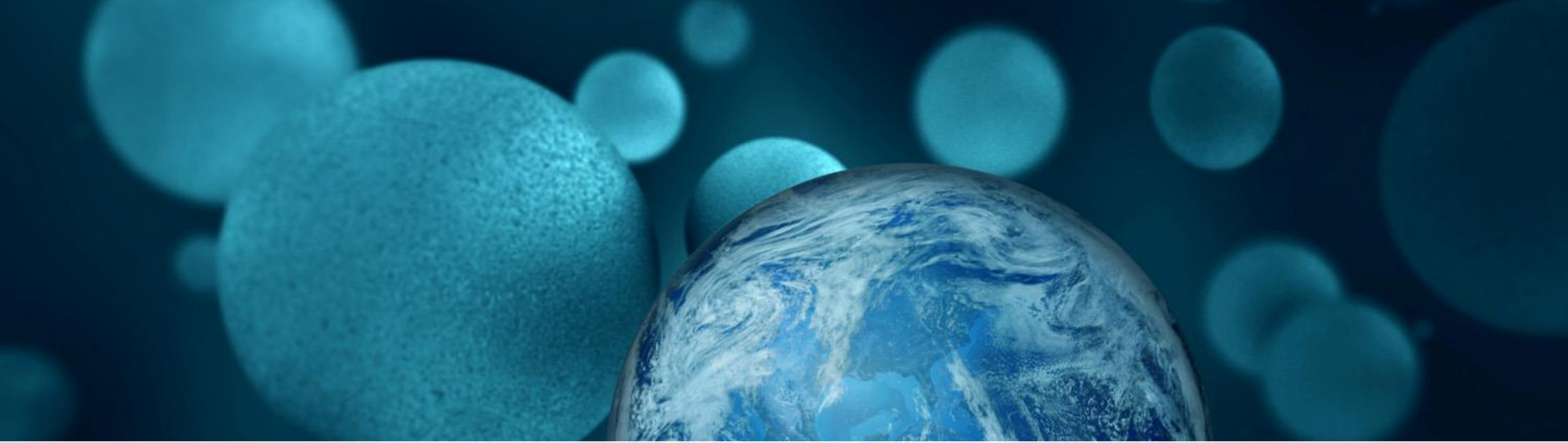

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Applied Biosystems™ PowerTrack™ SYBR™ Green Master Mix

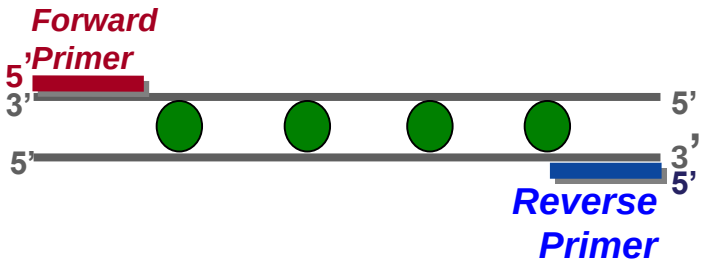
February 4, 2020

Table of Contents

qPCR Technologies: SYBR Green chemistry and TaqMan Assays

SYBR Green Chemistry

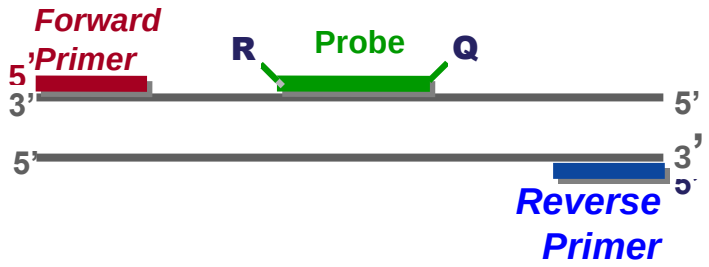
- Detection based on SYBR™ Green, a non-specific DNA binding dye
- Specificity based on **2** hybridization events: PCR primers



- Customer designed primers
- Plenty of sample
- Time to optimize primers
- Only 1 target per well
- Low priced (but takes time)

TaqMan Assays

- Detection based on 5' nuclease activity
- Specificity based on **3** hybridization events: two PCR primers & a probe and cleavage of the probe



- Accuracy & sensitivity for highest performance
- Multiplexing up to 4 targets per well
- Low sample input
- Convenient, Predesigned TaqMan assays
- Higher price (but saves time)

Recommended master mix by application

Chemistry	Application	Start with..
TaqMan®	Gene Expression	**TaqMan® Fast Advanced MasterMix** for best performance 2-step **TaqMan® Fast Virus Master Mix** for 1-step virus detection and gene expression
	Genotyping (see below)	
	Pre-amp	**TaqMan® Pre-AMP Master Mix** for customers with limited sample (e.g. FFPE)
SYBR®	Gene Expression	**PowerTrack™ SYBR™ Green Master Mix**
TaqPath (Clinical-Grade TaqMan) *General purpose reagent. For laboratory use.*	Genotyping and Copy Number Variation (CNV)	**TaqPath™ ProAmp™ Master Mix** **TaqPath™ ProAmp™ Multiplex Master Mix**
	Molecular Diagnostics Development	**TaqPath™ qPCR Master Mix** (DNA detection or 2-step Gene Expression) **TaqPath™ 1-step RT-qPCR Master Mix** **TaqPath™ 1-step Multiplex Master Mix** (RNA detection)

Requirements for a high quality SYBR Green assay experiment

1. Verify specificity (design/create primers)
2. Test dynamic range and efficiency
3. Achieve high reproducibility and fold discrimination
4. Determine sensitivity
5. Confirm performance robustness across variety of analytes
6.
   - Compatibility with workflow
   - Instruments performance
   - Reaction handling
7. Ensure no contamination

Ease of use and Flexibility at a Competitive Price

For SYBR Green gene expression, performance seekers looking for broad dynamic range and minimize pipetting errors, PowerTrack SYBR Green Master Mix offers

- TRACKING dye to reduce pipetting errors
- BROAD primer Tm and concentration allows flexibility and minimal optimization
- SUPERIOR specificity & sensitivity
- TIGHT reproducibility
- UNIVERSAL instrument compatibility

Reproducibility -- Specificity -- Sensitivity -- Compatibility -- Price

How does it work?

YELLOW sample buffer

BLUE Master Mix

Pipette BLUE Master Mix into the tubes/plates

Add YELLOW sample buffer to your cDNA/DNA sample (optional)

Indicates that sample has been added to Master Mix

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PowerTrack SYBR Green Target Applications

Gene expression

- Relative and absolute quantification of gene expression levels using SYBR

Validation

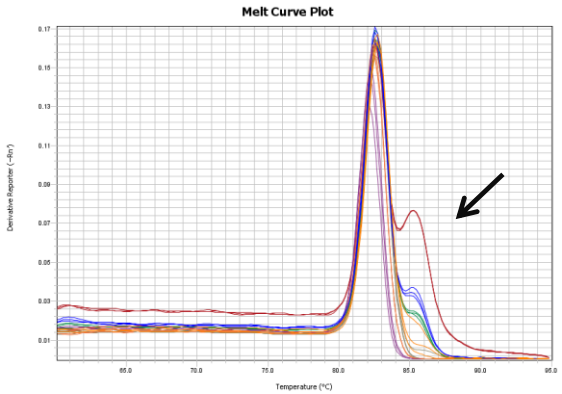
- Validation of subset of microarray gene targets
- Validation of subset of NGS gene targets

Screening

- Quick screening of cell lines for targets

Specificity is critical to SYBR Green Reactions

- Specific amplification is essential for valid data and part of the Minimum Information for Publication of Quantitative Real-Time PCR Experiments Guidelines (MIQE)
- Non-specificity can be observed as multiple MELT peaks following a dissociation curve analysis
- If non-specific amplification occurs, researchers can try to optimize reaction component concentrations or re-design primers.
- Of researchers using SYBR chemistry
  - 78% verify specificity of qPCR primers
  - 42% perform primer optimization at least 25% of the time
  - 15% redesign primers at least 25% of the time



Non-specificity compromises data quality and costs researchers extra time and money to address

Researcher challenges and benefits

PowerTrack SYBR Green Master Mix

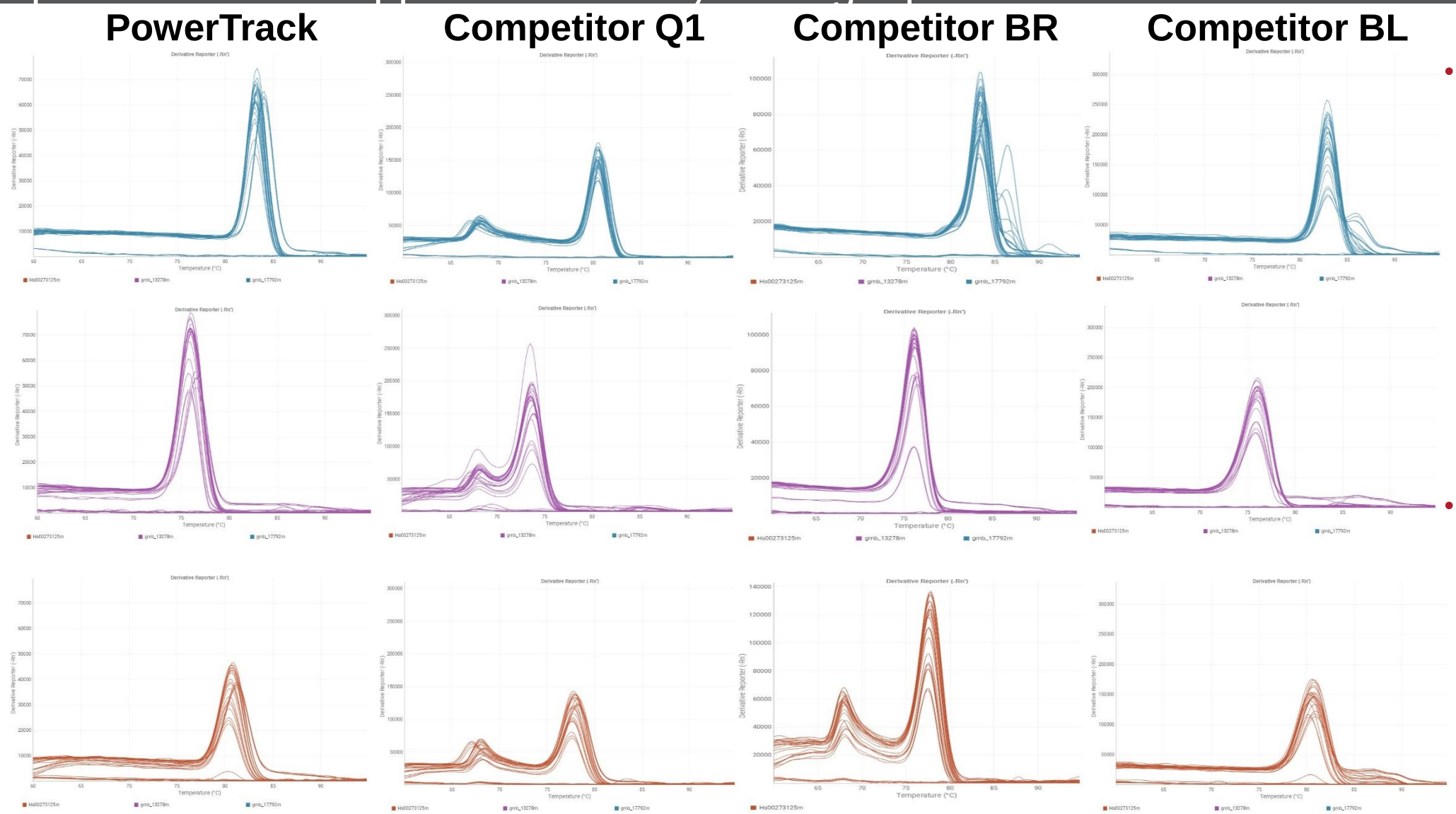
For gene expression analysis customers who need broad dynamic range and to **minimize pipetting mistakes** without compromising sensitivity and specificity, PowerTrack SYBR Green Master Mix contains a tracking dye allowing users to know where they have pipetted:

Challenge	Benefit	How
25% of poor real-time PCR data comes from pipetting errors	Helps reduce pipetting errors	Two-color tracking dye indicates where pipetting has occurred
Need for specific target amplification	Accurate and specific	Specific and tight reproducibility in Ct's over a broad dynamic range
Different targets have varying annealing temperatures	Flexible	Broad primer Tm and primer concentration compatibility allows qPCR reaction set up flexibility with minimal optimization
The need for high-yield of reverse transcriptase	Compatible with preferred RT kit	Designed to be used with SuperScript™ IV VILO™ Master Mix

Why ROX™ dye matters

- ROX dye is an inert reference dye used to normalize fluorescence across all samples. It removes fluorescent variations, such as those caused by bubbles from the data
  - For example if you have a bubble it can cause a difference in fluorescence, where ROX dye can normalize for this
- Unlike other suppliers, Applied Biosystems™ ROX dye is universal, therefore eliminating the need for ‘low’ or ‘high’ ROX dye versions to accommodate different instruments
- It will help improve your precision which can improve your data or reduce the number of replicates required

Melt curve data shows PowerTrack SYBR Green Master Mix has higher specificity compared to other suppliers shown by a single peak



- **PowerTrack amplifies specific product as shown by single peak vs. other suppliers mixes showing primer dimers and secondary peak.**
- **2nd peak is indicative of non-specific amplification in other suppliers mixes.**

- Thermal cycling performed using 60ºC primer annealing Tm with UHR cDNA in fast cycling mode.
- 10µl reactions run in quadruplicate on QuantStudio5 real time PCR instrument, 384 well block
- PGK1 – High expressor; ARL1 – Medium expressor; SNF 8 – Low expressor

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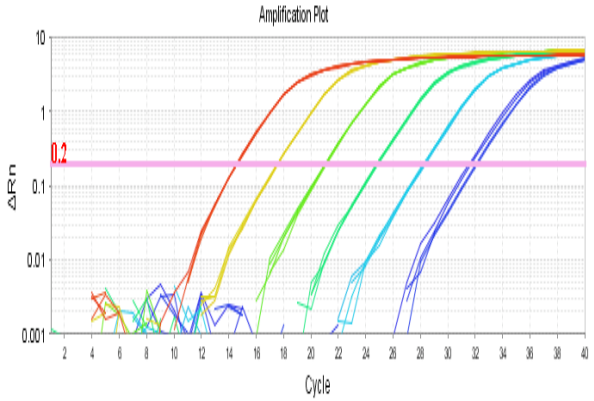
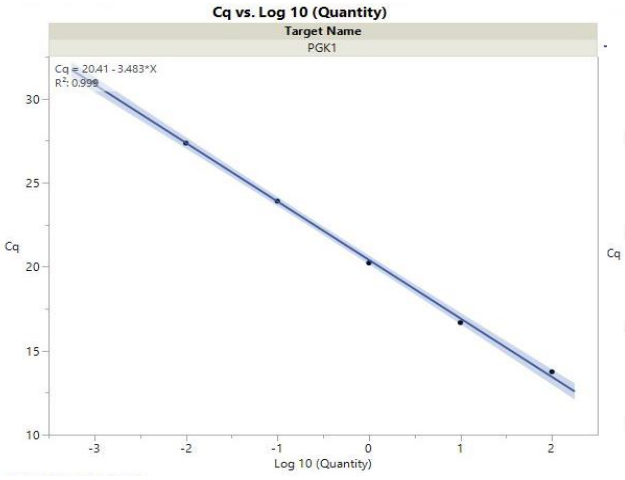
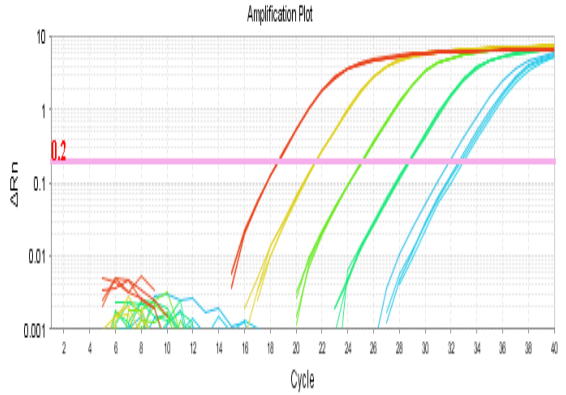
PowerTrack SYBR Green Master Mix powers through traditionally difficult targets

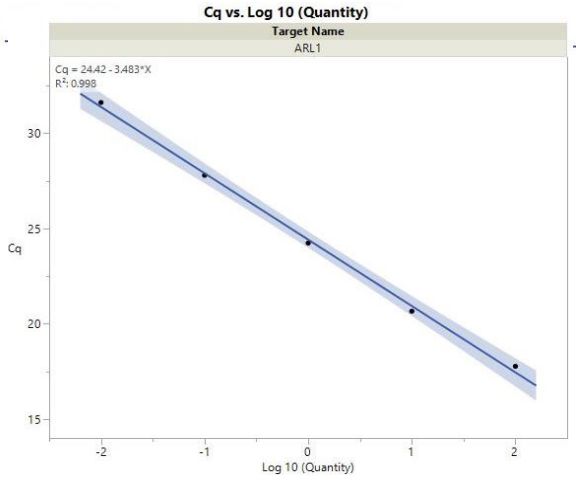
PGK1



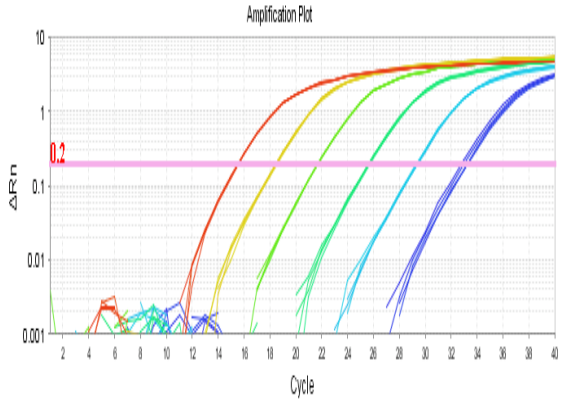


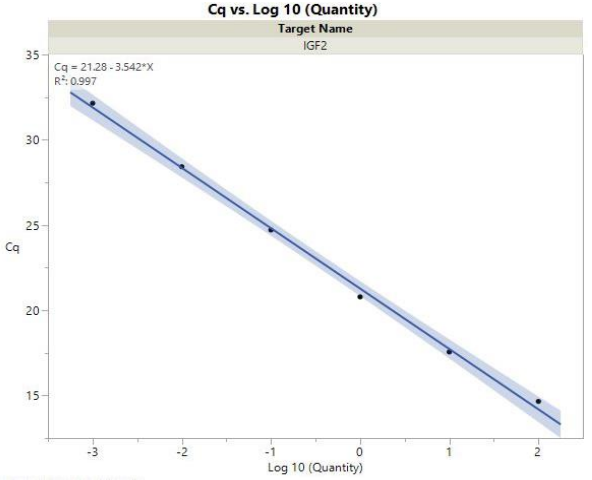
ARL1



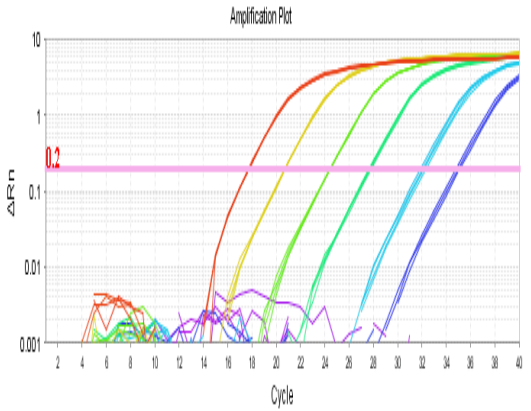


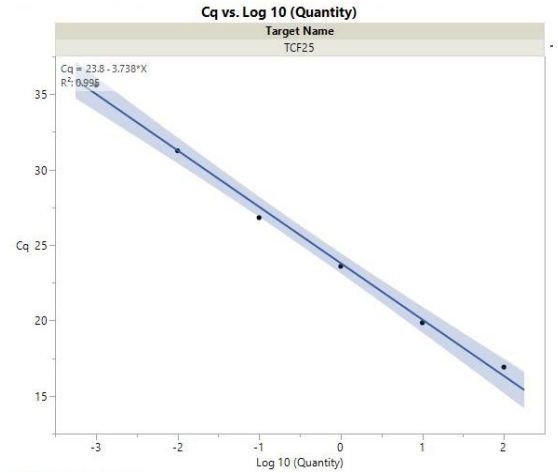
IGF2

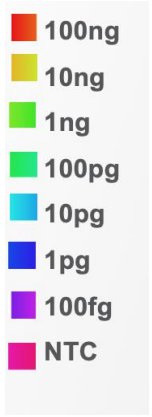




TCF25



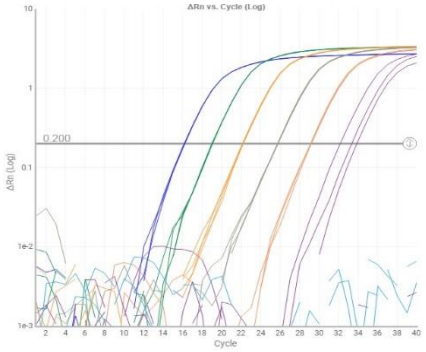


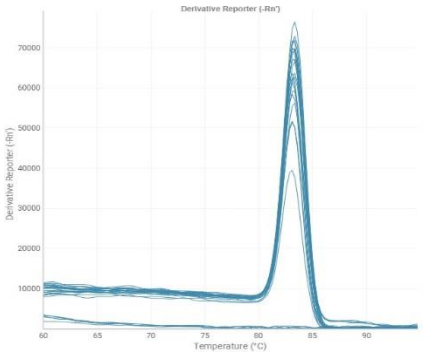


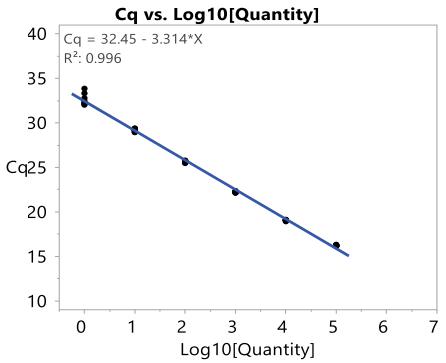
- Ten-fold dilutions of UHR cDNA ranging from 100ng to 100fg were run on Applied Biosystems QuantStudio5 Real time PCR instrument with 384 well block.
- 10 µl reactions run in quadruplicate using 60ºC annealing primer Tm. PGK1 – High Expressor, ARL1, IGF2 and TCF25 - Medium Expressor

PGK1; Invitrogen SuperScript IV VILO Master Mix reverse transcription

PowerTrack SYBR Green Master Mix

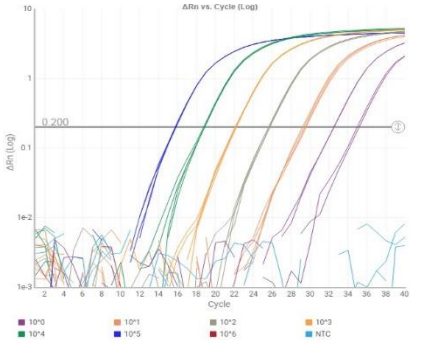


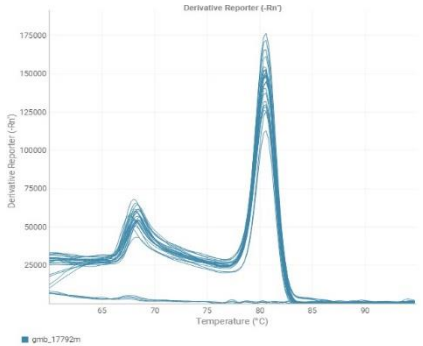


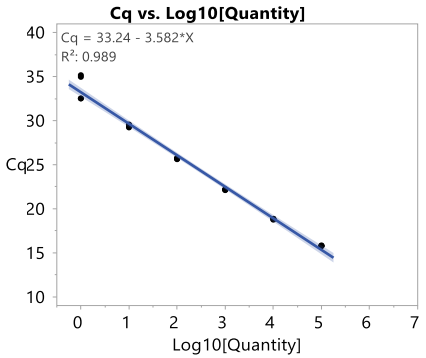


cDNA input		Ct Mean	Ct Std. Dev.	dCt
1.	100 ng/uL	0.00	0.00	
2.	10 ng/uL	16.22	0.07	
3.	1 ng/uL	19.05	0.06	2.83
4.	100 pg/uL	22.24	0.08	3.19
5.	10 pg/uL	25.65	0.11	3.41
6.	1 pg/uL	29.16	0.16	3.51
7.	100 fg/uL	32.69	0.68	3.54
8.	NTC	40.00	0.00	
Efficiency (%): 100.34				
Slope: 3.314				
R2: 1.00				

Qiagen QuantiNova SYBR® Green

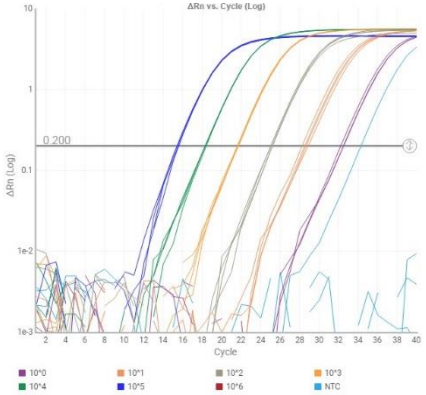


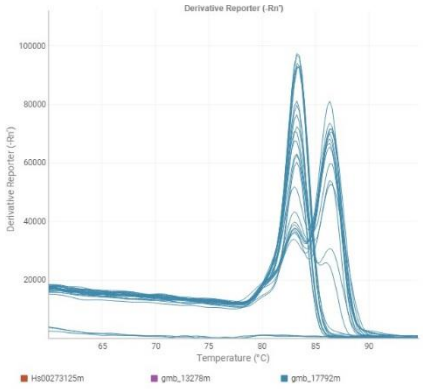


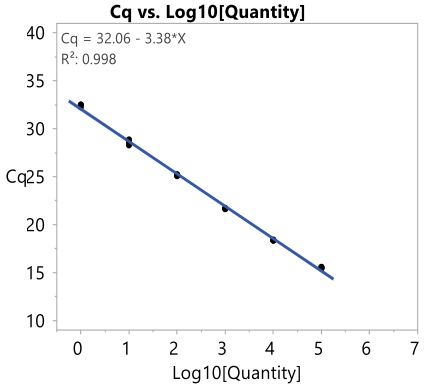


cDNA input		Ct Mean	Ct Std. Dev.	dCt
1.	100 ng/uL	0.00	0.00	
2.	10 ng/uL	15.79	0.04	
3.	1 ng/uL	18.83	0.05	3.04
4.	100 pg/uL	22.17	0.05	3.34
5.	10 pg/uL	25.70	0.07	3.53
6.	1 pg/uL	29.41	0.16	3.71
7.	100 fg/uL	33.80	1.47	4.39
8.	NTC	40.00	0.00	
Efficiency (%): 90.20				
Slope: 3.582				
R2: 0.99				

Bio-Rad SsoAdvanced™ Universal SYBR® Green Supermix







cDNA input		Ct Mean	Ct Std. Dev.	dCt
1.	100 ng/uL	0.00	0.00	
2.	10 ng/uL	15.50	0.08	
3.	1 ng/uL	18.38	0.04	2.87
4.	100 pg/uL	21.68	0.05	3.30
5.	10 pg/uL	25.15	0.09	3.48
6.	1 pg/uL	28.60	0.25	3.45
7.	100 fg/uL	32.43	0.15	3.83
8.	NTC	34.31	0.00	
Efficiency (%): 97.64				
Slope: 3.380				
R2: 1.00				

Other suppliers show sub-optimal results with SuperScript IV VILO even thought they claim to be ‘compatible’

2 Copy Study

- 3 Master Mixes
  - Applied Biosystems PowerTrack SYBR Green Master Mix
  - BioRad™ SSoAdvanced Supermix
  - Qiagen™ QuantiNova SYBR Green PCR Kit
- RNAseP Assay
- CEPH gDNA diluted down to 2 copies
- Run Protocol: Fast on QuantStudio™ 5 384-well block annealing Tm 60oC

Product configurations

PowerTrack SYBR Green Master Mix	Global List Price	List Price Per/ml
A46012 (1ml)	$91	$91
A46109 (5ml)	$387	$77
A46110 (2 x 5ml)	$709	$73
A46111 (5 x 5ml)	$1687	$70
A46112 (10 x 5ml)	$3358	$67
A46113 (50ml)	$3260	$65

Compatible with:

- Invitrogen SuperScript IV VILO Master Mix reverse transcription

PowerTrack SYBR Green Master Mix: Key Technical Specifications

Applications	Gene expression analysis (Microbe detection, ChIP DNA analysis)
Recommended master mix	Applied Biosystems™ PowerTrack SYBR™ Green Master Mix
Sensitivity	Best
Specificity	Better
Pipetting tracking dye	Yes
Hot start	Antibody-mediated
Cycling mode	Standard (~60 min) or Fast (~30 min)
Carryover contamination control	Includes heat-labile UNG and a blend of dTTP/dUTP
Instrument compatibility	Compatible with all Applied Biosystems™ real-time PCR instruments, Bio-Rad CFX™ (96 and 384), and Roche LightCycler™ 480,
Recommended primer concentration	300-800 nM nM
Recommended primer Tm	55 – 65C

To learn more and request a free sample*

Visit www.thermofisher.com/sybr

- Technical specifications
- See how PowerTrack SYBR Green Master Mix stacks up against other suppliers
- Request a free sample

*No purchase necessary. Available to life sciences professionals 21 years and older in most countries. Offer will apply to sample requests until promotional samples are depleted. Sample size: 1 mL. Limit one sample per laboratory. Offer void where prohibited by federal, state, provincial, or local laws or regulation or agency/institutional policy. Other restrictions may apply. View the privacy policy.

Appendix

- Experimental conditions
- Additional performance data

Experimental Conditions

SYBR Green Master Mixes
PowerTrack SYBR Green Master Mix
PowerUp™ SYBR™ Master Mix
Luminaris™ Color HiGreen qPCR Master Mix, low ROX
Bio-Rad SsoAdvanced™ Universal SYBR® Green Supermix
Takara TB Green® Premix Ex Taq™ II (Tli RNase H Plus)
ProMega GoTaq® qPCR Master Mix
KAPA SYBR FAST qPCR Kits; Low Rox
Qiagen QuantiNova SYBR® Green PCR Kit
Qiagen QuantiFast SYBR® Green PCR Kit
BioLine (Meridian Bioscience) SensiFAST™ SYBR® Lo-ROX Kit
Quanta Biosciences PerfeCTa® SYBR® Green FastMix® Low ROX Reaction Mixes
Toyobo SYBR® Green Realtime PCR Master Mix

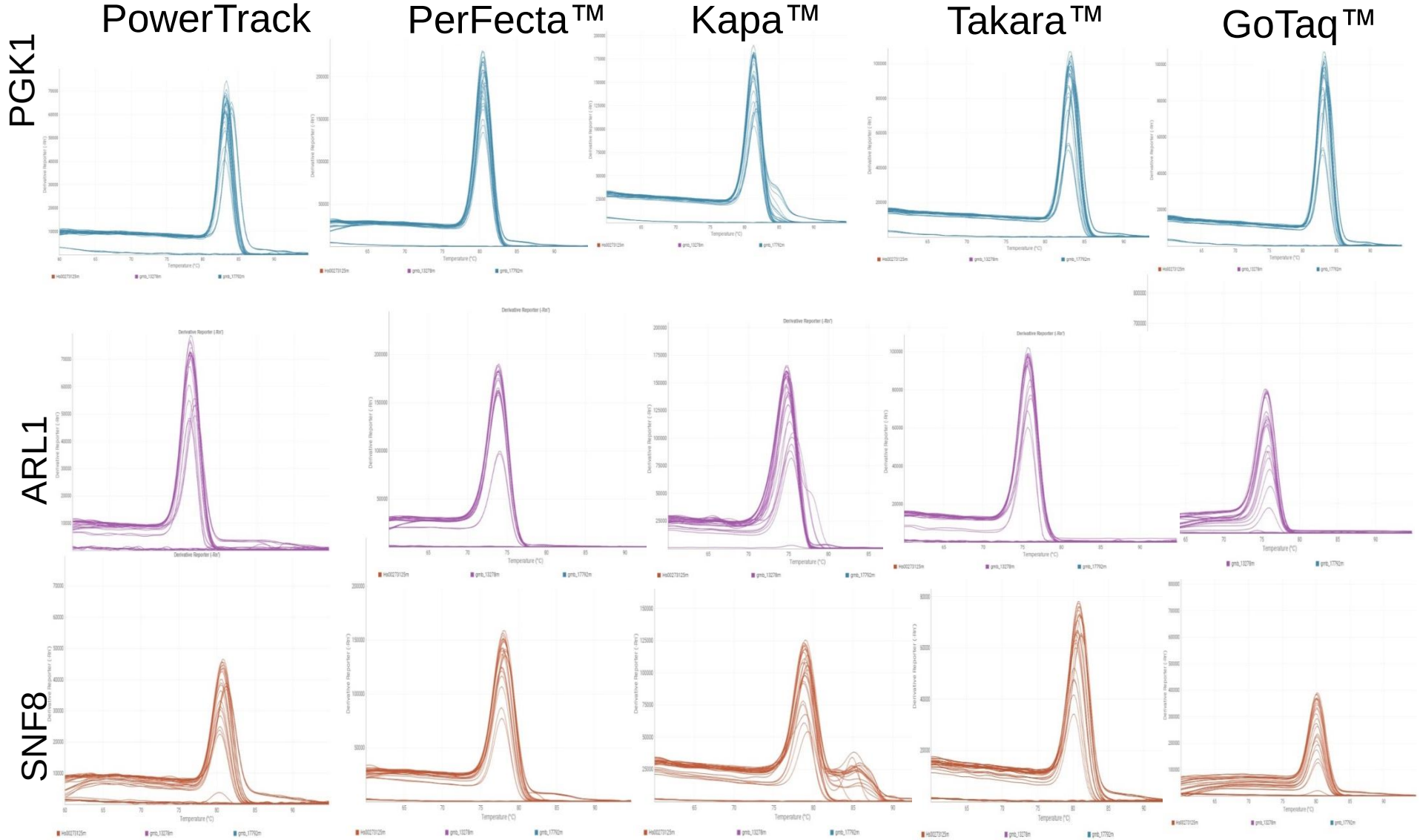
Samples
UHR cDNA concentration: 0.1 to 100,000 pg/reaction by factors of 10
10 μl reactions run in quadruplicate

4 cDNA Synthesis Kits	
SuperScript IV Vilo RT Kit	BioRad iScript
High Capacity cDNA Synthesis Kit	Qiagen QuantiTect RT Kit

4 Assays (400 nM final)
PGK1, ARL1, GAPDH, SNF8, DF, C1orf19

Instrument/Run Method
QuantStudio 5 Real-time PCR System with 384 well block
Thermal cycling performed as recommended by each manufacturer All assays had designed annealing temperature of 60 degrees

Melt Curve Data shows PowerTrack SYBR Green Master Mix is specific



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PowerTrack SYBR Green Master Mix works efficiently with SuperScript IV VILO Master Mix reverse transcription kit and performs better compared to competitor mixes.

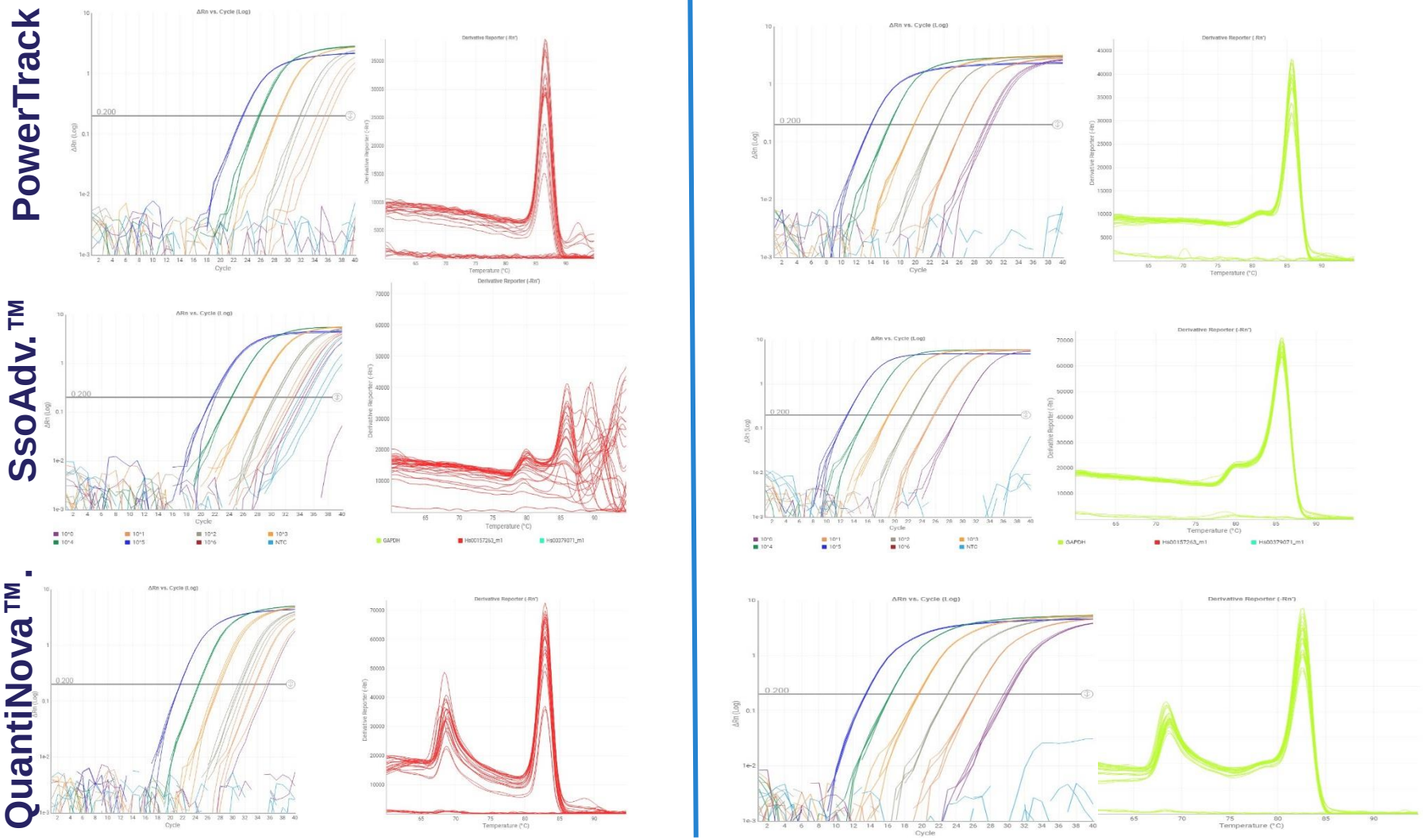
DF Assay

GAPDH Assay

PowerTrack

SsoAdv.™

QuantiNova™.



RT kit compatibility and Better Performance = Better data

Thank you

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