Roche

Diagnostics

www.roche-applied-science.com

High Resolution Melting: History, Technology, and Utility

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Field Applications Consultant

High Resolution Melting
What is it?

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- **Melting Curve Analysis is well established as a method to characterize amplicons with SYBR Green I, HybProbe (FRET) or SimpleProbe probes.**

- **High resolution melting analysis is an extension of melting curve analysis…**
  - **enables not only detection of SNPS but also their discovery**
  - **requires special fluorophores, a high-performance instrument (block homogeneity, suitable filters, optical sensitivity and resolution) and special analysis algorithms.**

History - Background

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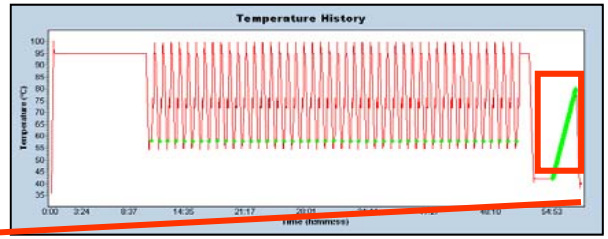
- Evolved from need to monitor sequence variation of entire amplicon
  - Single-strand conformation polymorphism
  - Heteroduplex migration
  - Denaturating gel electrophoresis
  - Temperature gradient gel electrophoresis
  - Enzymatic or chemical cleavage
  - Cycle sequencing and gel electrophoresis
  - Denaturing HPLC
  - Mass Spectrophotometry
  - Array analysis

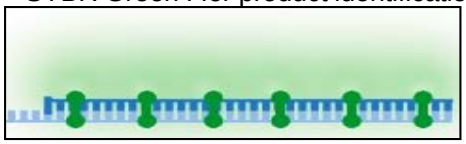
History – Melting Curve Analysis

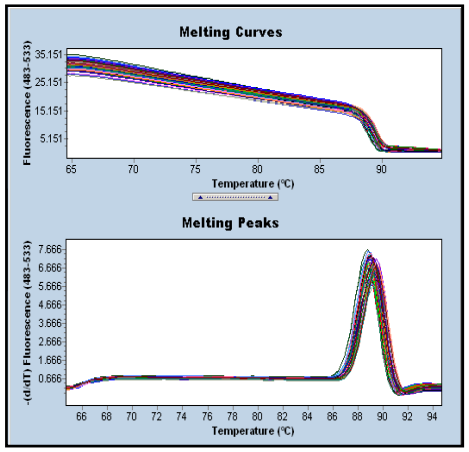
- **Melting Curve Analysis**
  - Introduced in 1997 in conjunction with real time PCR
  - With SYBR Green, provides a rough characterization of what product is amplified, and purity of product, indicating specificity of PCR reaction
    - Heterozygote detection possible only with addition of subsequent steps such as amplicon purification and addition of high concentrations or urea
  - With hybridization probes or ‘Simple probes’, can interrogate and detect specific regions of amplicon for sequence alterations
    - Difficult and expensive to screen for unknown mutations due to multiple probes required to span region
  - With a high resolution dye, can detect amplicon and oligonucleotide denaturation, allowing for product identification and SNP detection or discovery in same run.
    - Quantification not possible with HRM Dyes

Melting Curve Analysis
Established Applications



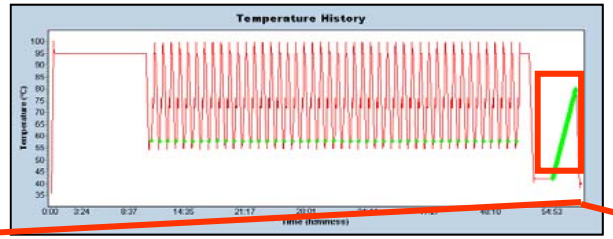
SYBR Green I for product identification



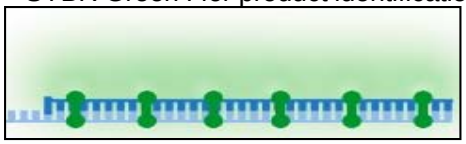


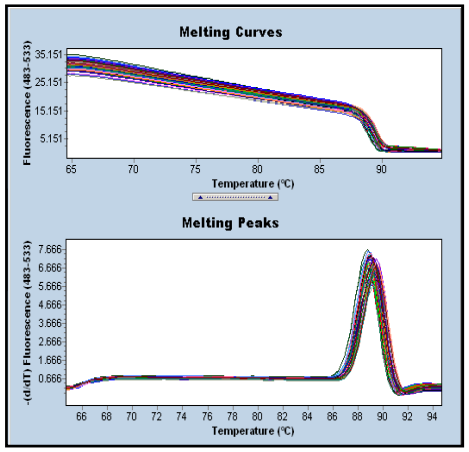
Melting Curve Analysis
Established Applications

Diagnostics

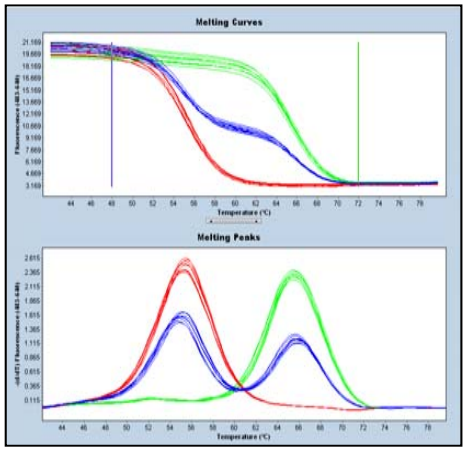


SYBR Green I for product identification



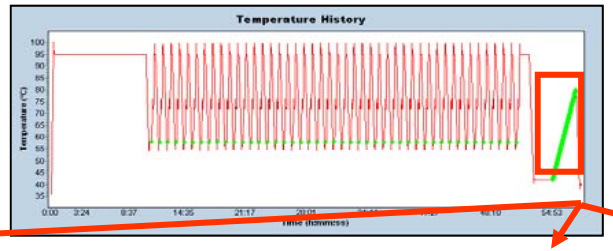


Fluorescence labeled Probes for Genotyping

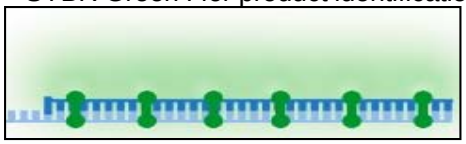


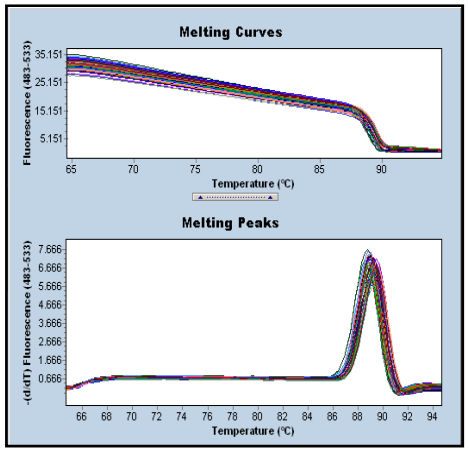
Melting Curve Analysis
Established and New Applications

Diagnostics

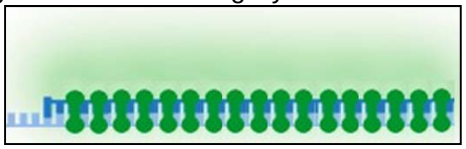


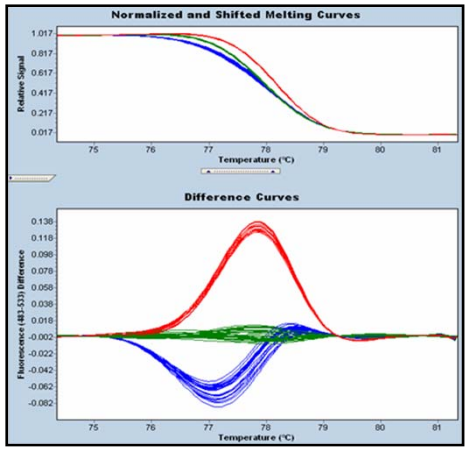
SYBR Green I for product identification



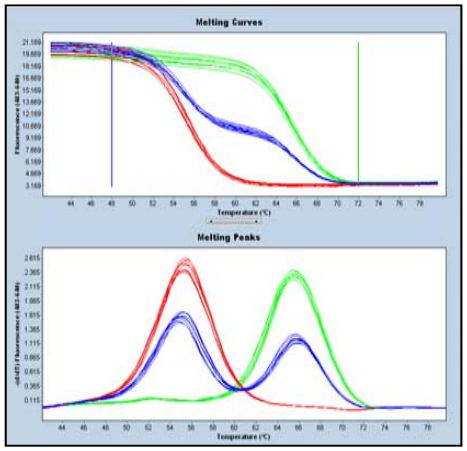


High Resolution Melting Dye for Gene Scanning





Fluorescence labeled Probes for Genotyping



History – High-Resolution Melting

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- Traditional genotyping methods versus high resolution melting
  - Ideal for screening 1000s of samples for sequence variations

Previous gene scanning techniques

dHPLC

Sequencing

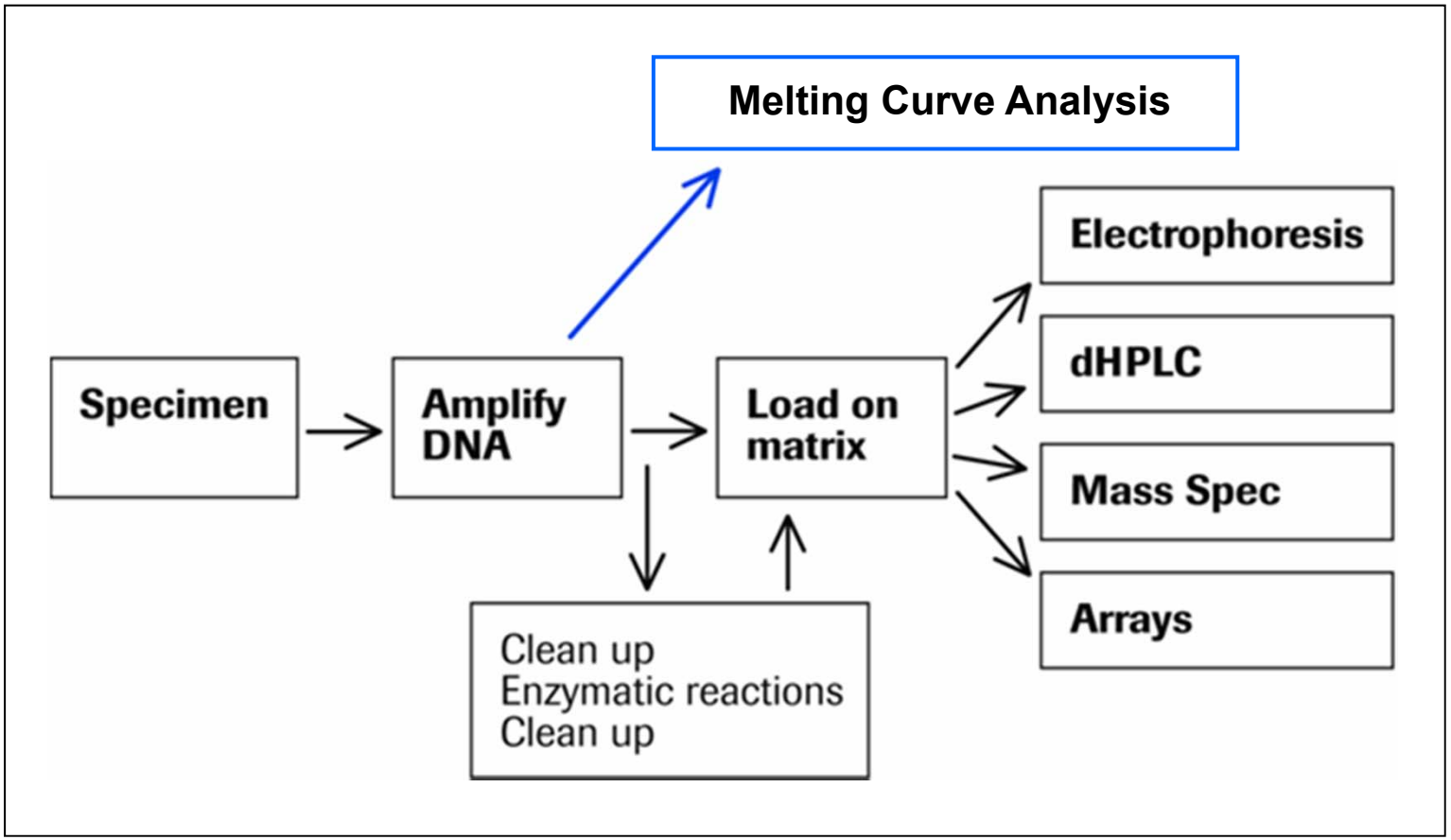
Real-Time PCR

•High throughput
•Low throughput

•Cost Effective
•Expensive

•Fast
•Time consuming

SNP Discovery and Genotyping Methods



History – High-Resolution Melting

Diagnostics

Why High Resolution Melting?

Robust, non-destructive closed-tube method with many applications; highly informative and flexible. More convenient and cost-effective than current technologies, such as sequencing or dHPLC.

High Resolution Melting - Technology

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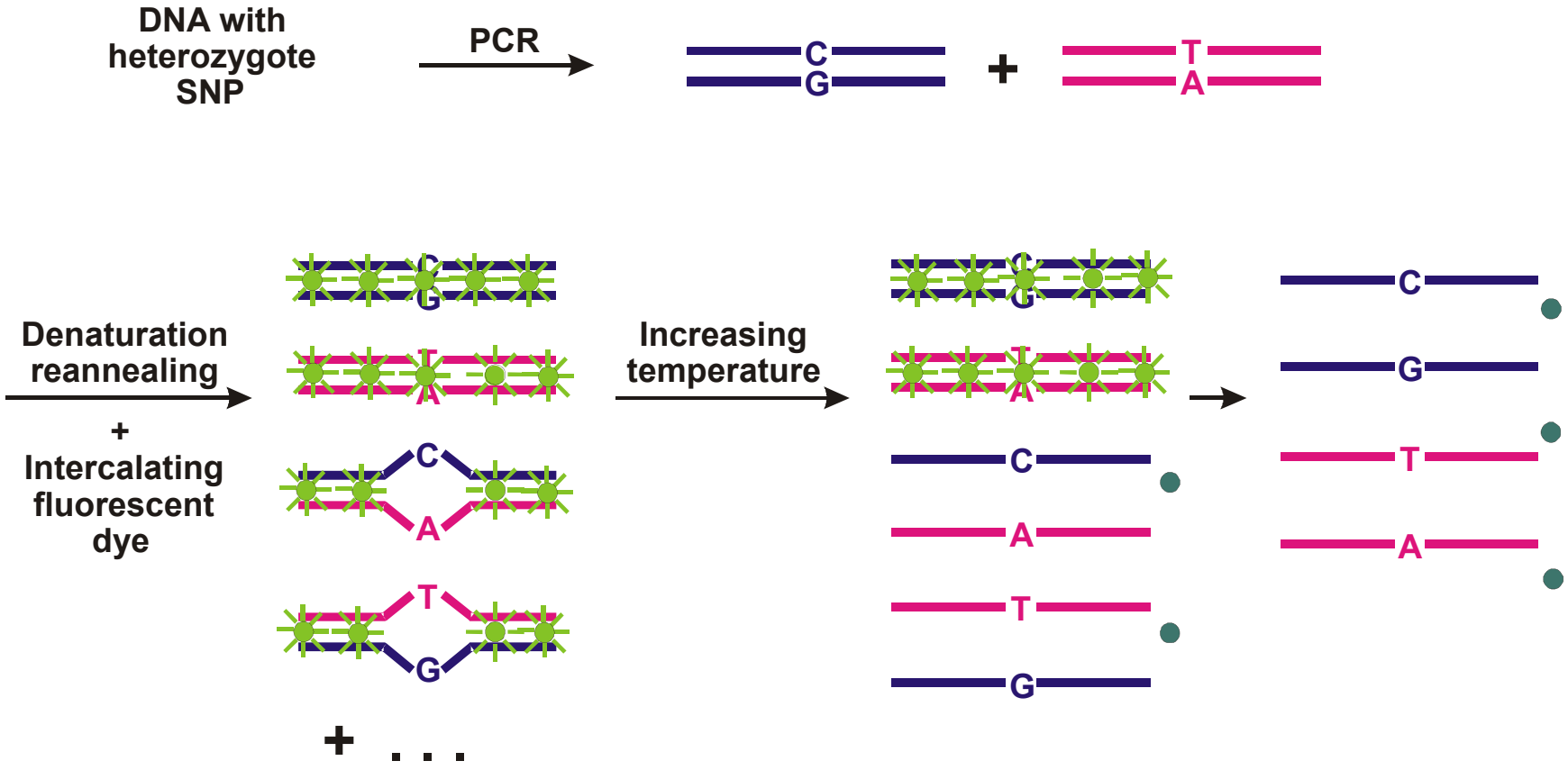
Diagnostics

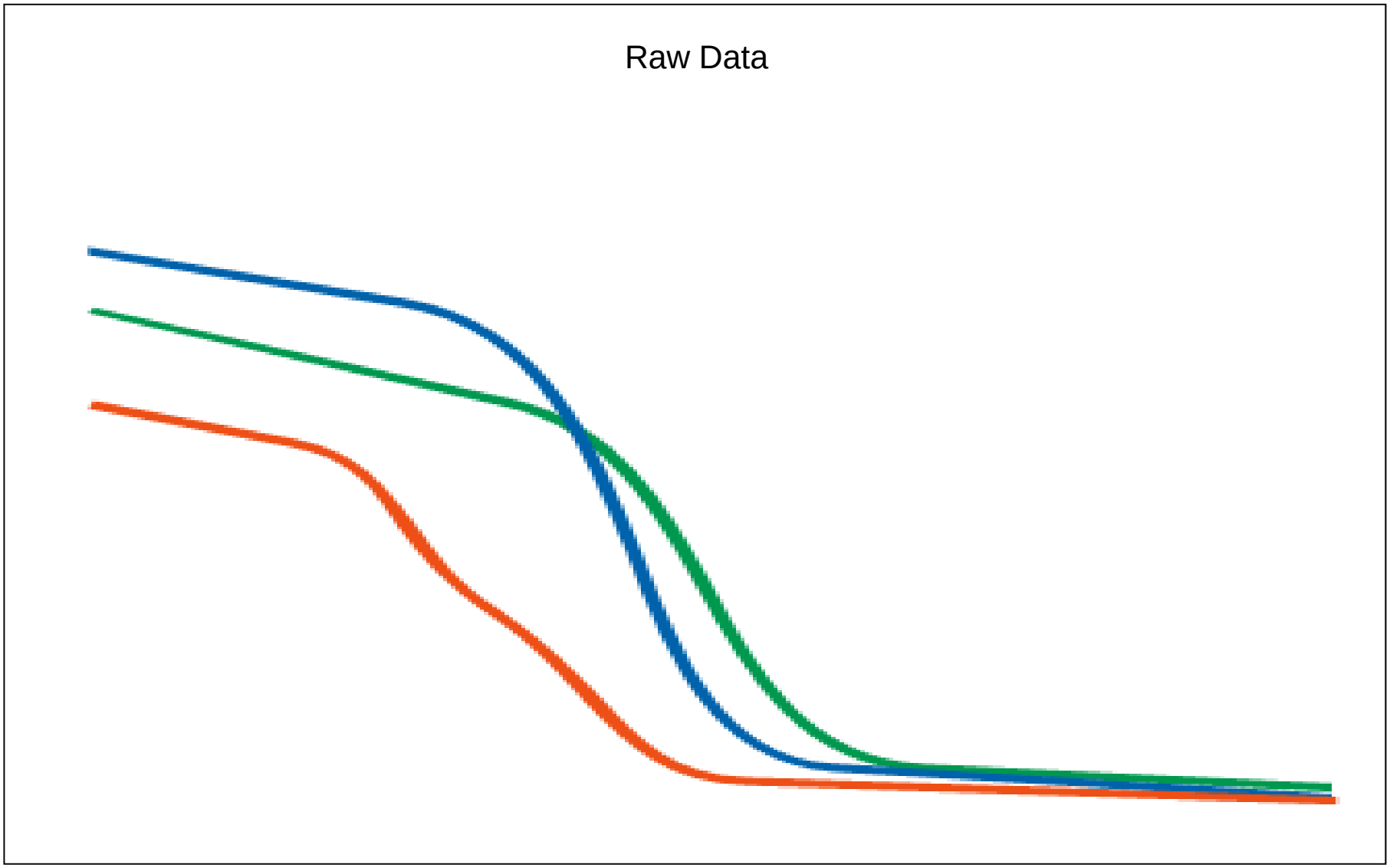
- Principles
- Prerequisites
- Dyes
- Instrumentation
- Data Analysis

High Resolution Melting

Amplicon Melting



High Resolution Melting



Diagnostics

Amplicon Melting

Variation in Melting Temperature (Tm)

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Diagnostics

- The Tm of an amplicon depends mainly on GC content. Alterations in the amplicon may influence the Tm.

Highest Stability											Lowest Stability
G:C	>	A:T	>	G:G	>	G:T = G:A	>	T:T = A:A	>	T:C	> A:C > C:C

- Amplicon Melting of homozygote samples (containing homoduplexes of wildtype or mutant DNA) give very similar curve shapes.
- Amplicon Melting of heterozygote samples (containing homo and heteroduplexes) give curve shapes which are highly distinct.

Technology - Prerequisites and Innovations
What Is Needed to Perform HRM?

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Diagnostics

- **Novel intercalating dye to identify heteroduplex DNA**
  - saturating, non-inhibitory, ds DNA binding without redistribution during melting

- **Precise Instrument** to allow genotyping and/or mutation scanning of whole PCR products.
  - homogenous temperature profile and temperature control
  - high sensitivity optical system (light source, filters and detection system)

- **Flexible Data Analysis Software**
  - Sensitive and specific algorithms to distinguish detected differences
  - Easy to use, easy to adjust
  - Melt-standard compatible

High Resolution Melting

Non-Saturating vs Saturating Dyes

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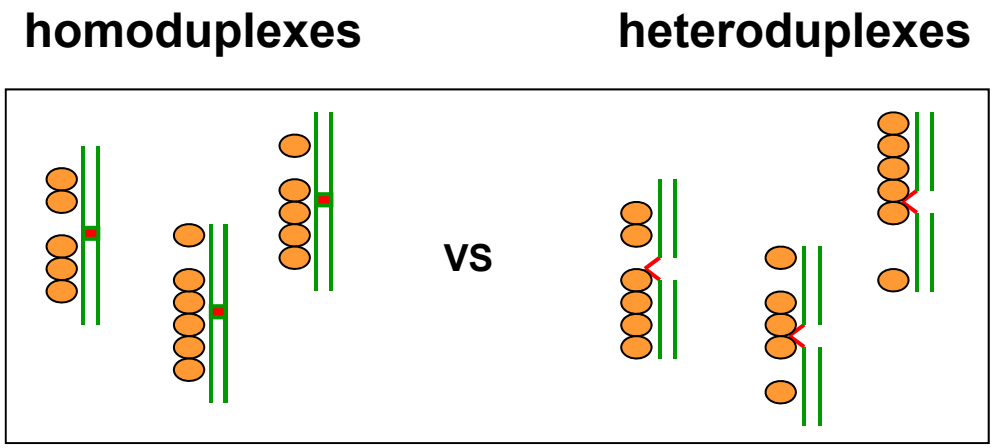
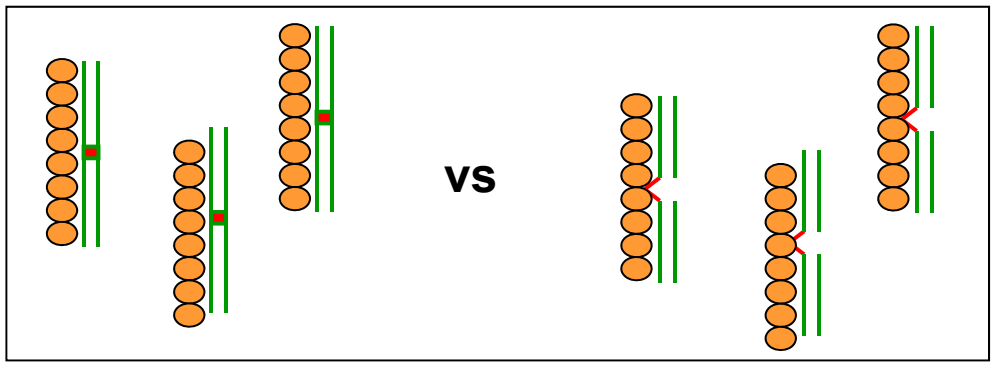
Diagnostics



Fluorescent ds-DNA specific dyes (e.g., SYBR Green I)
- **individual curves not sharp**
- **overlap is the same for homo- and heteroduplexes**



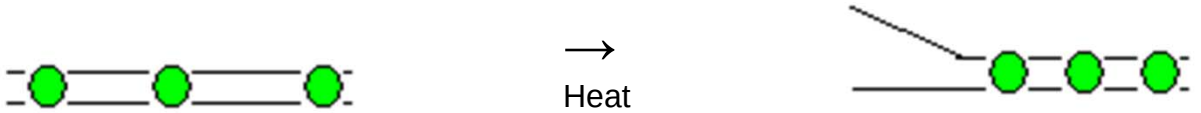
Saturating dye
- **uniform, sharp signals**
- **only sequence but not dye makes a difference**

High Resolution Melting

High Resolution Melting Dye in Action

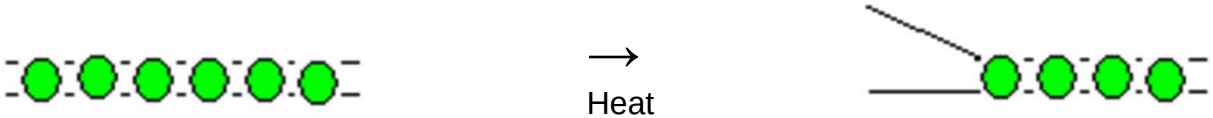
Diagnostics

Non-Saturating Dye- SYBR Green I



No decrease in fluorescence

Saturating Dye- LightCycler HRM Master



Decrease in fluorescence

High Resolution Melting

Dyes

- Gundry et al tried a number of common and uncommon dyes for HRM
  - SYBR Green 1
  - SYBR Gold
  - Ethidium bromide
  - Pico Green
  - TOTO-1
  - YOYO-1
- Requirements:
  - Saturating
  - non-inhibitory to PCR reaction
  - Sufficient fluorescent levels for detection
  - Allows heteroduplex detection

High Resolution Melting

Dyes

- Very few dyes meet the requirements
  - LC Green – Idaho Technologies – somewhat inhibitory
  - R27 – Biolight – limited heteroduplex detection
  - EvaGreen – Biotium – somewhat inhibitory, though less than SYBR
  - ResoLight – Roche
    - Signal 7x higher than LC Green
    - No PCR inhibition within 8x concentration range
    - Improved stability over LC Green or R27
    - Well suited to heteroduplex differentiation

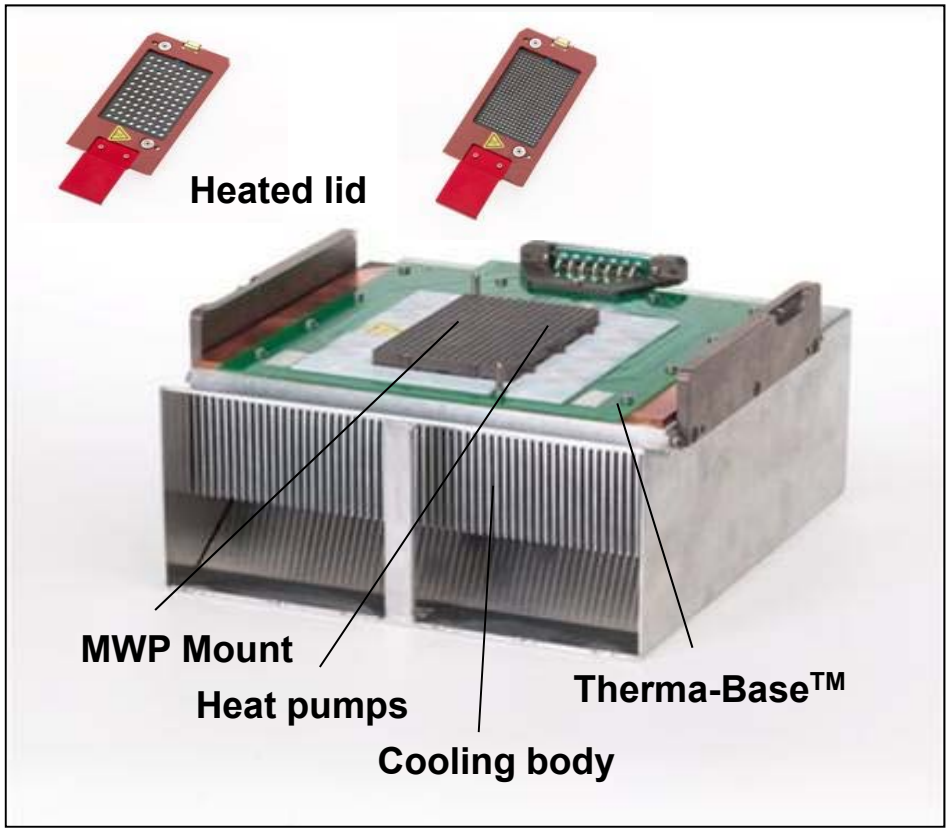
Prerequisites and Innovations

What Is Needed to Perform Hi Res Melt?

Diagnostics

- **Precise Instrument** to allow genotyping and/or mutation scanning of whole PCR products.
  - homogenous temperature profile and temperature control
  - high sensitivity optical system (light source, filters and detection system)

LightCycler® 480 System
Thermocycler

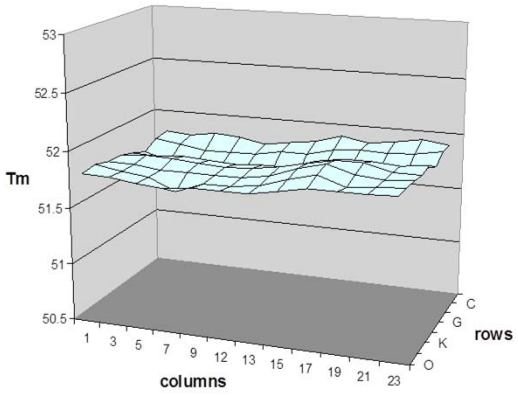


- Six Peltier elements: semi-conductors where direction of current either cools or heats the thermoblock.
- Includes Therma-Base™ for optimized heat exchange which results in excellent overall temperature homogeneity.
- Allows to finish a PCR run:
  96 wells in < 1 hour
  384 wells in < 40 min.
- New technology for thermocyclers
- **Unique to LC480**

Diagnostics

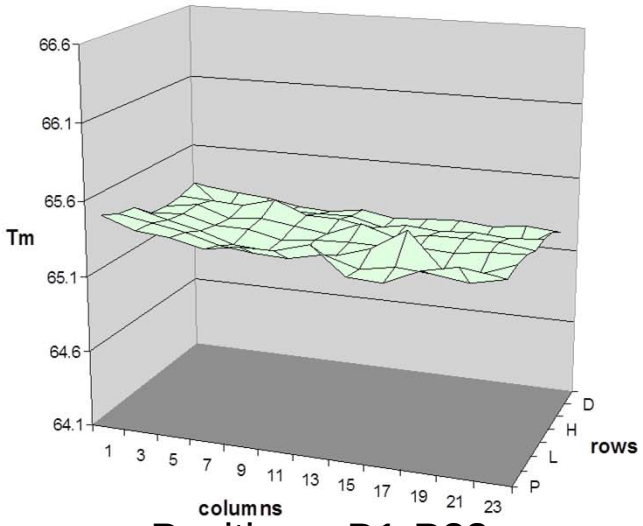
Thermal Uniformity
Intra-Run Reproducibility of 96 Replicates

Diagnostics



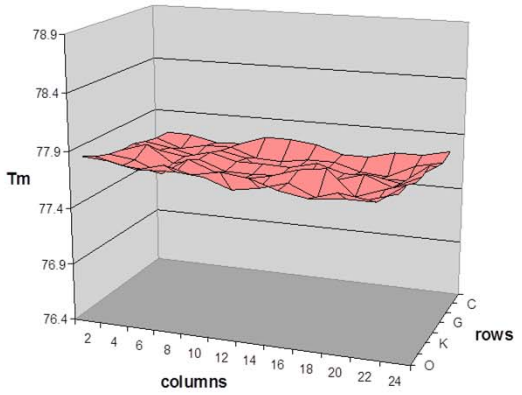
Positions: A1-O23

	values / °C
average	51,80
min	51,63
max	51,96
delta	**0,33**
SD	0,0722



Positions: B1-P23

	values / °C
average	65,31
min	65,14
max	65,52
delta	**0,38**
SD	0,0731



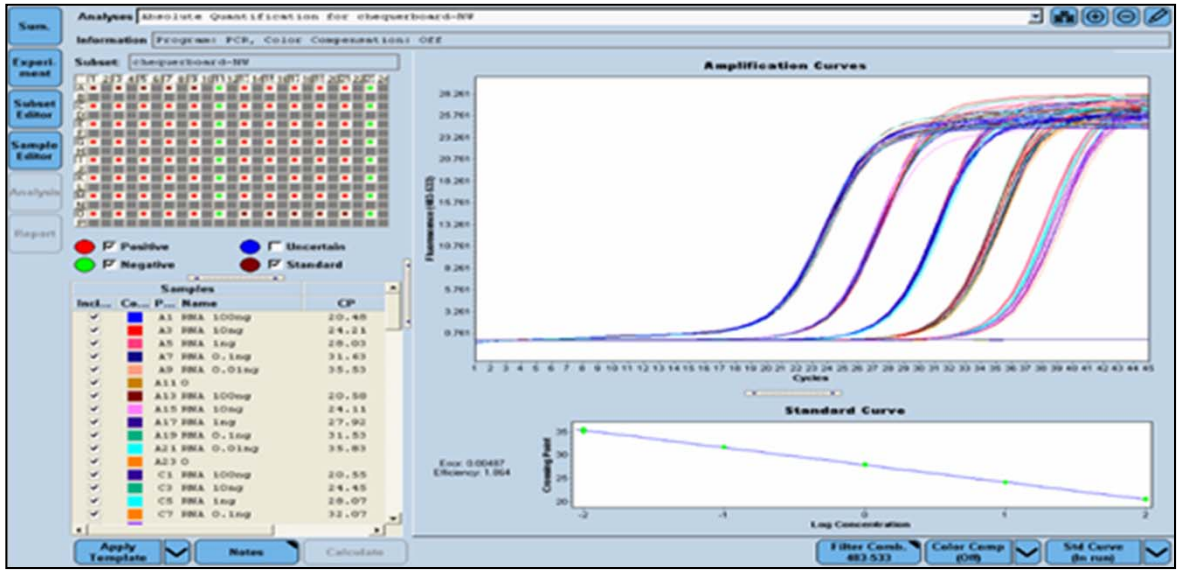
Positions: A2-O24

	values / °C
average	77,69
min	77,46
max	77,87
delta	**0,41**
SD	0,0801

LightCycler® 480 Performance
Absolute Quantification, SYBR Green I

Diagnostics

Total RNA per reaction	100ng	10 ng	1 ng	100 pg	10 pg	H2O
mean	20,57	24,11	27,91	31,69	35,49	-
SD	0,083	0,163	0,14	0,186	0,337	-
max	20,71	24,45	28,17	32,07	35,96	-
min	20,42	23,83	27,69	31,44	34,9	-
delta Cp	0,29	0,62	0,48	0,63	1,06	-



2-step RT-PCR
Target: h-HPRT

Thermal Uniformity
Instrument Comparison -96 wells

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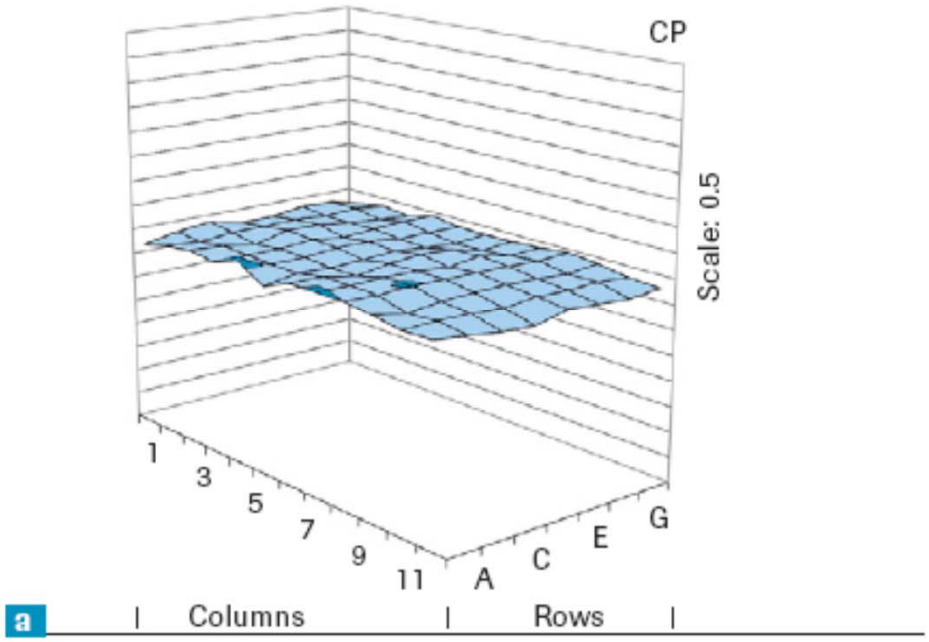
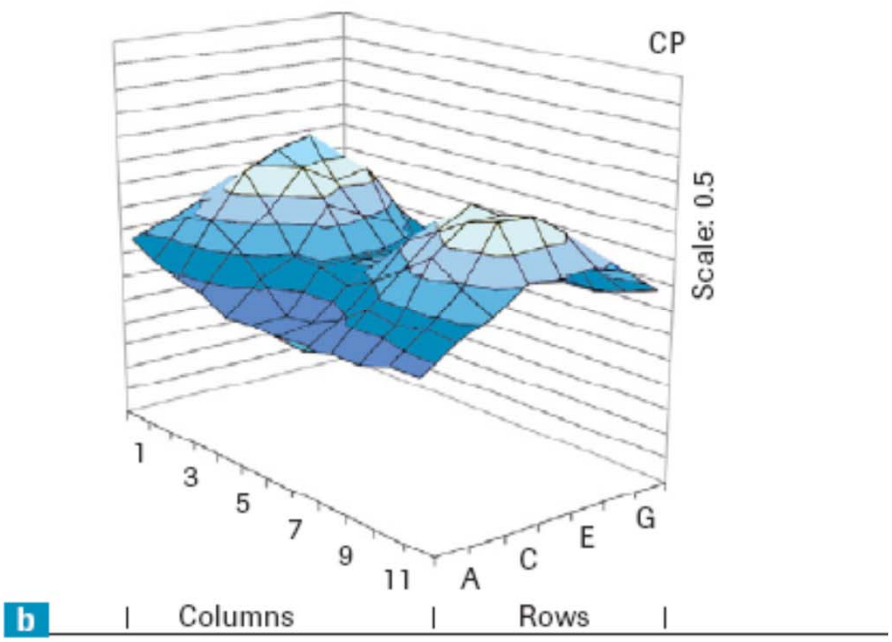
Diagnostics

LightCycler® 480 Instrument



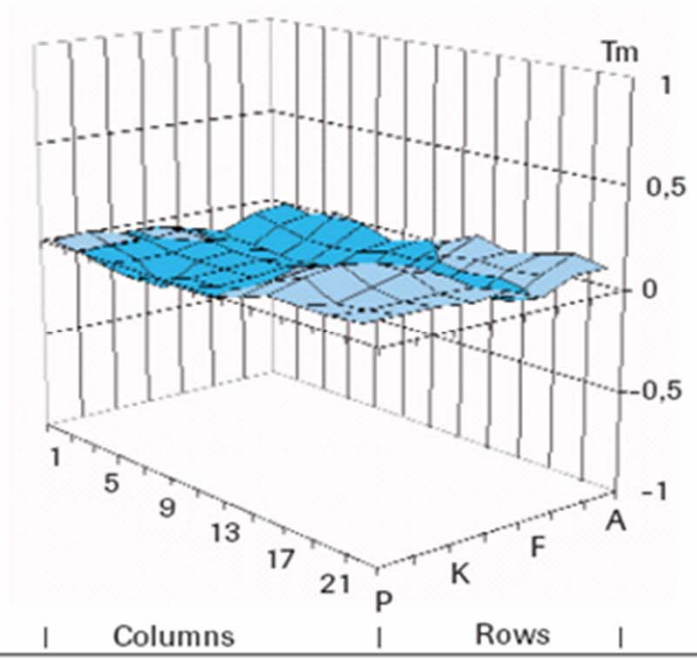
Standard Instrument



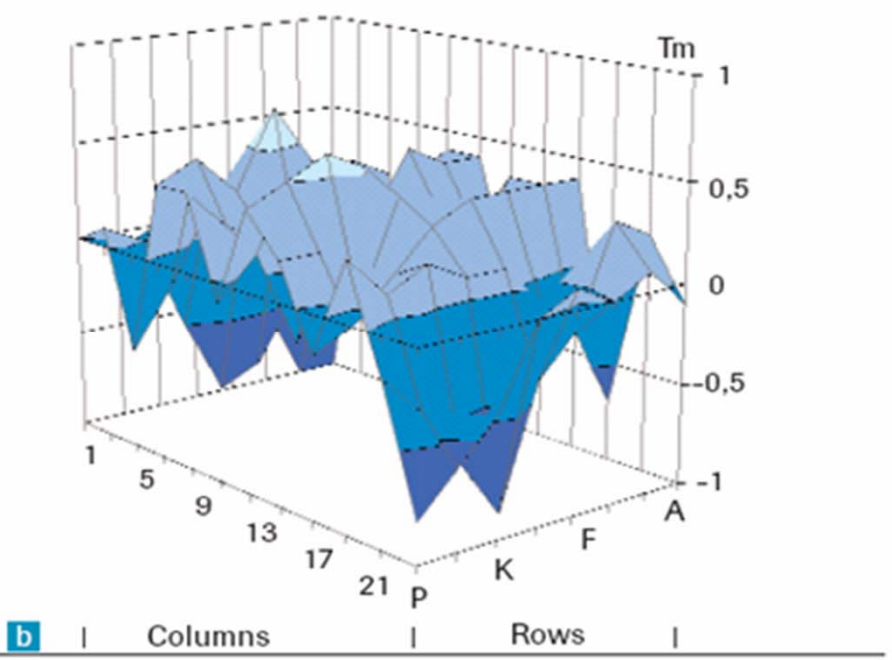
Thermal Uniformity
Instrument Comparison – 384 wells

Diagnostics

LightCycler® 480 Instrument

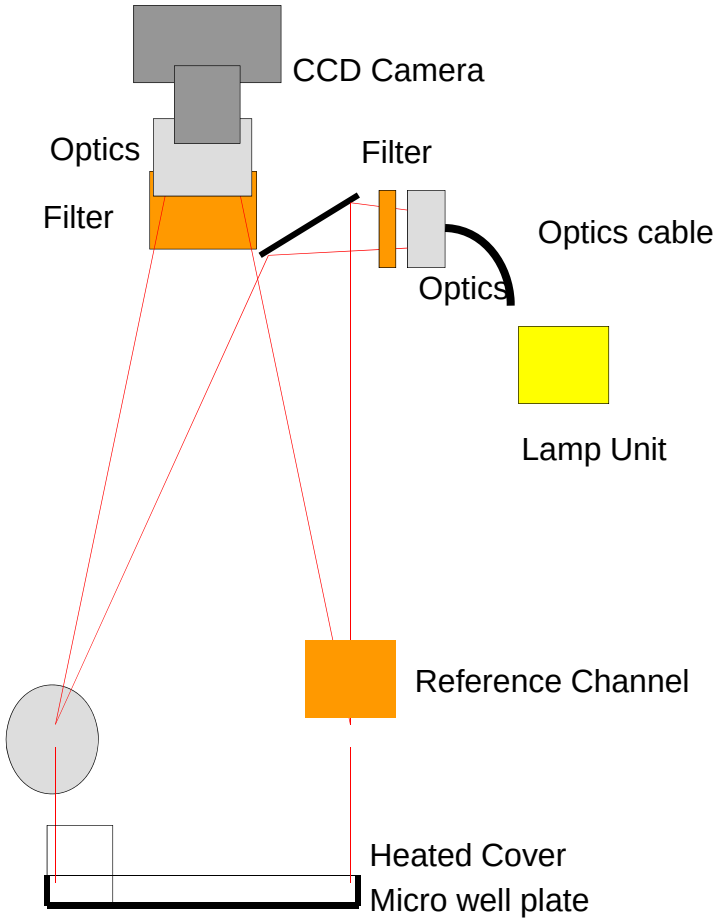


Standard Instrument

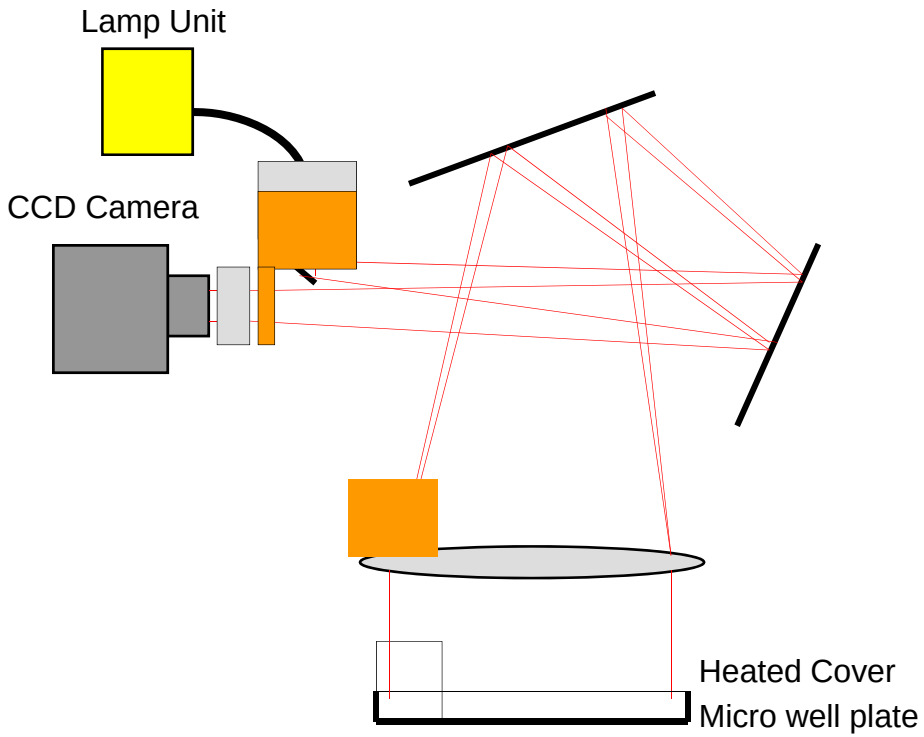


LightCycler® 480 Instrument
Optical System - Lightpath

Diagnostics



Folded optical path to reduce height



LightCycler® 480 Optical System
Sensitivity and Homogeneity

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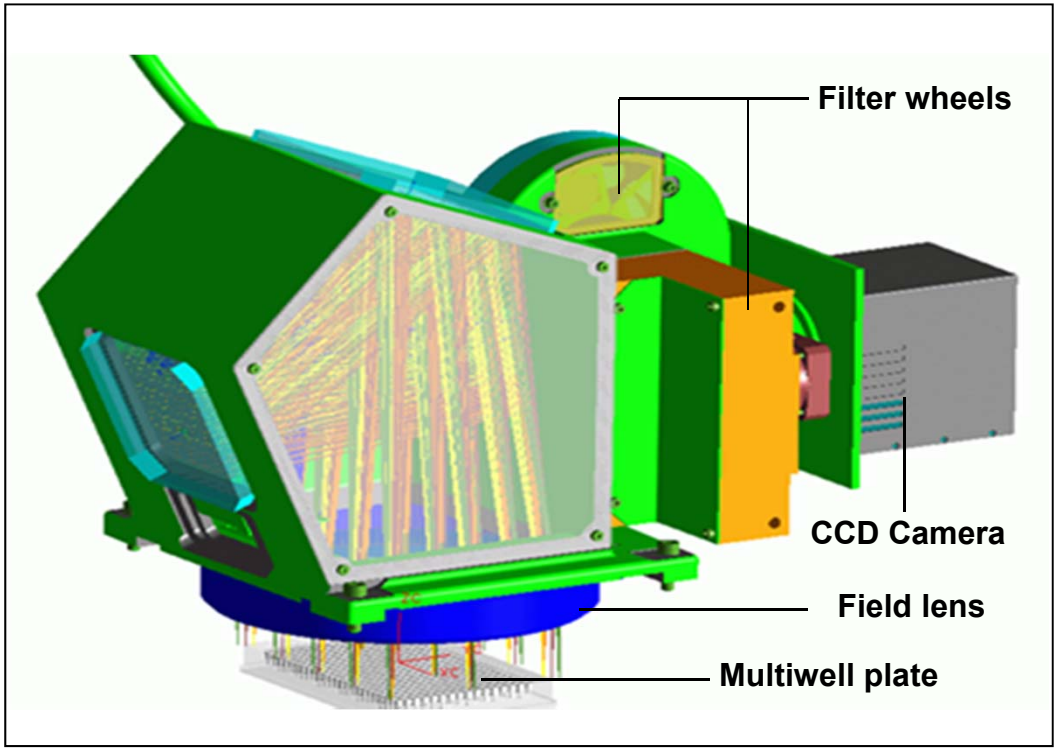
Diagnostics



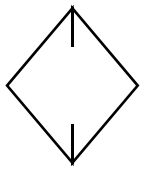
- Xenon lamp
- CCD camera
- Five excitation filters
- Six detection filters
- Optimized arrangement of optical components
- Homogeneous excitation and fluorescence detection

LightCycler® 480 Instrument
Optical Properties

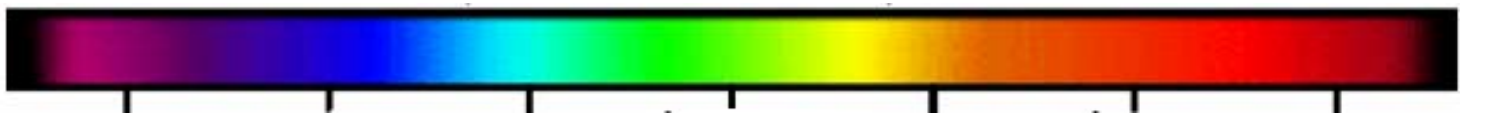
Diagnostics

- **Light source: high intensity xenon lamp**
- **Highest intensity of light over a broad electromagnetic spectrum**
- **Degrades (ages) in linearly, without spectral shift.**

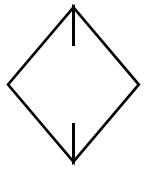
Excitation filters



450, 483, 523, 558, 615 nm



Emission filters



500, 533, 568, 610, 640, 670 nm

LightCycler® 480 System
Assay Formats and Dyes

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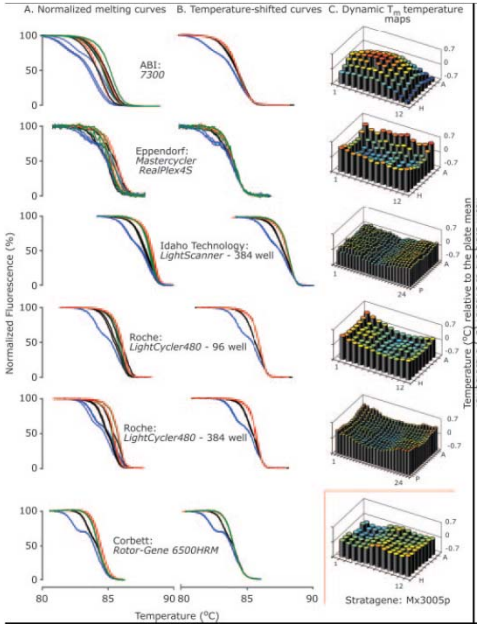
Diagnostics

Excitation filters	450	483	483	523	558		615
Emission filters	500	533	533	568	610	640	670
Dyes *(Examples)*	**LightCycler® Cyan 500**	**SYBR Green I**	**Fluorescein (Fluos / FAM)**	**HEX (VIC)**	**LightCycler® Red 610**	**LightCycler® Red 640**	**Cy5**
Detection formats	Hydrolysis probes (R), HybProbe probes (D)	SYBR Green I	Hydrolysis probes (R), HybProbe probes (D), SimpleProbe probes (R)	Hydrolysis probes (R), HybProbe probes (A)			

Legend: Reporter (R), Donor (D), Acceptor (A).

External evaluation
ARUP (Salt Lake City) study of hardware features



Herrmann, M. G. et al. (2007). "Expanded Instrument Comparison of Amplicon DNA Melting Analysis for Mutation Scanning and Genotyping." Clin Chem; **June 2007**

- Heterozygote scanning: LightCycler® 480 equals LightScanner
- LightCycler® 480 advantages: data density, signal-to-noise ratio, melting rate, speed

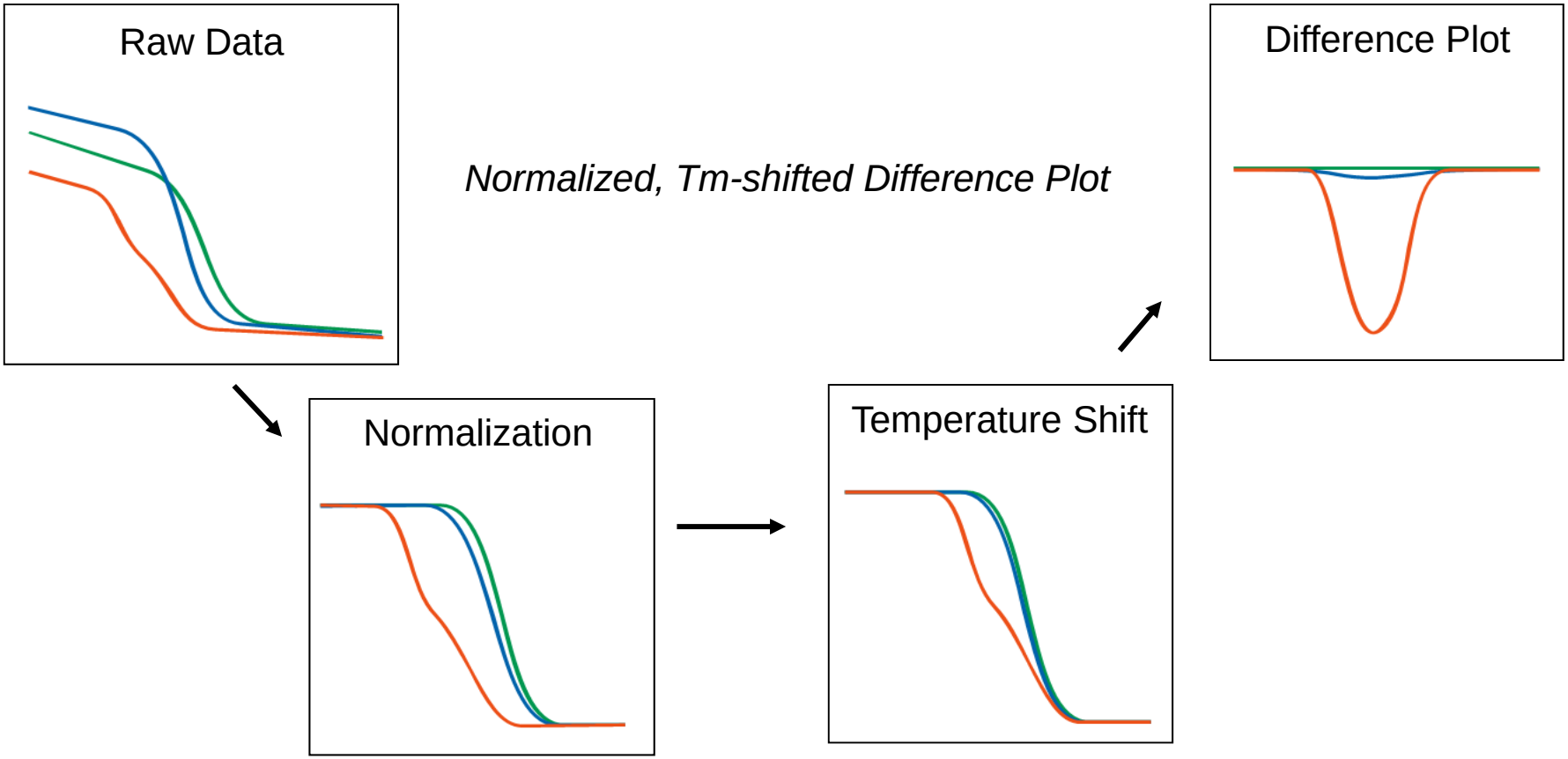
High Resolution Melting

Software and Data Analysis

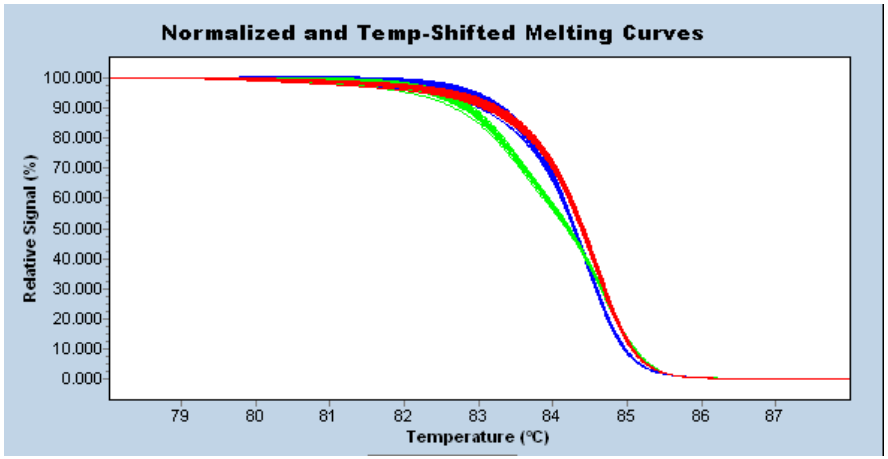
- Wittwer et al (2003) demonstrated a useful and robust analysis methodology that has the capability to reveal both homo- and hetero-duplex DS DNA configurations
- Utilizes fluoresence normalization, temperature shift adjustment, and derivative melting curve plots
- Can reveal extremely minor differences in DS DNA melting curve shape
- Allows for comparison and adjustment to use melting standards for genotyping

High Resolution Melting

Data Analysis



Wt/Homo/Heterozygote Differentiation



Example:

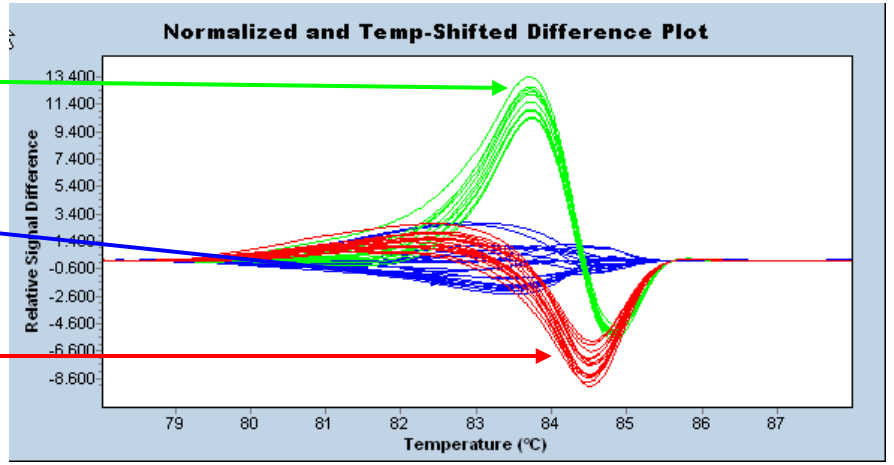
Sequence variations (SNP G→T) in the LPLH3 gene

72 samples, 164 bp amplicon

heterozygous (homo and heteroduplexes)

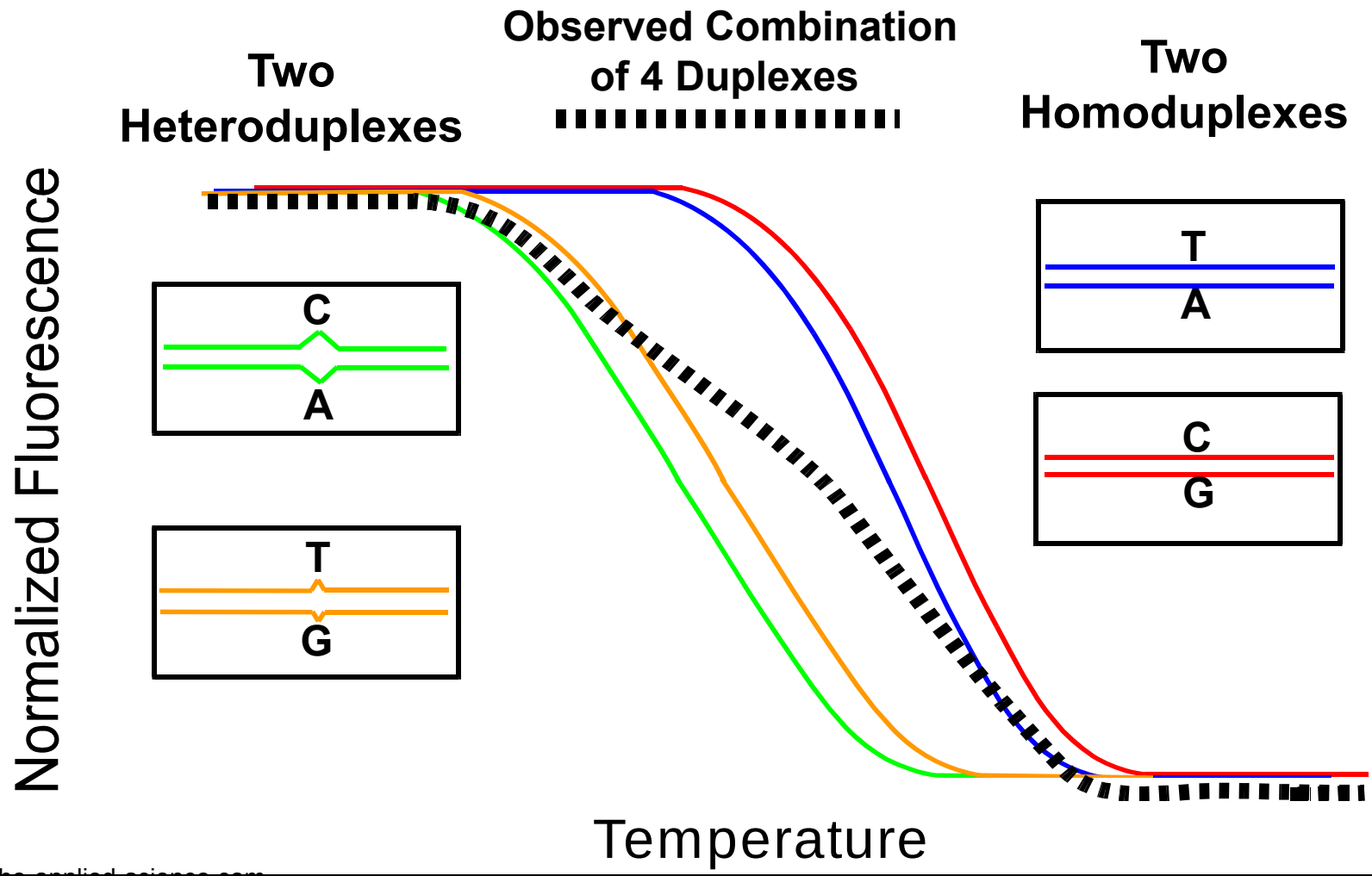
homozygous wildtype (homoduplexes)

homozygous mutant (homoduplexes)

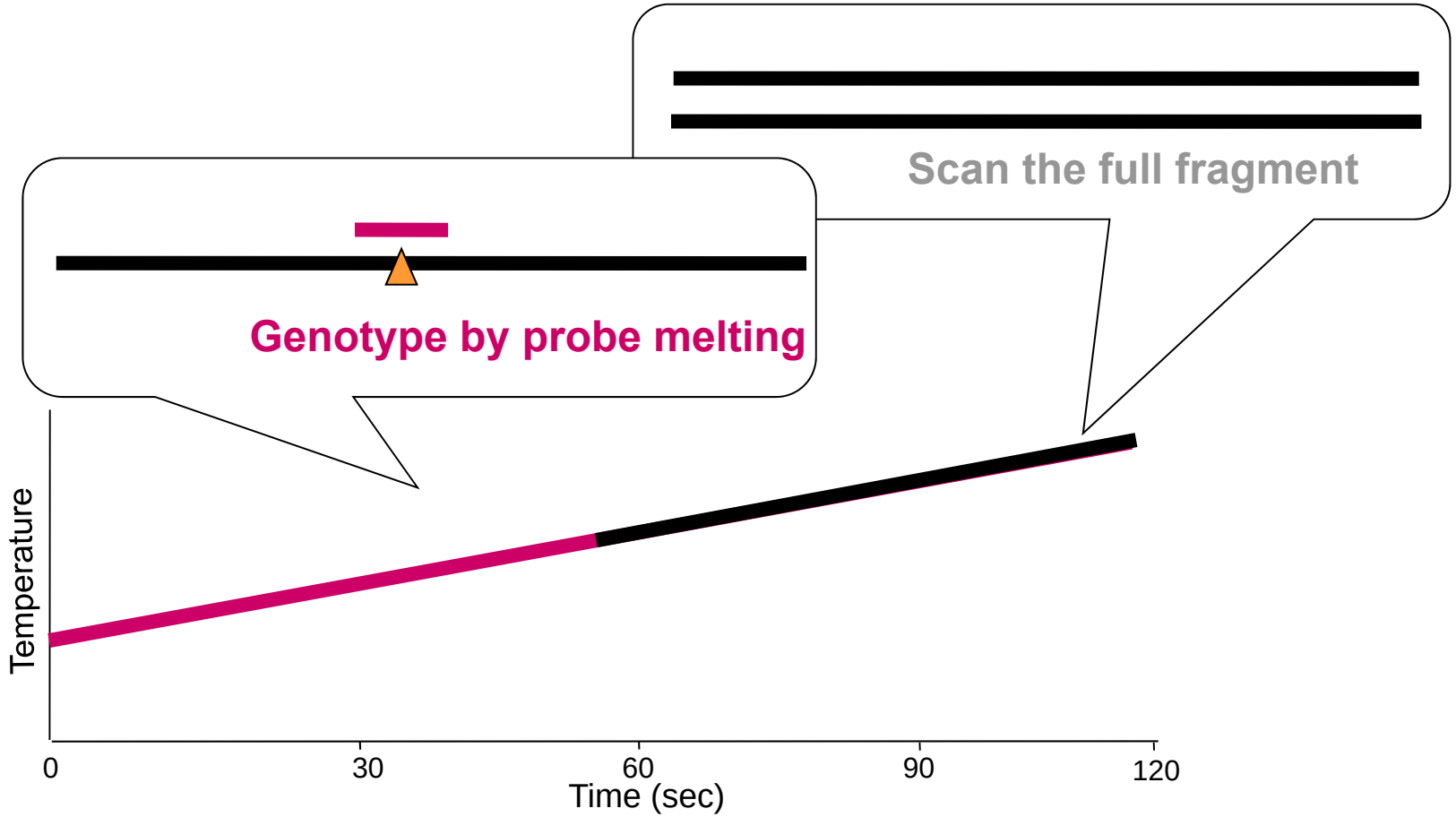


Melting Curves

Heterozygote Amplification

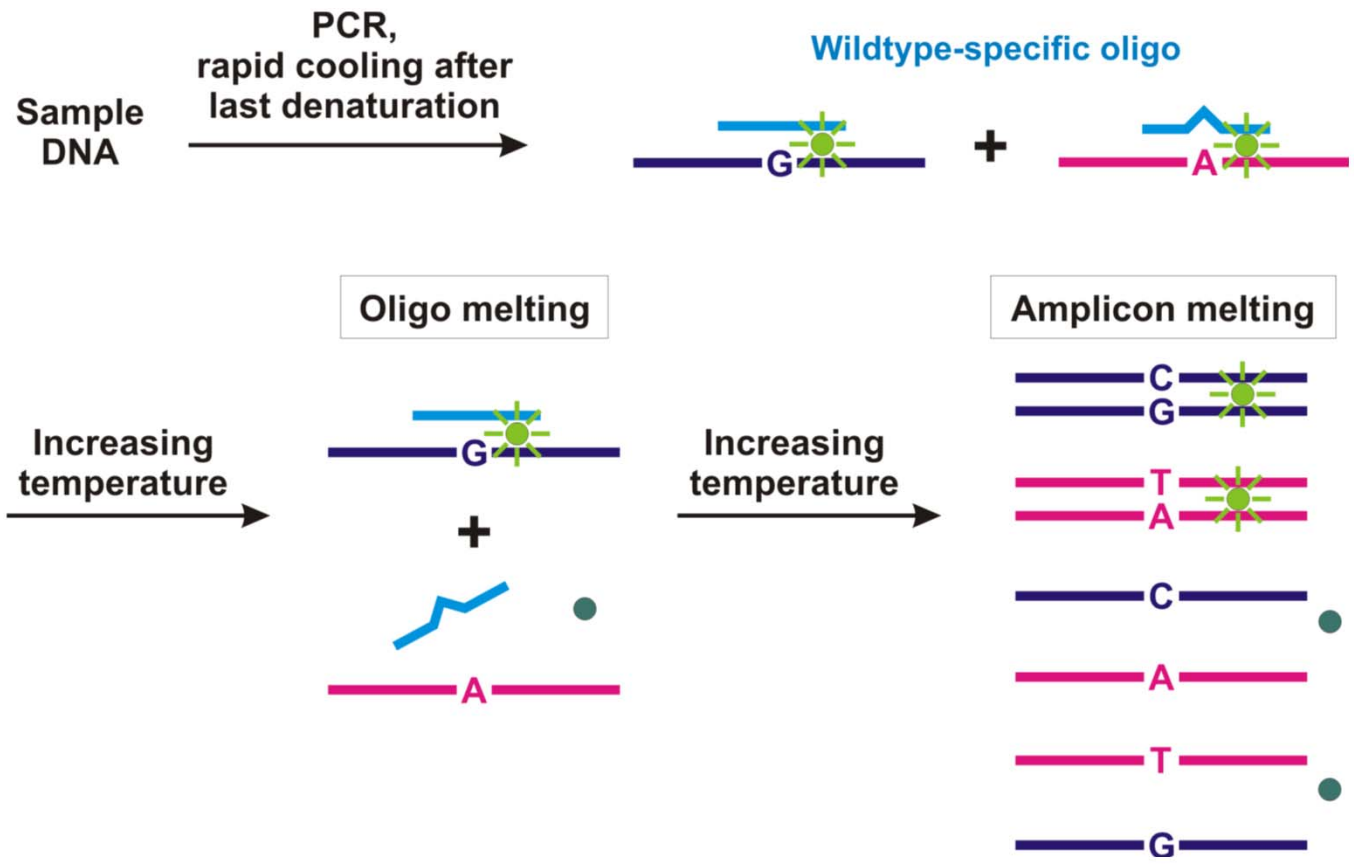


Unlabeled Probe Genotyping and Amplicon Melting *Simultaneous genotyping and scanning*



Unlabeled Probe Melting

Principle of Genotyping by Hi Res Melt



High-Resolution Melting with intercalating dye and unmodified oligo specific for known mutation site

Diagnostics

Combined Unlabeled Probe and Amplicon Melting

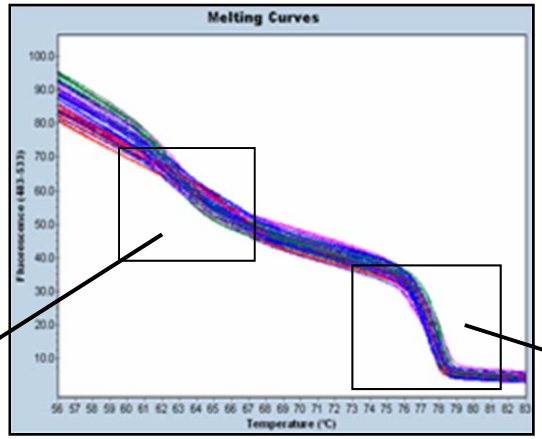
Example 1: TNFα

Diagnostics

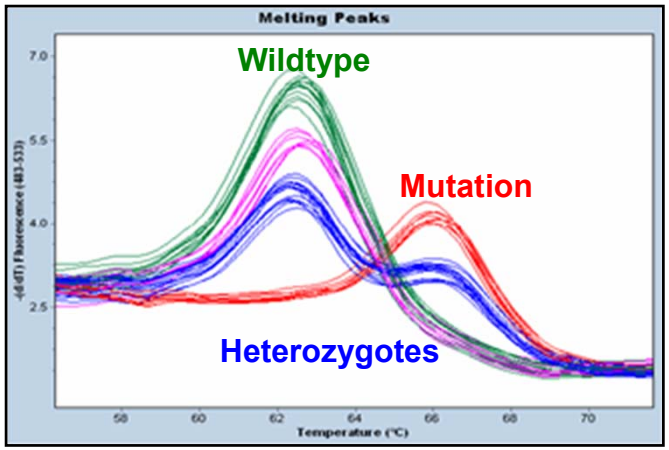
Probe for SNP
C→T

Amplicon 136 bp

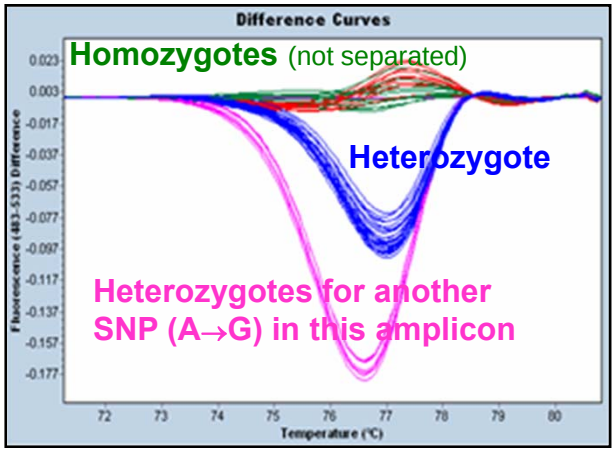
96 samples



1st Derivative



Normalization, Difference Plot



High Resolution Melting

Utility

- Optimization requirements
- Data and Results
- Possibilities
- References and Papers

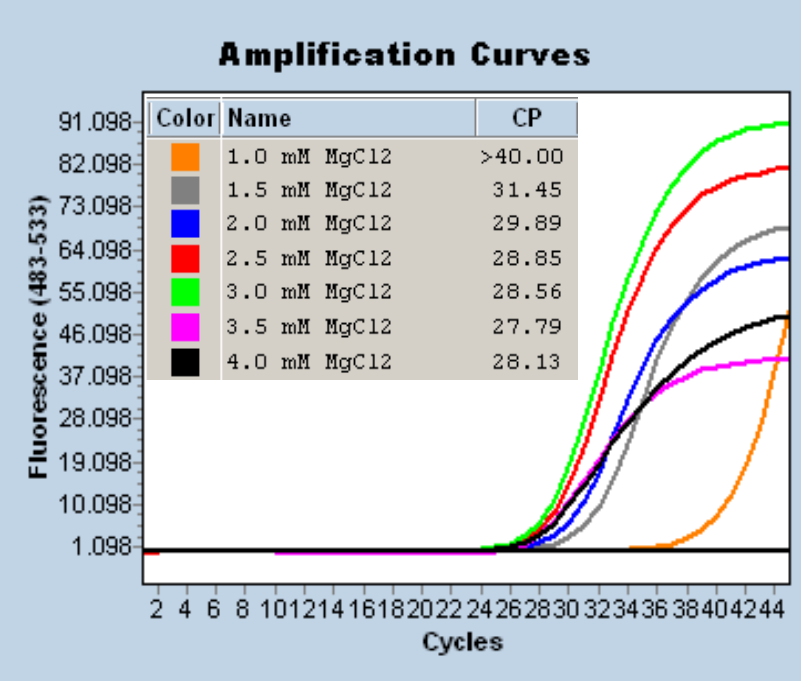
High Resolution Melting

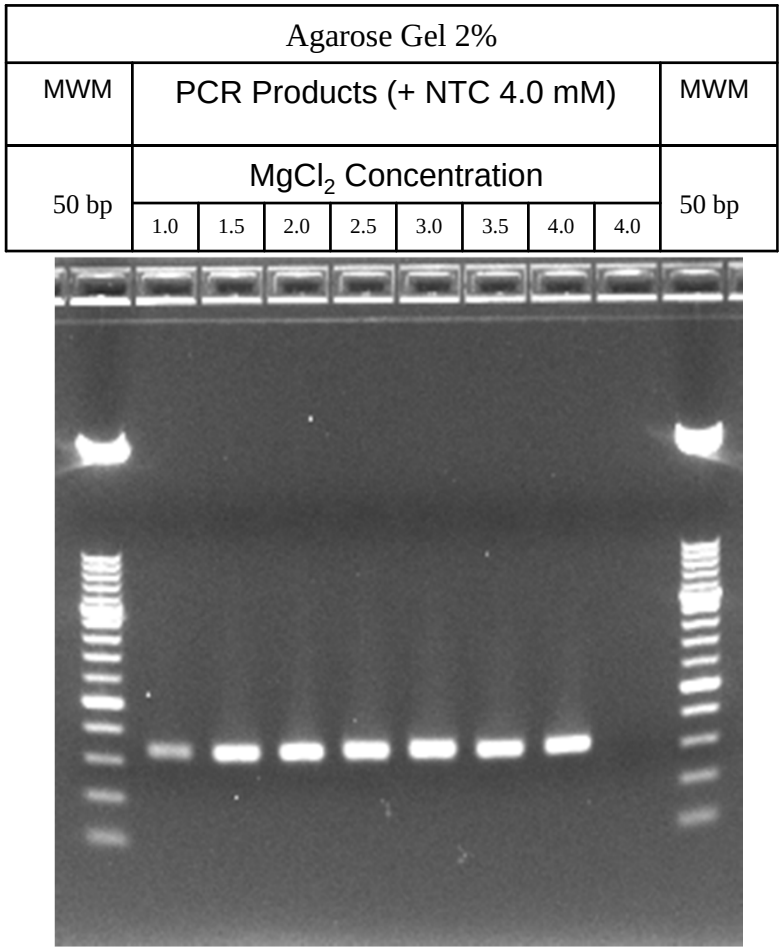
Utility

- **Optimization requirements**
- Data and Results
- Possibilities
- References and Papers

Optimizing a Gene Scanning Experiment
$MgCl_2$ Concentration

Diagnostics





167 bp PCR Fragment
$MgCl_2$ Titration 1.0 – 4.0 mM
PCR Primers: 200 nM each
Touchdown PCR Protocol (64 – 54° C)

Optimizing a Gene Scanning Experiment
Sample Material

Diagnostics

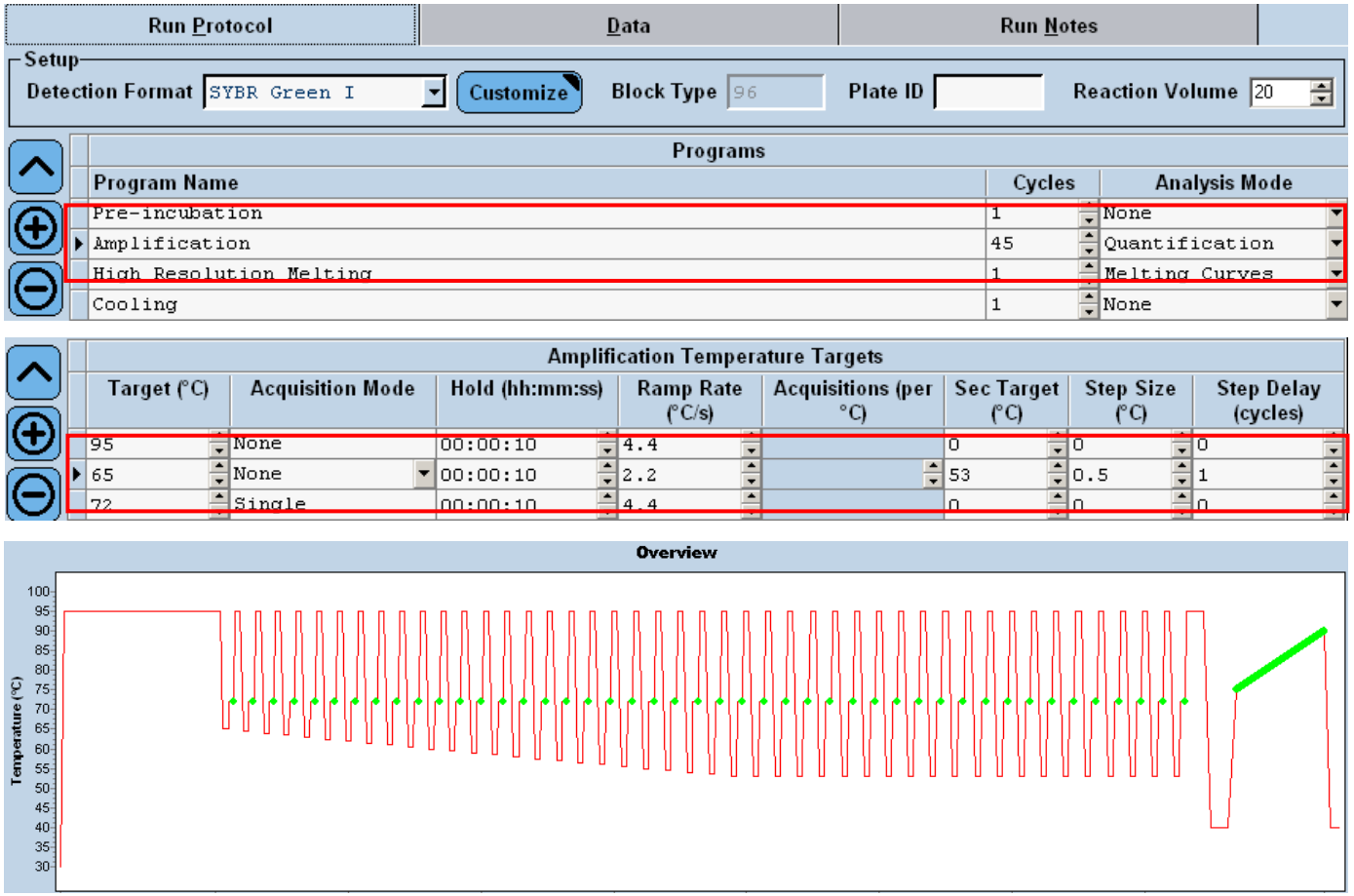
- Use consistent extraction protocols for all samples to be analyzed via High Resolution Melting.
- Quantify DNA samples using spectrophotometry. Adjust them to the same concentration prior to PCR
- Use the same amount of template in each reaction (5 to 30 ng template DNA in a 20 µl reaction). Amplification plots should produce a crossing point value of < 30.
- Crossing points (aka C_T) should be within 5 cycles of each other

Optimizinging a Gene Scanning Experiment
PCR Primers

Diagnostics

- Design PCR primers that have annealing temperatures around 60˚ C and produce short amplicons, ideally100–250 bp.
- Use a software package to design primers
  - *Primer3* (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi)
  - *LightCycler® Probe Design Software 2.0.*
- BLAST (http://www.ncbi.nlm.nih.gov/BLAST) the primer sequences to ensure they are specific for the target species and gene.
- Use primers that have been purified by HPLC.
- Use low primer concentrations (*e.g.,* 200 nM each) to avoid primer-dimer formation.

Optimizing a Gene Scanning Experiment
PCR Programs: Amplification

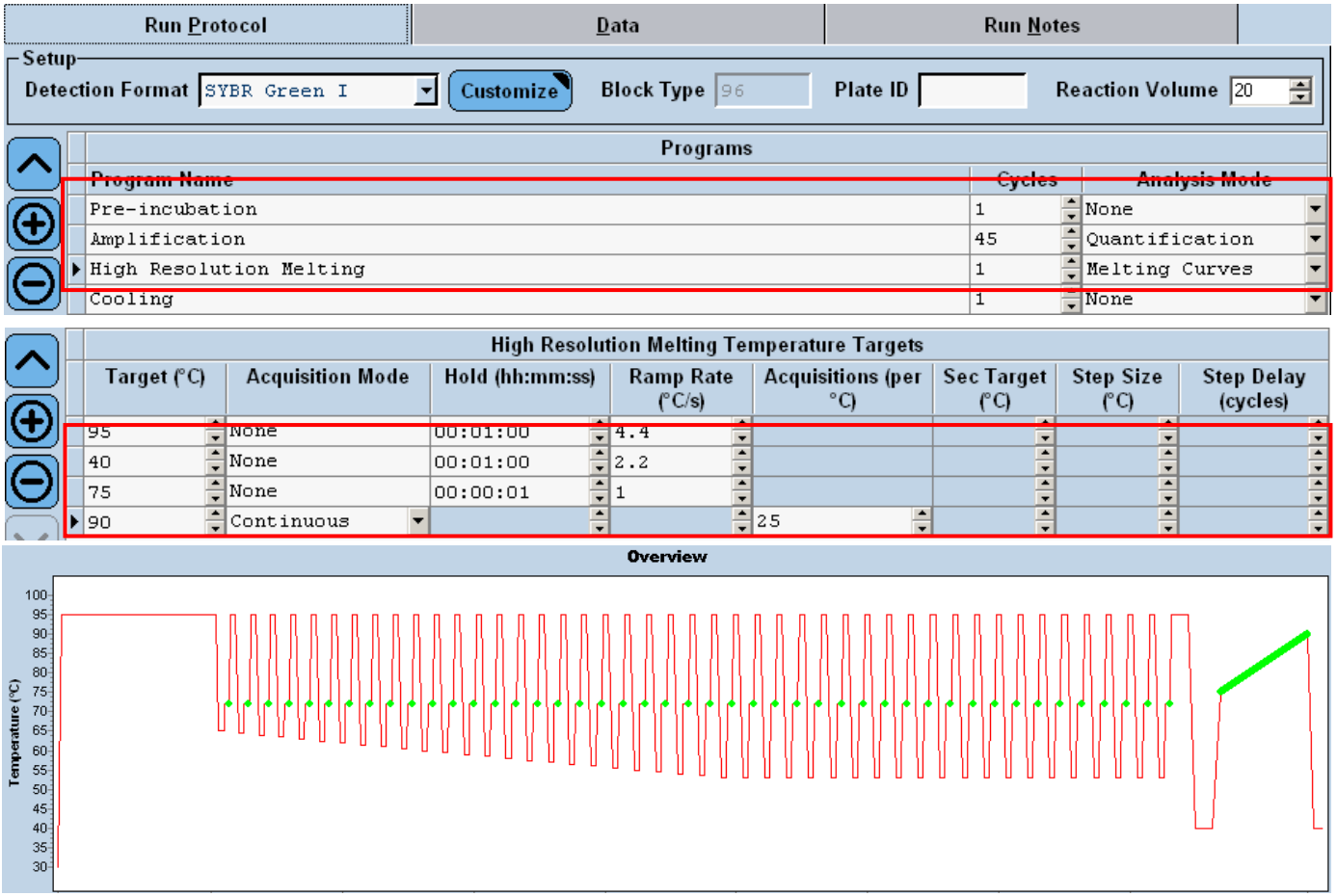


Example:

Touchdown PCR

Optimizing a Gene Scanning Experiment
PCR Programs: High Resolution Melting

Diagnostics



Example:

HRM program

Optimizing a Gene-Scanning Experiment

Roche

Diagnostics

Controls

- Negative Controls – ensure PCR products not result of carryover
- Positive Controls – may be eliminated if known reference standards are used
- Known Reference Genotypes – „Melt Standards“
  - Especially useful when only a few samples are compared or when unlabeled probes are used and designed against a specific sequence variant
- Replicates?
  - Biological replicates can be used to provide an estimate of variation within a genotype
    - Replicates of individual samples not required
  - „experimental“ replicates used to confirm extraction / pipetting / PCR repeatability

Guidelines for successful HRM Assays

1. Analyze small DNA fragments

   There will be a bigger effect of a single base variation on a small amplicon.

2. Analyze a single pure product

   Primer-dimers and non-specific products make HRM difficult to interpret.

3. Use sufficient pre-amplification template

   Make sure the product has a C_p (C_T) no more than 30 cycles. Samples that amplify later than this produce variable HRM results due to amplification artifacts.

4. Check for aberrant amplification plots

   Check the qPCR plots carefully for log-linear plots that are not steep, jagged, or reach a low signal plateau. This can indicate poor amplification, incorrect reaction setup, etc.

Guidelines for successful HRM Assays

Diagnostics

5. Keep post-amplification sample concentrations similar

   The concentration of a DNA fragment affects its T_M. Try to keep DNA concentrations as similar as possible. Make sure every reaction reached a plateau.

6. Ensure sample-to-sample uniformity

   All samples must be of equal volume and should contain the same concentration of dye.

   DNA melting behavior is affected by salts in the reaction mix so make sure the buffer, Mg and other salts is the same in all samples. Use identical tubes or plates for all comparisons.

7. Allow sufficient data collection for pre-and post-melt phases

   Collect HRM data points over about a 10^o C window centered on the observed T_M.

Optimizing a Gene-Scanning Experiment Troubleshooting – Montgomery et al (2007)

Roche

Diagnostics

TABLE 1 | Troubleshooting table.

Problem	Possible reasons	Solution
Extraneous melting transitions or poor curve clustering	Secondary PCR products	Optimize PCR conditions to obtain clean product
	Low PCR yield	Optimize PCR to enhance product yield
	Inconsistent genomic DNA preparation	Ensure that the genomic DNA concentration and buffer is consistent
Amplicon and probe melting transitions not visible or are very small	Probe *Tm* too high, preventing PCR extension	Redesign probe with lower T_m, use and exonuclease-positive *Taq* or add the probe after PCR
	Amplicon too long	Design primers for shorter amplicon length
	Low PCR yield	Optimize PCR to enhance product yield
PCR product *Tm* too high	High GC content	Add DMSO, betaine or glycerol to the PCR buffer

Optimizing a Gene-Scanning Experiment

Roche

Diagnostics

LightCycler® 480 High Resolution Master

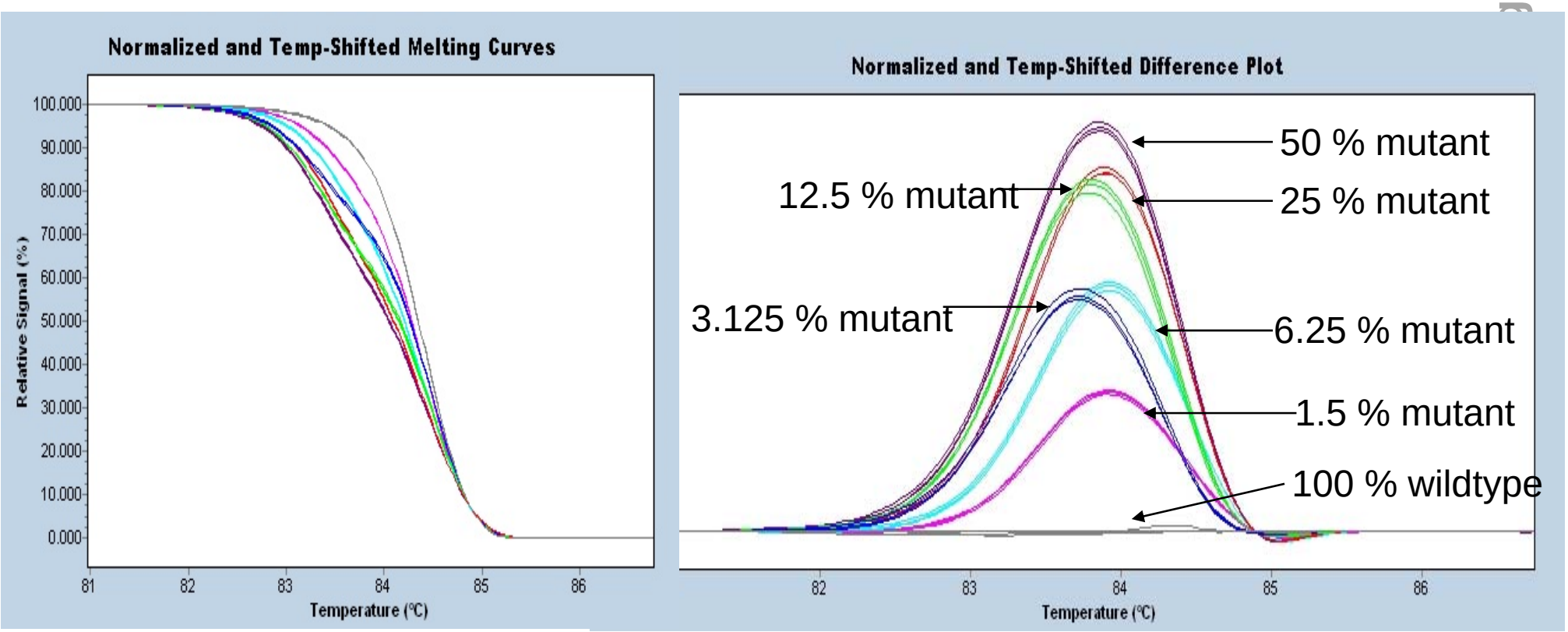
- Cat. No. 04 909 631 001 Kit for 5 x 100 reactions (20µL)
- Contents:
  - Master Mix 2 x conc.
    contains FastStart Taq DNA Polymerase, reaction buffer, dNTP mix (with dUTP instead of dTTP), and **ResoLight**
  - $MgCl_2$, 25 mM to adjust $MgCl_2$ concentration
  - H_2O, PCR-grade to adjust the final reaction volume
- Application
  For amplification and detection of a specific DNA sequence (with suitable primers) followed by high resolution melting curve analysis for detection of sequence variants among several samples.

Utility

- Optimization requirements
- **Data and Results**
- Possibilities
- References and Papers

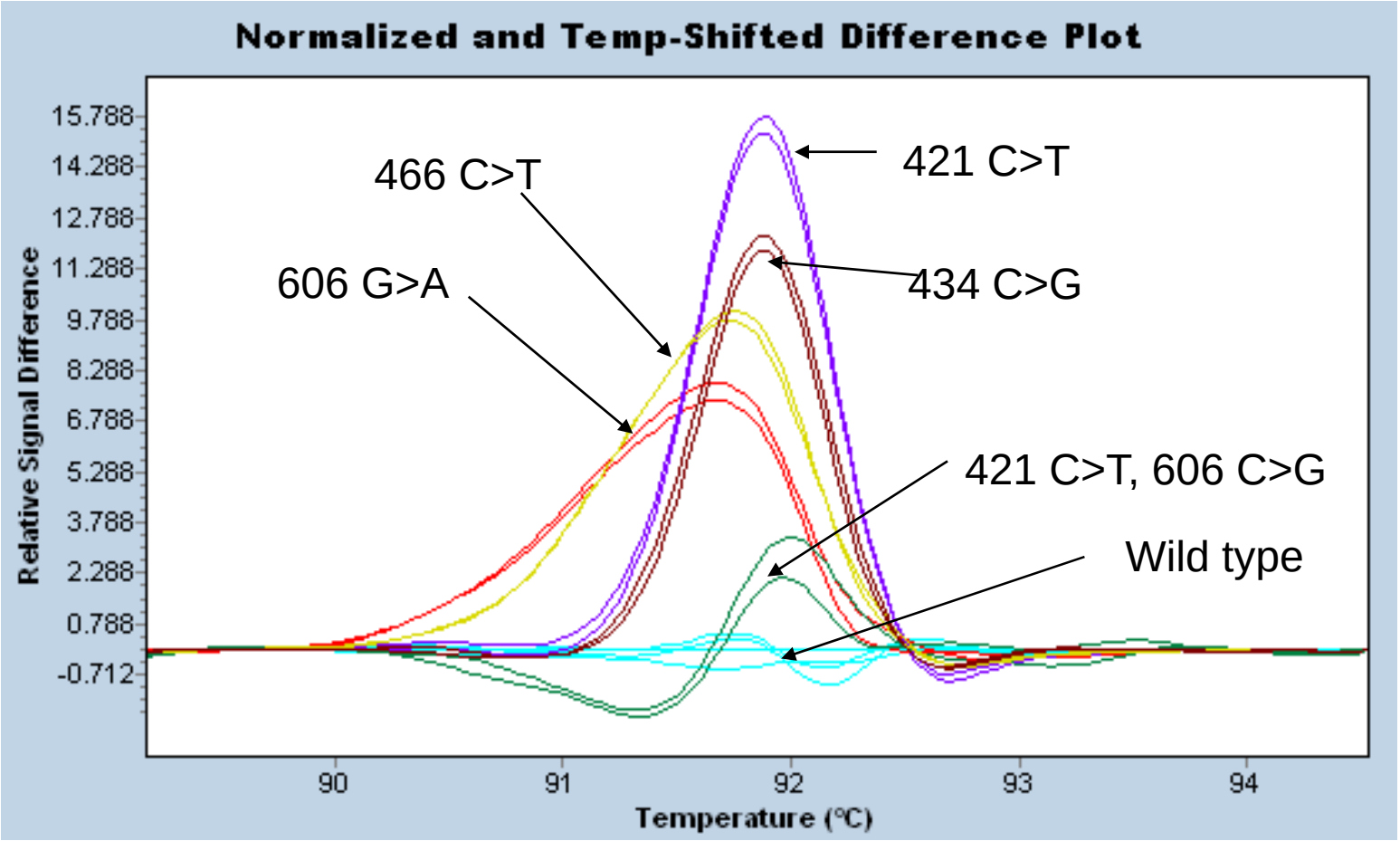
HRM 2 - Sensitivity testing

Dilution series of wild type/mutant mixes

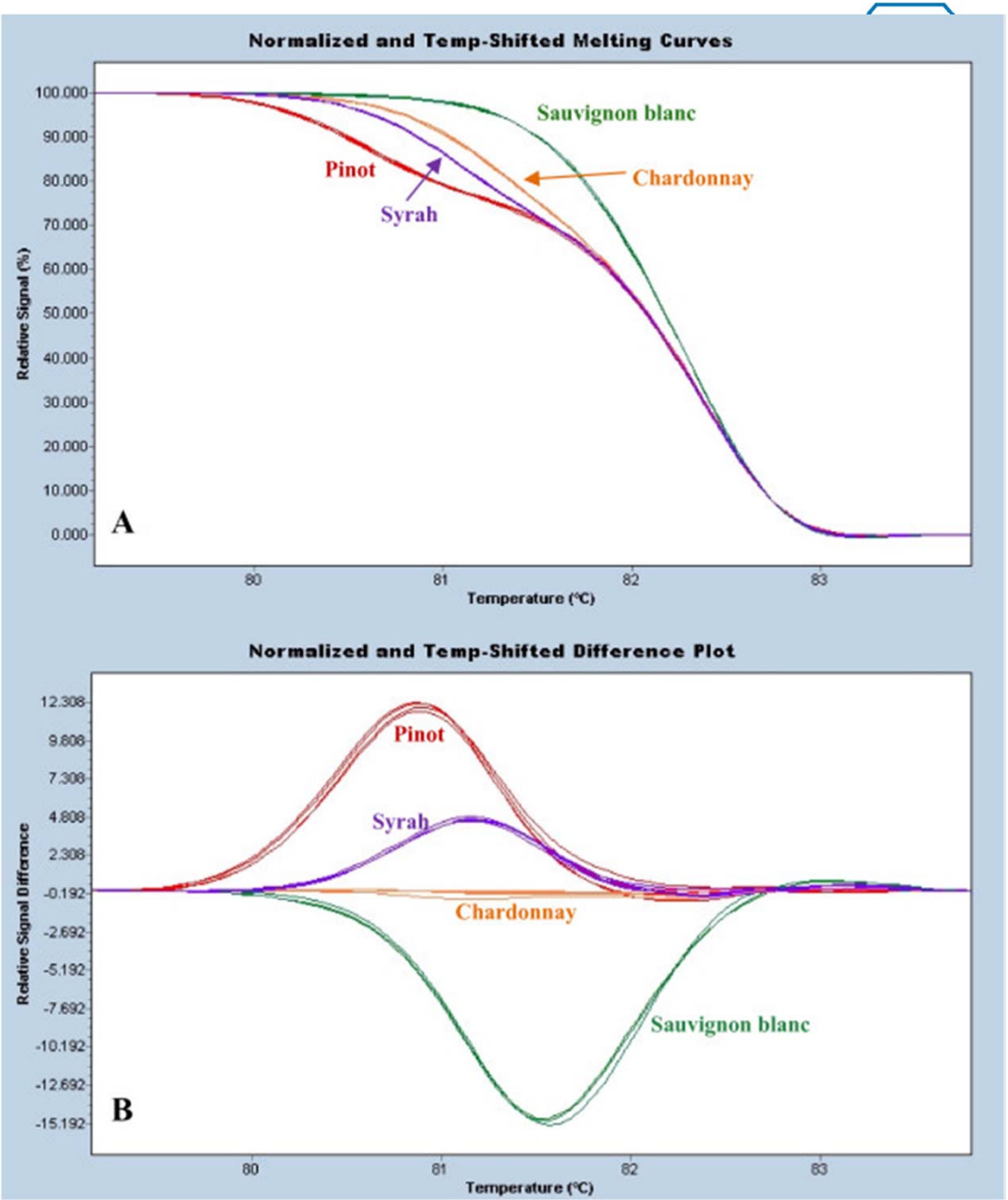


HRM 3 - Sensitivity testing

Mutations identified in 650bp product
(samples shown in replicates)



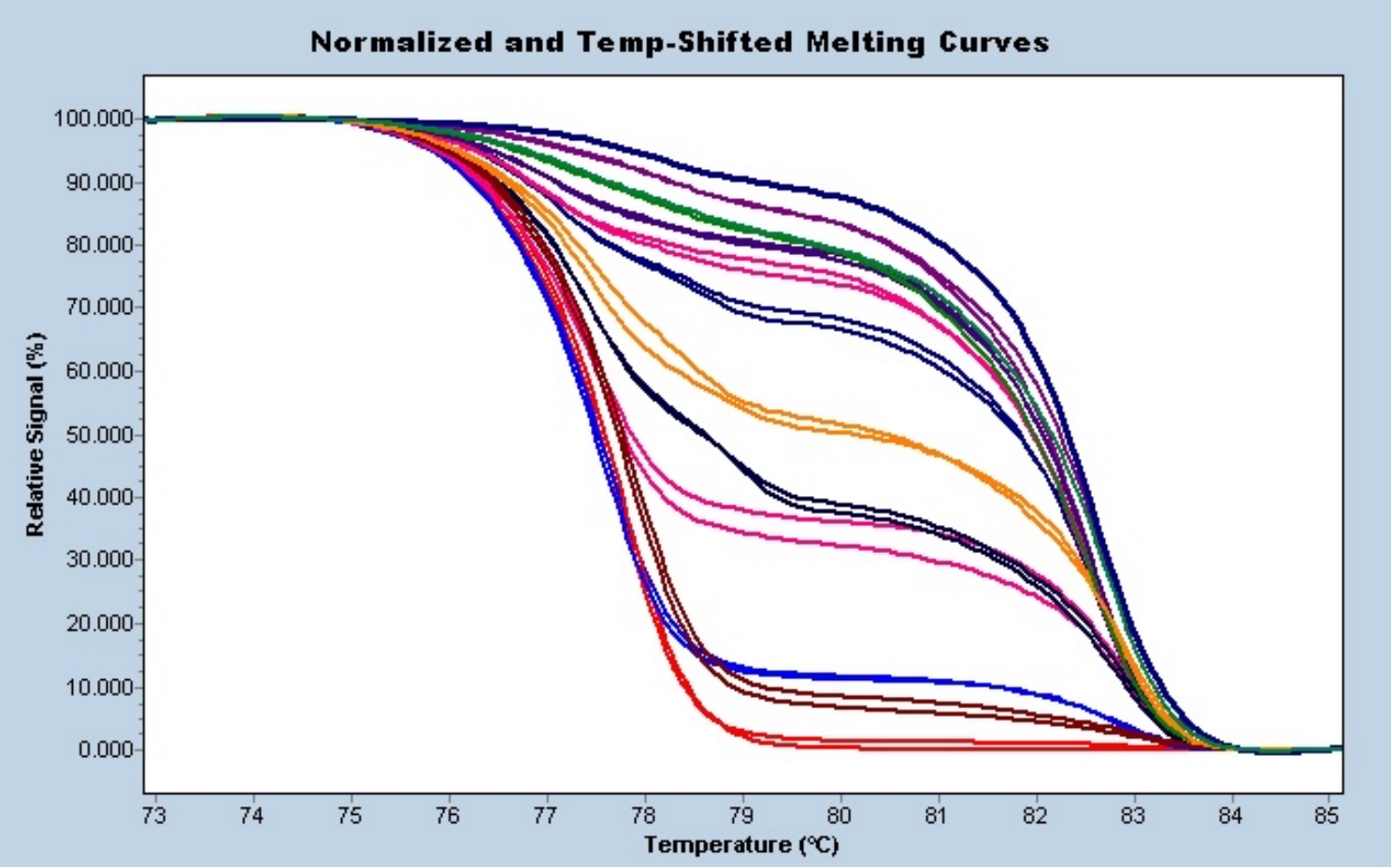
HRM of grape varieties

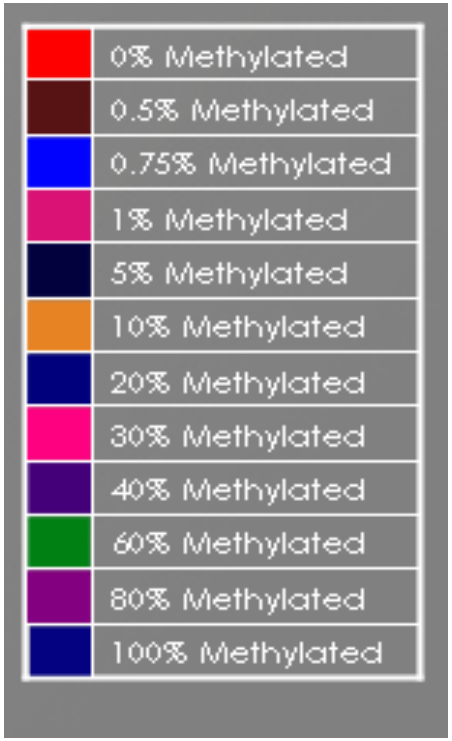


From: Plant Methods. 2008; 4: 8.

HRM Data – 5

DNA Methylation

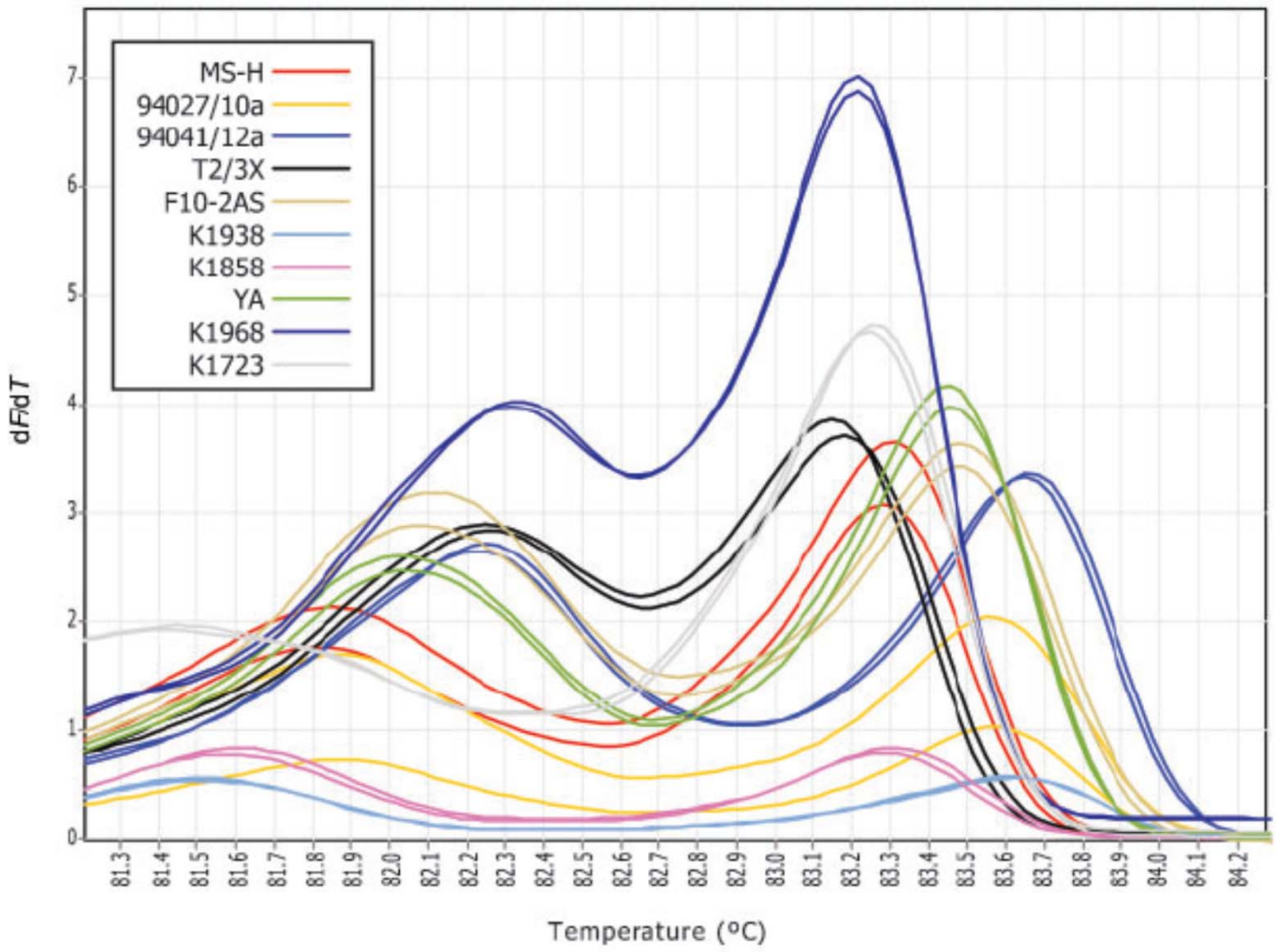




www.roche-applied-science.com

HRM Data – 6

Mycoplasma synoviae strain identification – Jeffery et al (2007)



Roche

Diagnostics

Utility

- Optimization requirements
- Data and Results
- **Possibilities**
- References and Papers

High Resolution Melting
Key applications

- Scan genes to discover SNPs and/or somatic mutations
- Genotyping of known SNPs
- Characterization of haplotype blocks – “hap maps”
- DNA methylation analysis
- DNA mapping
- Species identification/taxonomy
- HLA compatibility
- Screening for loss of heterozygosity
- Association (case/control) studies
- Allelic prevalence in a population
- Identification of candidate predisposition genes

Roche

Diagnostics

Utility

- Optimization requirements
- Data and Results
- Possibilities
- **References and Papers**

HRM References

- Gundry CN, Vandersteen JG, Reed GH, Pryor RJ, Chen J, Wittwer CT. Amplicon melting analysis with labeled primers: a closed-tube method for differentiating homozygotes and heterozygotes. Clin Chem. 2003 Mar;49(3):396-406.
- Wittwer CT, Reed GH, Gundry CN, Vandersteen JG, Pryor RJ. High-resolution genotyping by amplicon melting analysis using LCGreen. Clin Chem. 2003 Jun;49(6 Pt 1):853-60.
- Zhou L, Myers AN, Vandersteen JG, Wang L, Wittwer CT. Closed-tube genotyping with unlabeled oligonucleotide probes and a saturating DNA dye. Clin Chem. 2004 Aug;50(8):1328-35.
- Zhou L, Wang L, Palais R, Pryor R, Wittwer CT. High-resolution DNA melting analysis for simultaneous mutation scanning and genotyping in solution. Clin Chem. 2005 Oct;51(10):1770-7.
- Jeffery N, Gasser R, Steer P, Noormohammadi A. Classification of Mycoplasma synoviae strains using single-strand conformation plolymorphism and high-resolution melting-curve analysis of the vlhA gene single-copy region. Microbiology 2007 153, 2679-2688.

HRM References

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- Montgomery J, Wittwer CT, Palais R, Zhou L. Simultaneous mutation scanning and genotyping by high-resolution DNA melting analysis. Nat Protoc. 2007;2(1):59-66.

HRM References

- von Ahsen, N. Two for typing: homogeneous combined single-nucleotide polymorphism scanning and genotyping. Clin Chem 2005 51, 1761-1762.
- Herrmann, M.G., Durtschi, J.D., Bromley, L.K., Wittwer, C.T. & Voelkerding, K.V. Amplicon DNA melting analysis for mutation scanning and genotyping: cross-platform comparison of instruments and dyes. Clin Chem 2006 52, 494-503
- Dujols V, Kusukawa N, McKinney JT, Dobrowolsky SF, Wittwer CT. High-resolution melting analysis for scanning and genotyping., in Real Time PCR. Tevfik D, ed., Taylor and Francis, Abingdon, 2006.
- Reed GH, Wittwer CT. Sensitivity and specificity of single-nucleotide polymorphism scanning by high-resolution melting analysis. Clin Chem. 2004;50:1748-54.
- Reischl U. Melting of the ribosomal RNA gene reveals bacterial species identity: a step toward a new rapid test in clinical microbiology. Clin Chem 2006 52(11): 1985-7.

History – High-Resolution Melting

Diagnostics

Why High Resolution Melting?

Robust, non-destructive closed-tube method with many applications; highly informative and flexible. More convenient and cost-effective than current technologies, such as sequencing or dHPLC.

Why HRM on the LightCycler® 480 System?

Only plate-based Real-Time PCR HRM platform offering high-throughput HRM as a highly versatile, integrated system (hardware, software, reagents).

Utility

- Optimization requirements
- Data and Results
- Possibilities
- References and Papers
- **What this means for other real time Applications**

Real Time PCR, HRM, and Quantification

Diagnostics

- The technological and biochemical requirements for accurate and meaningful HRM studies are fulfilled by the LC 480 system.
- HRM Scanning is another software module that expands the capabilities of the LC 480, the premier real time PCR system on the market.
- The technologies that enable HRM also provide unsurpassed accuracy and consistency for the amplification, producing excellent quantitative data and results.

The LightCycler® 480 System

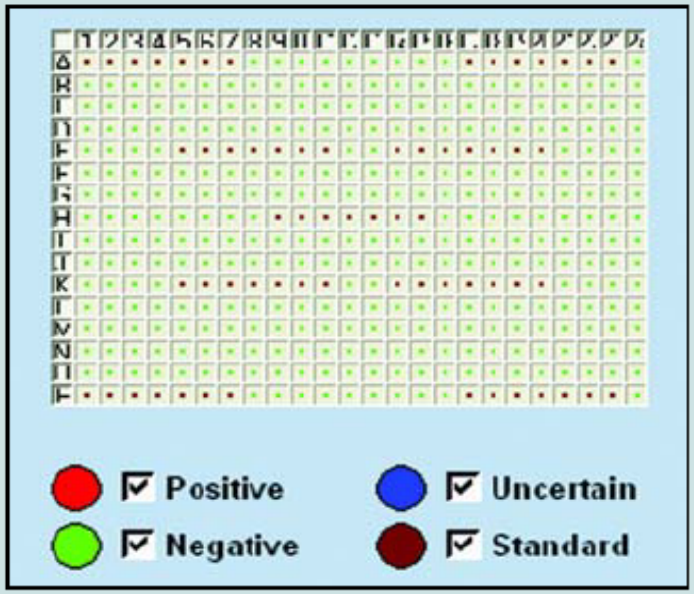
Data Homogeneity

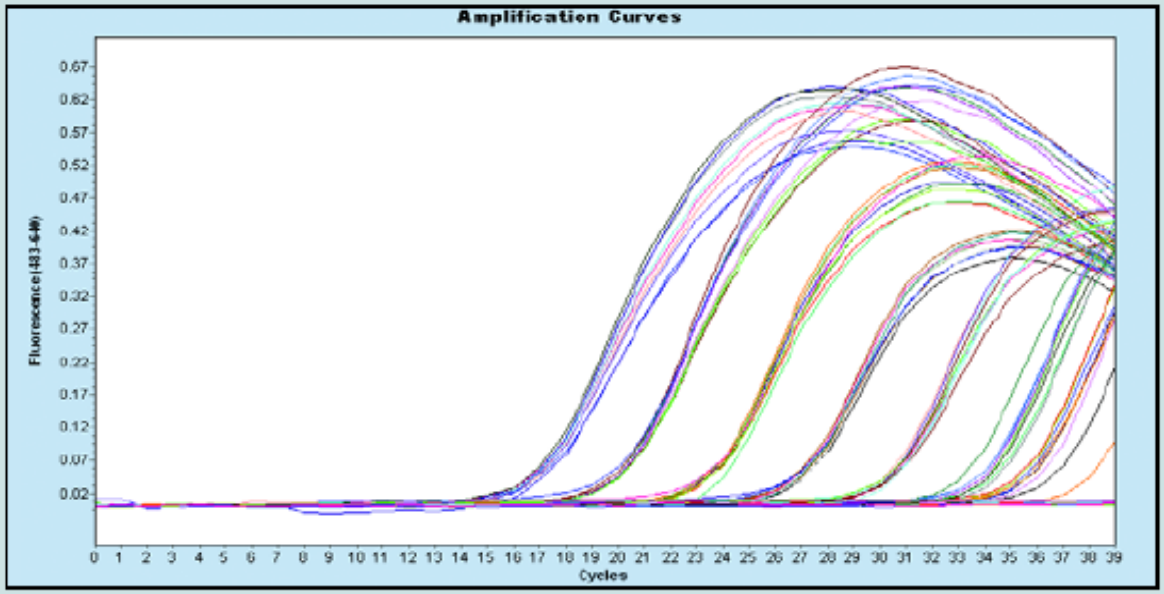
Roche

agnostics

Quantification analysis

"A Walk Around the Block"





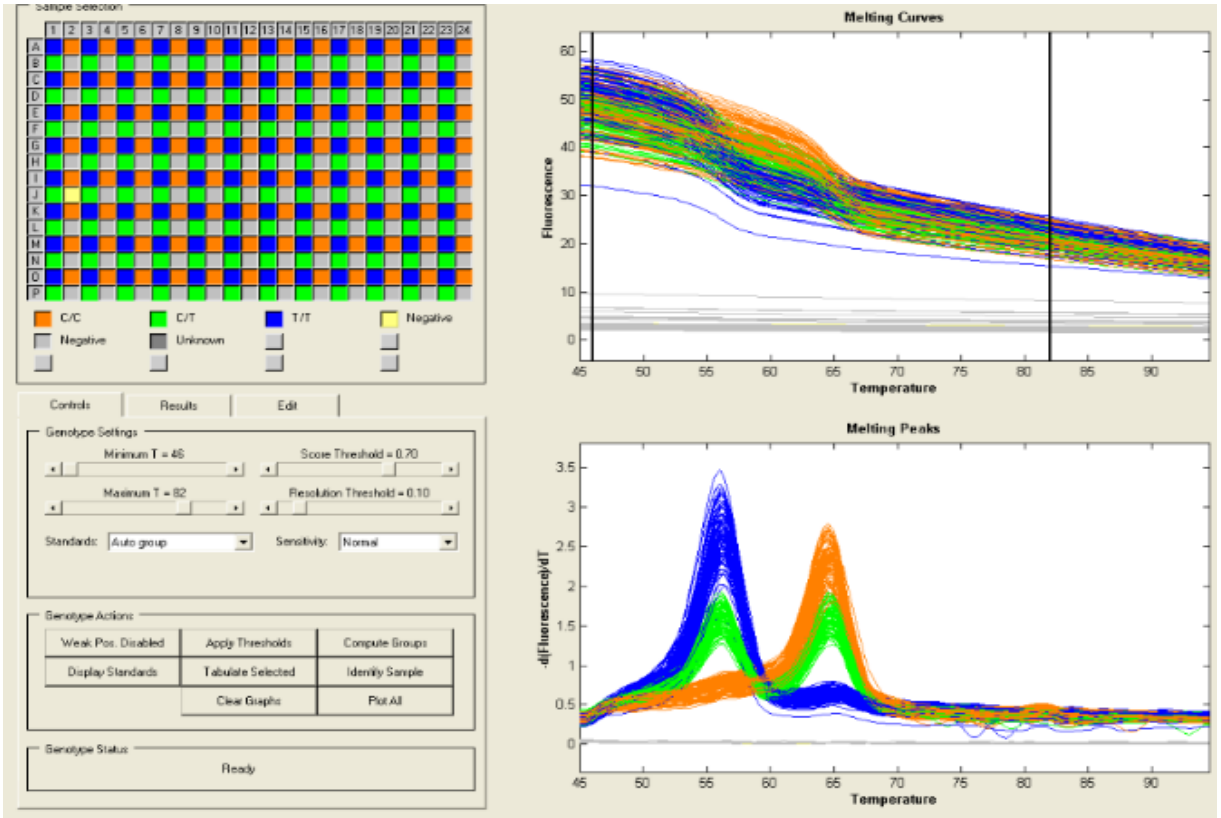
▲ **Figure 2: Reproducibility and sensitivity of real-time PCR on the LightCycler® 480 Instrument.** Serial dilutions of a viral target sequence (seven steps, 10^6 to 10^0 copies/20 µl) were amplified via PCR and detected with HybProbe probes. The whole dilution series was assayed in nine replicates positioned on different areas of the plate (*e.g.*, wells A1 to A7 corresponding to one of the nine replicates). The graphic illustration of the amplification curves shows that the position of a sample in the plate has no significant influence on the results, thus demonstrating very high well-to-well homogeneity over the entire block (*e.g.*, standard deviation for 100 copies: 0.11).

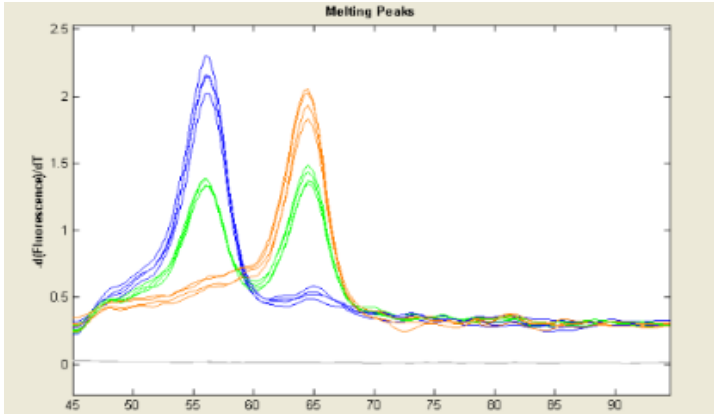
LightCycler® 480 Instrument

Temperature Homogeneity

Diagnostics

96-fold replicates of 3 genotypes



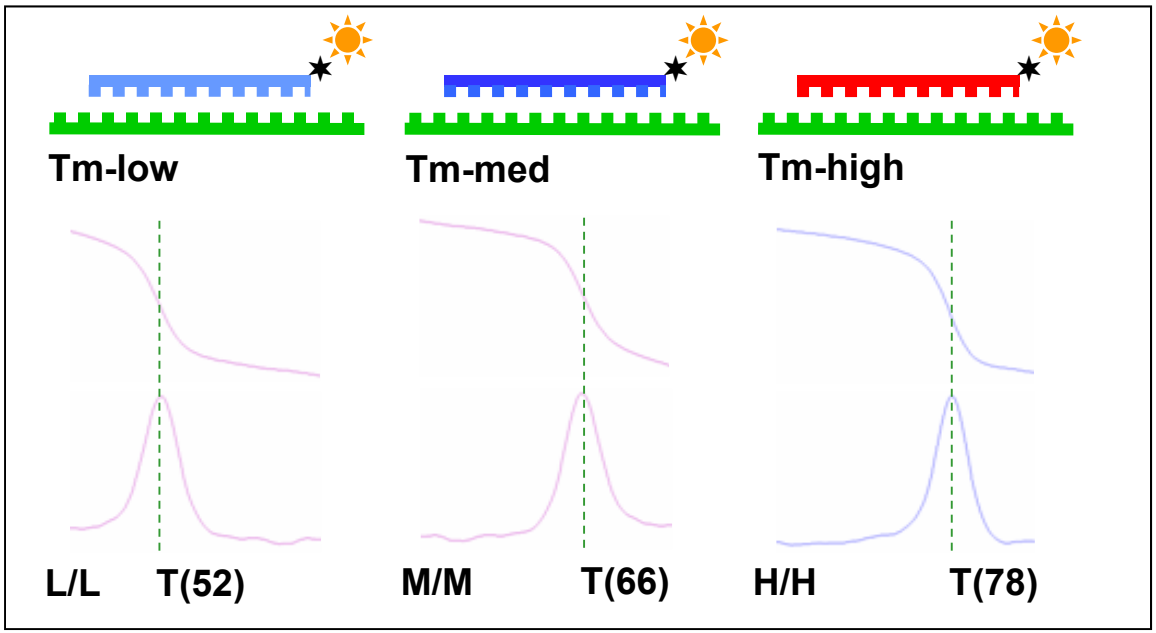


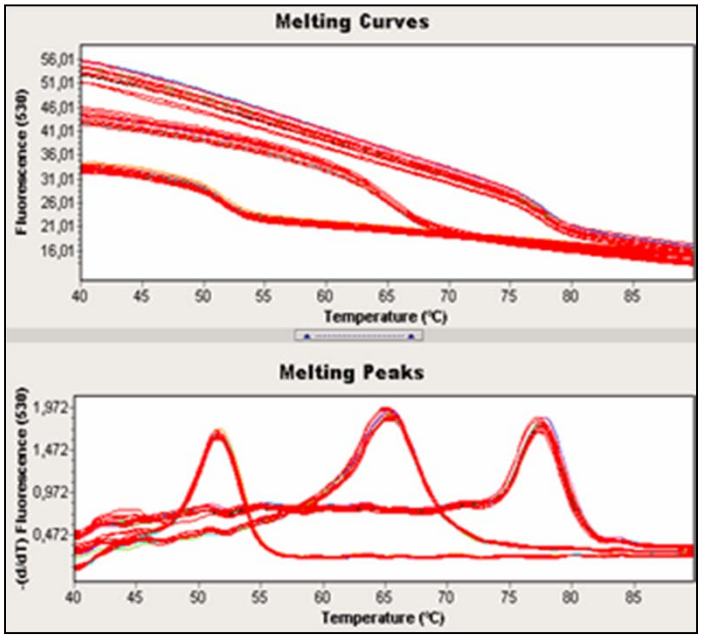
	Tm(1) / ° C	Tm(2) / ° C
average	**56.47**	**64.88**
minimum	**56.14**	**64.67**
maximum	**56.85**	**65.4**
delta	**0.71**	**0.73**
SD	**0.1612**	**0.1801**

Prototype Software

Thermal Homogeneity
Demonstration by Melting Curve Analysis

Diagnostics



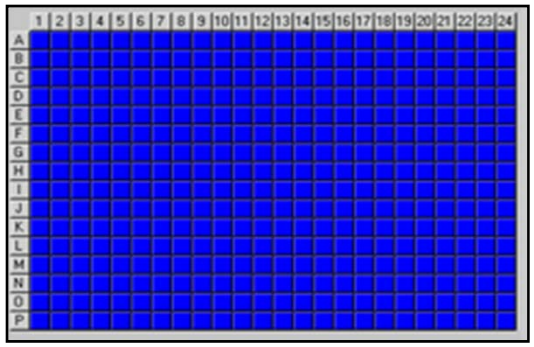


SimpleProbe probes, FAM-label

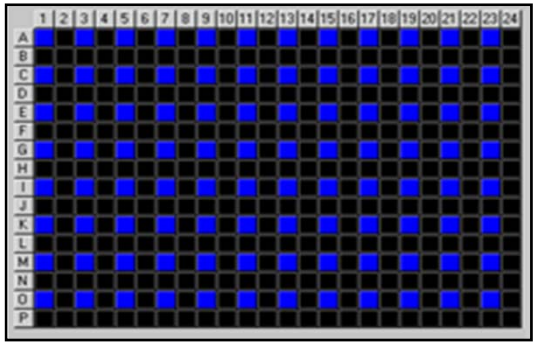
Thermal Homogeneity - Experimental Setup

Analysis of four 96-well Plate Subsets

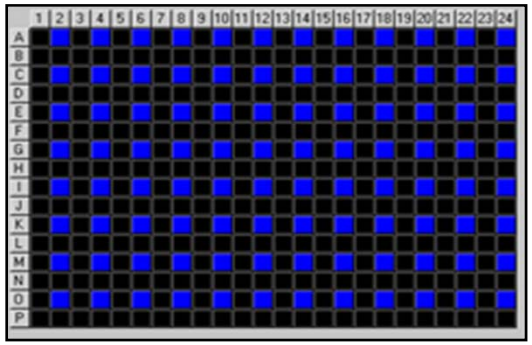
Diagnostics



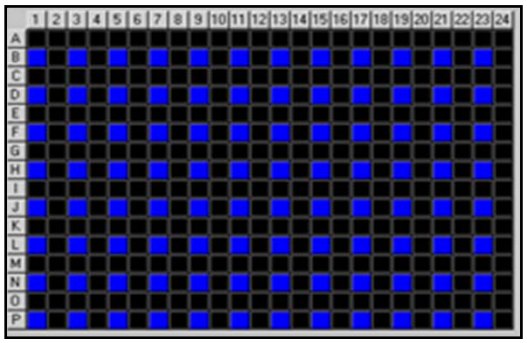
Total - 384 samples



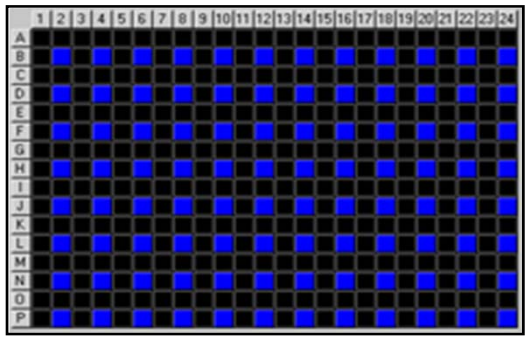
A1: 96 x Tm-low (52° C)



A2: 96 x Tm-high (78° C)



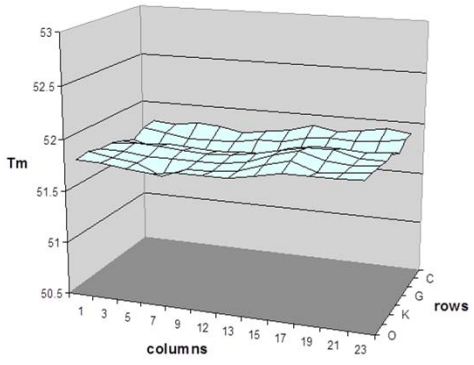
B1: 96 x Tm-med (66° C)



B2: 96 x negative control

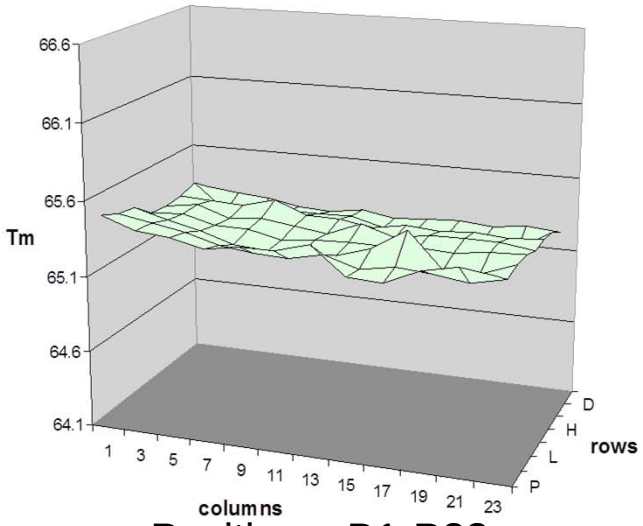
Thermal Homogeneity – LightCycler 480
Intra-Run Reproducibility of 96 Replicates

Diagnostics



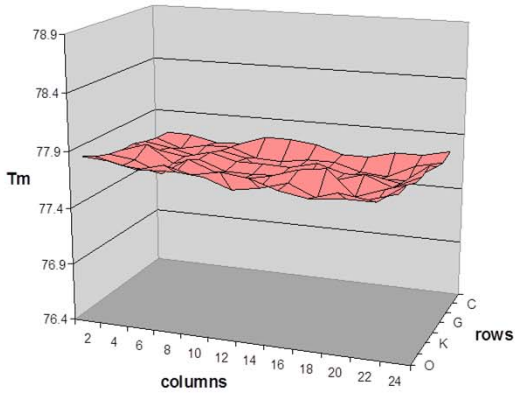
Positions: A1-O23

	values / °C
average	51.80
min	51.63
max	51.96
delta	**0.33**
SD	0.0722



Positions: B1-P23

	values / °C
average	65.31
min	65.14
max	65.52
delta	**0.38**
SD	0.0731

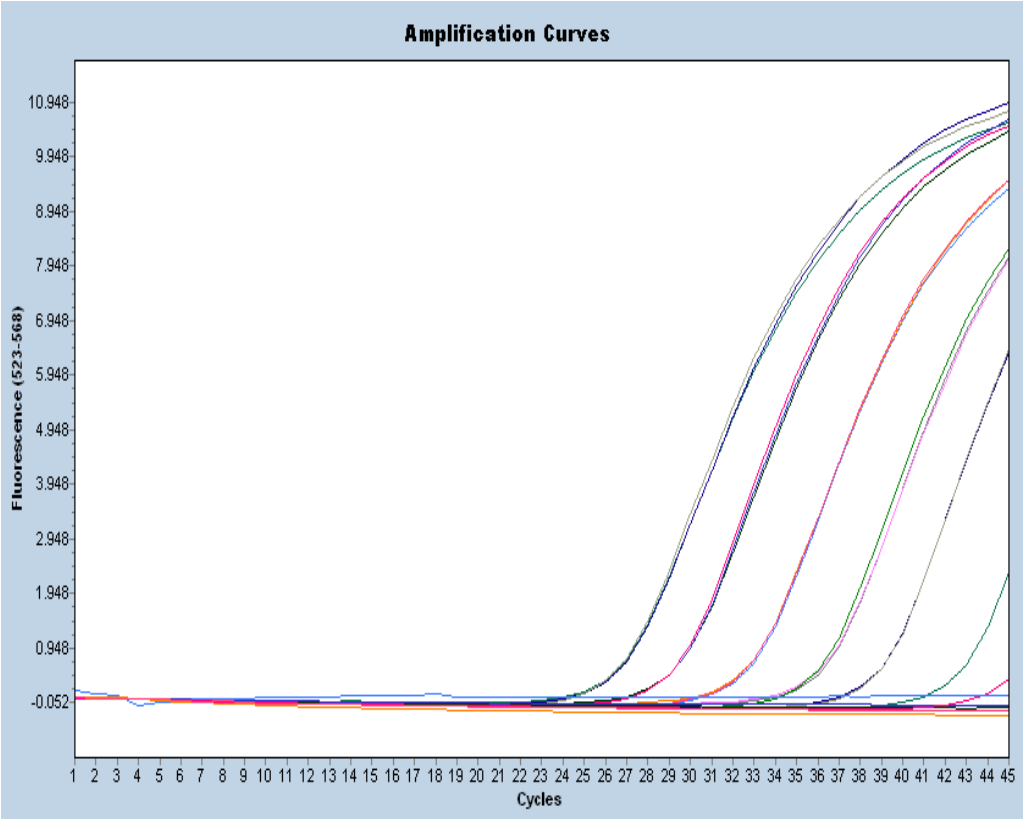


Positions: A2-O24

	values / °C
average	77.69
min	77.46
max	77.87
delta	**0.41**
SD	0.0801

Data Uniformity

Dilution Series/Neighboring Wells – 165 bp target



Experiment:

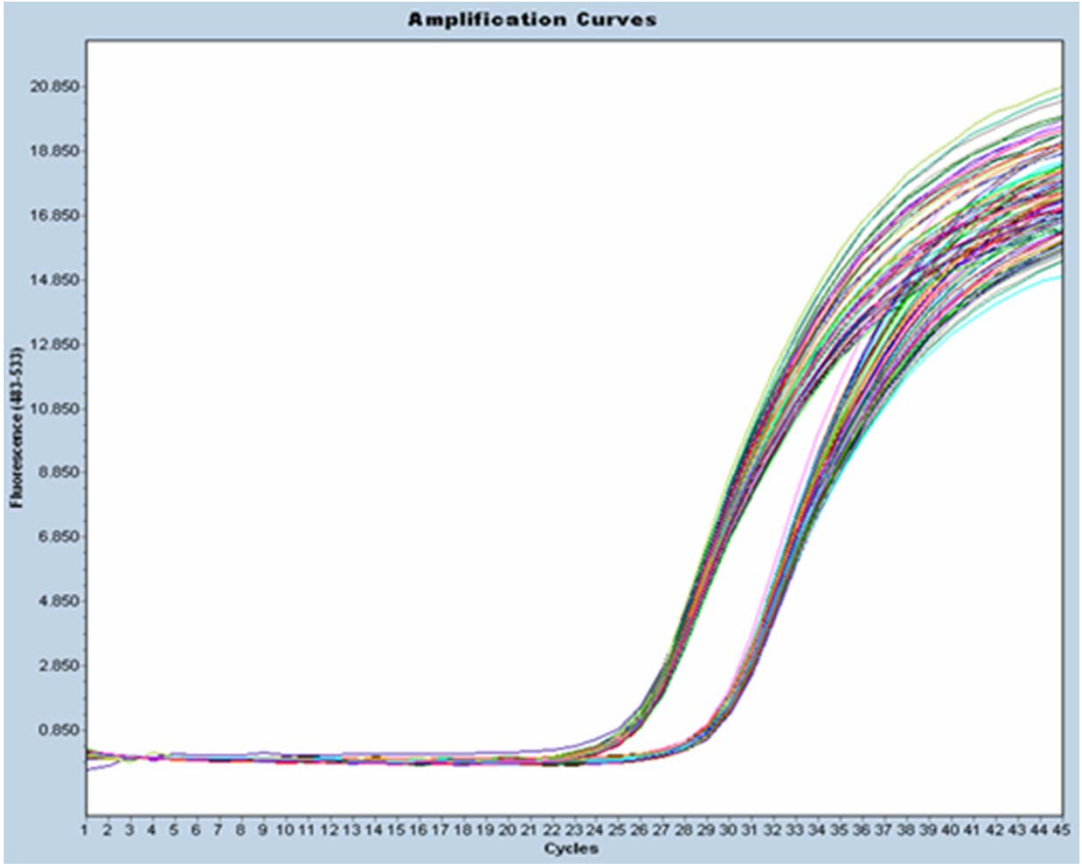
- Serial 10-fold dilutions
- 3 replicates
- Target: Cyp2C9.2; 165 bp long fragment
- Fast & Standard protocol (Hydrolysis Probe Format)
- Samples in neighboring wells

LightCycler® 480 (96): 55 min

Data Uniformity

Two Copy Numbers/Spread Across Plate – 442 bp target

Diagnostics



Experiment:

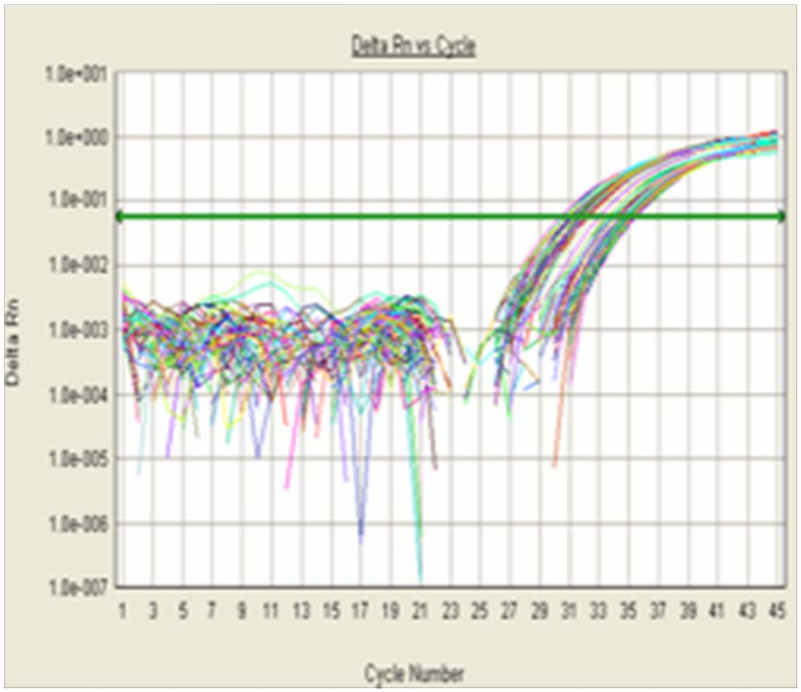
- Samples in checkerboard pattern
- 1000 & 100 copies
- 48 replicates
- Target: CycA; 442 bp long fragment
- Fast & Standard protocol (Hydrolysis Probe Format)
- Samples in neighboring wells

LightCycler® 480 (96): 55 min

Data Uniformity

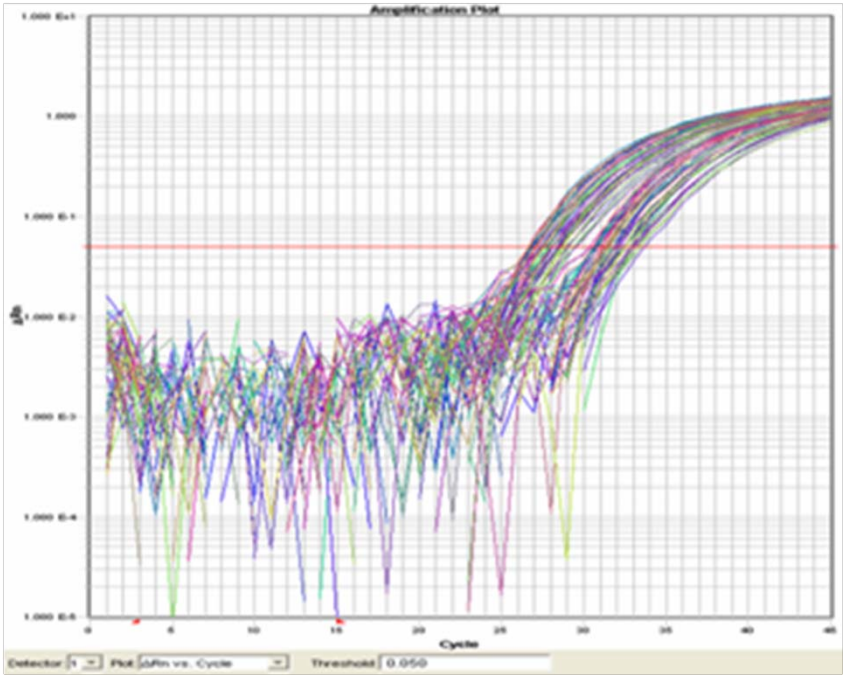
Two Copy Numbers/Spread Across Plate – 442 bp target

Diagnostics



ABI 7900 (96): 90 min

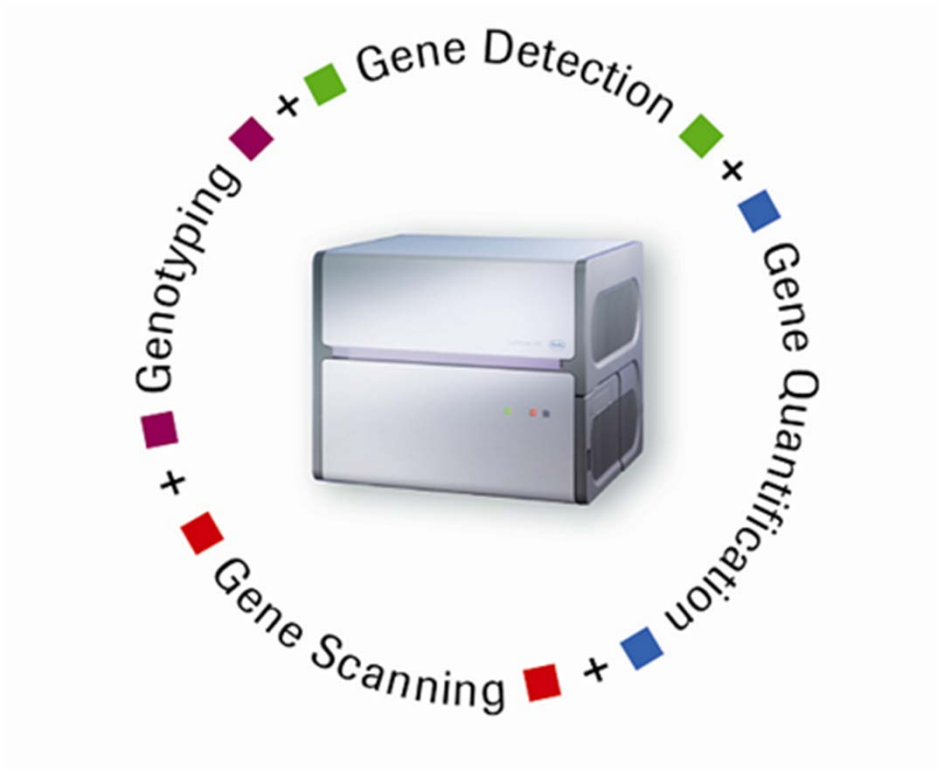
AB 7900 (96) Fast: 44 min



Experiment:

- Samples in checkerboard pattern
- 1000 & 100 copies
- 48 replicates
- Target: CycA; 442 bp long fragment
- Fast & Standard protocol (Hydrolysis Probe Format)
- Samples in neighboring wells

LightCycler® 480 System Applications



- Gene Detection: Detecting *e.g.*, bacteria in sample material
- Gene Expression: Analyzing expression level of gene of interest
- Genotyping: Detecting known variants
- Gene Scanning: Finding new variants

Diagnostics

Credits

- **Special thanks for contributions for this presentation:**
  - **Natalie Barnes** – RAS Australia Systems Account Representative
  - **Dr. Michael Hoffman** – RAS Global Marketing Manager
  - **Roche Applied Science US Technical Support**
    - **Bill Demyan, Ph.D**
    - **Joe Donnenhofer**
    - **Alex Pierson**
    - **Michelle Moore**
    - **Duane Marks**
  - **Dr. Oliver Geulen** – RAS Global Training and Applications
  - **Steve Hurwitz** – RAS US LightCycler Manager
  - **John Ogden, Ph.D** – RAS US Genomics Marketing Manager

HRM Genotyping – History, Technology, and Utility

Diagnostics

- Questions?
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