Suggestions for the Trainer...

- This is the system overview presentation for both QS3 and QS5
- Please prepare yourself according to the type of the SSO
  - Half day SSO (mostly for QS3) – the time for this intro is 20 minutes
  - Check the slides and reduce the content as needed
  - Full one day SSO (mostly for QS5) – the time for this intro is 60 minutes

- The slide deck contains two parts
  - About the instrument
  - About the software (Design & Analysis)

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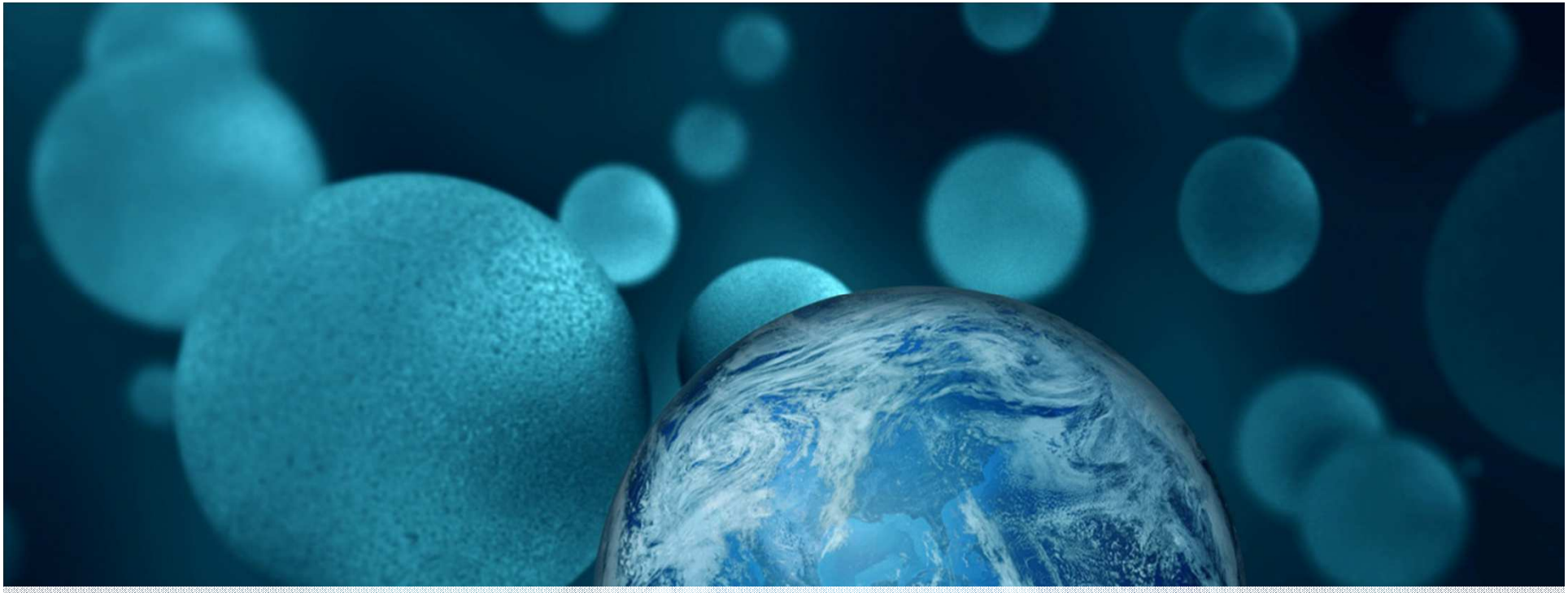

ThermoFisher SCIENTIFIC

QuantStudio™ 3 & 5 Real Time PCR Systems

Letizia Gerace
Senior qPCR/CE Application Scientist

Instrument Features

- Touchscreen
- 10 GB of Onboard Memory (2,000-5,000 run files)
- Wi-Fi connectivity, enabling remote monitoring
- Low maintenance
- Factory calibrated for Applied Biosystems™ reagents
- Browser Based Software (Cloud), enabling PC/MAC compatibility

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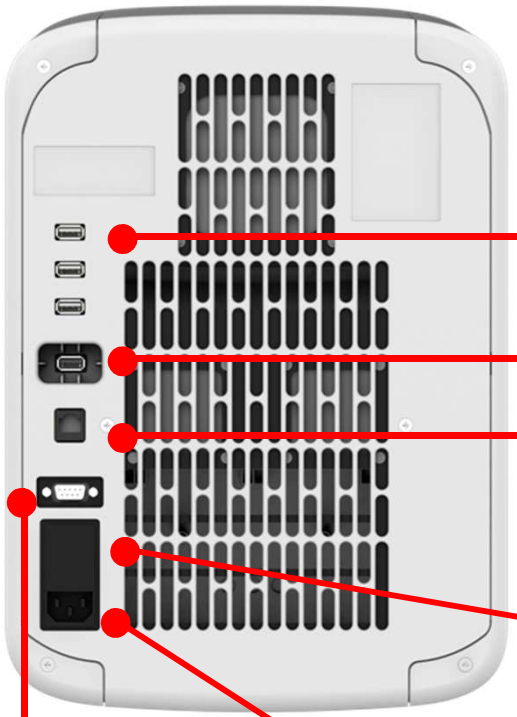
Instrument Front & Rear Features

Touchscreen (stand-alone capabilities, PIN-protected user accounts, and dye calibration/RNaseP functionality)

USB port for template upload and data download

Motorized block drawer (controlled by touchscreen)



USB ports

WiFi adapter port (optional use)

Ethernet port : RJ45 (10/100Mbps)

Fuse cover

Power port: 100/240 VAC

RS232 port (Service only)

ThermoFisher SCIENTIFIC

Technical Specifications

	QuantStudio™ 3 Real Time PCR System	QuantStudio™ 5 Real Time PCR System
Block configurations	96-well 0.1 ml block :10-30 µl 96-well 0.2 ml block : 10-100 µl	96-well 0.1 ml block :10-30 µl 96-well 0.2 ml block : 10-100 µl 384-well: 5-20 µl
Run time	<30 minutes	96-well block: <30 minutes 384-well block: <35 minutes
Excitation source	Bright white LED	Bright white LED
Optical Detection	4 coupled filters	96-well block: 6 decoupled filters 384-well block: 5 coupled filters
Temperature Zone Function	3 VeriFlex zones	96-well block: 6 VeriFlex zones 384-well block: N/A
Temperature Accuracy and Uniformity	0.4 ^{0}C & 0.2 ^{0}C	0.4 ^{0}C & 0.2 ^{0}C
Max block ramp rate	96-well 0.1 ml block: 9 ^{0}C/sec 96-well 0.2 ml block: 6.5^{0}C/sec	96-well 0.1 ml block: 9 ^{0}C/sec 96-well 0.2 ml block: 6.5^{0}C/sec 384-well block: 6.0 ^{0}C/sec
21 CFR p11 enablement	No	Yes, with no additional fees
Detection Sensitivity	10 log dynamic range sensitivity 1 copy 1.5 fold differences in target quantities	10 log dynamic range sensitivity 1 copy 1.5 fold differences in target quantities

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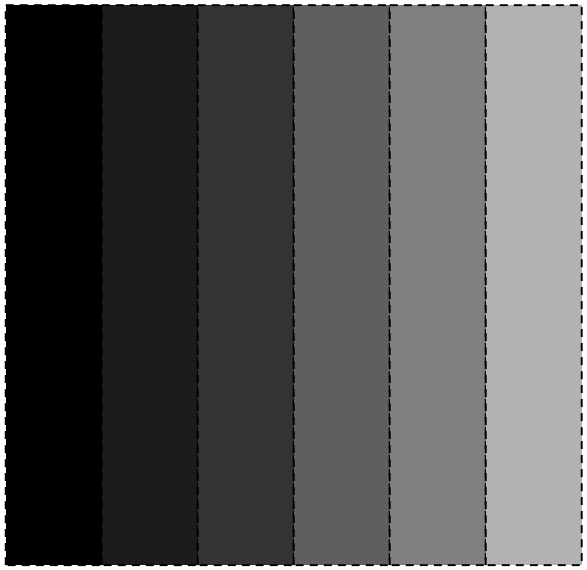
VeriFlex™ Blocks

- Independent temperature control in each zone (more precise than gradient)
- Can program at will, including multiple zones with same temp (Temp. difference between adjacent zones <5°C)
- Great for optimization and also running multiple assays at the same time

QuantStudio™ 3

60°C 61°C 62°C

QuantStudio™ 5 96-well block



60°C 61°C 62°C 63°C 64°C 65°C

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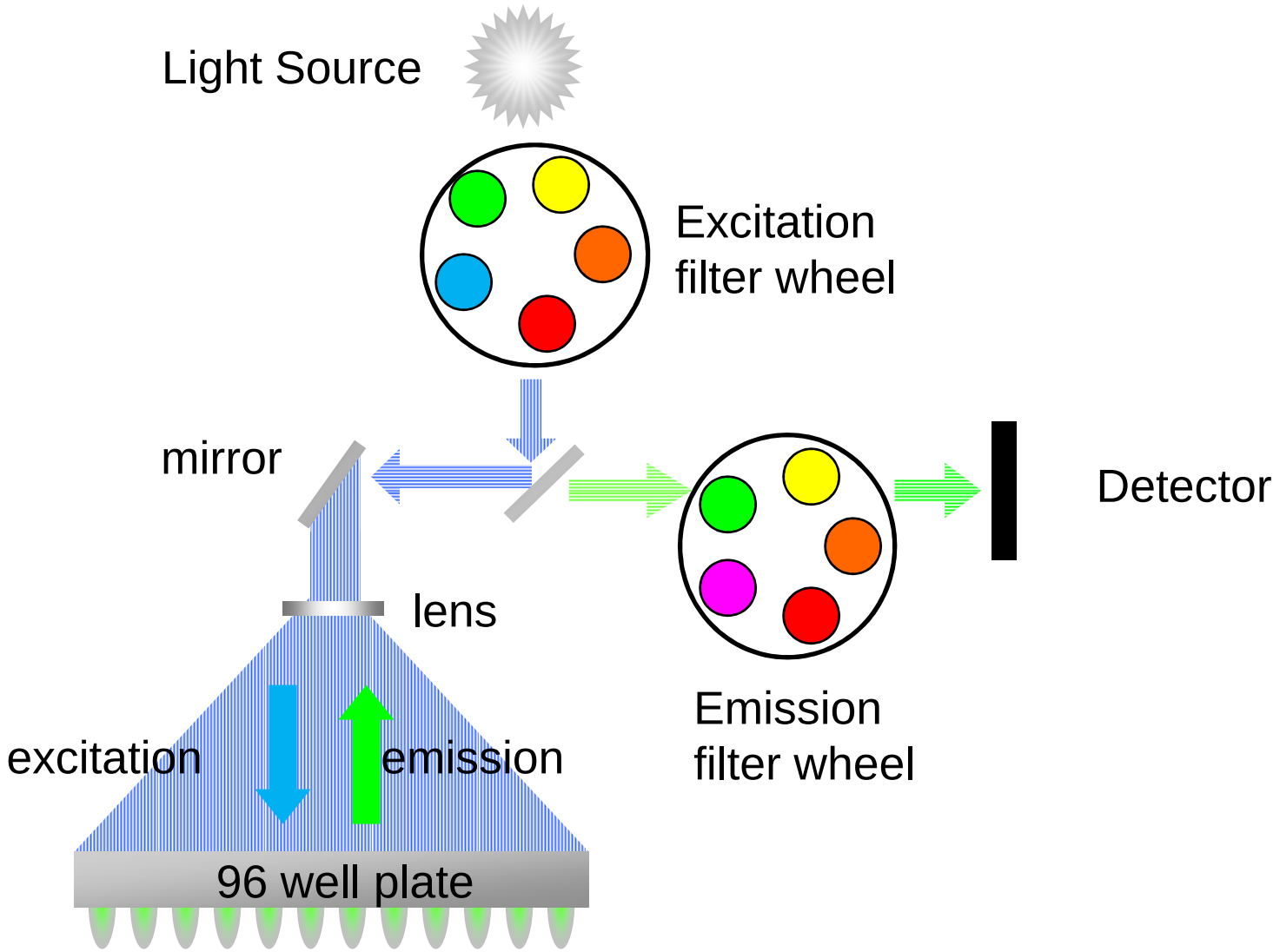
OptiFlex™ System with Bright White LED



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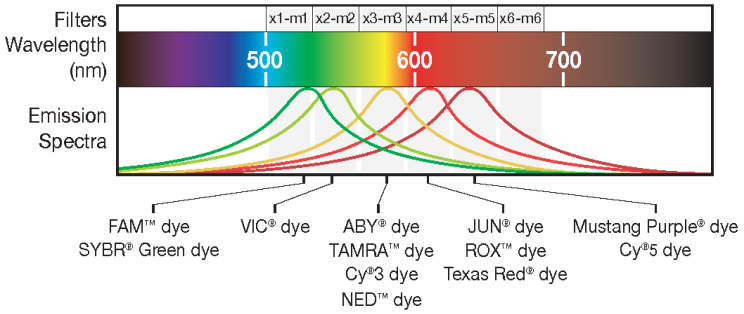
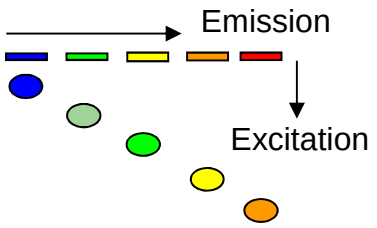
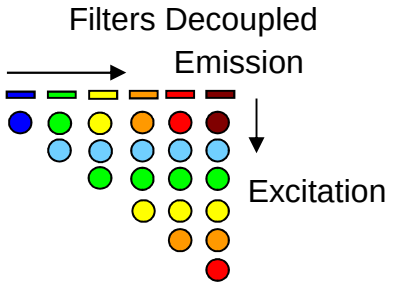
Multiplex Capabilities

Channel	Dye Examples	Excitation Filter	Emission Filter	QuantStudio™3	QuantStudio™5 384w-block	QuantStudio™5 96-block
x1-m1	FAM™ and SYBR Green	470 ± 15nm	520 ± 15nm	✔	✔	✔
x2-m2	VIC™, JOE™, TET™, HEX™	520 ± 10nm	558 ± 12nm	✔	✔	✔
x3-m3	TAMRA™, NED™, ABY™	550 ± 10nm	586 ± 10nm	✔	✔	✔
x4-m4	ROX™, JUN™, Texas Red™	580 ± 10nm	623 ± 14nm	✔	✔	✔
x5-m5	Mustang Purple™, LIZ™, Cy®5	640 ± 10nm	682 ± 14nm		✔	✔
x6-m6	Cy®5.5, Alexa Fluor™	662 ± 10nm	711 ± 12nm			✔





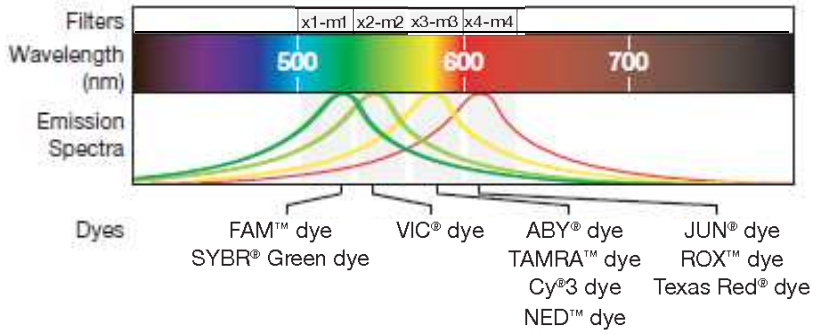


ThermoFisher SCIENTIFIC

Multiplexing Capabilities - QuantStudio™ 3

- OptiFlex™ System with Bright White LED
- Four color locked filter system
- Factory calibrated

Peak channel	Color	Filter wavelength (nm)[1]		Pre-calibrated dyes	Example custom dyes
		Excitation	Emission		
x1-m1	Blue	470 ± 15	520 ± 15	FAM™ and SYBR® Green	SYT09
x2-m2	Green	520 ± 10	558 ± 12	VIC®	HEX™, TET™, and JOE™[2]
x3-m3	Yellow	550 ± 10	587 ± 10	ABY®, NED™, and TAMRA™	Cy®3
x4-m4	Orange	580 ± 10	623 ± 14	JUN® and ROX™	Texas Red®



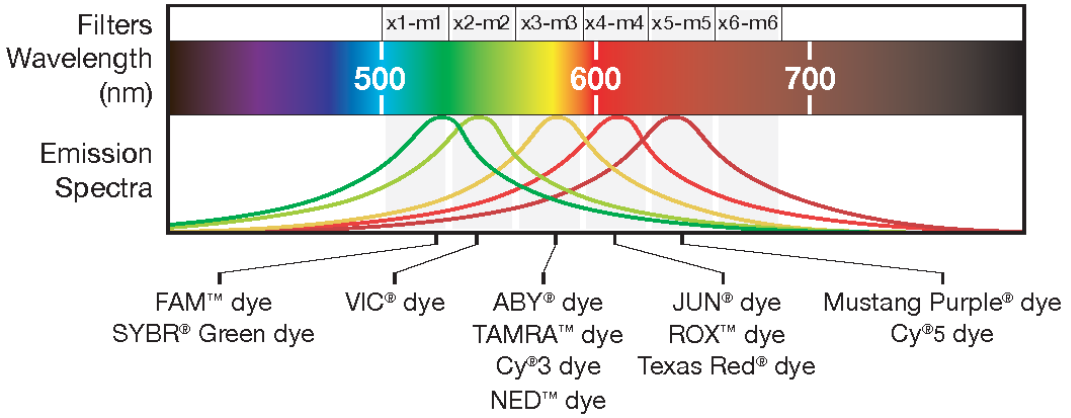
ThermoFisher SCIENTIFIC

Multiplexing Capabilities - QuantStudio™ 5

- OptiFlex™ System with Bright White LED
- Six color unlocked filter system
- Factory calibrated

Peak channel	Color	Filter wavelength (nm)[1]		Pre-calibrated dyes	Example custom dyes
		Excitation	Emission		
x1-m1	Blue	470 ± 15	520 ± 15	FAM™ and SYBR® Green	SYT09
x2-m2	Green	520 ± 10	558 ± 12	VIC®	HEX™, TET™ and JOE™
x3-m3	Yellow	550 ± 10	587 ± 10	ABY®, NED™, and TAMRA™	Cy®3
x4-m4	Orange	580 ± 10	623 ± 14	JUN® and ROX™	Texas Red®
x5-m5	Red	640 ± 10	682 ± 14	Cy®5 and MUSTANG PURPLE®	LIZ®
x6-m6	Deep-Red	662 ± 10	711 ± 12	None*	Cy®5.5

*This filter set currently does not support any dyes supplied by Thermo Fisher Scientific

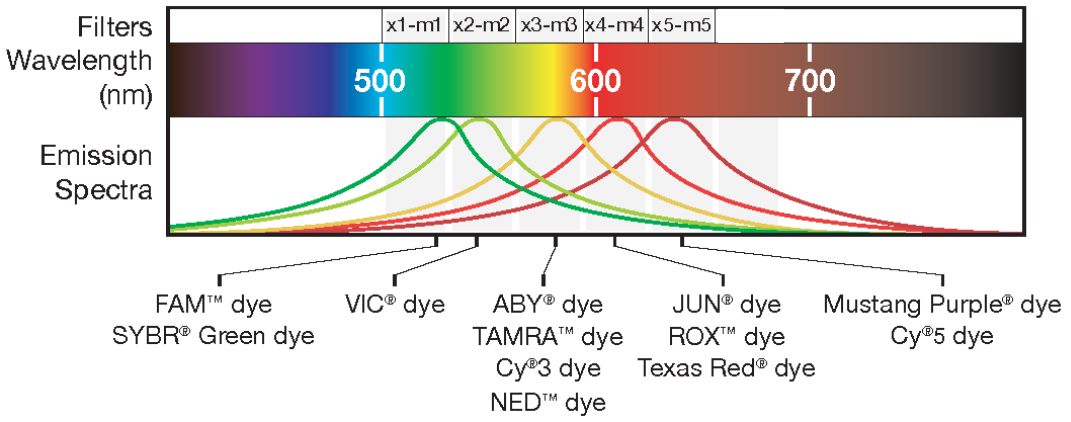


ThermoFisher SCIENTIFIC

Multiplexing Capabilities - QuantStudio™ 5 – 384w

- OptiFlex™ System with Bright White LED
- Five color locked filter system
- Factory calibrated

Peak channel	Color	Filter wavelength (nm)[1]		Pre-calibrated dyes	Example custom dyes
		Excitation	Emission		
x1-m1	Blue	470 ± 15	520 ± 15	FAM™ and SYBR® Green	SYT09
x2-m2	Green	520 ± 10	558 ± 12	VIC®	HEX™, TET™ and JOE™
x3-m3	Yellow	550 ± 10	587 ± 10	ABY®, NED™, and TAMRA™	Cy®3
x4-m4	Orange	580 ± 10	623 ± 14	JUN® and ROX™	Texas Red®
x5-m5	Red	640 ± 10	682 ± 14	Cy®5 and MUSTANG PURPLE®	LIZ®



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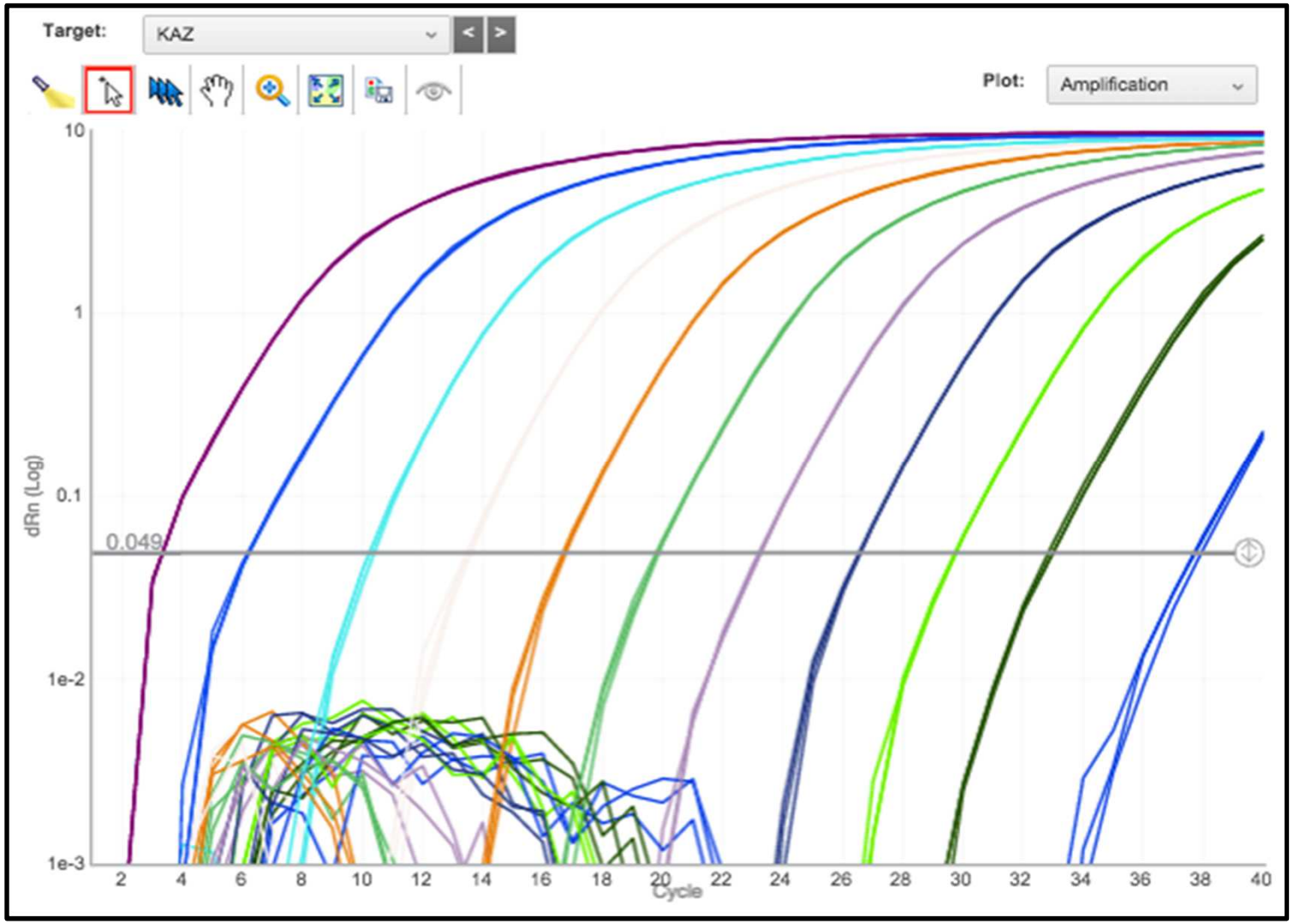
10-Log Dynamic Range Sensitivity



Data courtesy of System Verification and Validation

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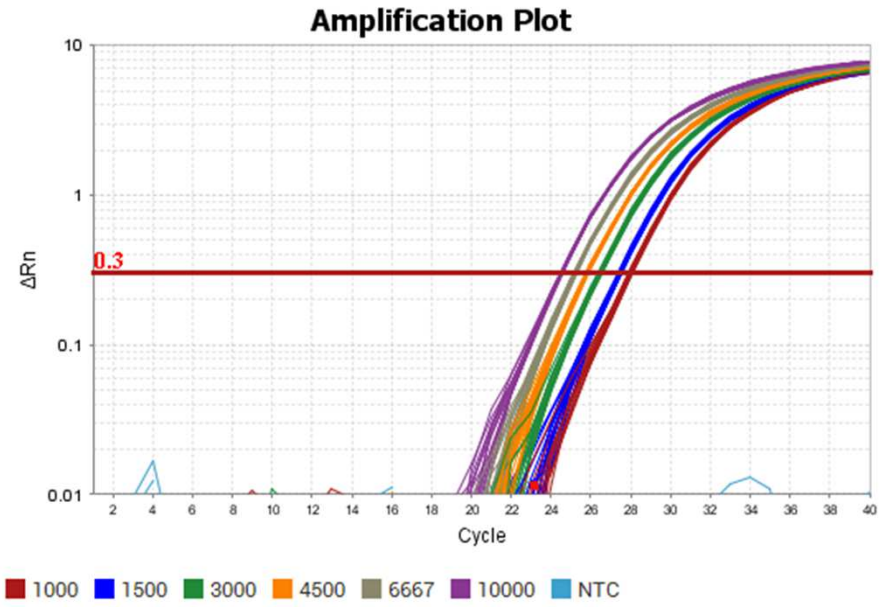
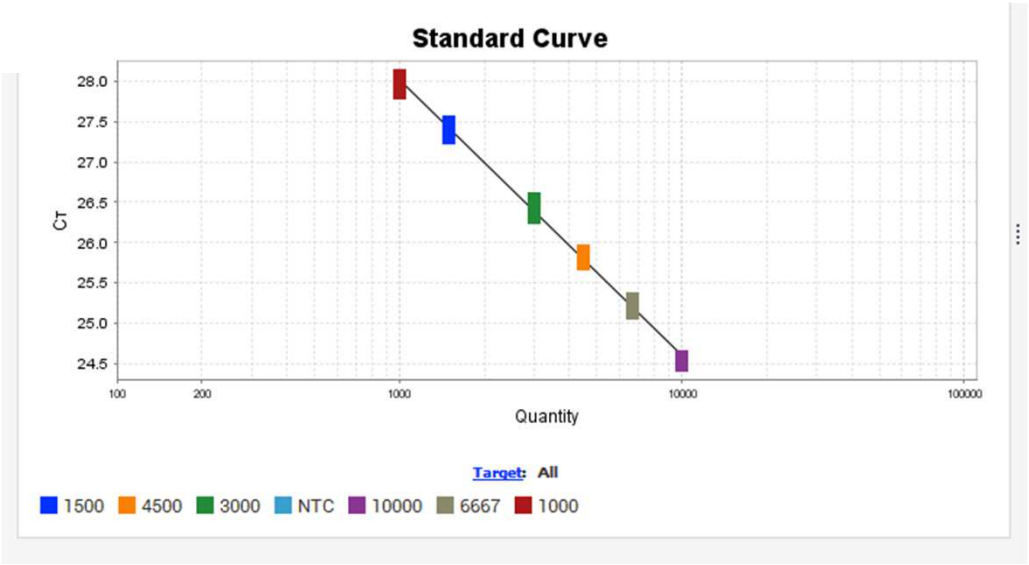
QuantStudio™ 3 & 5 Enable 1.5-Fold Discrimination



Amplification plots for 1.5-fold dilutions of KAZ plasmid amplified with PE2 TaqMan™ assay under standard Fast run conditions using the TaqMan Fast Advanced Master Mix.

Quantity	C_T	Std Dev
1000	27.9	0.063
1500	27.45	0.059
3000	26.40	0.060
4500	25.80	0.047
6667	25.20	0.049
10000	24.50	0.041



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Instrument Configurations: Stand-alone, Desktop, or Online

Connected Laptop with QuantStudio Design and Analysis desktop software

ThermoFisherCloud

WiFi

LAN

Connect to the Design and Analysis Cloud software using any device with a compatible web browser

USB

Note: You can start an experiment run only from the instrument touchscreen or from the Desktop Software

Ability to Connect Multiple Instruments

- Single software to connect and control all QuantStudio 3/5 instruments in the lab
- Seamless integration with instruments that helps minimize manual data transfer

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How the Cloud is utilized with QuantStudio™ 3 & 5

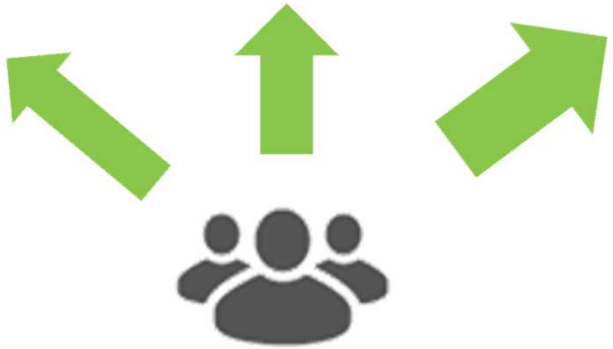
ThermoFisherCloud

- Access/share data
- Analysis Apps
- Browser Based
- Storage and Backup

- Create Studies
- Analyze Data with Apps
- Setup run/plate
- Monitor Run
- View Results

- Load run/plate
- Perform Run
- Monitor Run
- View Results
- Calibration

- Monitor Run
- View Results



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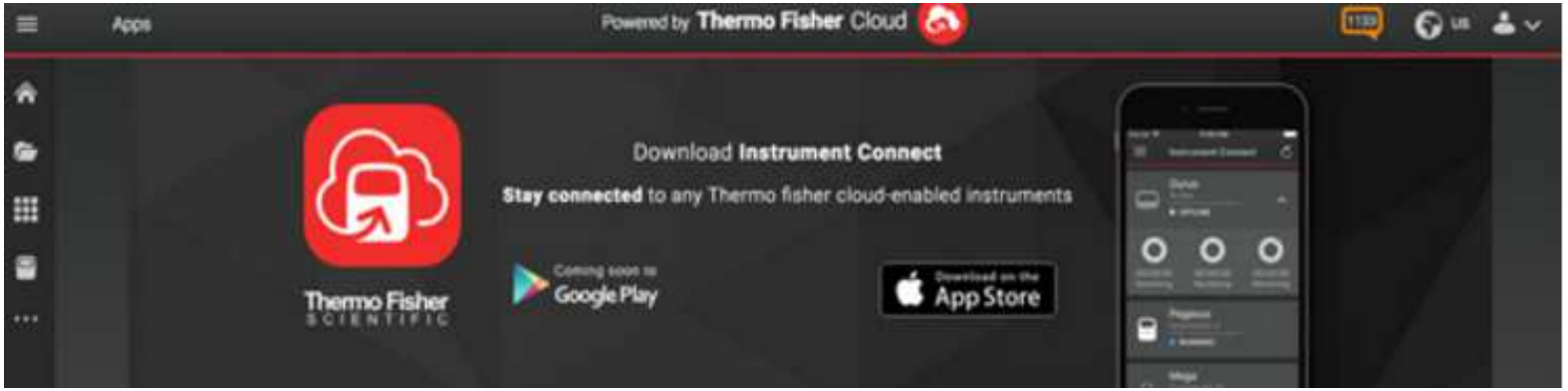
Instrument Connect Mobile App



Monitor your runs on multiple devices

ThermoFisher SCIENTIFIC

Interactive Touch Screen

Open/Close door

Help

Sign In or Create a Local User Account

Connection type

Settings:
- Calibration
- Runs
- Logs
- Ship Prep

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Instrument Local User Accounts

Create local pin-protected account on instrument

Connect to online Thermo Fisher Cloud account

ThermoFisherCloud

Optional but highly recommended for full capabilities

- Create individual accounts for multiple users
  - PIN-protected accounts help keep protocols and data safe and stop “accidental” run interruptions
- Instrument users can be designated as “Admin” or “Standard” users
  - First user defaults to “Admin” status but can create other Admins, as needed

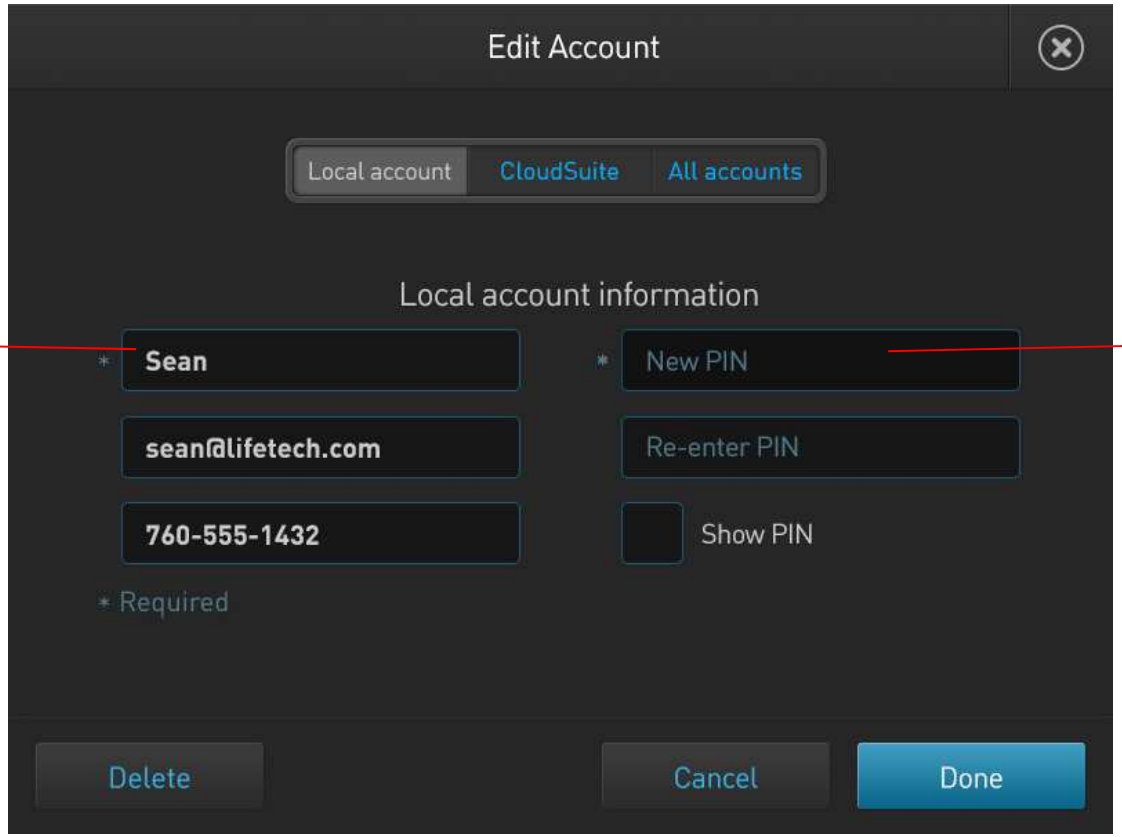
Administrator Only Tasks

- Enable SAE module (QS 5 only)
- Require Sign-In
- Enable Remote Instrument Monitoring
- Update Instrument Software
- Manage/View all Instrument Profiles
- Select Cloud Region
- Manage Sign-Out Timer and Instrument Name

After logging in, Standard Accounts start and save run files in their own folders.

Create Local User Account and Link to Cloud

Enter User Name



Set PIN
(and remember it!)

Why link to the Cloud?
The Cloud enables you to download run files from the cloud and automatically upload them when complete

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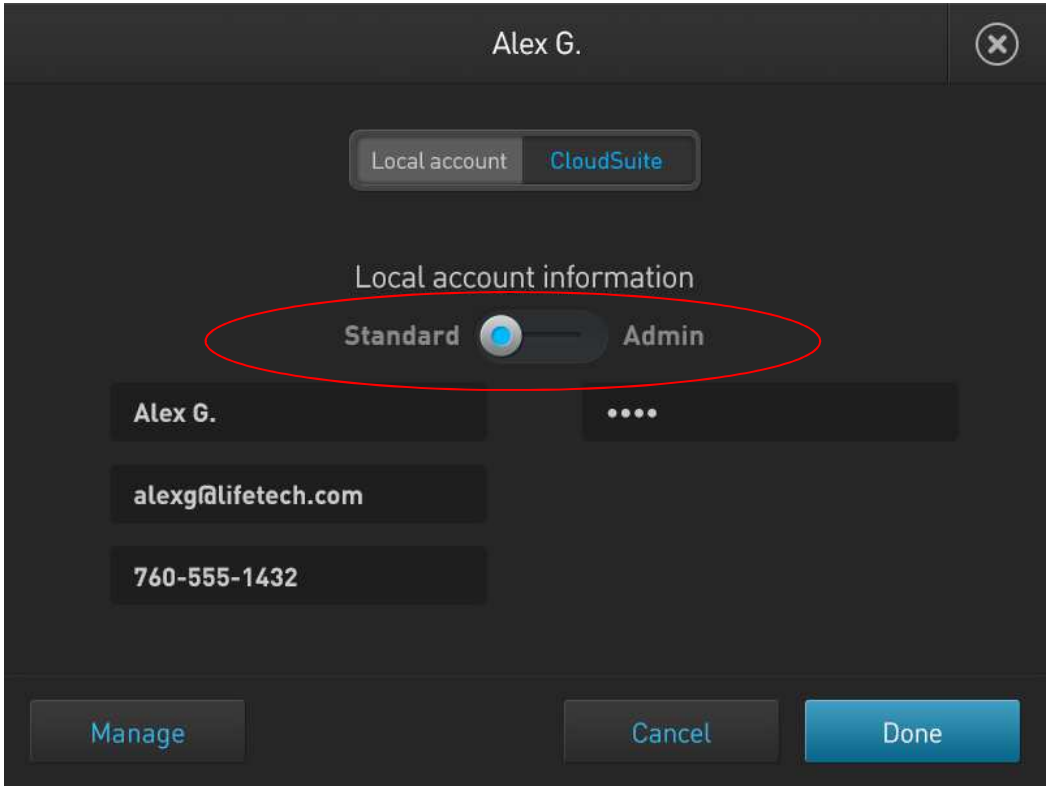
Admin and Standard User Accounts



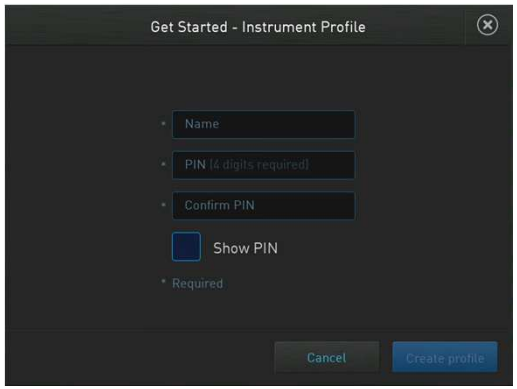
Note: the first user with “Admin” status to connect to the Cloud will be the Cloud Admin for that linked instrument

ThermoFisher SCIENTIFIC

Account Setup on Touchscreen

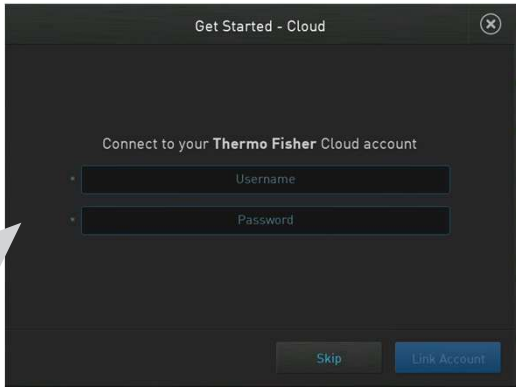
One Step
Instrument Profile Setup

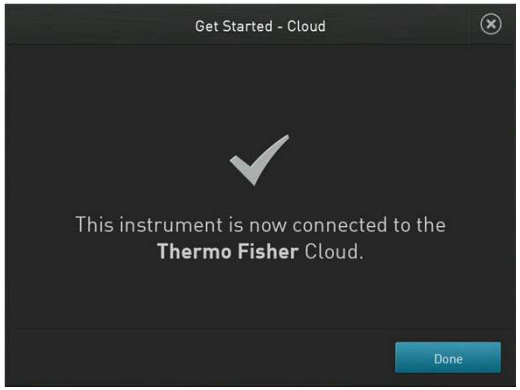
- Enter Name &PIN
- Select “Create Profile”



Optional Step to link to Thermo Fisher Cloud Account

- Enter Thermo Fisher Cloud account credentials & select “Link Account”
- Or select “Skip” to complete setup without linking to cloud account





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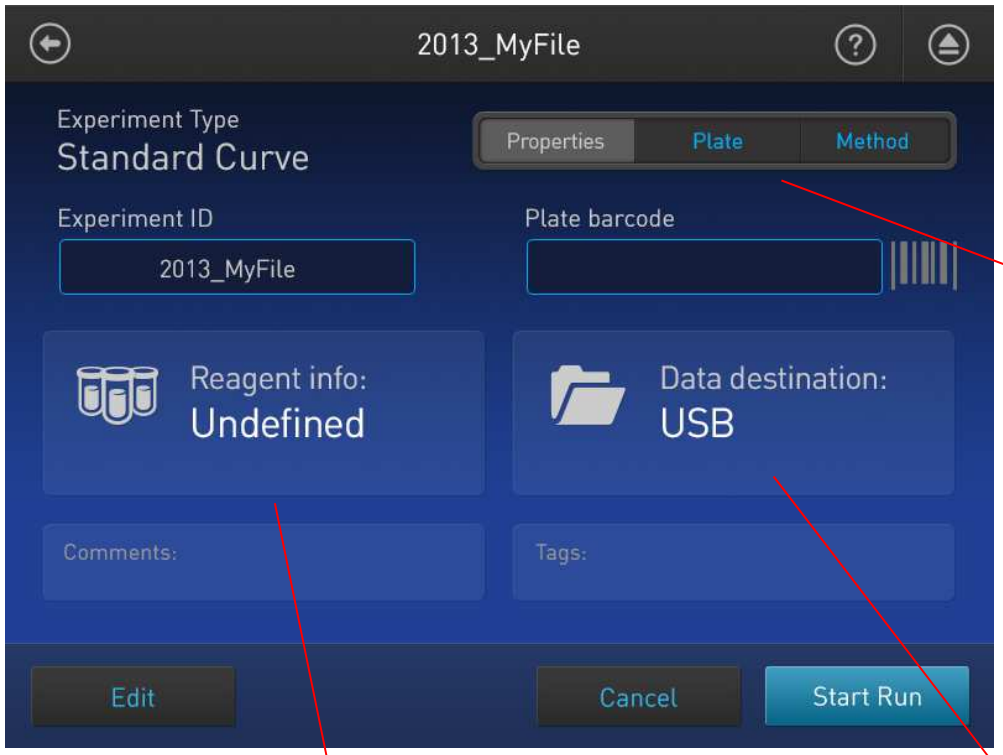
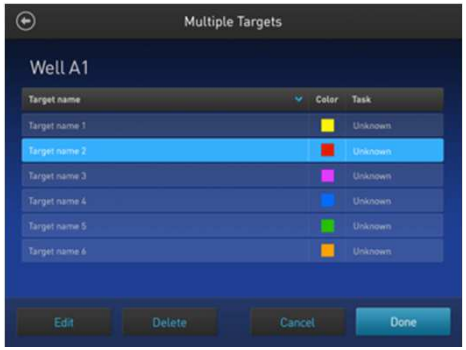
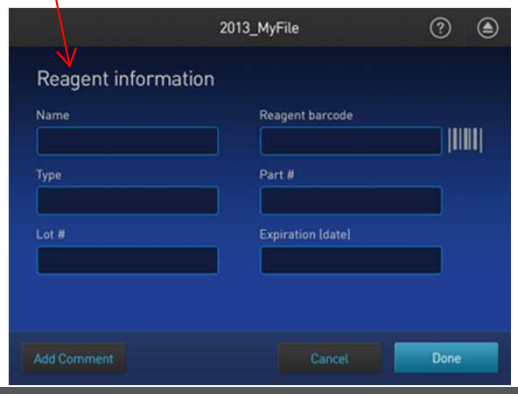
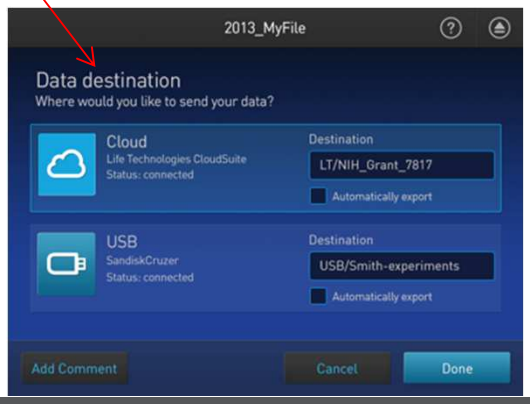
Use touchscreen to edit reagent info, destination, and plate setup









2D reagent barcodes supported for Applied Biosystems reagents!

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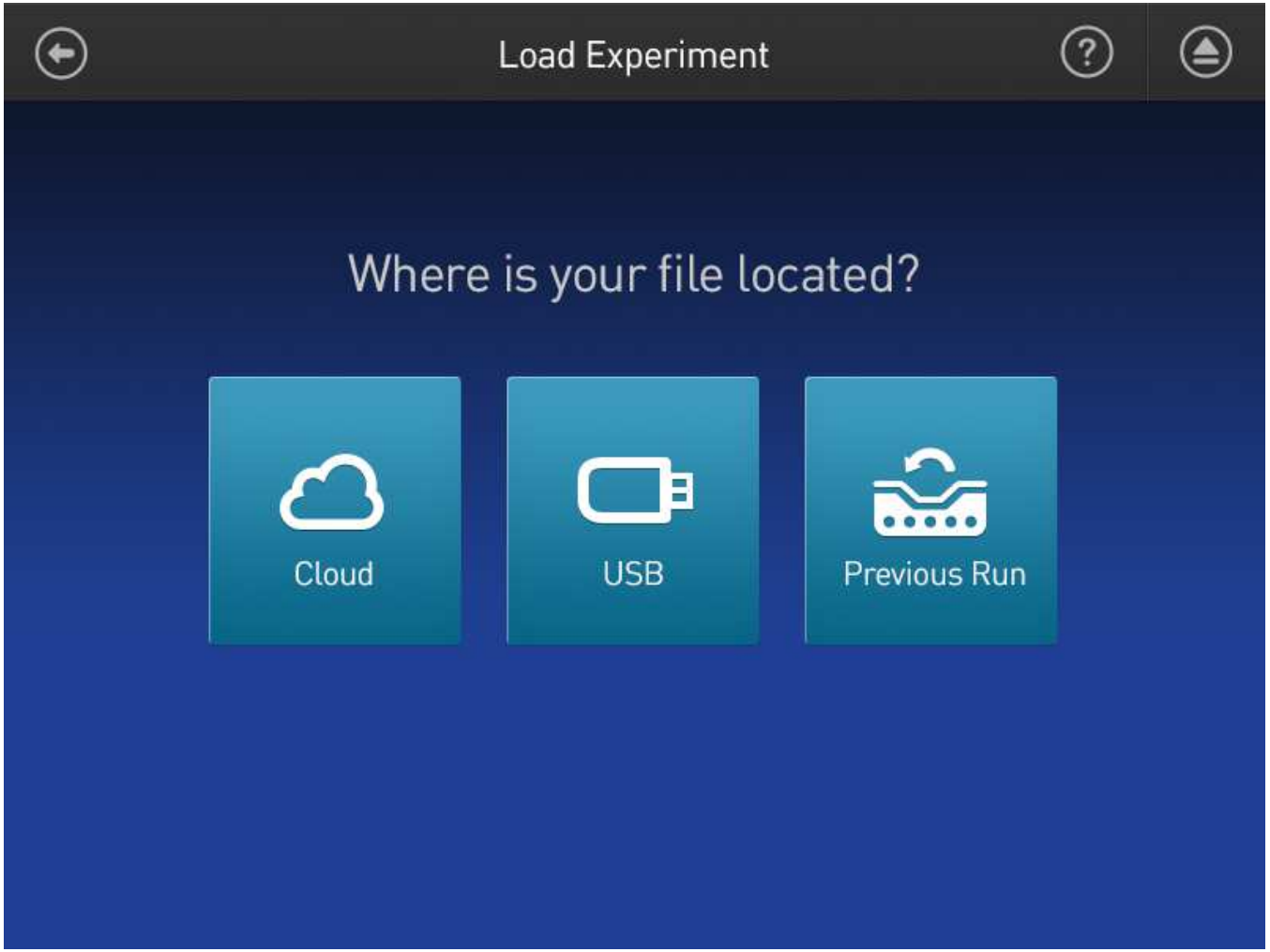
Access run files from multiple locations



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Edit run protocol

Full method editing capabilities on the touch screen, including VeriFlex, Pause, and Melt

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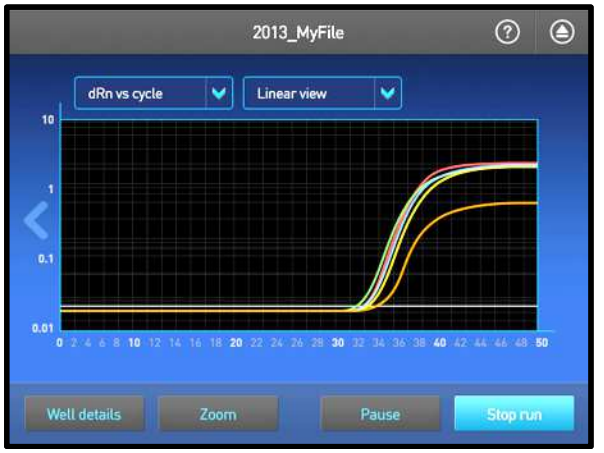
Monitor Progress During the Run

Time Remaining

Thermal Protocol Status

Live Amplification Curves



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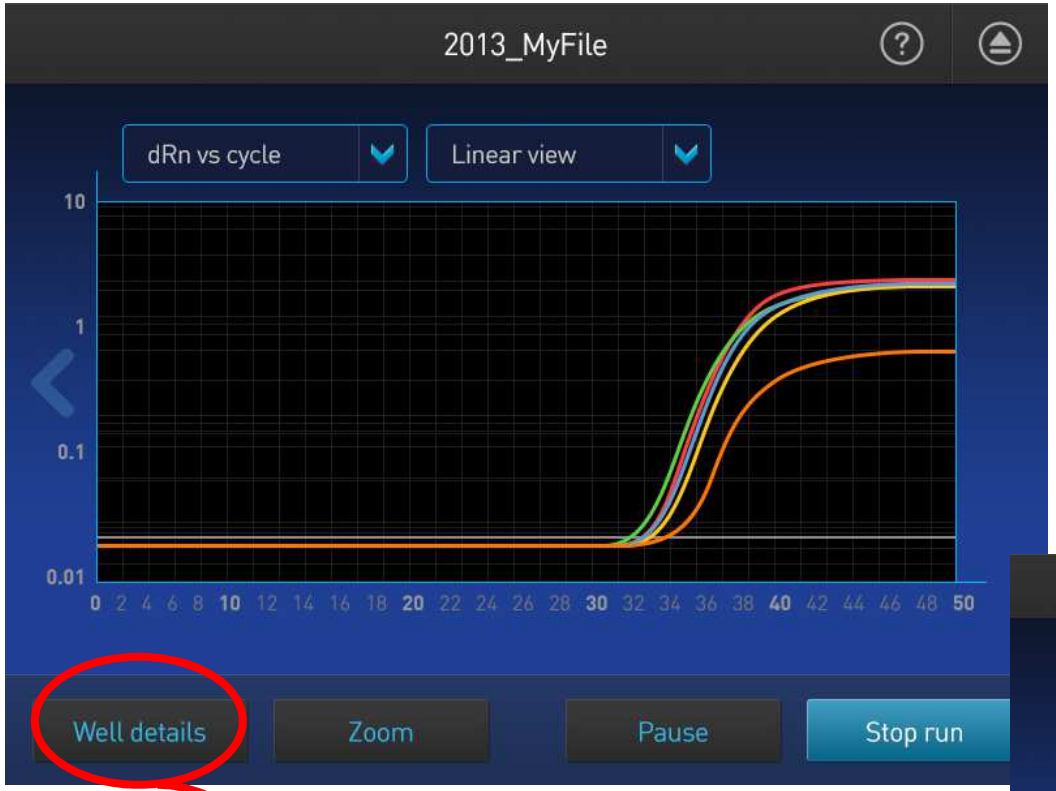
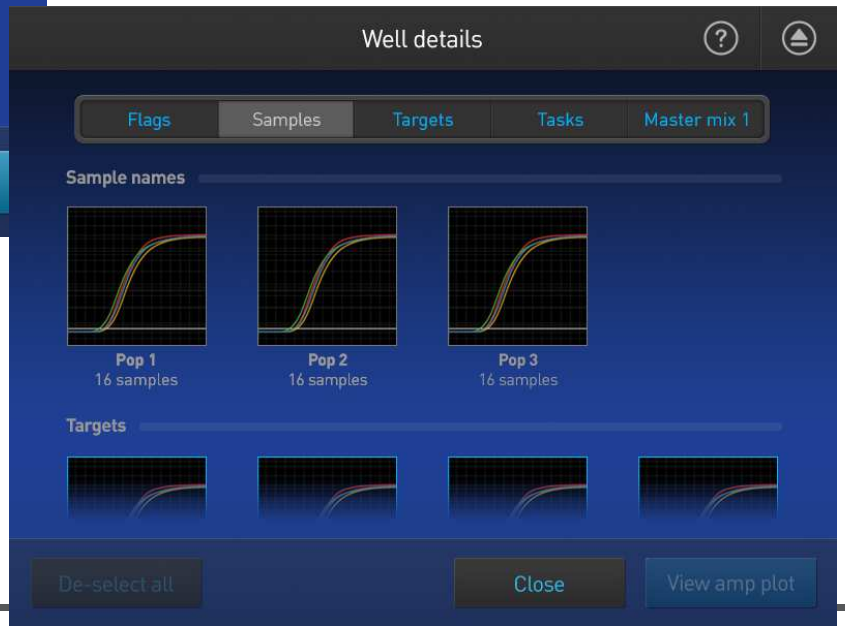
Review amp plots in real time





Review well details and select amplification plots to view by Target, Sample, Task, or Master Mix

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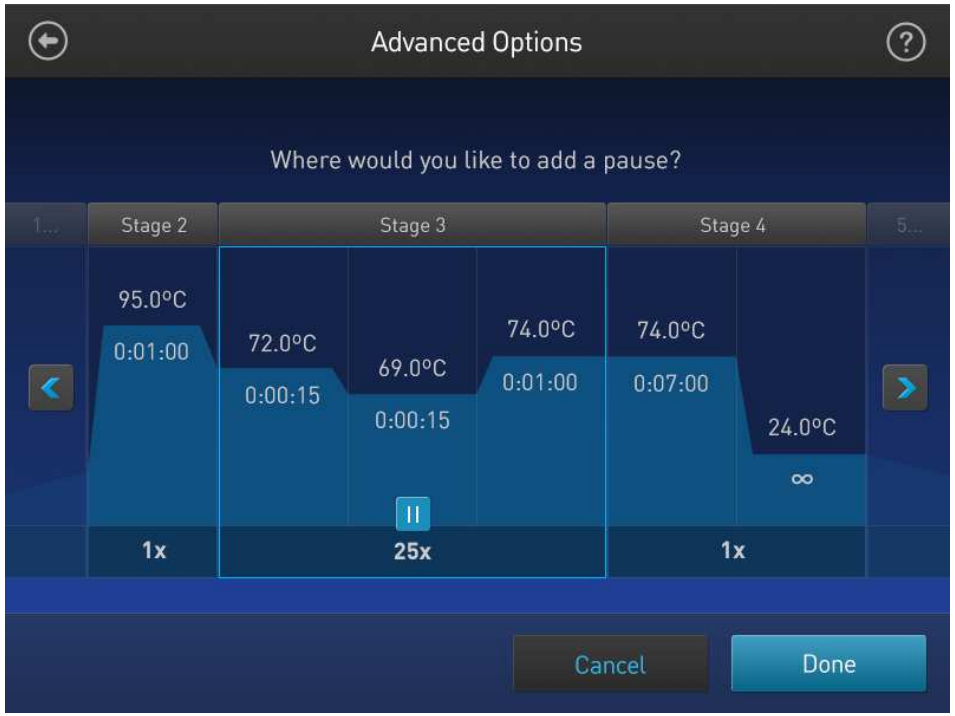
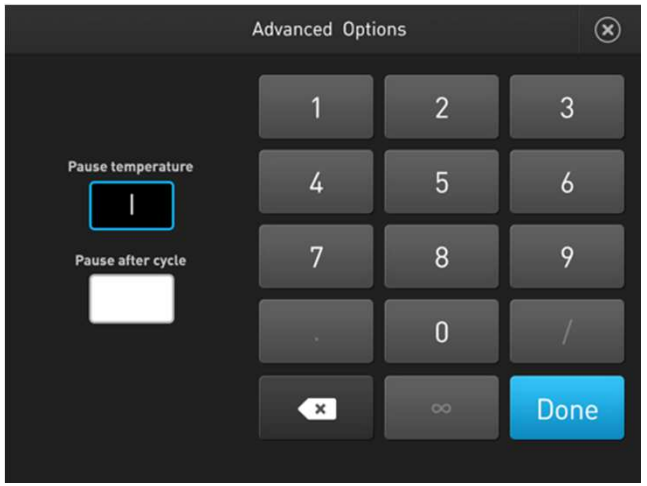
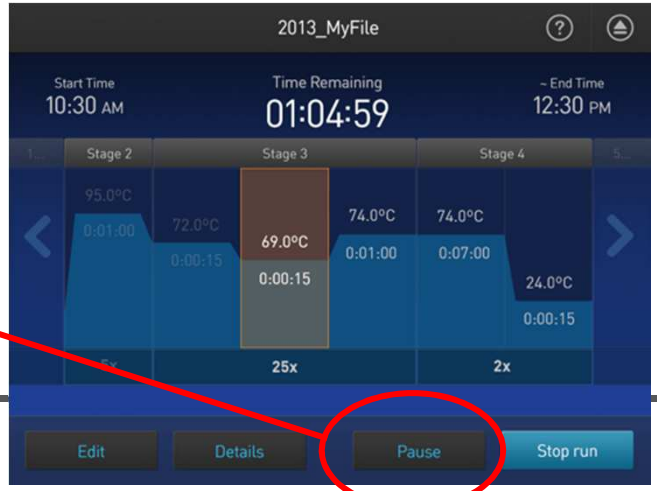
New Feature: Run Pause



Program a Pause into the run: define which step and at what temperature to pause



Or pause a run on the fly



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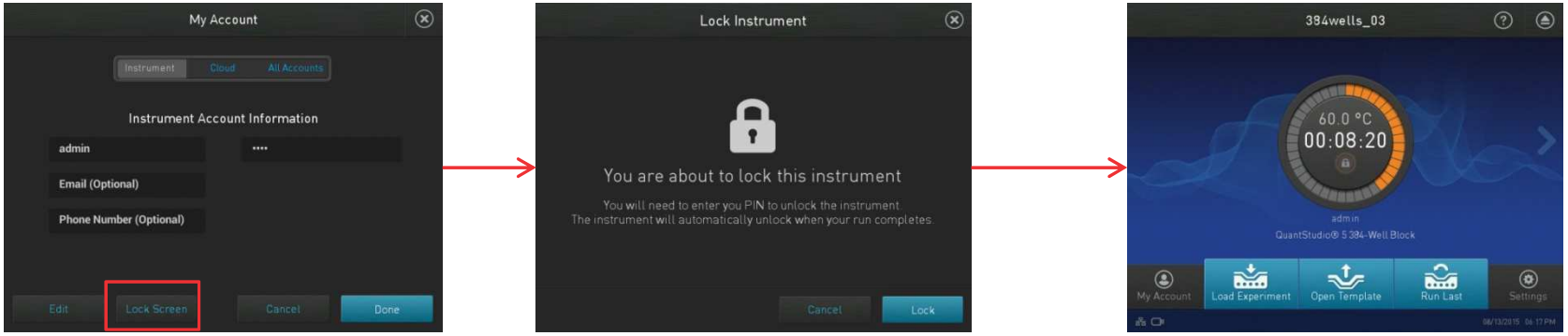
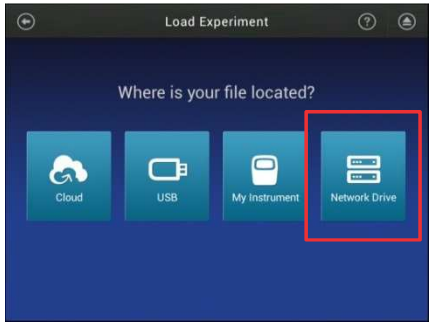
Enhanced Instrument Touchscreen

- Ability to lock instrument touchscreen during run to prevent run interruptions
  - Only current user and admin can unlock during the run.
  - Anyone can unlock and access instrument after run is completed.



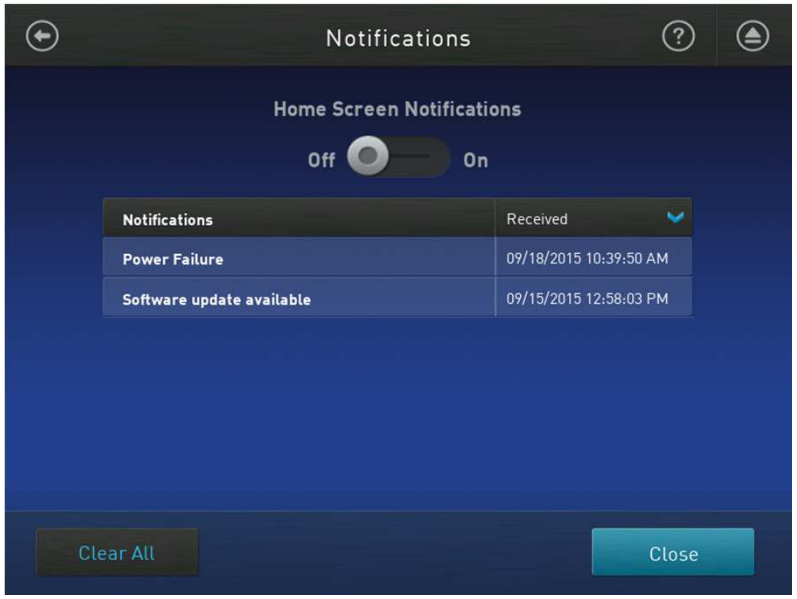
- Ability to transfer run data to/from a ‘Network Location”

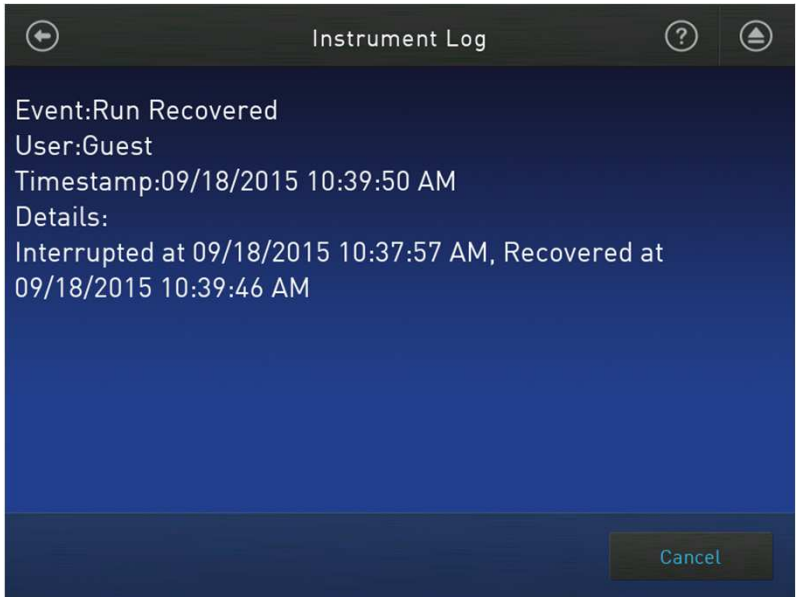


ThermoFisher SCIENTIFIC

Enhanced Instrument Touchscreen (2)

- Power Failure Mode
  - On-going run resumed automatically within 30’
  - On screen notifications, Run log & instrument log





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Options to Upload Data

1. **Cloud = Data saved to user's online account**
2. **USB = Data saved to attached USB drive**
3. **Desktop = Data automatically saves back to desktop if run started from desktop**

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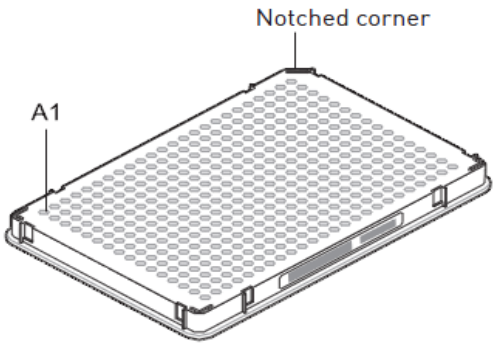
QuantStudio™ 3 & 5 consumables

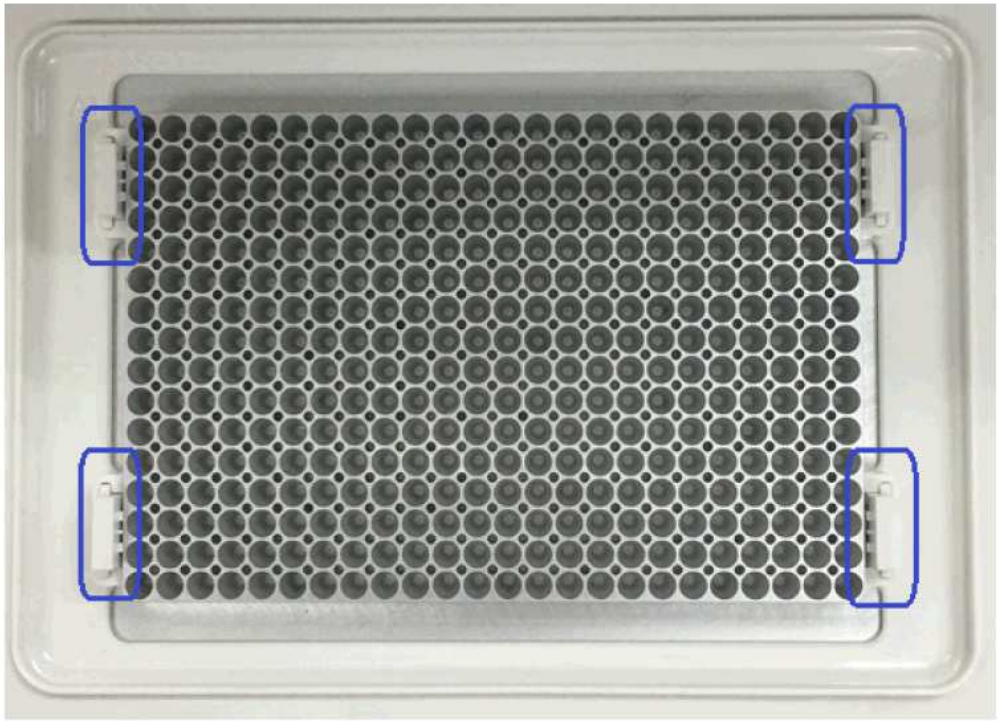
Disposables – 384 well

Sample block	Consumable	
384-well plate		• MicroAmp® Optical Adhesive Film • MicroAmp® Optical 384-Well Reaction Plate with Bar Code

- Use the proper Plate Adaptor for 384-well block - Ref for reordering 4457087
- Supported volume on 384-well block: 5 to 20µl

ThermoFisher SCIENTIFIC

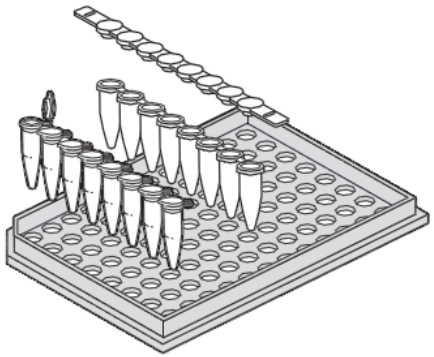
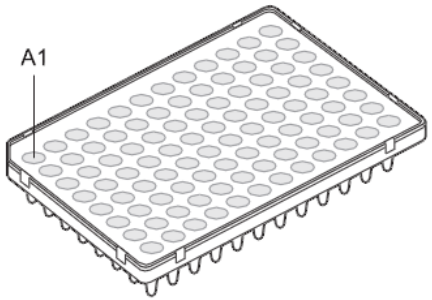
Plate Ejector for 384w block



- Plate ejector is part of the machine
- Do not remove.
- No Tubes/Strips available for 384w block

ThermoFisher SCIENTIFIC

Disposables – 96-well 0.2 ML

Sample block	Consumable	
96-well plate, 0.2 mL		• MicroAmp® Optical 8-Cap Strip • MicroAmp® 8-Tube Strips (0.2-mL) • MicroAmp® Reaction Tubes without Caps (0.2-mL) • MicroAmp® 96-Well Tray/ Retainer Set **4381850**
		• MicroAmp® Optical Adhesive Film • MicroAmp® Optical 96-Well Reaction Plate with Bar Code

- Supported volume on 0.2 ML 96-well block: 10 to 100µl

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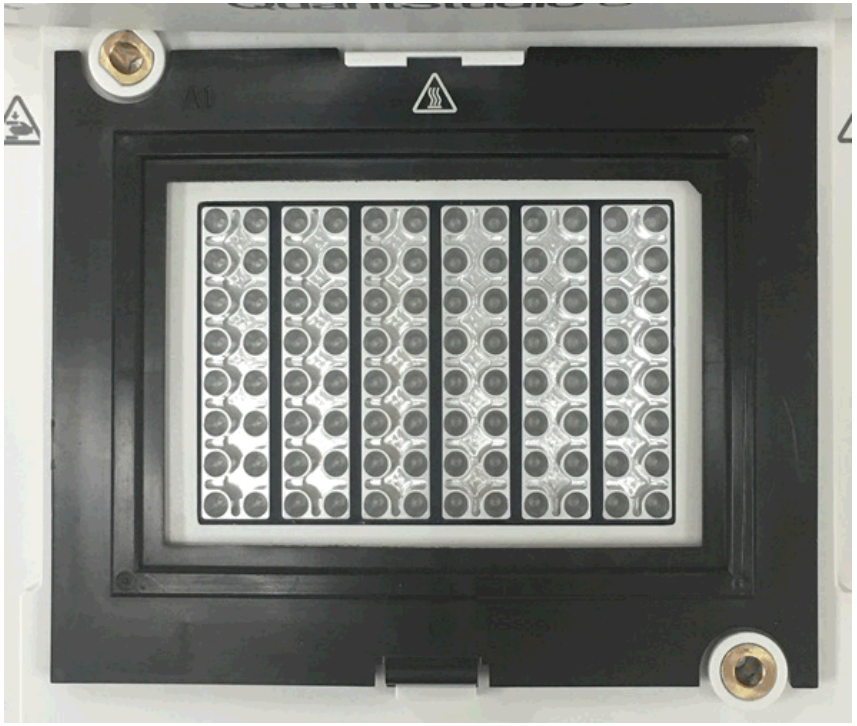
Plate Ejector & Tray retainer (for 96w 0.2ML block)



- Plate ejector is part of the machine
- Do not remove.

- Tubes/Strips to combine with 4381850
- Tray/retainer set for 96w 0.2 ML block
- Box of 10 tray/retainer set

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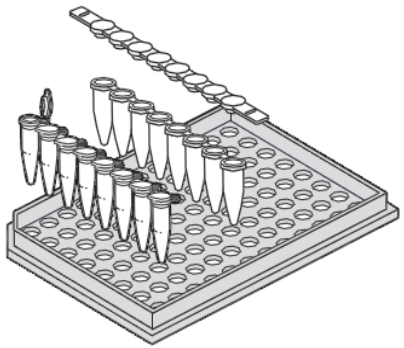
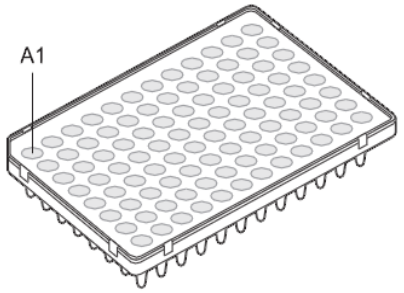
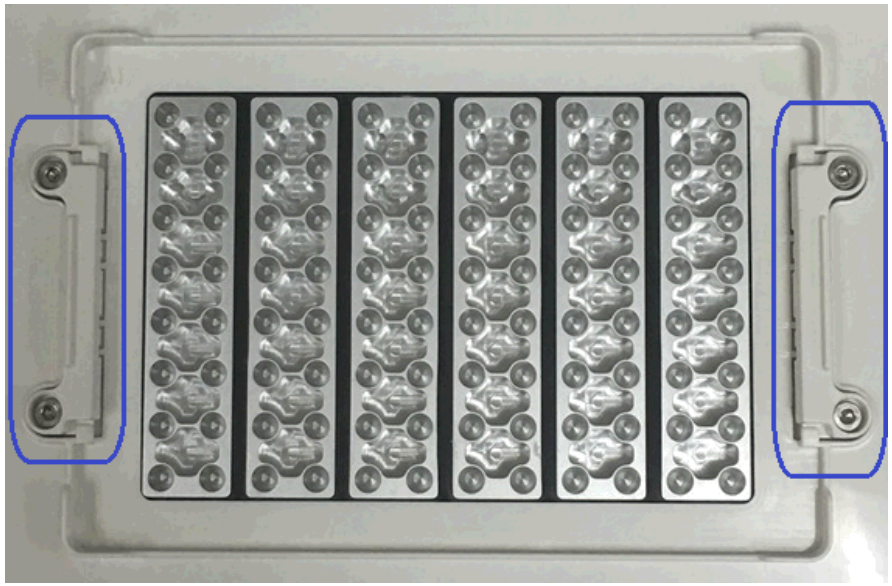
Disposables – 96-well 0.1 ML

Sample block	Consumable	
96-well fast plate, 0,1ml		• MicroAmp® Optical 8-Cap Strip • MicroAmp® Fast 8-Tube Strips • MicroAmp® Fast Reaction-Tube w/ caps • MicroAmp® Fast 96-Well Tray /Retainer **4379983**
		• MicroAmp® Optical Adhesive Film • MicroAmp® Optical 96-Well Fast Reaction Plate with Bar Code

- Supported volume on 0.1ML Block: 10 to 30µl

ThermoFisher SCIENTIFIC

Plate Ejector & Tray (for 96w 0.1ML block)



- Plate ejector is part of the machine
- Do not remove.

- Tubes/Strips to combine with 4379983
- Tray for 96w 0.1 ML block
- Box of 10 trays

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How to use single tubes and tube-strips

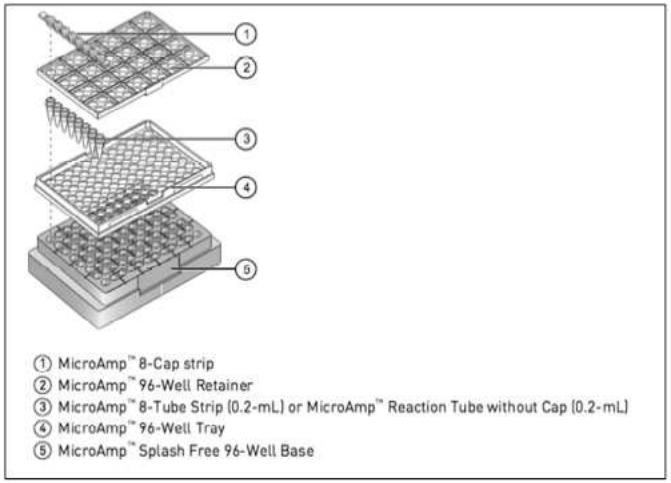
appliedbiosystems

Using Applied Biosystems™ MicroAmp™ Tubes and Strips with a Tray/Retainer on the Applied Biosystems™ QuantStudio™ 3 and 5 Real-Time PCR Systems

When using individual 0.1/0.2ml tubes and strips on the QuantStudio 3 and 5 Real-Time PCR 96-well Systems the following adaptations should be made :

1. Assemble the MicroAmp tubes/strip and caps on the Blue Tray/Retainer (SKU 4381850 for 0.2ml or SKU 4379983 for 0.1ml) following the instructions available online at:

https://tools.thermofisher.com/content/sfs/manuals/100033471_MicroAmpReactionPlates_TubeStrips_Tubes_UB.pdf



① MicroAmp™ 8-Cap strip
② MicroAmp™ 96-Well Retainer
③ MicroAmp™ 8-Tube Strip (0.2-mL) or MicroAmp™ Reaction Tube without Cap (0.2-mL)
④ MicroAmp™ 96-Well Tray
⑤ MicroAmp™ Splash Free 96-Well Base

2. Once the tubes/strips are securely capped, place the blue Tray/Retainer on the instrument block with the black adapter on the block:

- Plastics should be optical
- Seal properly with the required tools
- Use the proper tray/adapter
- Balance the tube positions

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Recommendations

- Prepare the 96-plates in a MicroAmp Splash Free 96 well base
- This base can protect plate wells from the bottom
- Also can hold the plate fix while pipetting
- Catalog number to order: 4312063 (box of 10 bases)

- Centrifuge the plate 1 min x 900g
- Do not use markers on the plates/tubes

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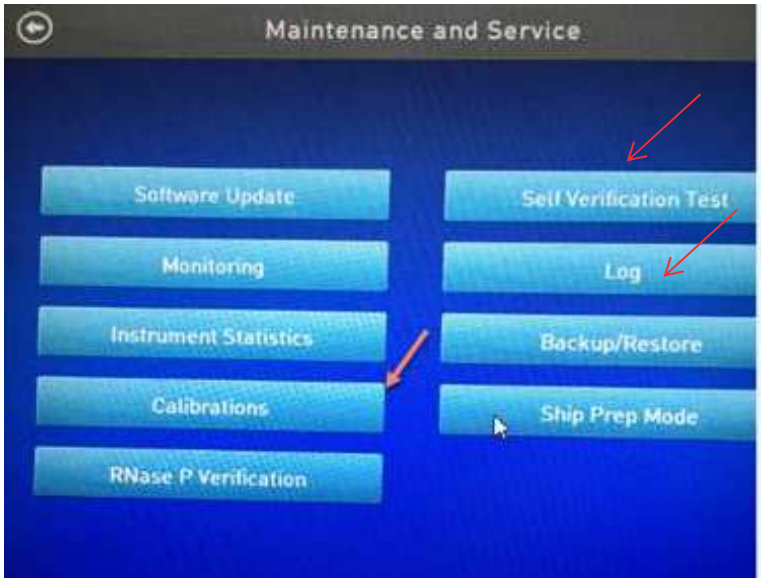
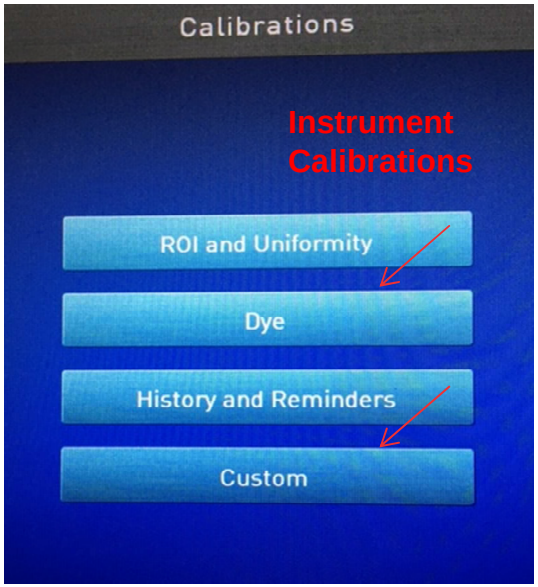
Maintenance & Service

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
	Replace the instrument lamp

Background, Dye calibrations and Log files





- For instrument malfunctions, please perform the self verification test and export the log files

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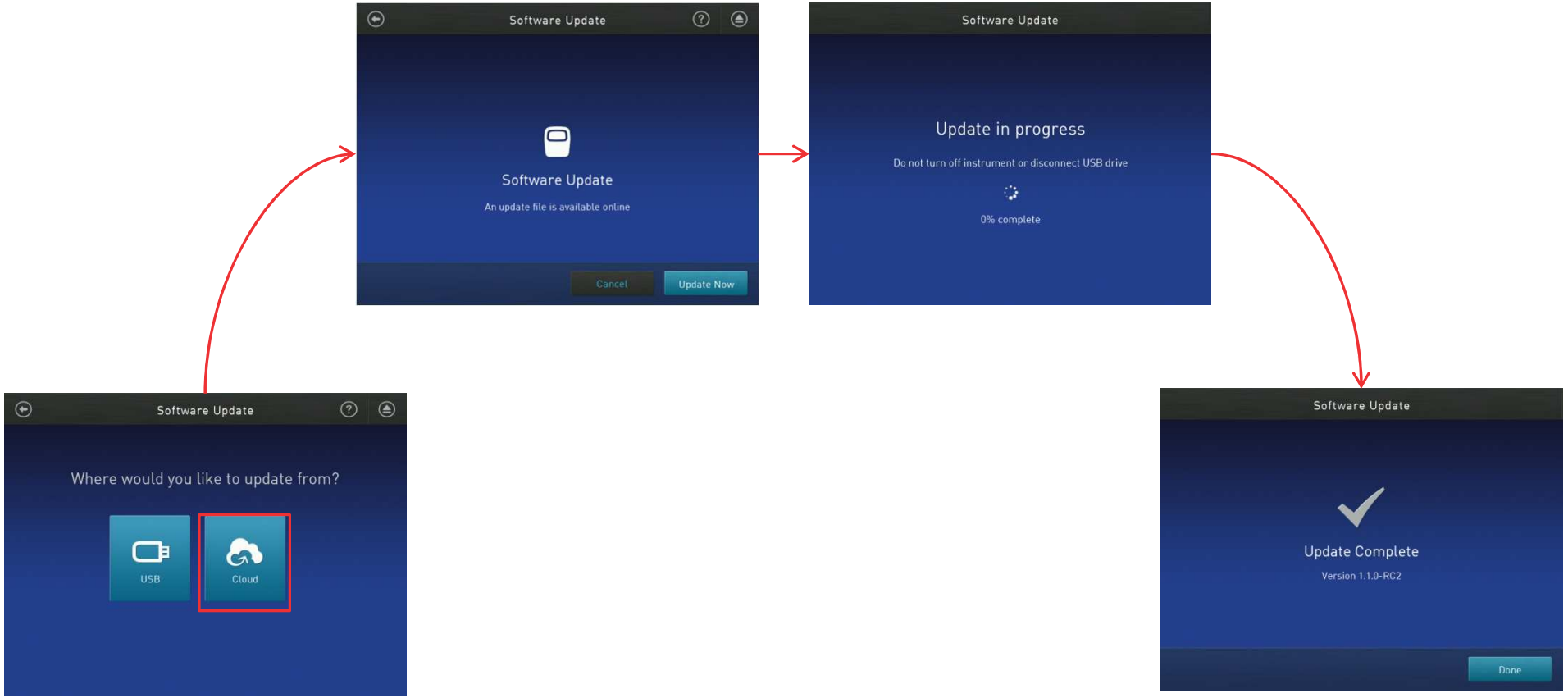
Firmware upgrade from the cloud



On eGUI, Firmware Upgrade for a cloud connected instrument:
Settings>Maintenance and Service>Software Update

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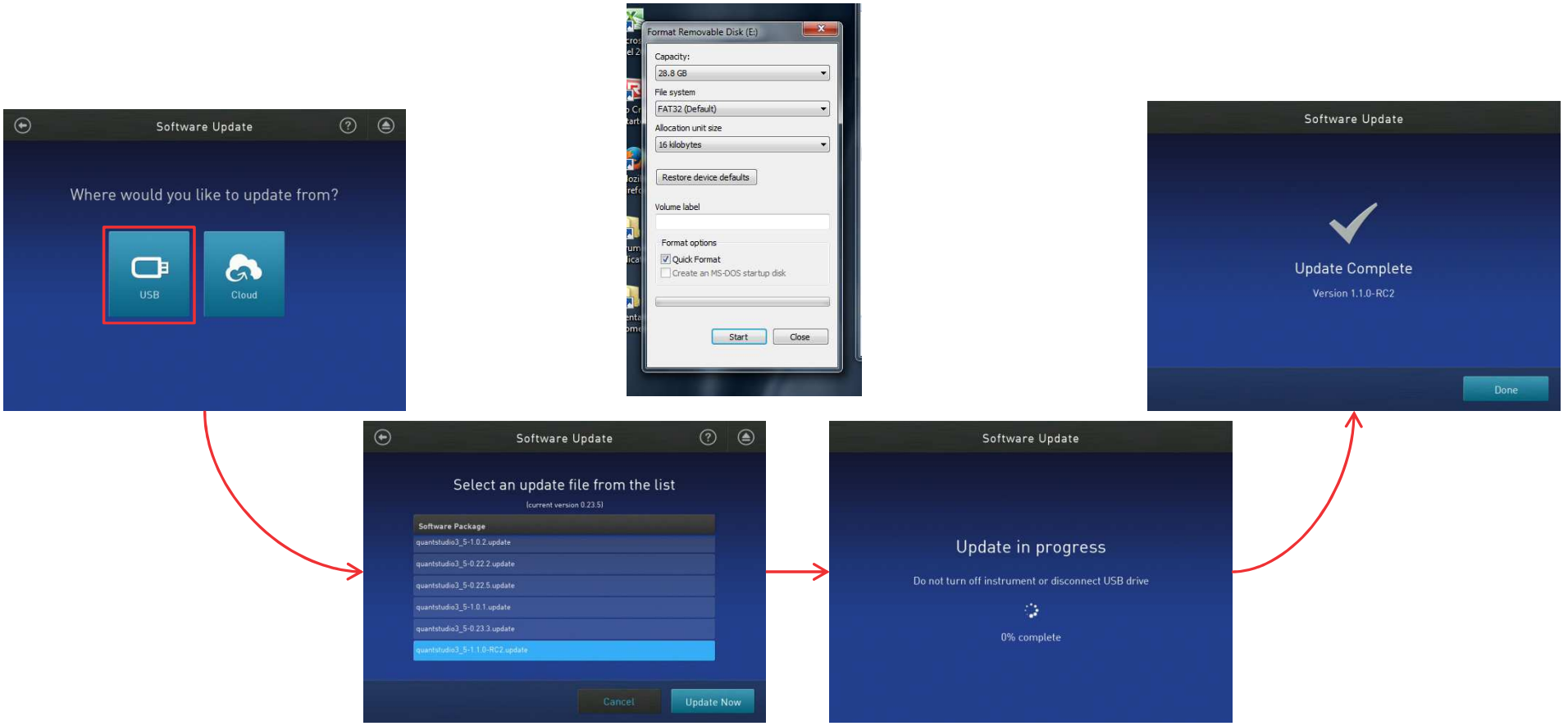
Firmware upgrade via USB

- Go to [QS D& A Software Download Webpage]
- Click on “Download” next to the “Firmware” option and download file to USB drive
- Attach USB drive to instrument and, on eGUI, Settings>Maintenance and Service>Software Update



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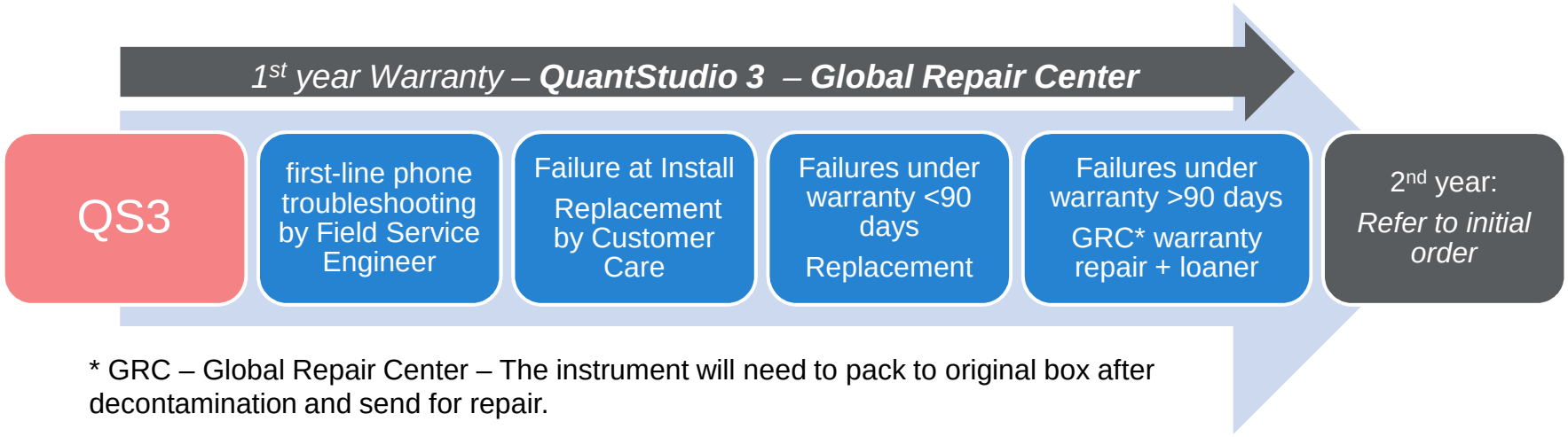
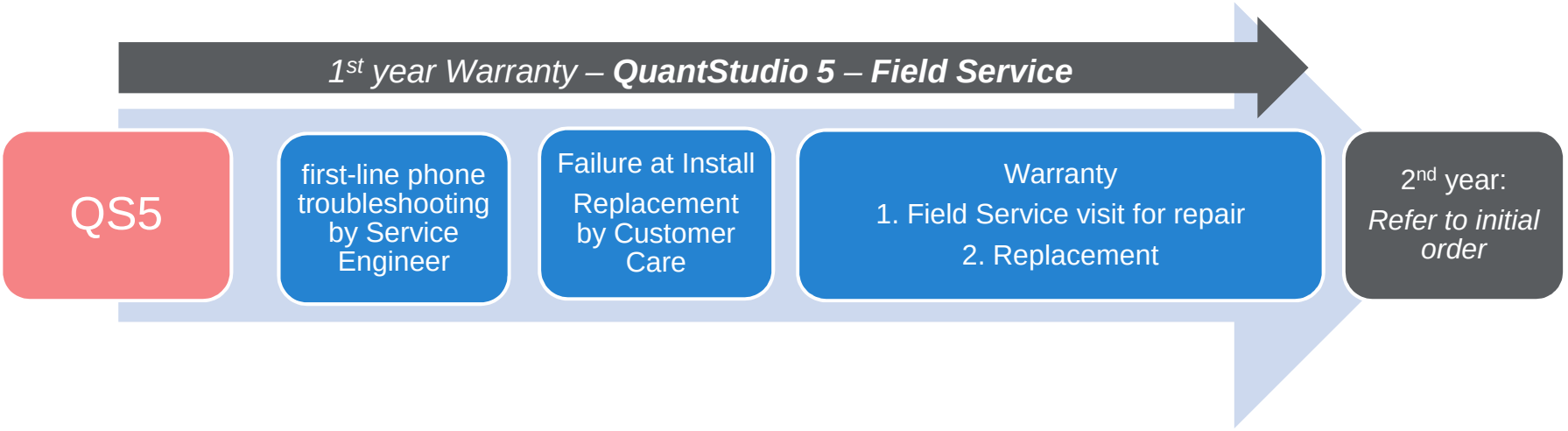
QuantStudio 3 and 5 - Service Plans



* GRC – Global Repair Center – The instrument will need to pack to original box after decontamination and send for repair.



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Service Plans

Service Plans at a glace	Repair Center service plans		On-site service plans			
	AB Repair Center Support Plus	AB Repair Center Support Plus Care	AB Maintence	AB Maintenance Plus	AB Assurance	AB Complete
On-site Response Time			Target 2 business days*	Target 2 business days*	Guaranteed 2 business days*	Guarnanteed next business day*
Scheduled On-site Planned Maintenance (PM)		✓	✓	✓	✓	✓
Remote Diagnostics	✓	✓	✓	✓	✓	✓
Parts, Labor & Travel for Repair, included	✓	✓		10% discount optional add-on in select regions	✓	✓
Computer repair & replacement, included	✓	✓			✓	✓
Priority access to Tech Support Mon-Fri, 8am - 5pm, local time	✓	✓			✓	✓
Priority access to Remote Service Engineer Mon - Fri, 8am - 5pm, local time	✓	✓			✓	✓
Re-qualification post PM & critical repairs						✓
Field Application Scientist Consult						✓
Loaner instrument issued during repair (Repair Center plans only)	✓	✓				

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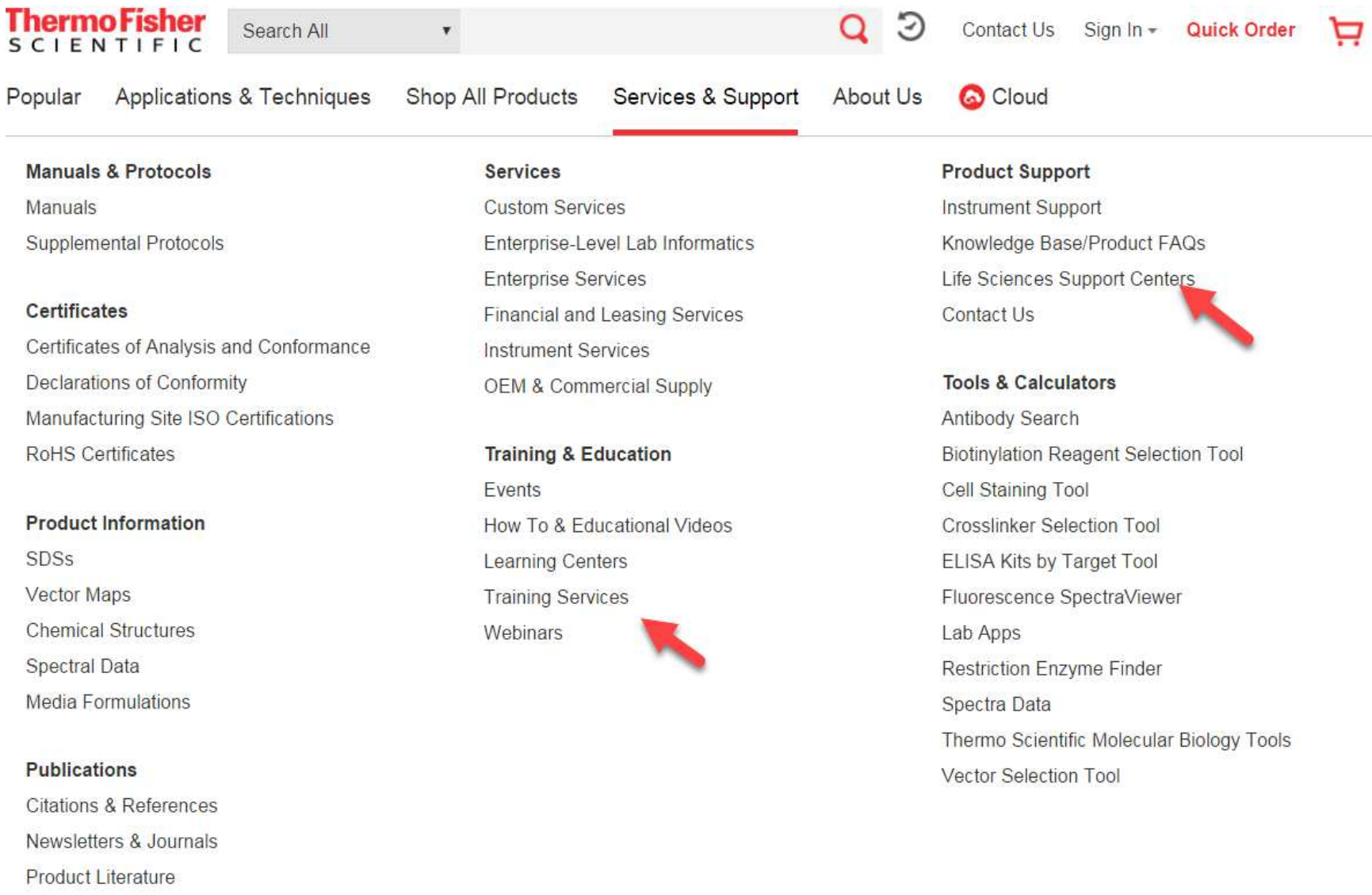
Where can I find technical help?

Thermo Fisher Scientific Services and Support



Instrument and Application Support Centers

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Support Centers

Technical Resources

- Contact Us
- Product FAQs
- Manuals & Protocols
- MSDS, COA & other Support Documents
- Instrument Repair
- Software Downloads
- Order Support
- Order & Web FAQs
- Events
- Training

General Resources

- How-To & Educational Videos
- Life in the Lab
- Mobile & Desktop Apps
- Newsletters & Journals
- Product Selection Guides & Tools

- Product Documentation
- Product FAQs
- Selection Guides & Tools
- Webinars
- Contact Us

Support centers by application

Resources and tips for getting started, and troubleshooting help all in one location. Browse the support centers by application for technical support.

Antibodies and immunoassays

- Antibodies
- ELISA Kits and Antibody Pairs
- Luminex Assays
- **View all ›**

Capillary electrophoresis applications

- Fragment Analysis
- Sanger Sequencing
- **View all ›**

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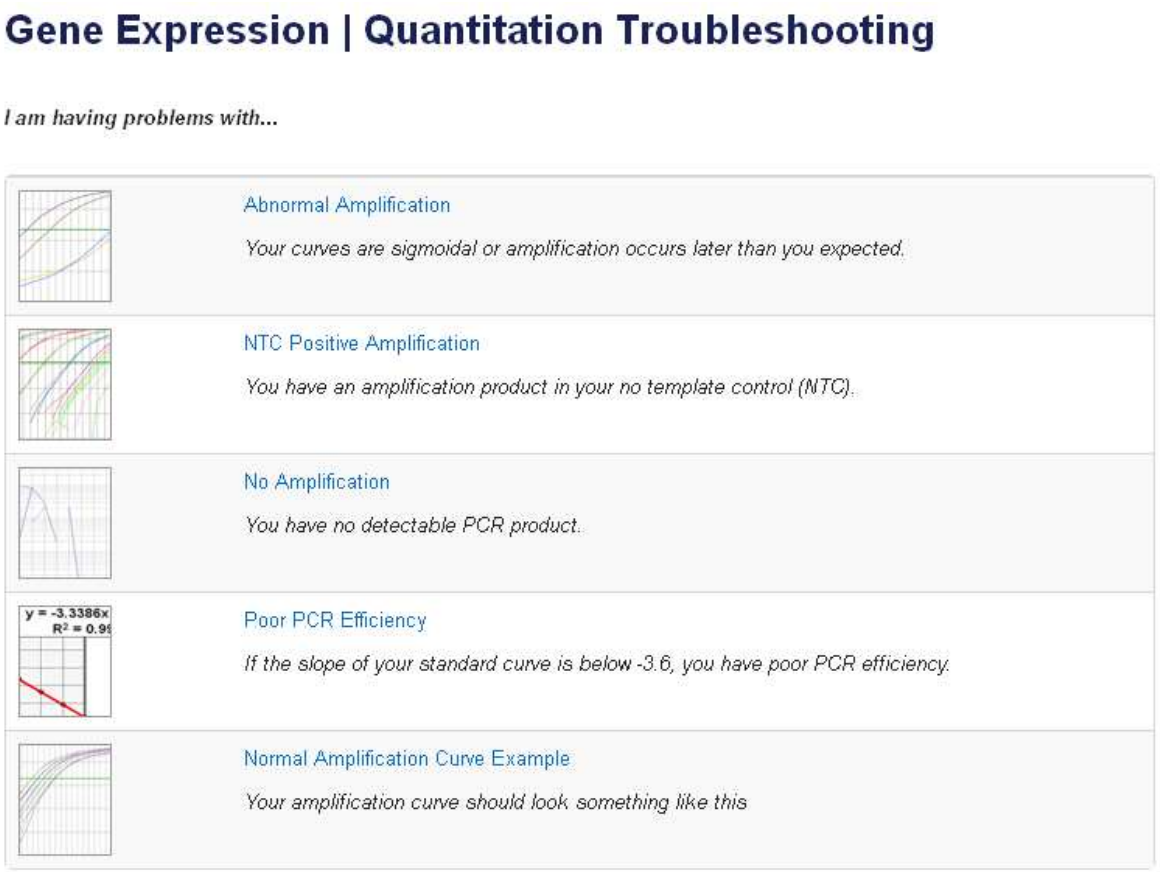
What if the qPCR does not work?

http://www.lifetechnologies.com/fr/en/home/life-science/pcr/real-time-pcr/qpcr-education/real-time-pcr-troubleshooting-tool/gene-expression-quantitation-troubleshooting.html

- Real-Time PCR Troubleshooting Tool



Gene Expression | Quantitation Troubleshooting

I am having problems with...

Abnormal Amplification

Your curves are sigmoidal or amplification occurs later than you expected.

NTC Positive Amplification

You have an amplification product in your no template control (NTC).

No Amplification

You have no detectable PCR product.

Poor PCR Efficiency

If the slope of your standard curve is below -3.6, you have poor PCR efficiency.

Normal Amplification Curve Example

Your amplification curve should look something like this

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Events

Get hands-on lab experience and further your working knowledge of applications and instrument operation with our Application Training Courses, available at our Customer Training Centers located throughout the world. Our centers are state-of-the-art laboratories equipped with the latest Thermo Fisher Scientific instruments and products, computer-based interactive training, and the opportunity to collaborate with application scientists.

An award-winning team of application scientists delivers courses bringing hundreds of years of collective experience in using, training, and troubleshooting sequencing, real-time PCR, cell culture, and molecular biology. This knowledge, combined with our world-class facilities, makes us the ideal choice for all of your training needs.

Technical Support Hotline – Contact Center

00 800 5345 5345

www.thermofisher.com/askaquestion

eurotech@thermofisher.com

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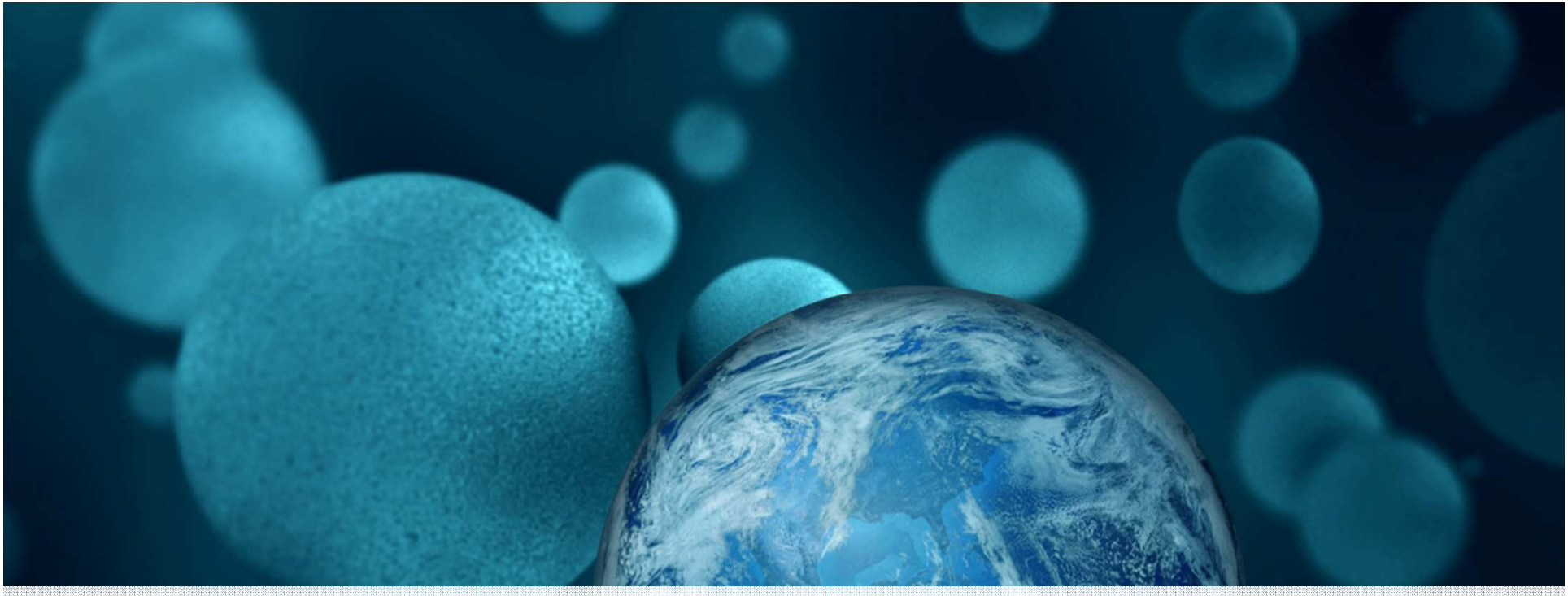

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QuantStudio™ Design and Analysis Software

The world leader in serving science

Desktop and Web Browser-Based Software Available

Features	Web	Desktop
Plate Set Up - Compatible with 7500, 7500 Fast, Step One, Step One Plus, and QuantStudio™ 6 and 7 setup files - Custom attributes for sample - VeriFlex™ Support	✔	✔
Edit Thermal Protocol	✔	✔
Locked Template*	✔	✔
Start Run	✖	✔
Programmable Pause*	✔	✔
Single plate analysis	✔	✔
Multiple plate analysis	✔	✖
Remote monitoring	✔	✔
SAE (available for QuantStudio 5 only)	✖	✔

QuantStudio™ Design and Analysis Software

- The QuantStudio Design and Analysis Software supports a variety of analysis methods, including:
  - Absolute Quantitation
    - Standard Curve
  - Relative Quantitation
    - Relative Standard Curve
    - Comparative CT (ΔΔ CT)
    - Multiplate ΔΔ CT Studies
  - Presence/absence (Plus/Minus) assays with an internal positive control
  - Melt curve analysis
  - Genotyping (including real-time amplification)
- Multiplate GEx analysis available online on the QuantStudio Design and Analysis **Cloud** Software

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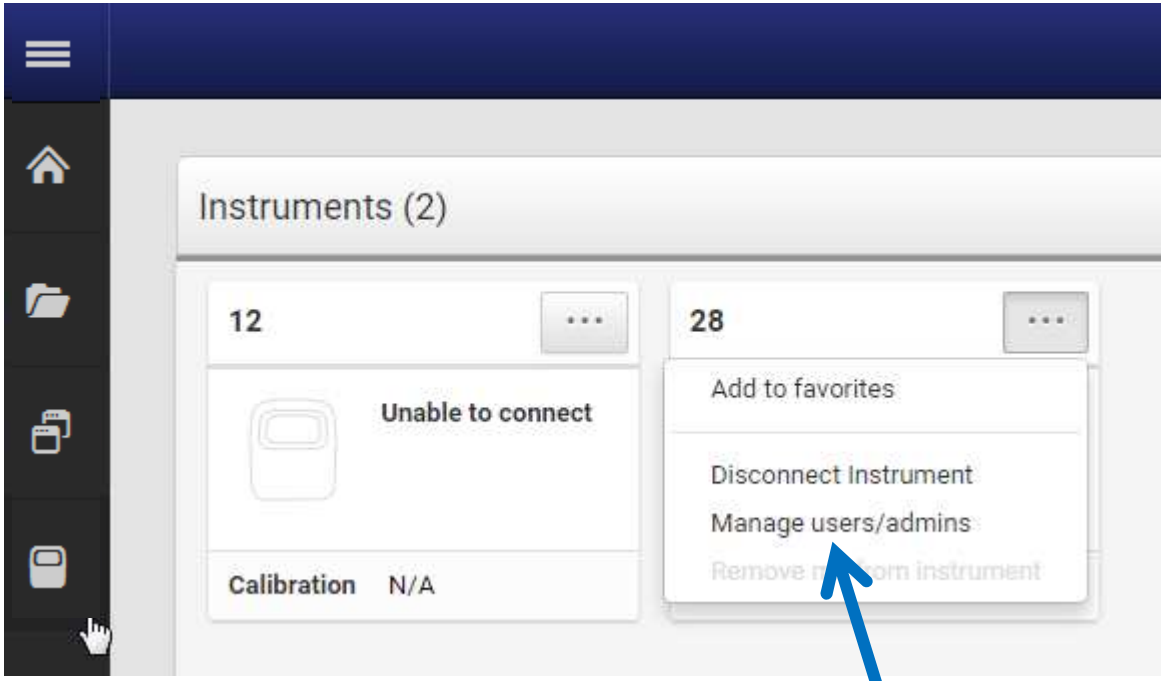
QuantStudio™ Design and Analysis Cloud software: Instrument Admin? (Optional)

Manage connected instruments



Note: If a Cloud admin is not setup at install, any lab user who connects the instrument online will become the admin!

Allow others to connect

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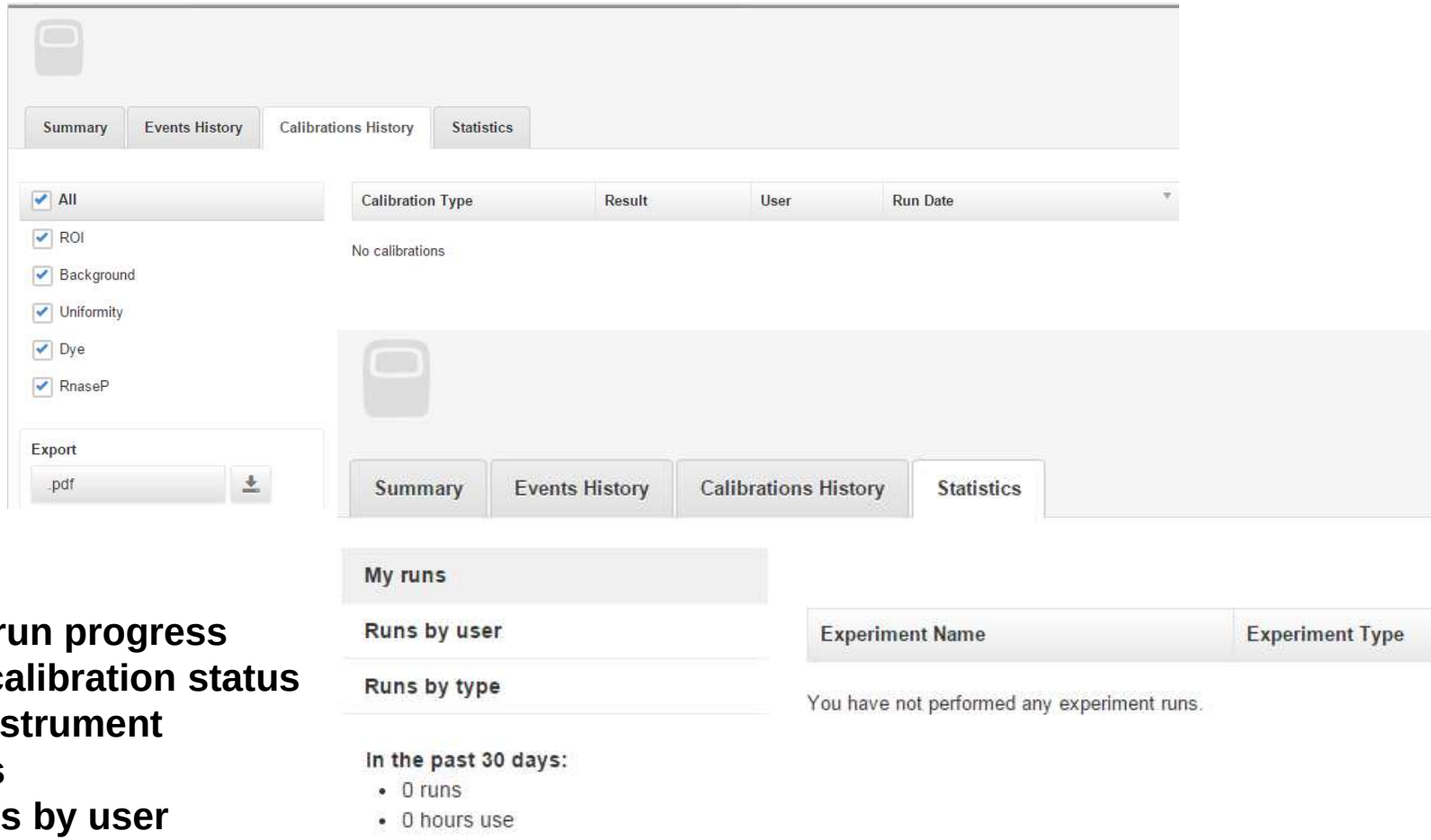
Connected Instrument



1. **Monitor run progress**
2. **Review calibration status**
3. **Check instrument statistics**
   a. **Runs by user**
   b. **Runs by type**

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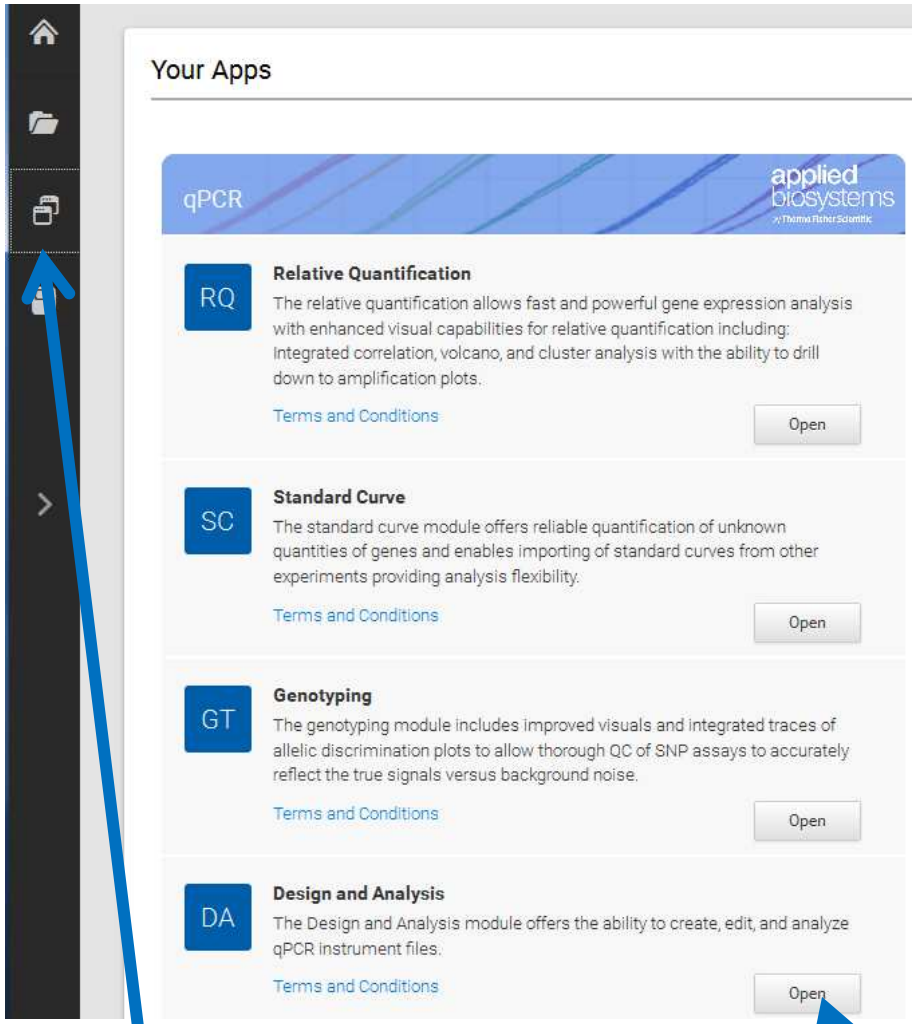
Start Run or Open a Run File



“Your Apps”

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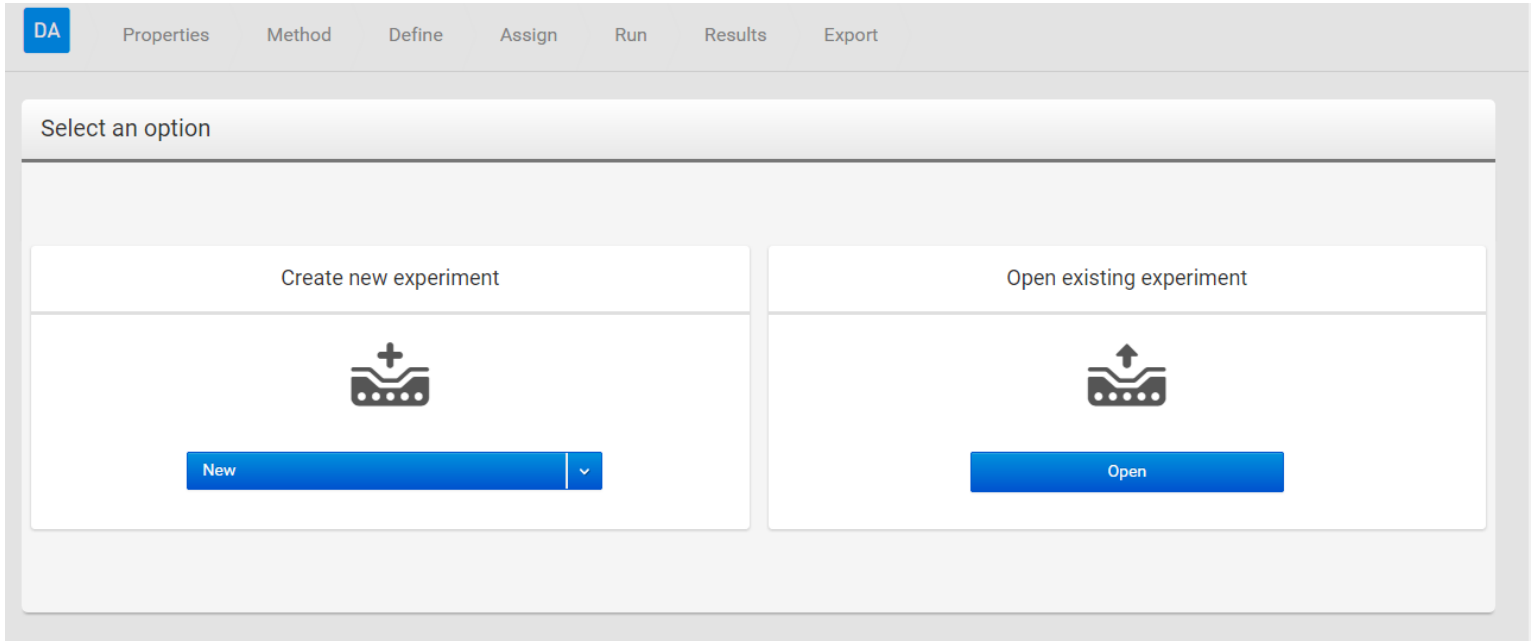
Start Run or Open a Run File



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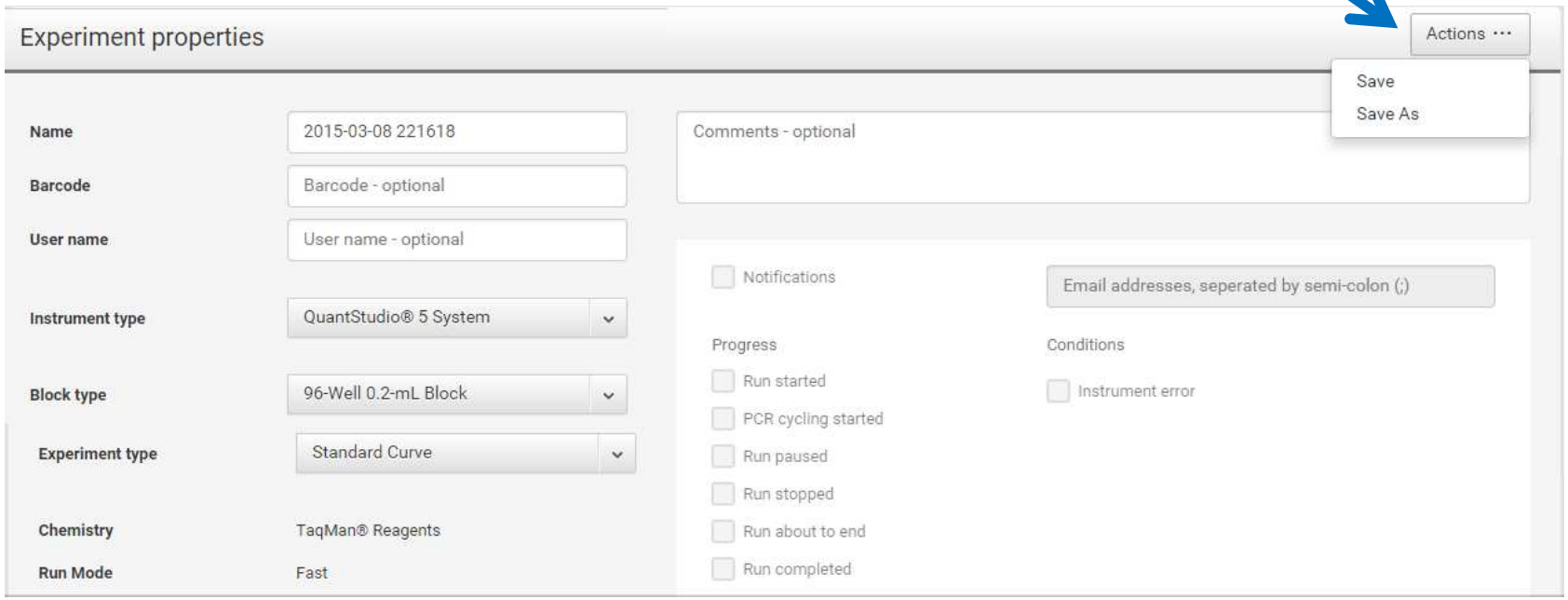
Remember to Save!



- When a run file is created, it will exist as an .edt file until it is run
- The .edt file will remain, even after the .eds file is generated

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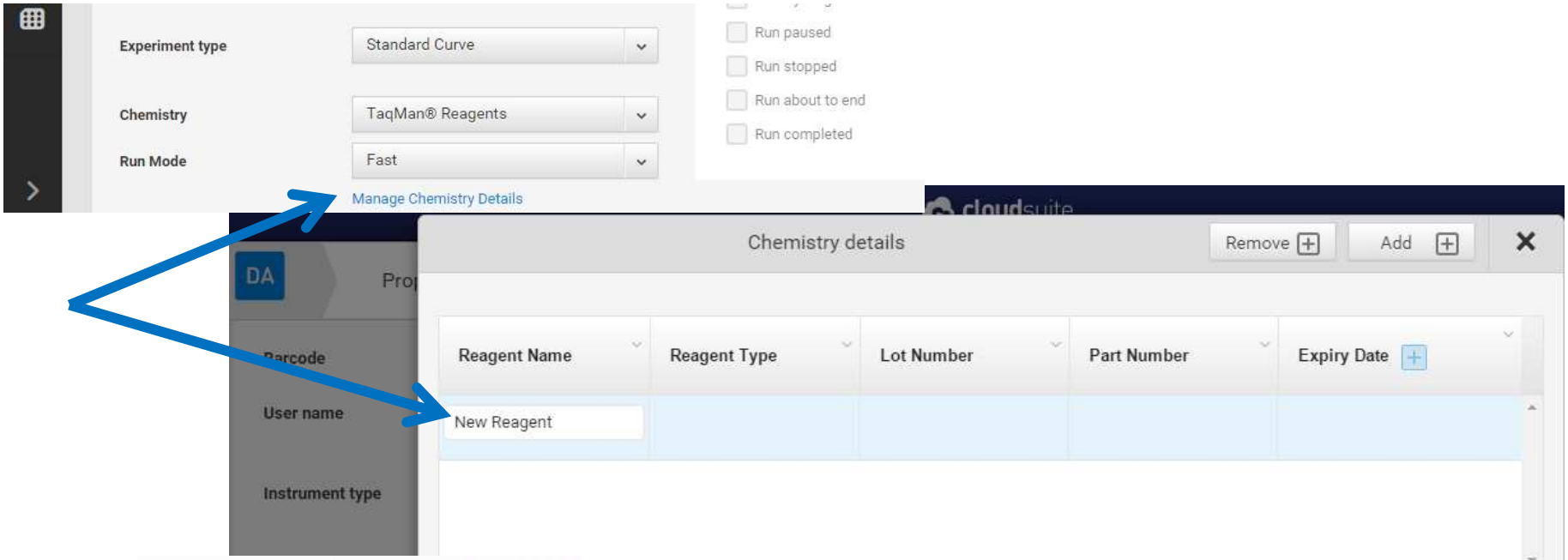
Applied Biosystems™ 2D Barcodes



Use a 2D barcode scanner to track reagent details:

- Part Number
- Lot Number
- Expiration Date

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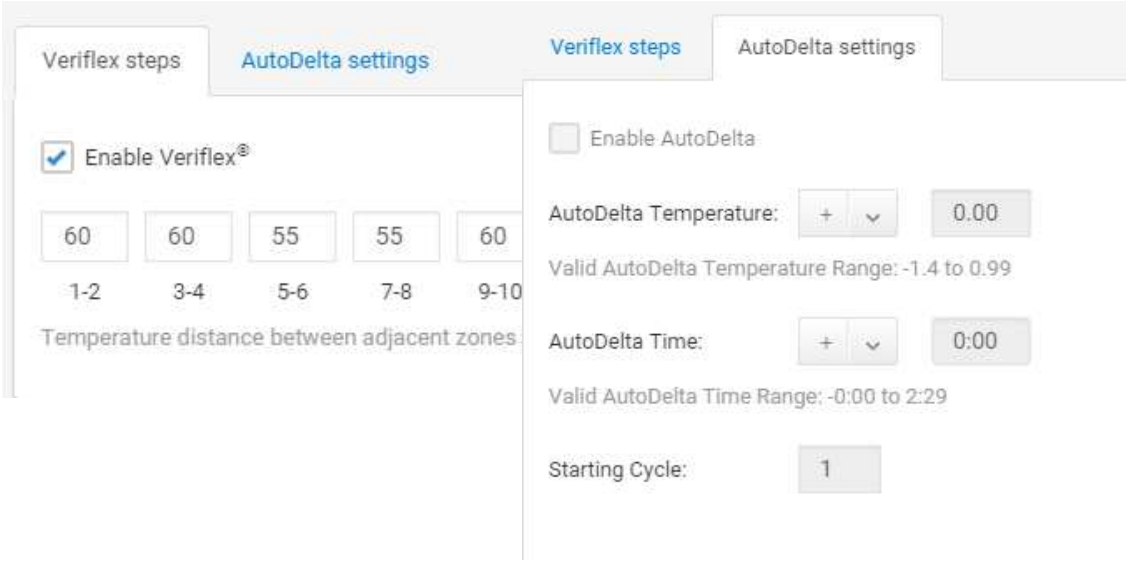
Programmable Pause

Settings

- VeriFlex™ blocks
- AutoDelta



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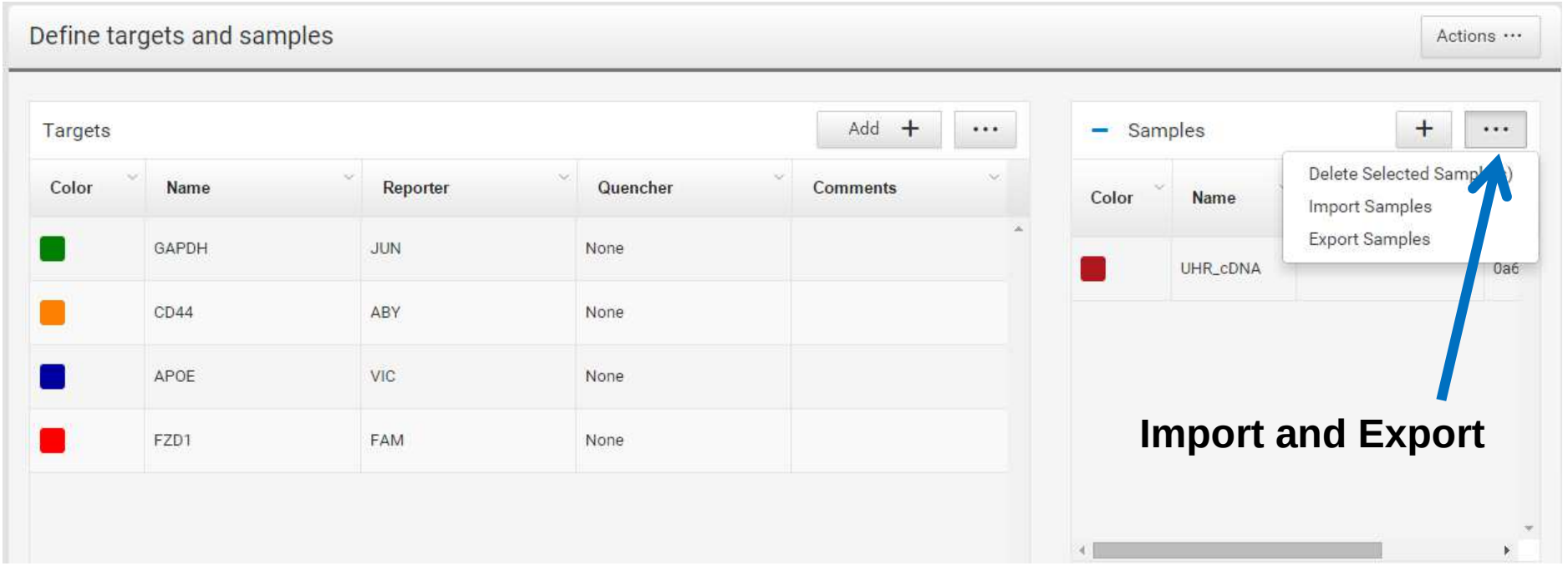

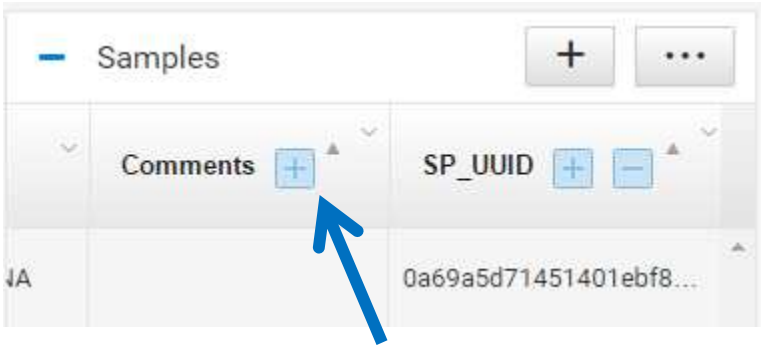

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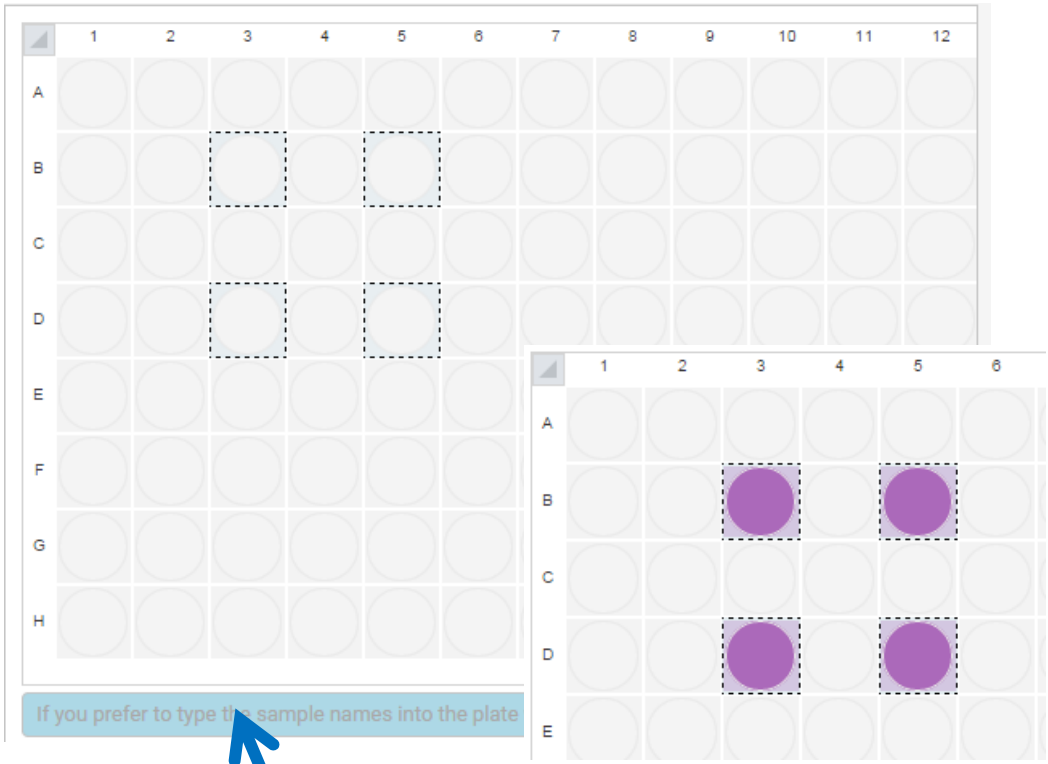
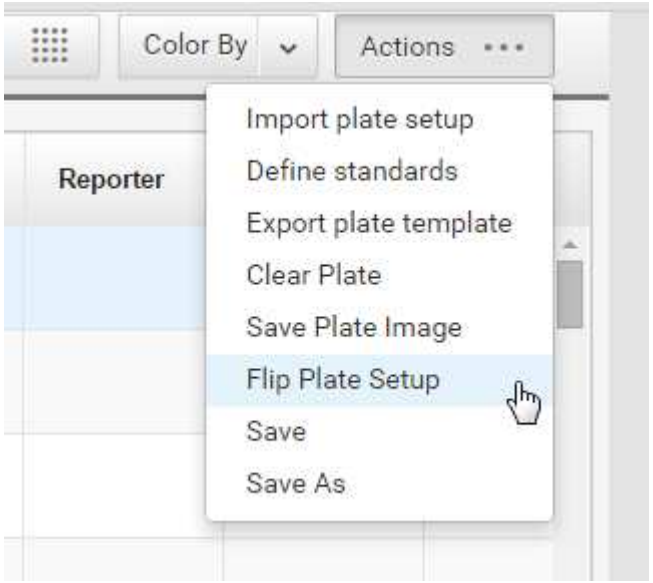


Select well and type sample names



- **Import & Export**
- **Save plate image**
- **Flip plate setup**

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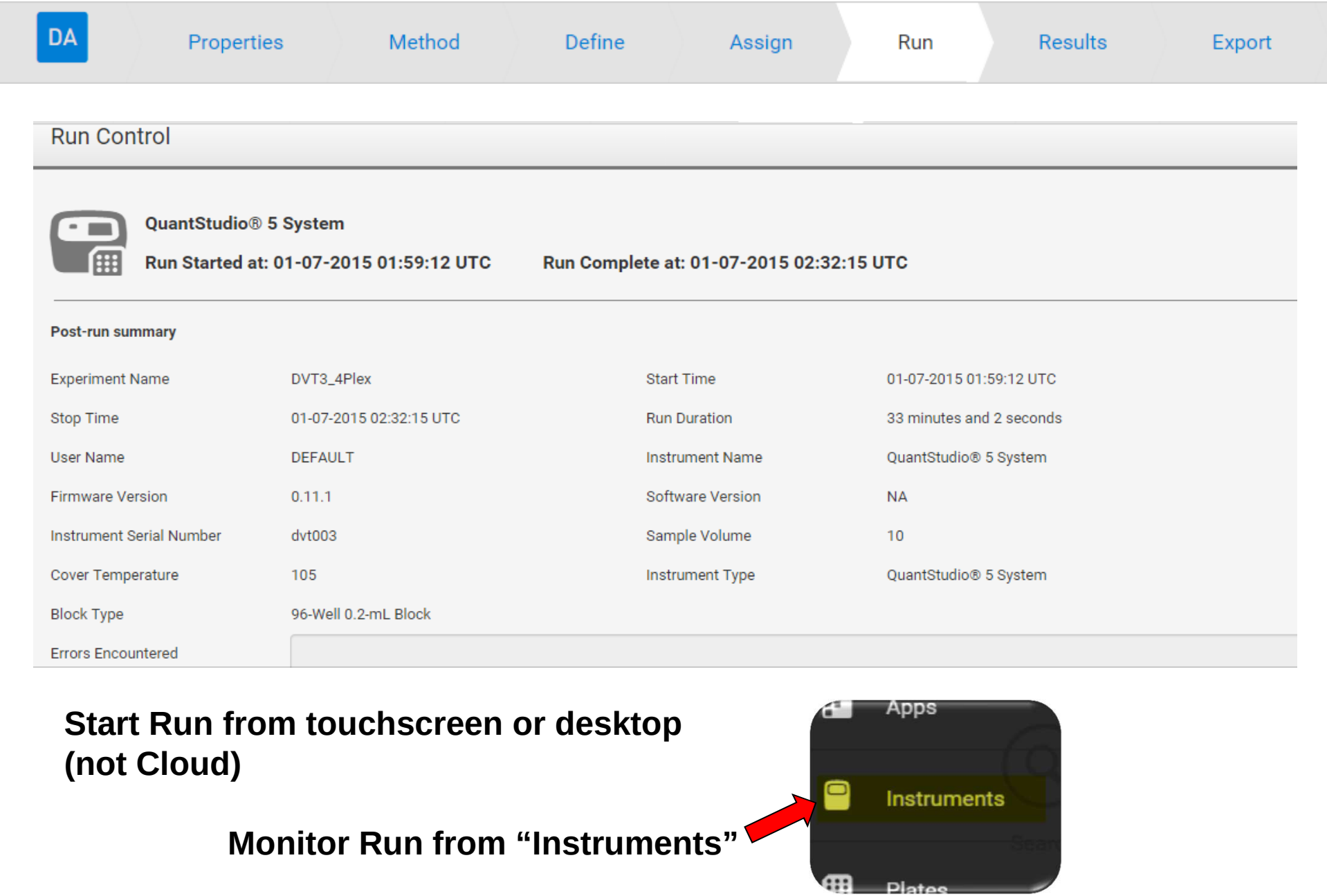

Start Run from touchscreen or desktop (not Cloud)

Monitor Run from "Instruments"

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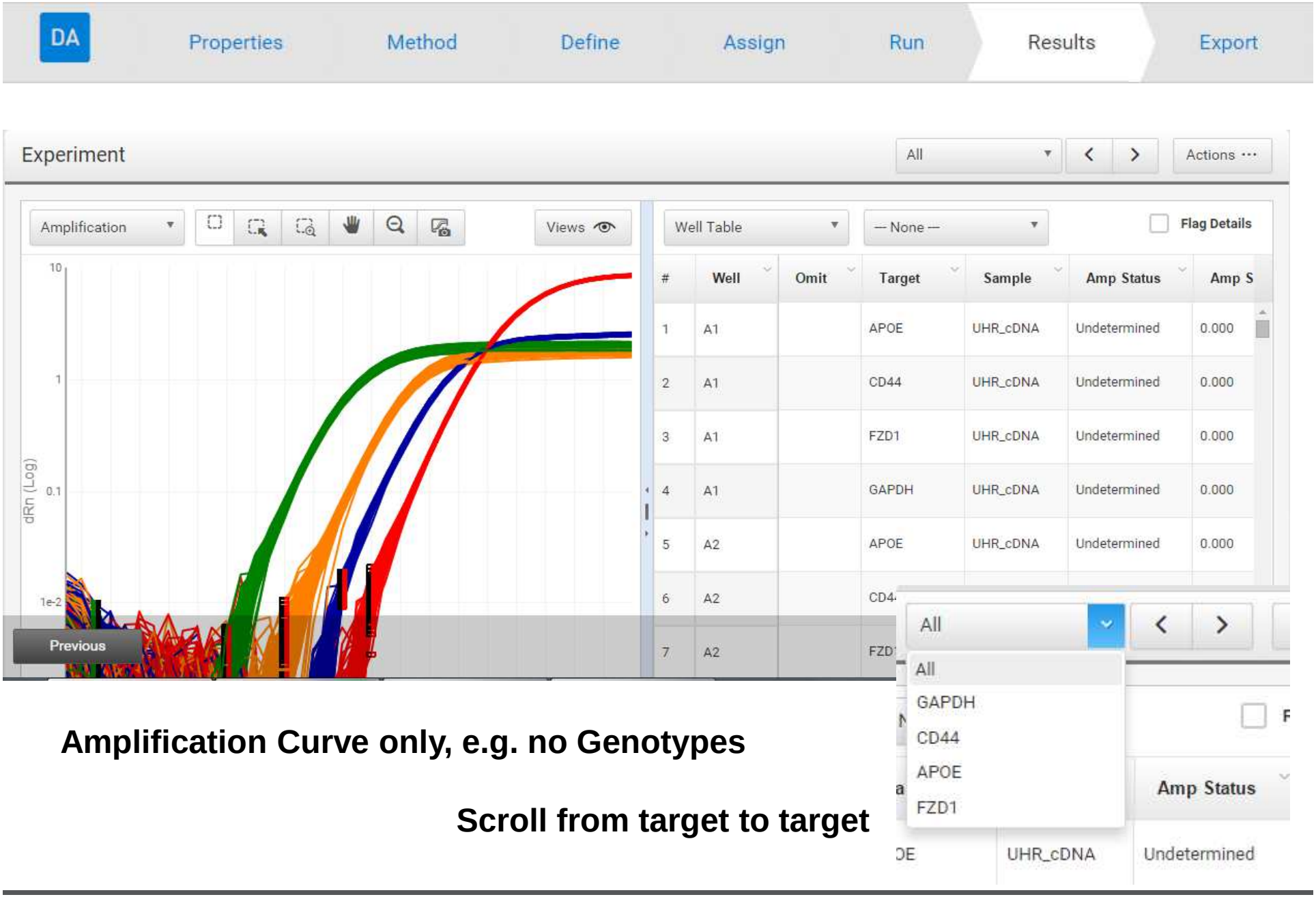

Amplification Curve only, e.g. no Genotypes

Scroll from target to target

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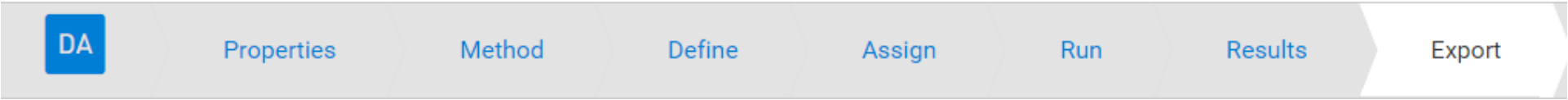
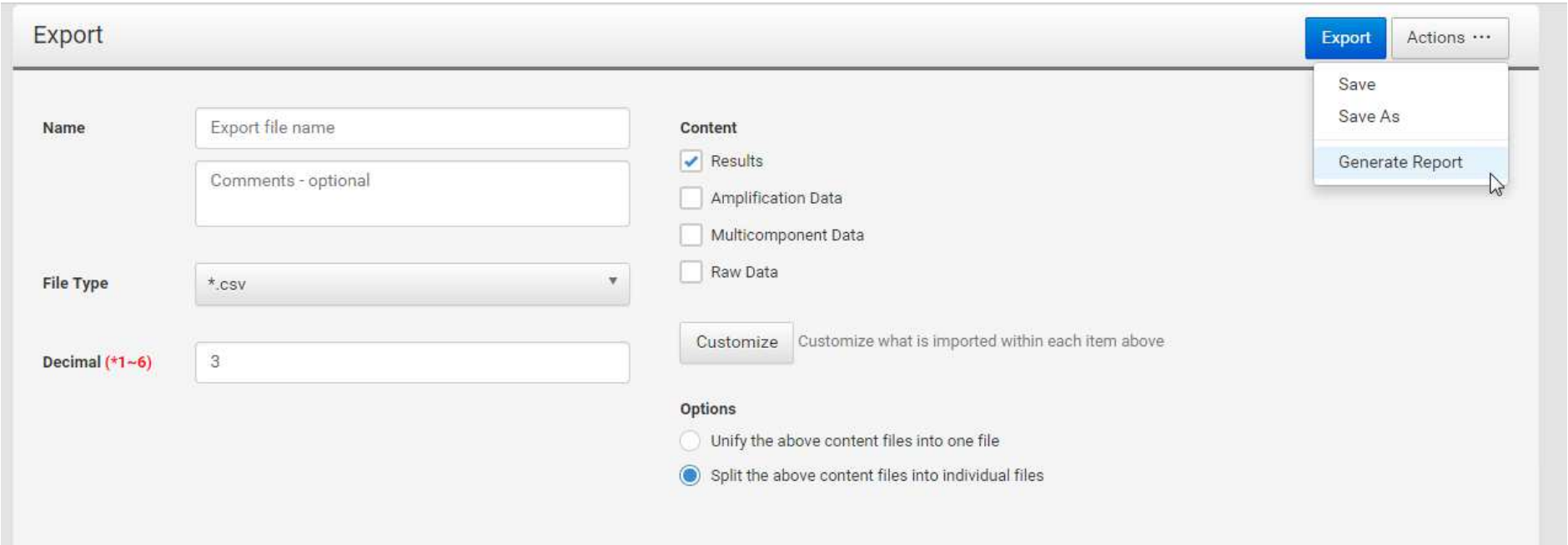
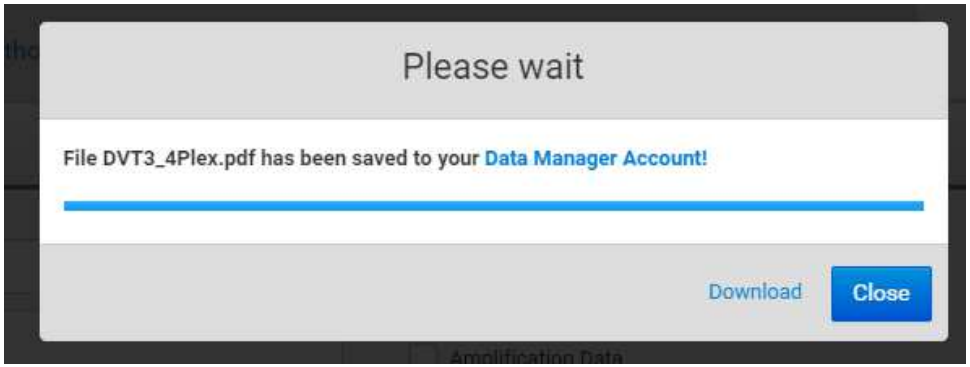




Export to local computer: Text, csv, Excel™, and RDML (xml)

Report saved online, option to download as PDF



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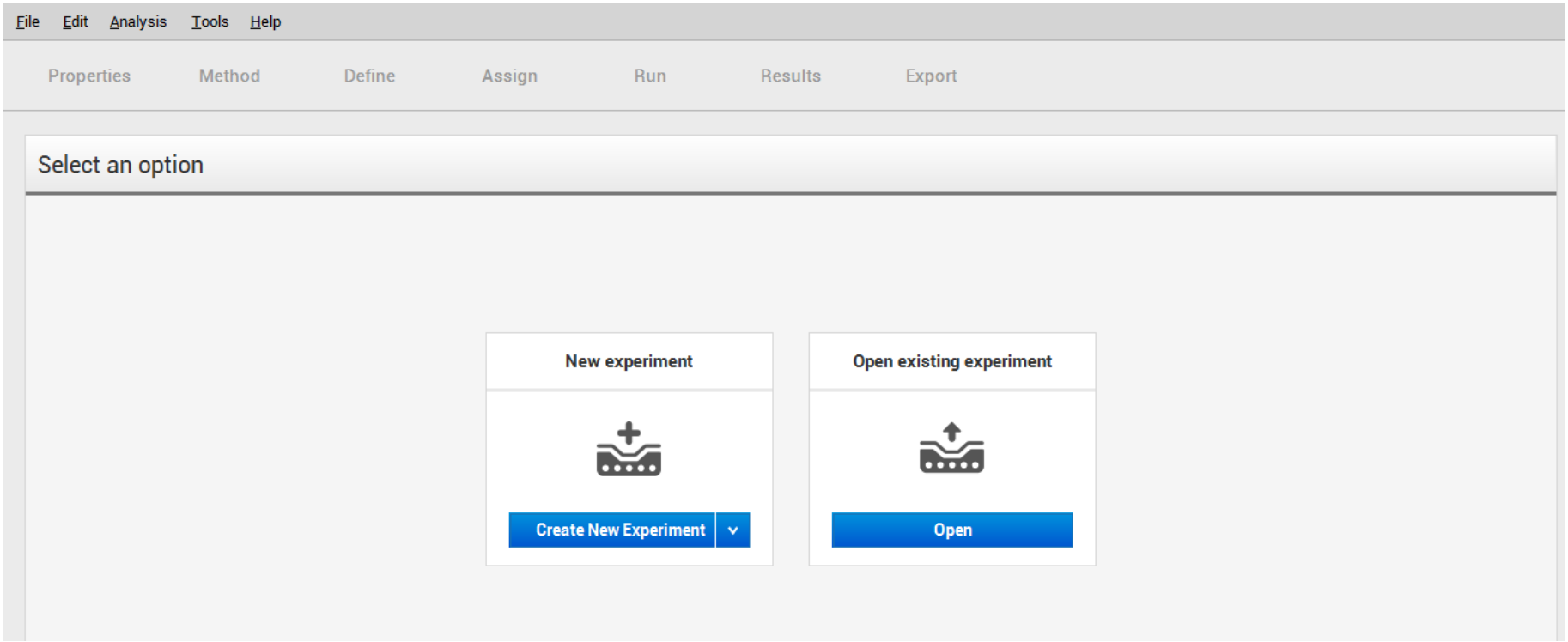
Desktop – For those who can not be online



- Similar look and feel as online software

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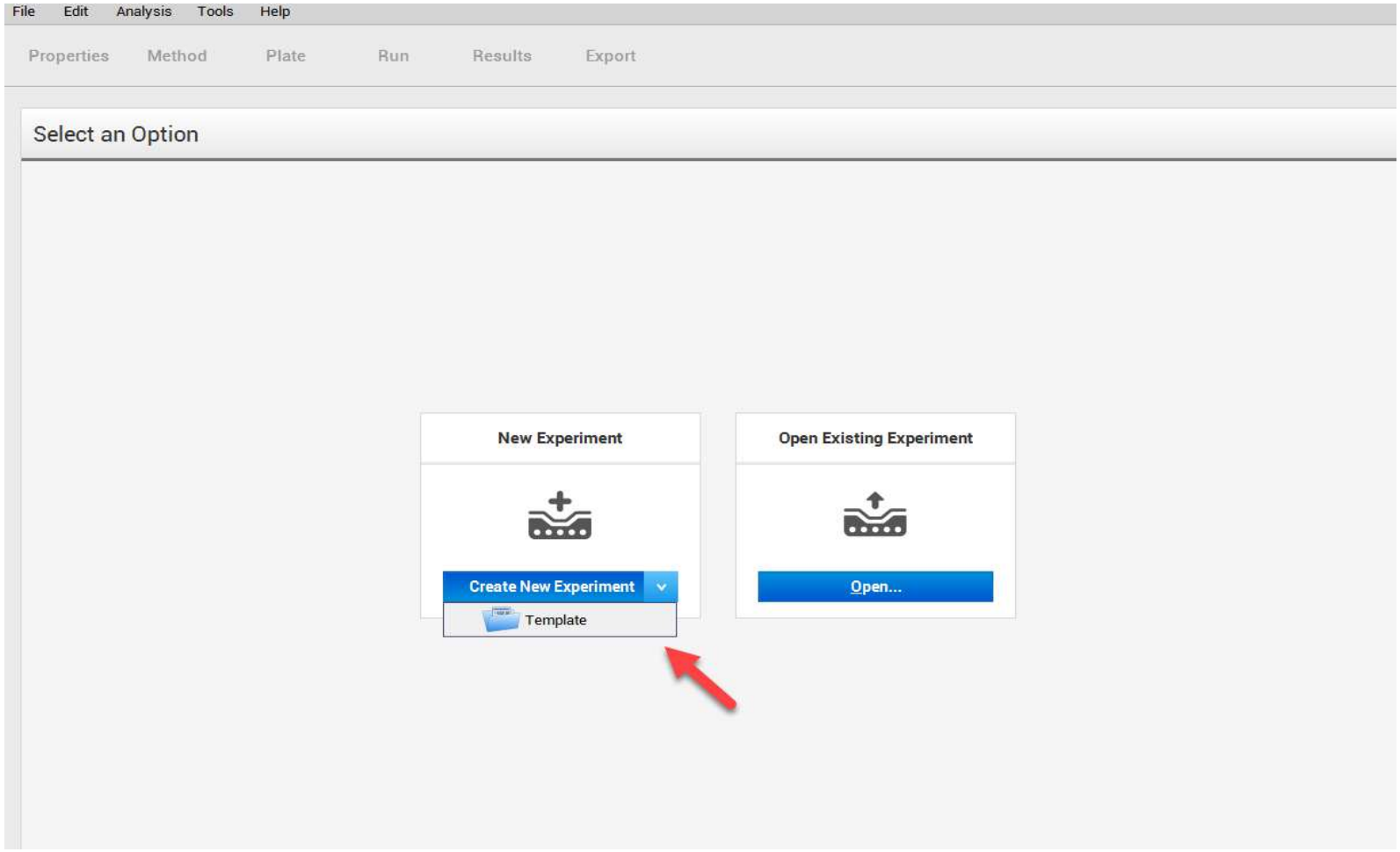
Experiment Setup



- Create your experiment or start it from a template

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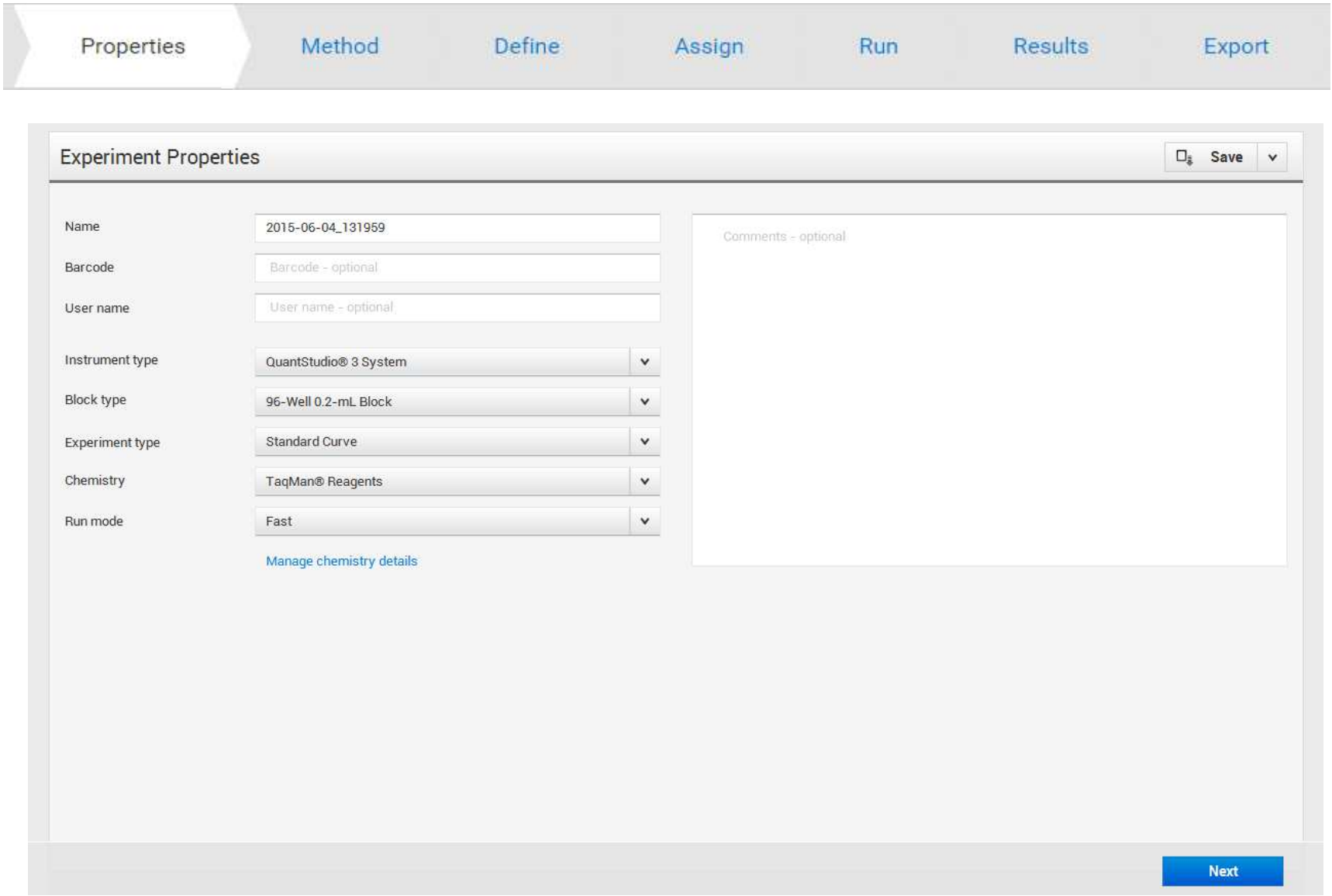
Experiment Properties



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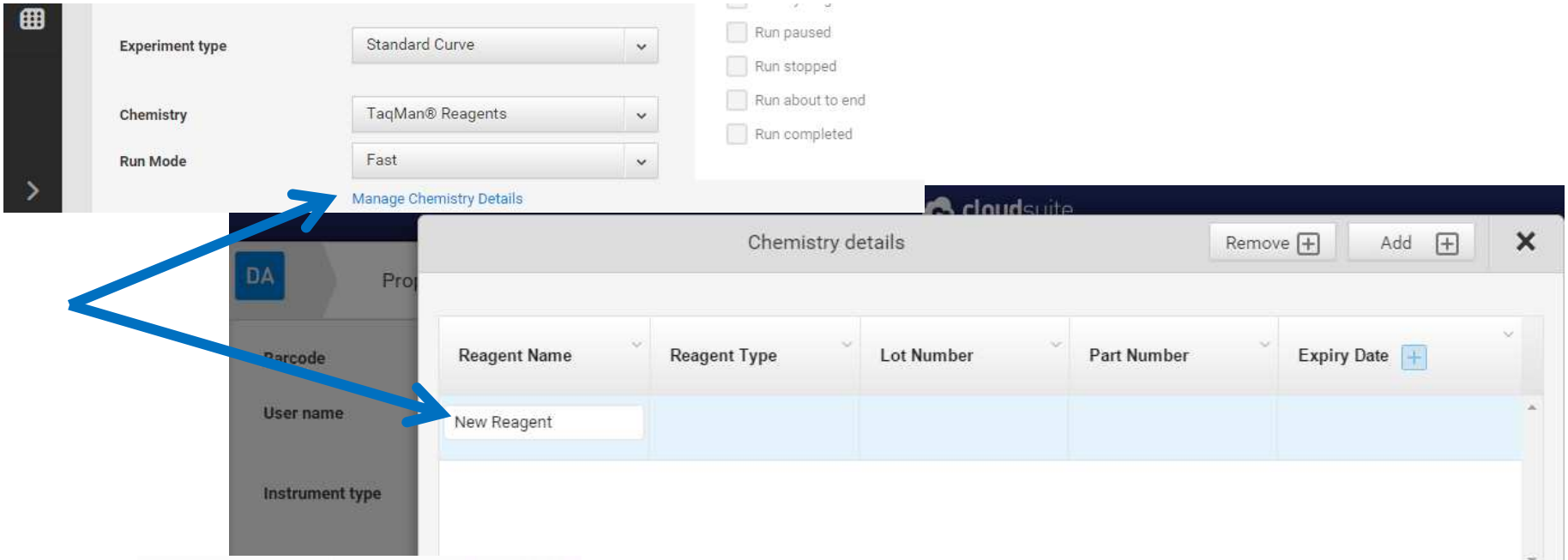
Applied Biosystems™ 2D Barcodes



Use a 2D barcode scanner to track reagent details:

- Part Number
- Lot Number
- Expiration Date

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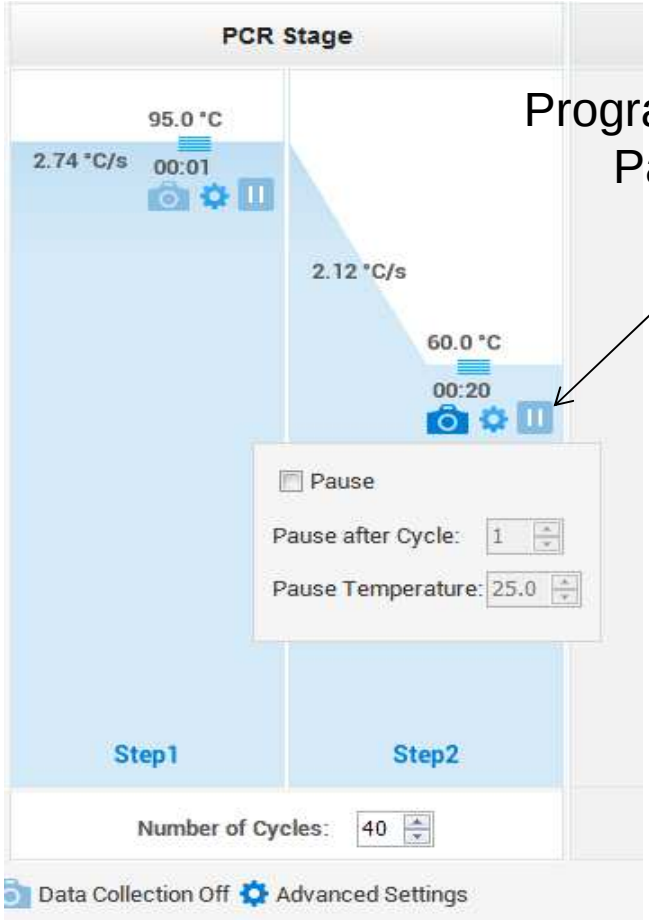
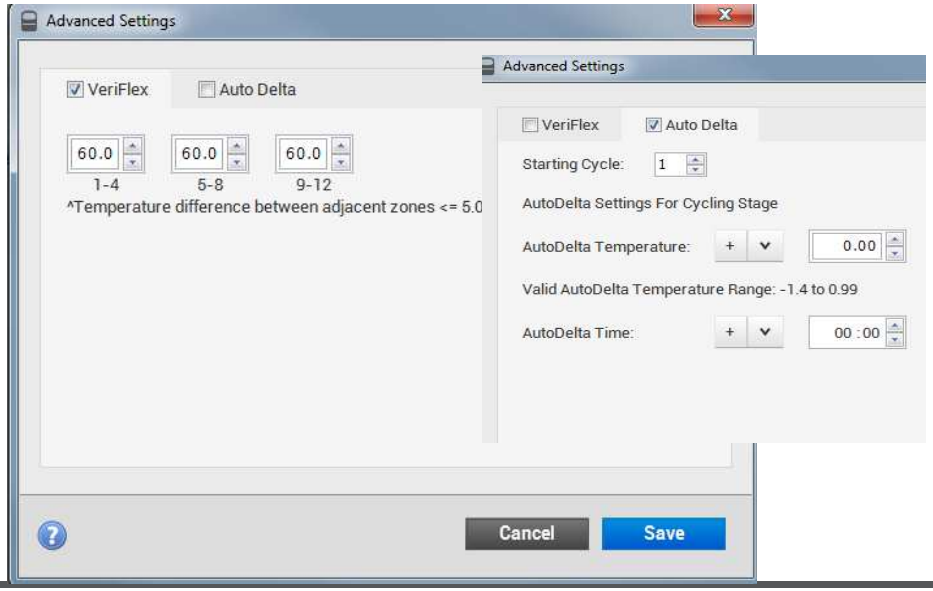
Run Method



Programmable Pause

Advanced Settings

- Veriflex™
- AutoDelta



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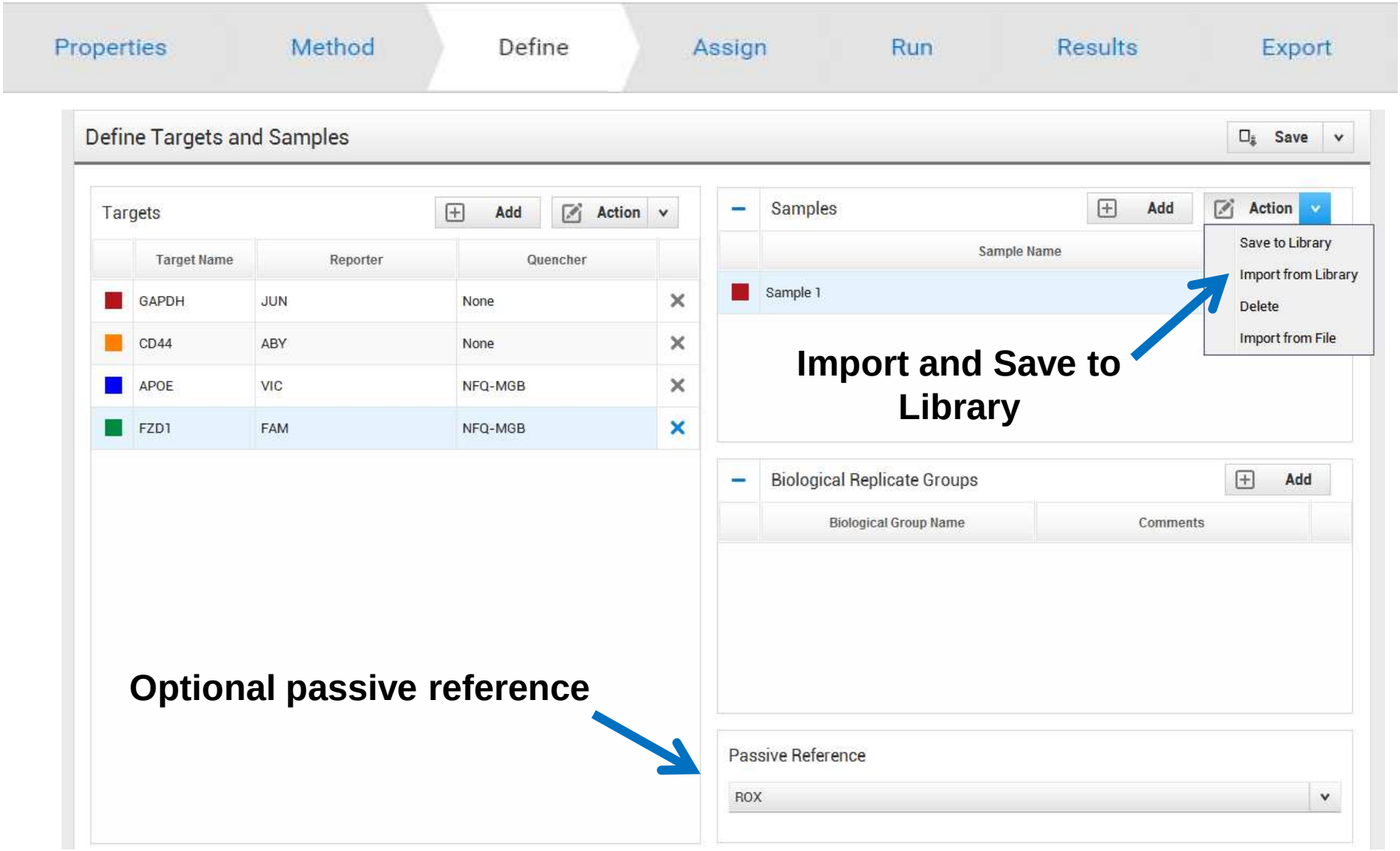
Define Samples and Targets



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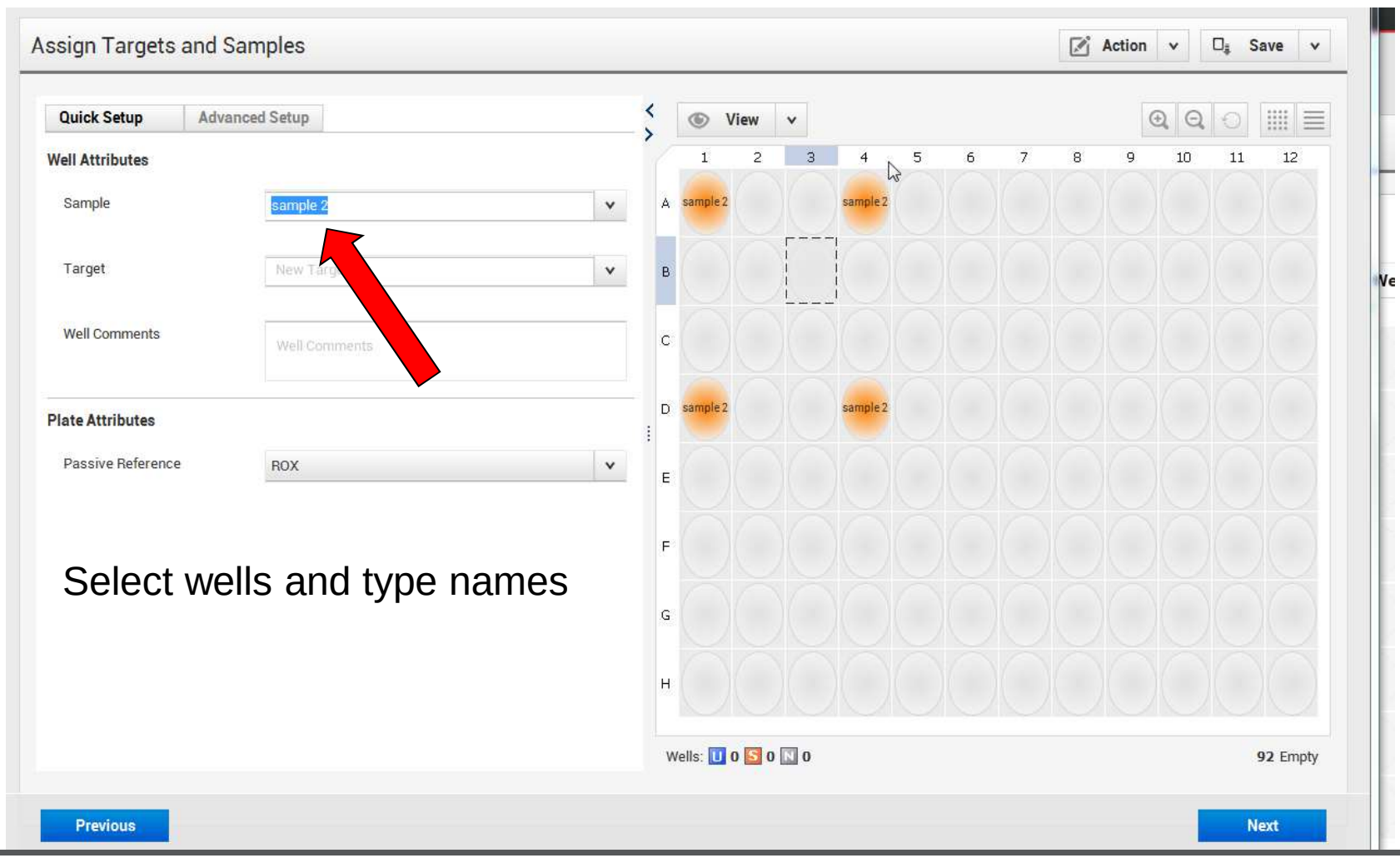
Assign plate information



Select wells and type names

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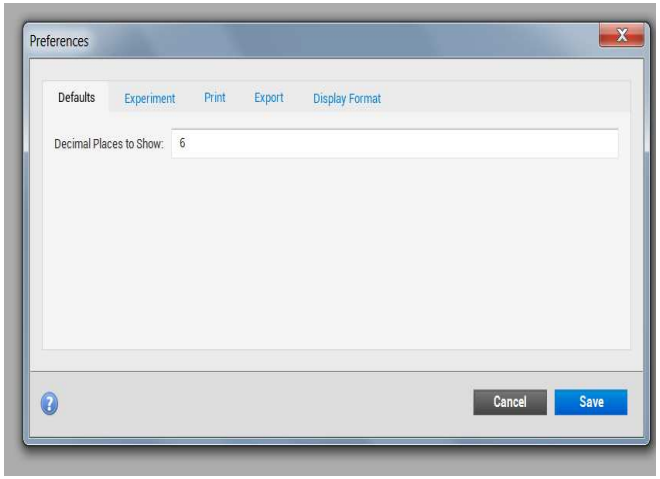
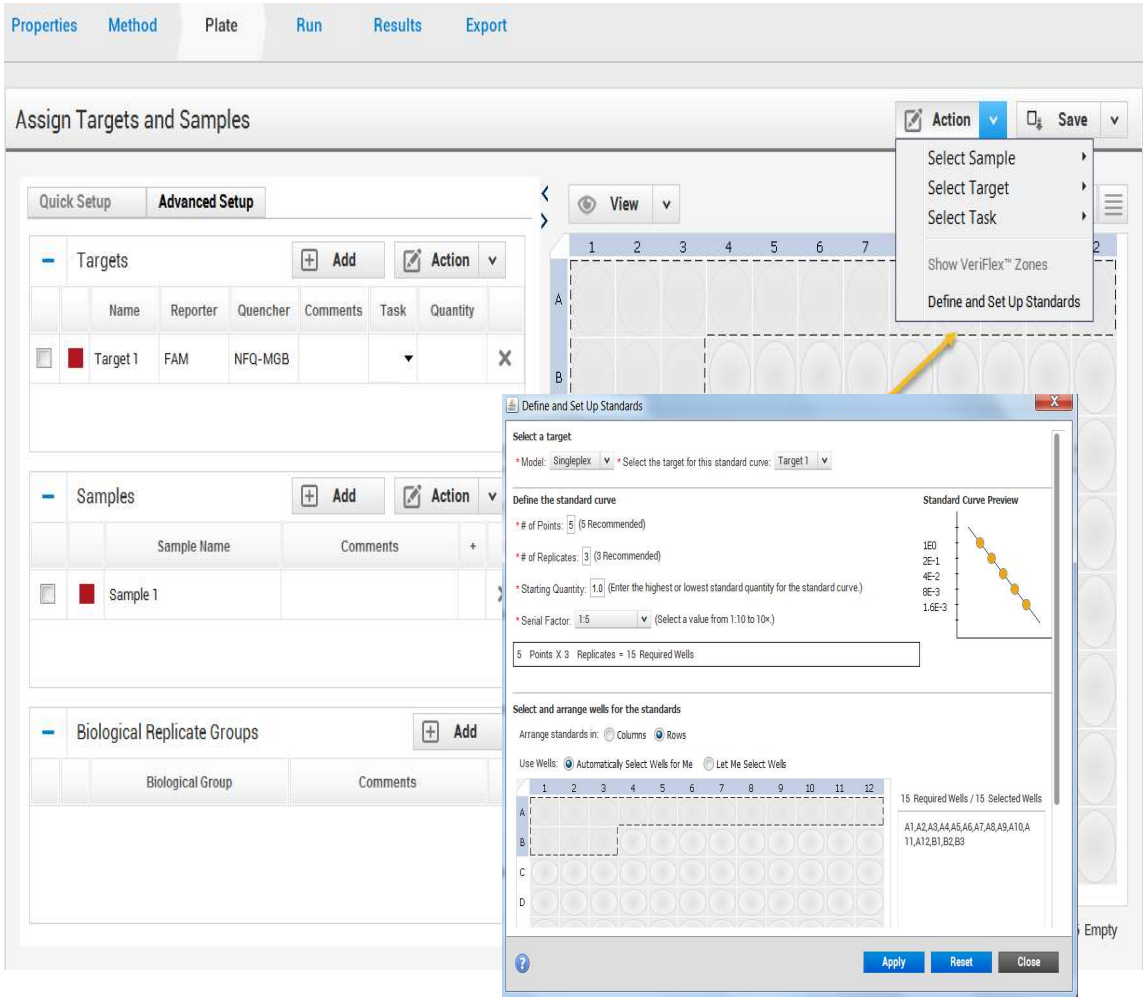
Standard Curve: Define and Setup Standards Wizard

Tools> Preferences> Defaults



Set your decimals



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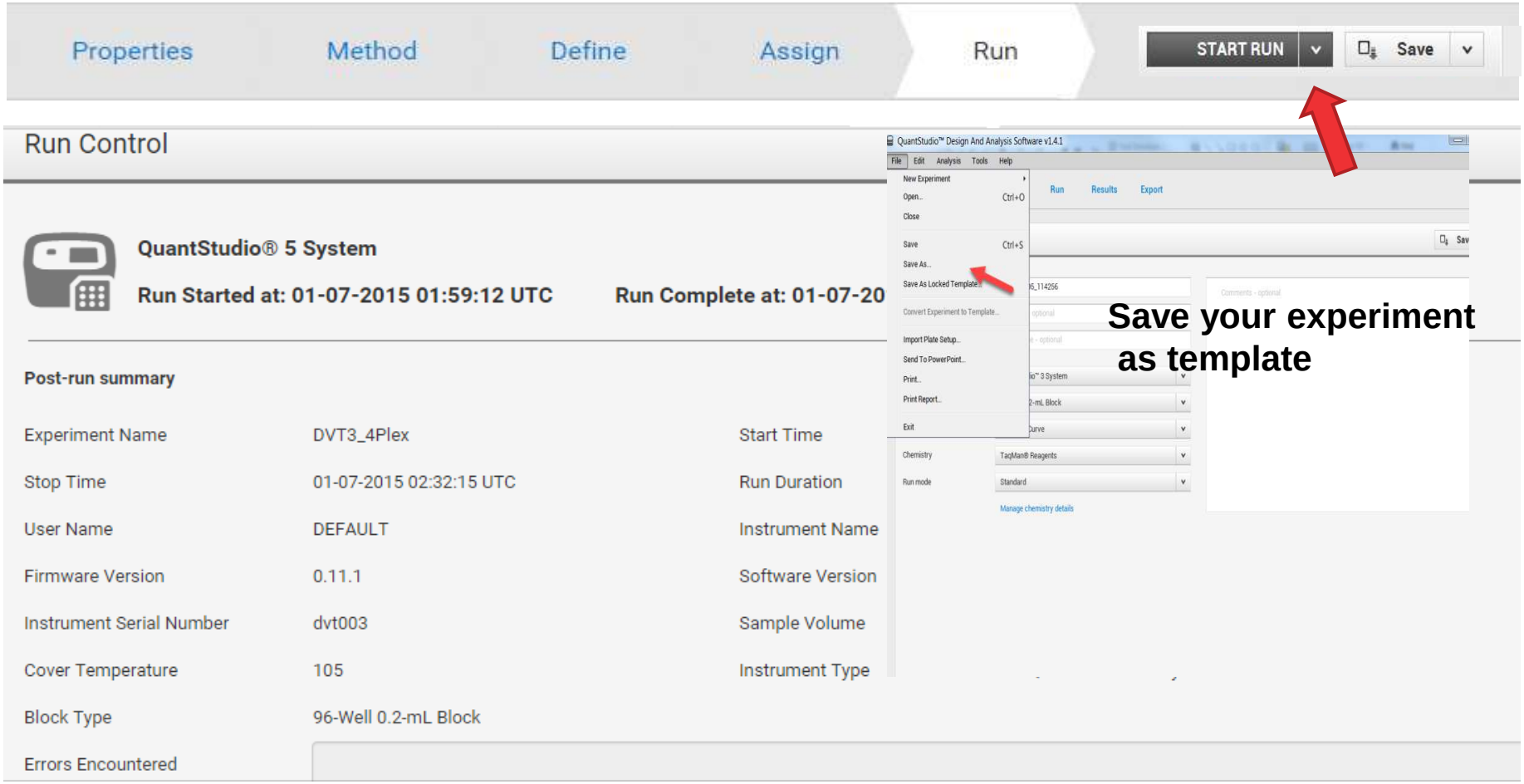
Start your run



Start Run from touchscreen or desktop

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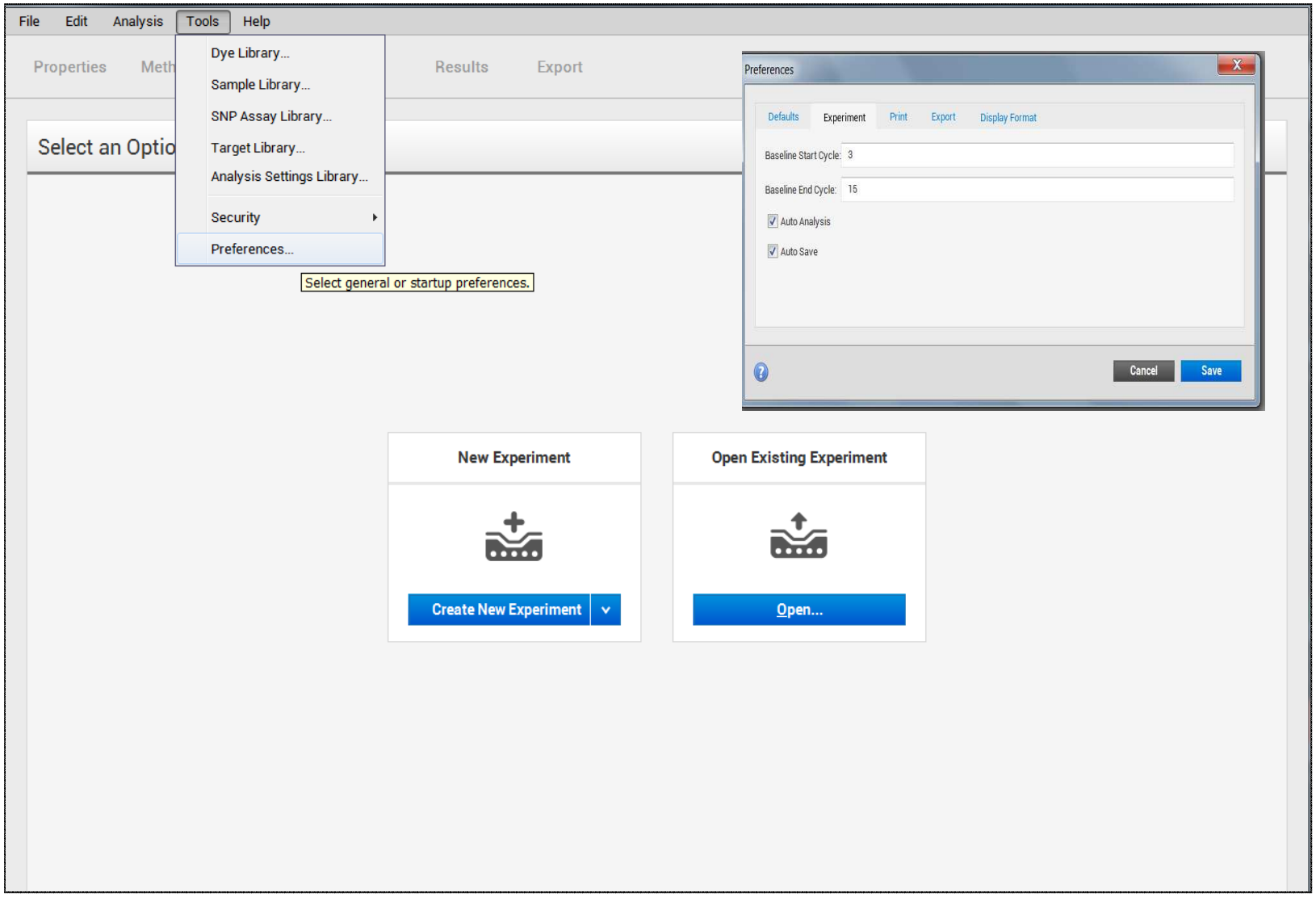
Analysis Settings: Defaults



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Changing threshold and baseline for single target

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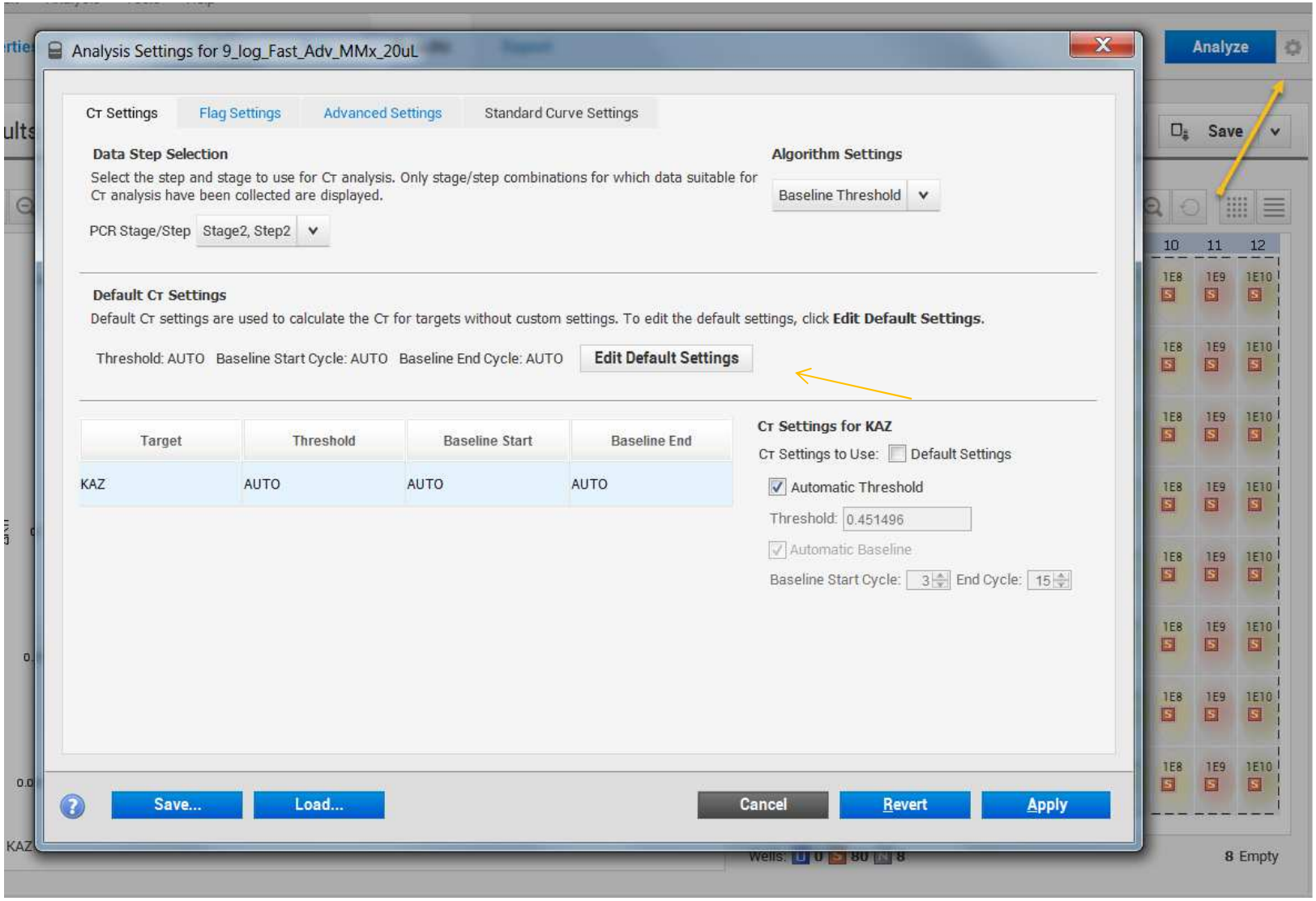
Changing thresholds and baselines for all targets



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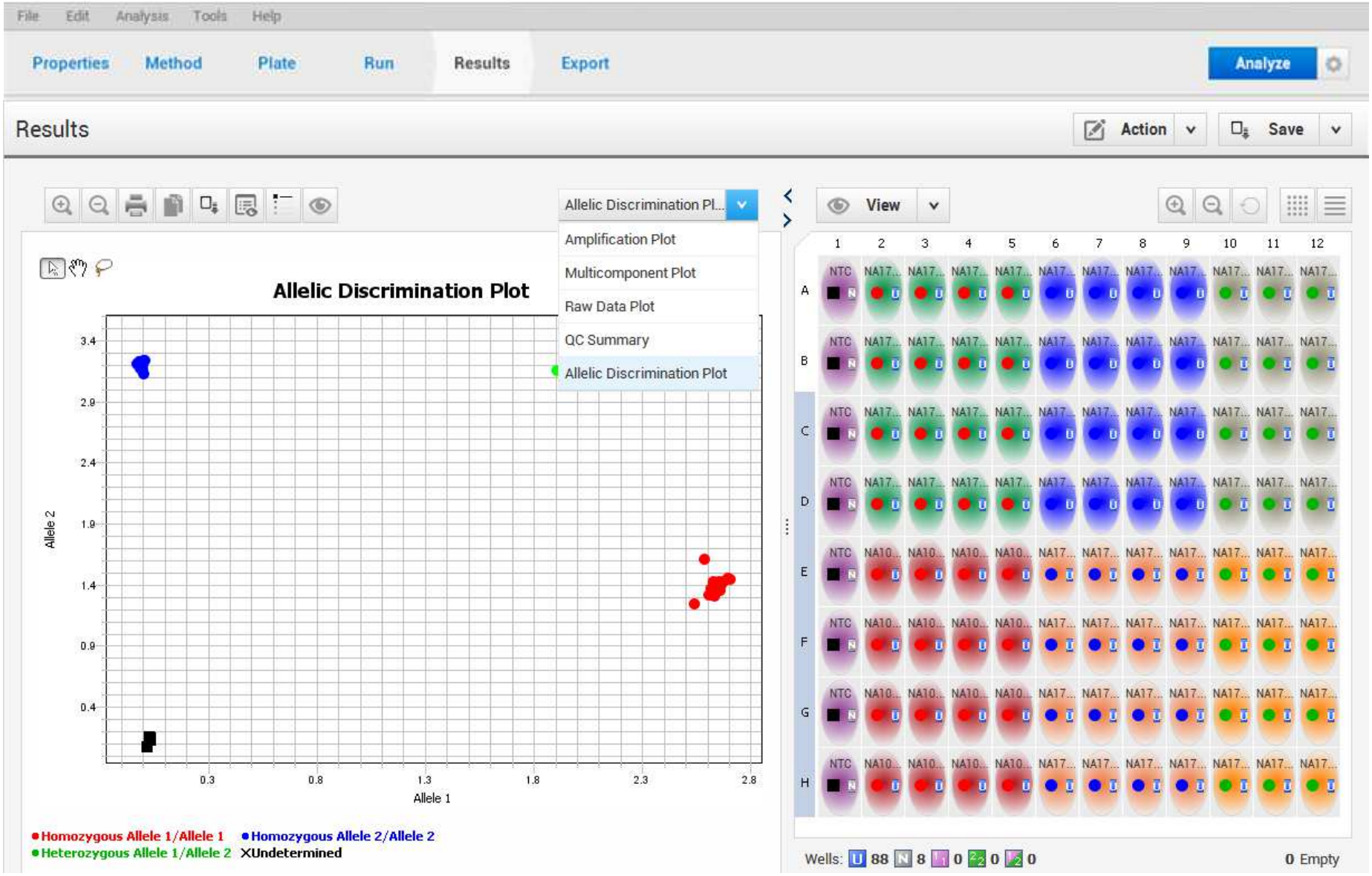
SNP Genotyping Results



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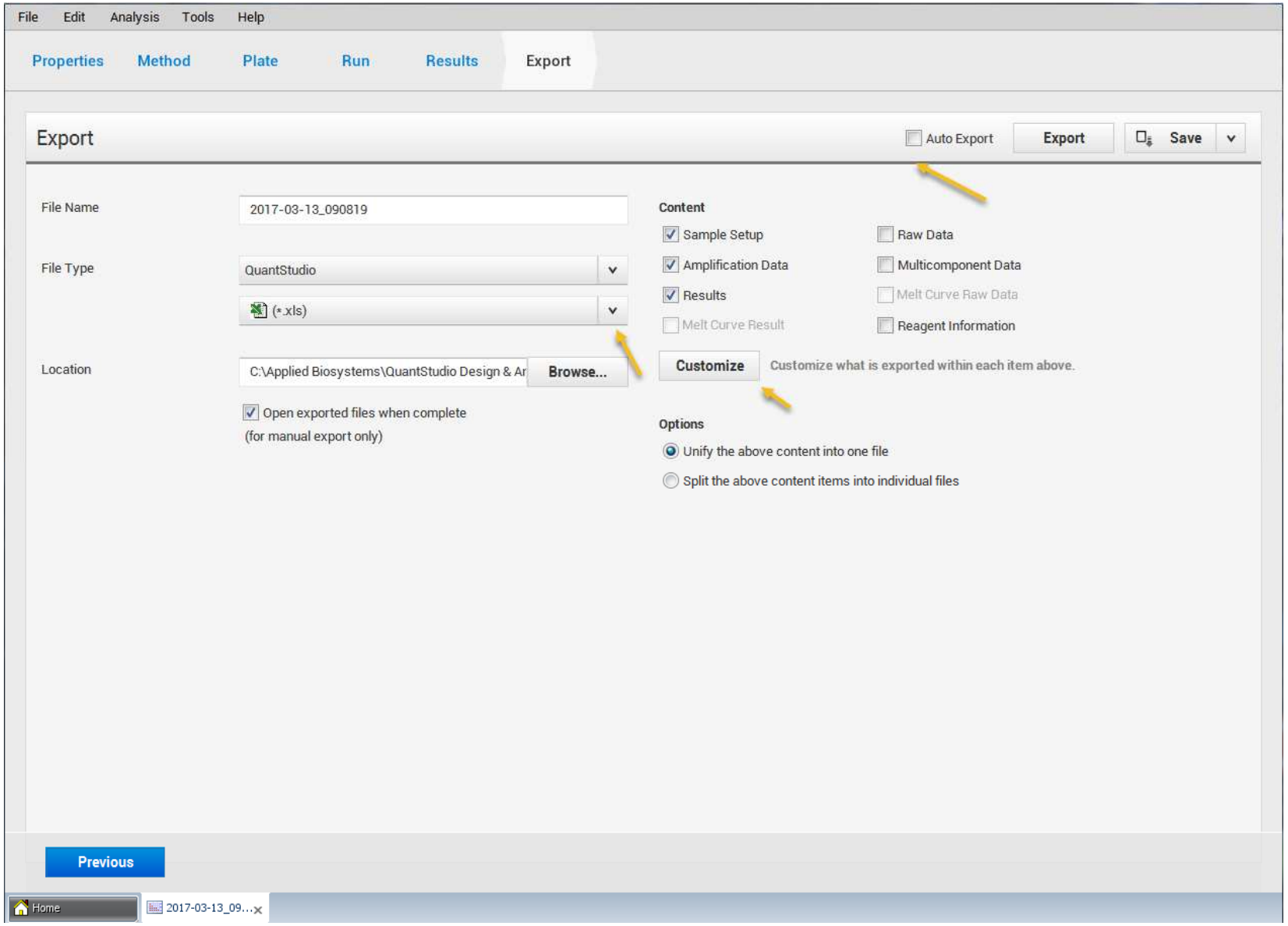
Export Settings



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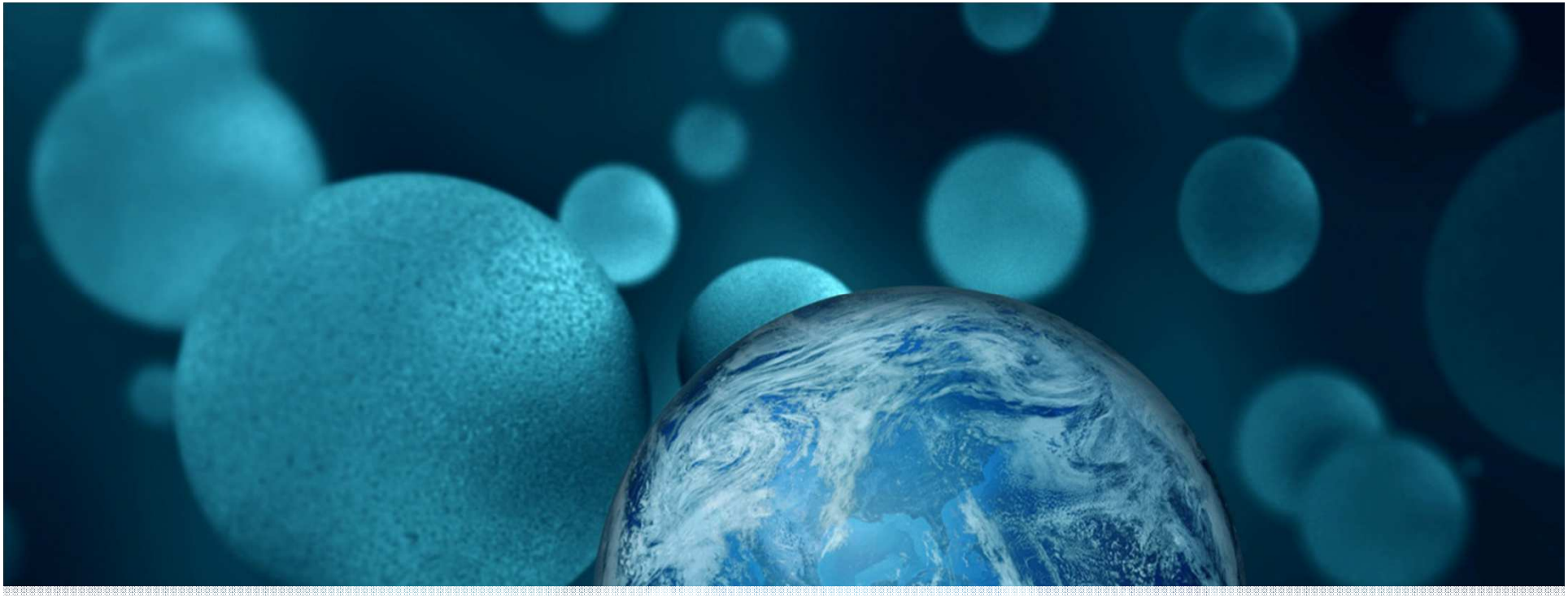

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QUESTIONS?

Appendix

Ramp rate

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Block Ramp rate – Technical specifications

Re: Applied Biosystems™ QuantStudio™ 3 and 5 Real-Time PCR System Peak Block Ramp Rates

Dear Valued Customer:

Thank you for your inquiry related to our Applied Biosystems™ QuantStudio™ 3 and 5 Real-Time PCR System Peak Block Ramp Rates. The table below outlines the peak Up and Down Ramp Rates for these systems by block type:

Block Type	Peak (Up Ramp)	Peak (Down Ramp)
96-well 0.2ml	6.5C/s	5.4C/s
96-well 01.ml	9.0C/s	7.7C/s
384-well	6.0C/s	4.8C/s

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Sample ramp rate – User can set this in the software

- User can set two parameters
  - Reaction volume
  - Sample ramp required

... and actual block ramping is calculated by a proprietary algorithm.

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Maximum programmable sample ramp rate per volume

96well 0.2ml

Vol	UR	DR
1	3.66	2.87
10	3.48	2.70
50	2.74	2.12
100	1.94	1.80

96well 0.1 ml

Vol	UR	DR
1	4.81	3.71
10	4.50	3.44
20	4.14	3.16
30	3.76	2.90

384well

Vol	UR	DR
1	2.92	2.15
5	2.57	1.99
10	2.27	1.87
20	1.99	1.76

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