ Unknown ■ Unknown (Flagged)

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

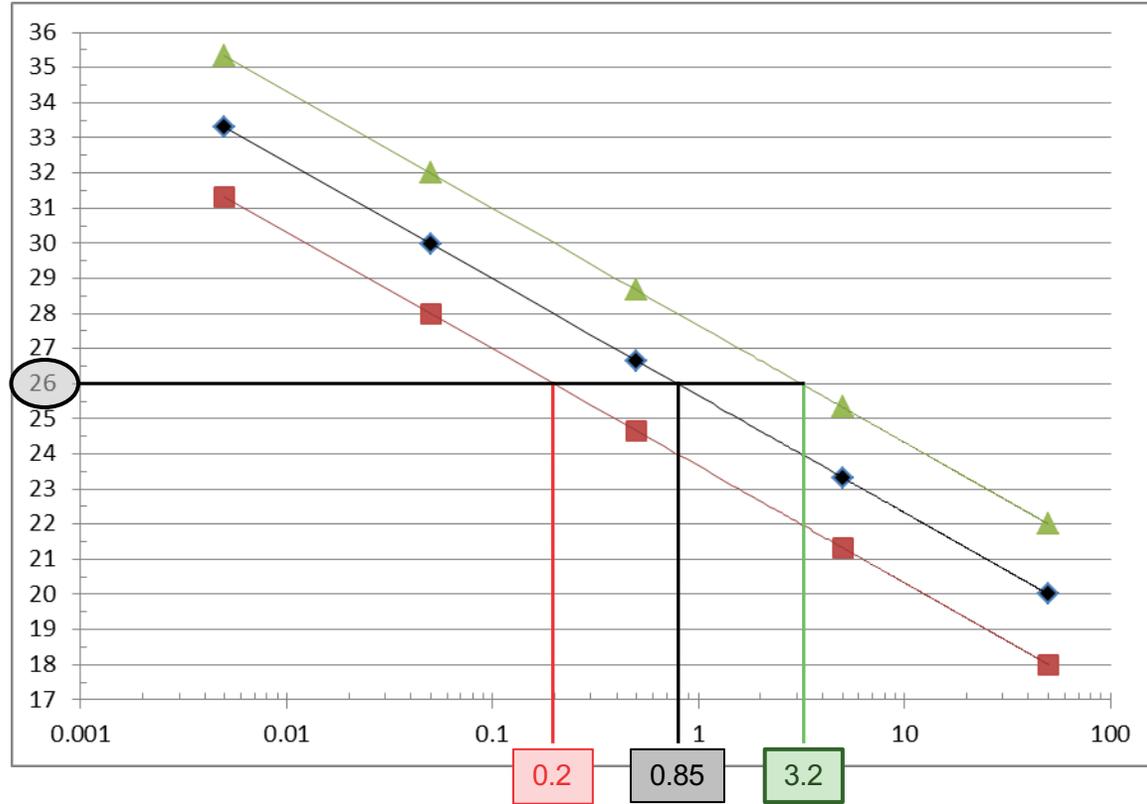
- Should be 50ng, 5ng, 0.5ng, 0.05ng...
- Actually 50ng, 6ng, 0.7ng, 0.08ng...
 - Ct values decrease due to more DNA than expected
 - Slope is less negative (-2.90)

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

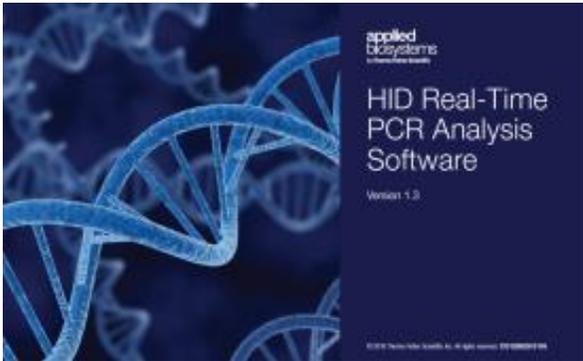
- Should be 50ng, 5ng, 0.5ng, 0.05ng...
- Actually 52ng, 5.2ng, 0.52ng, 0.052ng...
 - Ct values decrease due to more DNA than expected
 - Y-intercept is lower
 - Unknown sample concentration falsely low

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



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)

The screenshot shows a 'Create New Standard Curve' dialog box. At the top, it says 'Enter all the information for the new Virtual Standard Curve, then click OK to save. * = Required'. The form contains several fields: 'Virtual Standard Curve *' with a text box containing 'Standard 1'; 'Is Standard Curve Default?' with an unchecked checkbox; 'Expiration Date *' with a dropdown menu showing 'Feb 16, 2017'; 'Select Kit *' with a dropdown menu showing 'Quantifiler Trio' and a list of other kits including 'Quantifiler Trio', 'Quantifiler HP', 'Quantifiler Duo', and 'Quantifiler Human'; 'Targets *' with two sections: 'T.Y' and 'T.Small Autosomal'. Each section has 'Y-Intercept' and 'Slope' input fields, all containing '0.0'. There is also a 'Comments' text area at the bottom. At the very bottom of the dialog are 'Reset Fields', 'OK', and 'Cancel' buttons.

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.



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