

# LightCycler 2.0 System

*Built on experience, designed for the future*

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## | The LightCycler 2.0 System



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## LightCycler System for

100µl / 20µl Capillaries

Multicolor Detection / Multiplex-PCR

Fast and Accurate Quantification

Flexible Detection Formats

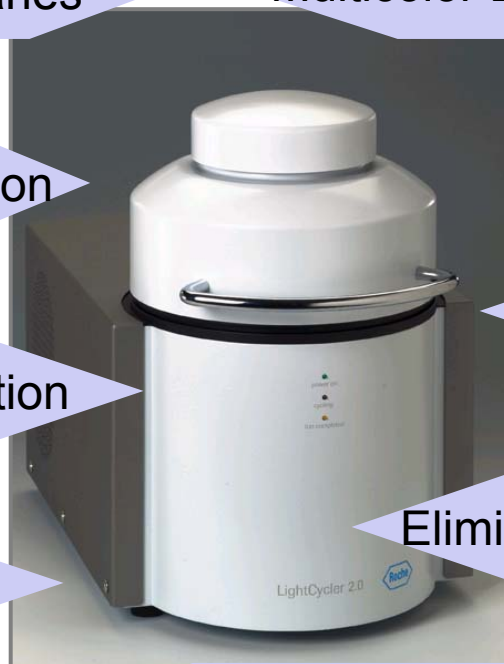
Mutation & SNP Detection

Generic Reagents

Research & Food Safety

Elimination of Contamination Risk

Rapid Real-Time PCR



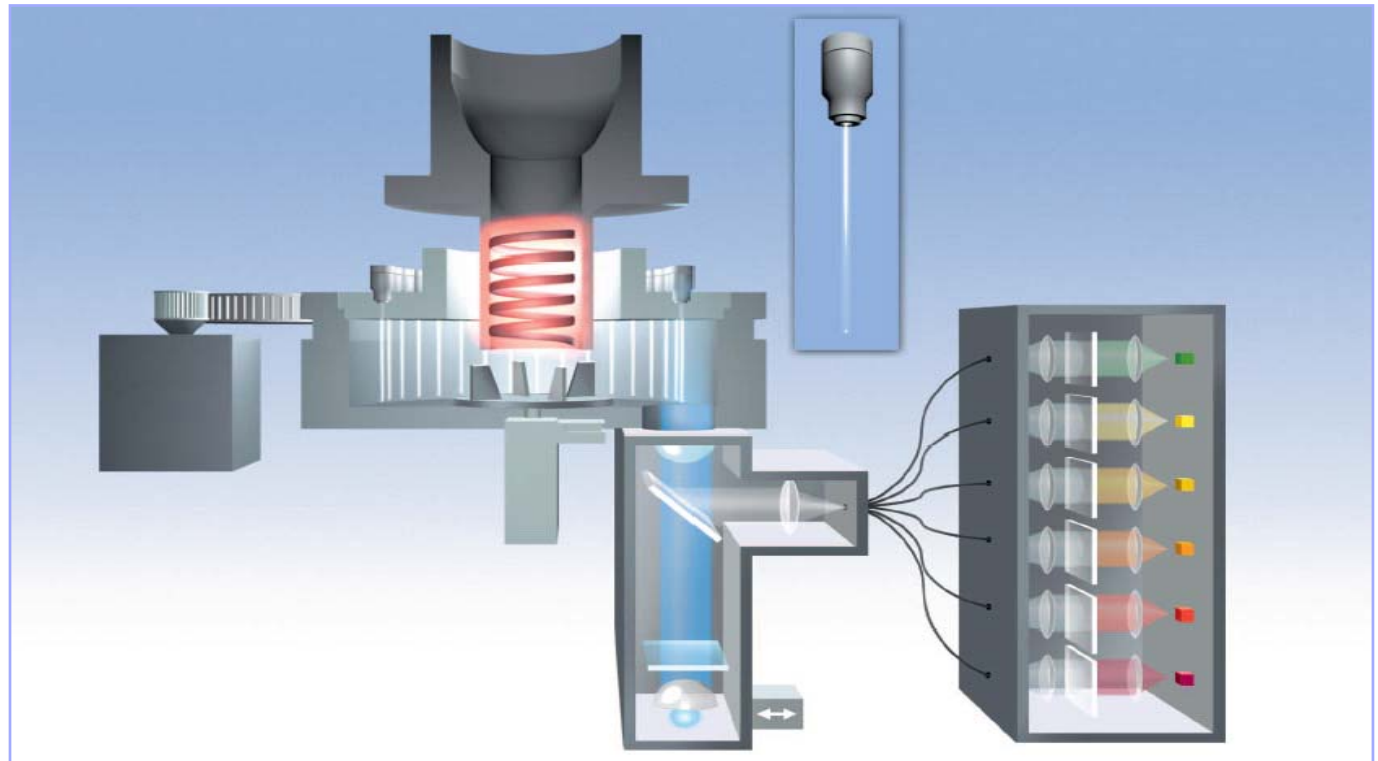
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## Schematic of LightCycler Instrument



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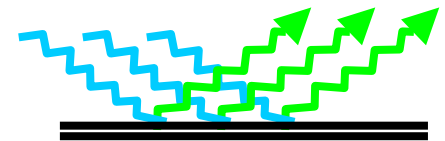
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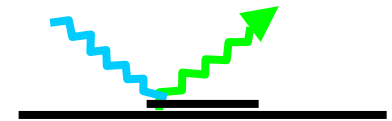
## Entering into a new dimension: LightCycler Assay Formats



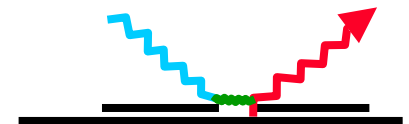
- SYBR Green I



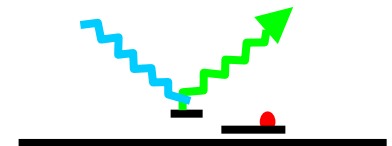
- SimpleProbe Probes



- HybProbe Probes



- Hydrolysis Probes



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## LightCycler Assay Formats, Detection Channels, Dyes and Applications

Assay Format	Detection Channel	Reporter Dye	Application
SYBR Green I	530	SYBR Green I	Product Characterization Quantification
Hybridization Probes (HybProbe)	610 640 670 710	LightCycler RED 610 LightCycler RED 640 LightCycler RED 670 LightCycler RED 705	Quantification Mutation Analysis
Hydrolysis Probes (TaqMan)	530 560	FAM VIC, HEX	Quantification (Mutation Analysis)
Single Label Probes (SimpleProbe)	530	Fluorescein	Mutation Analysis

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## Improve Your Results with SW 4.0

- **Integration of data and User Management into the Software:**
  - data base instead of files, no access to raw data
  - flexible user management
- **Improved Workflow:**
  - modular organisation of experiments matching typical workflow
  - creation of templates and macros
- **Expansion of Analysis Options:**
  - analysis methods available as modules
  - performed analysis saved within the experiment
  - easier interpretation of results

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Choose the Application Type to create



## Amplification Analysis

- Absolute Quantification
- Qualitative Detection
- Relative Quantification-Dual Color
- Relative Quantification-Monocolor

## Melting Curve Analysis

- Genotyping
- T<sub>m</sub> Calling

## Other Methods

- Color Compensation
- Nucleic Acid Quantification

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## Modules for Data Acquisition during Amplification

- Automatic pos/neg differentiation for improved Cp calling
- Optional Control Concept, e.g.:
  - Run Controls  
Positive Control, Negative Control, Standard can be defined  
Only if these controls are “Success“, the results are called as “Valid“
  - Internal Control  
use of a control in the same capillary to verify the reaction  
(dual color experiment)

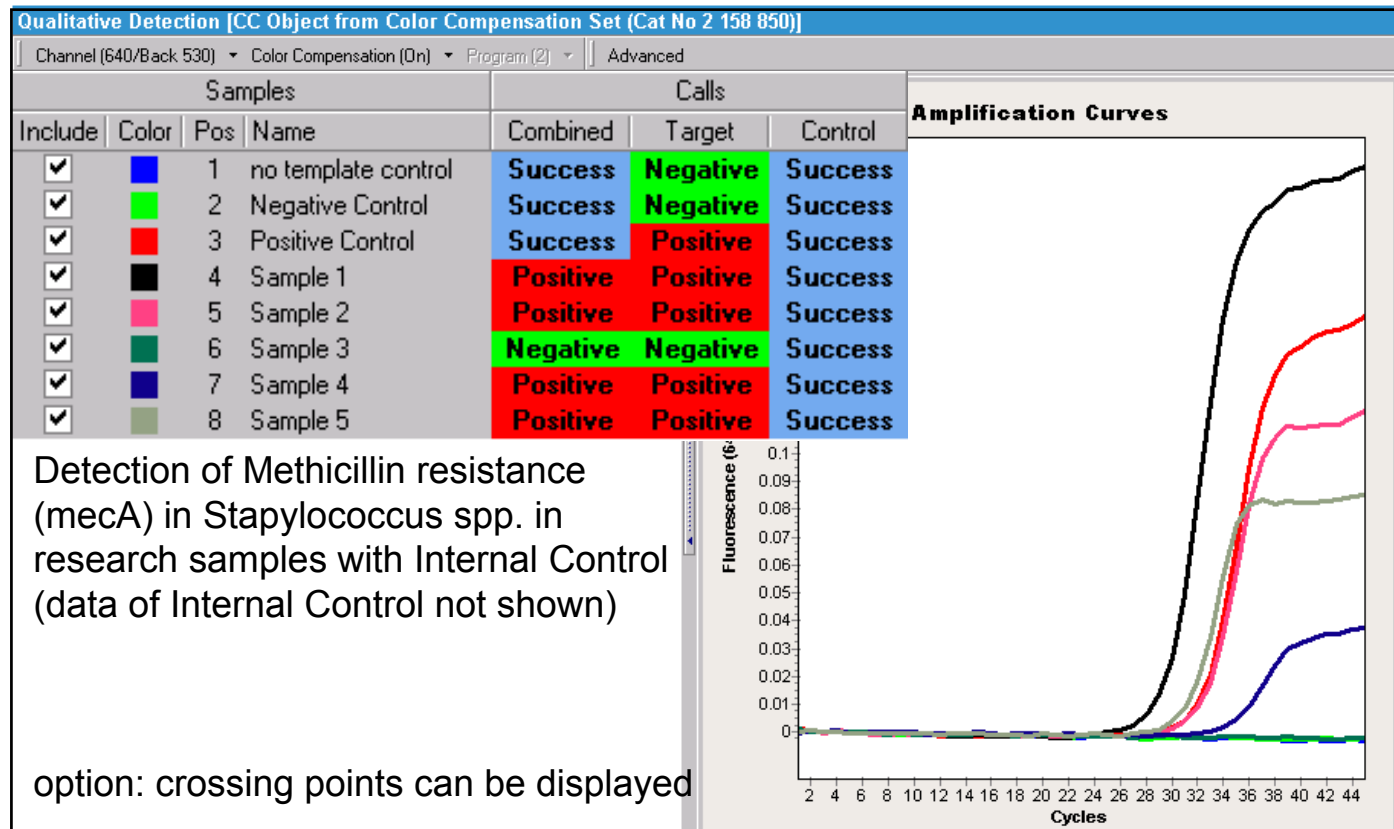
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## Example: Qualitative Analysis with Internal Control

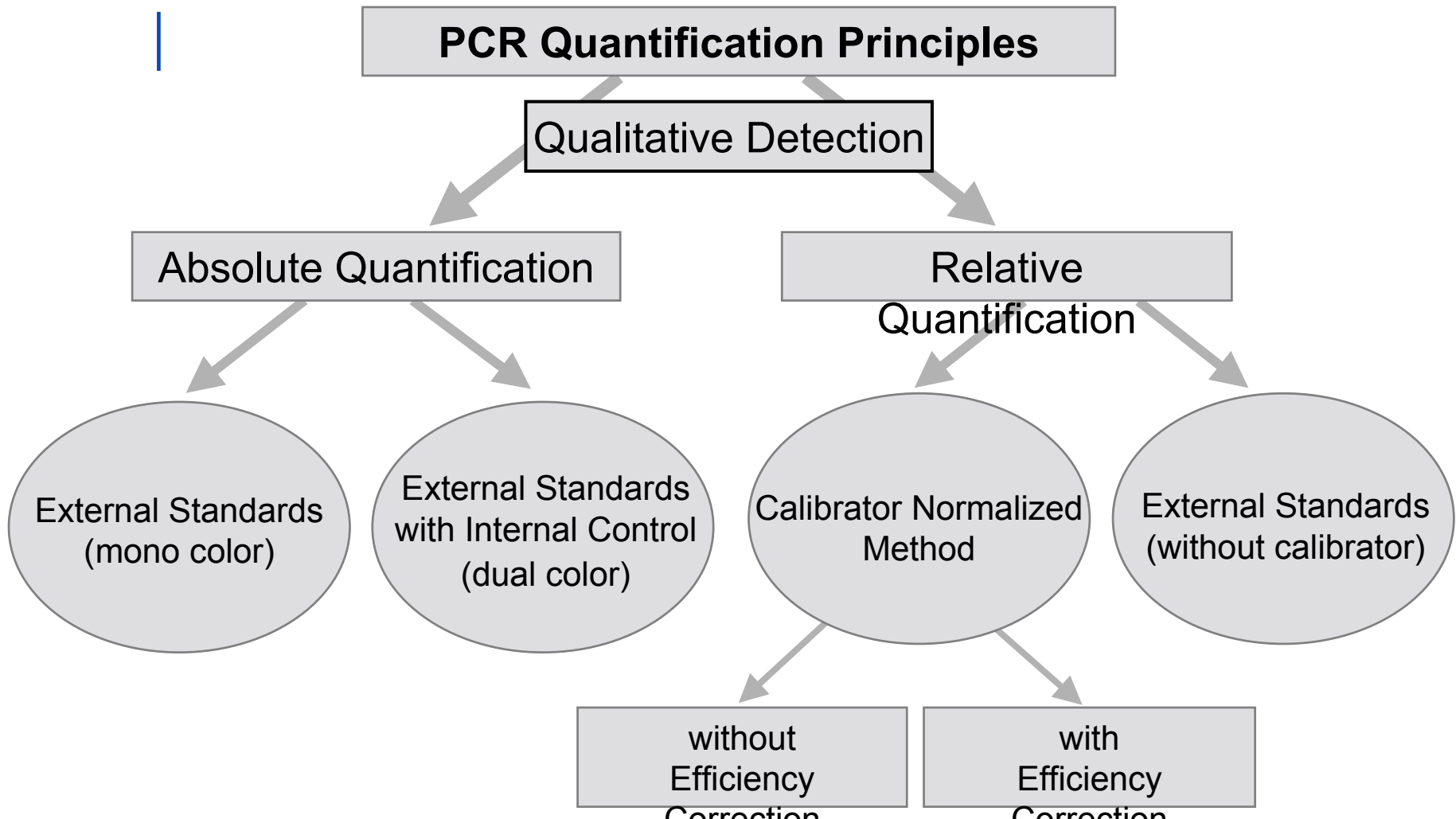


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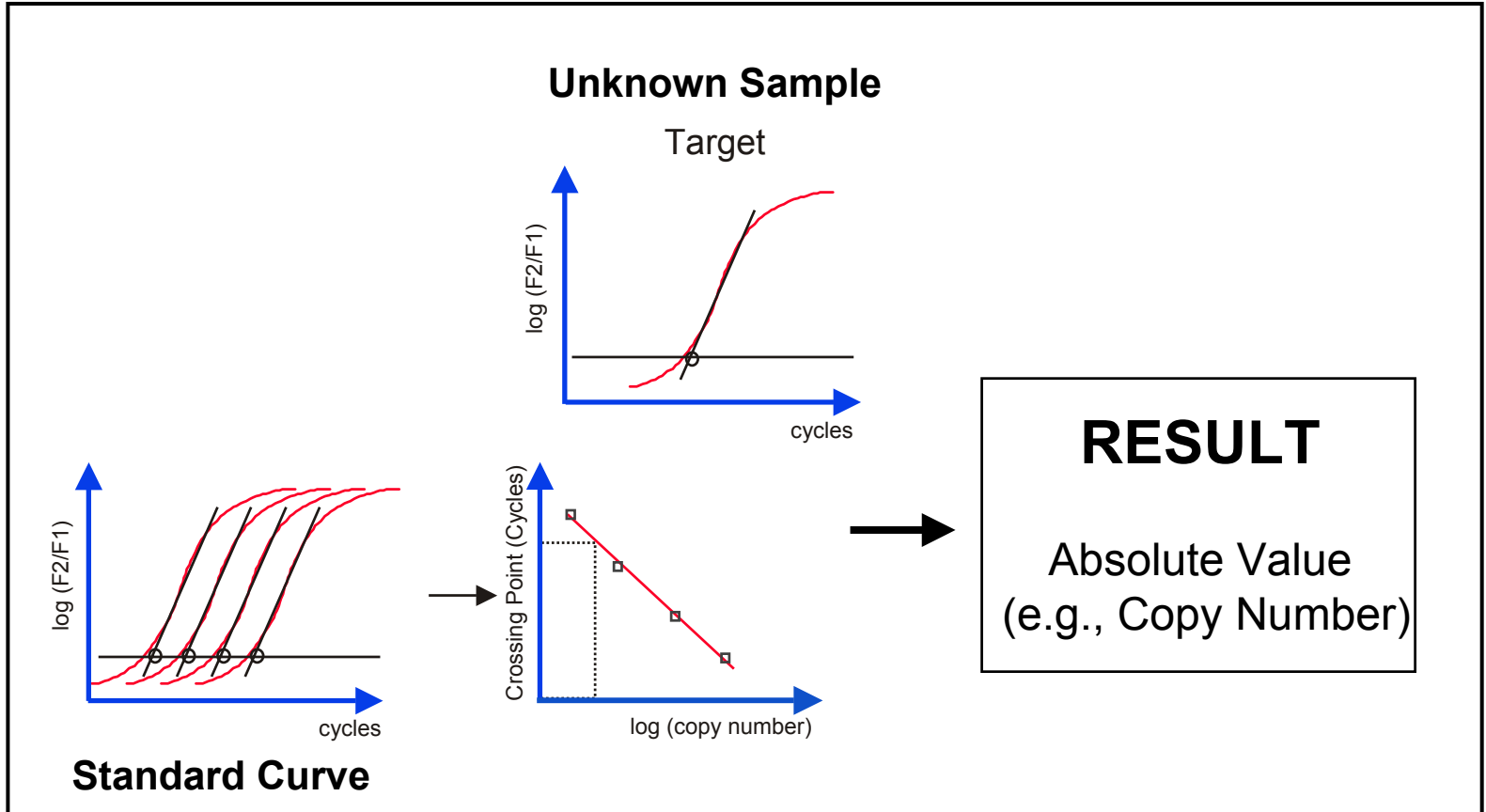
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## Absolute Quantification with External Standards: Principle



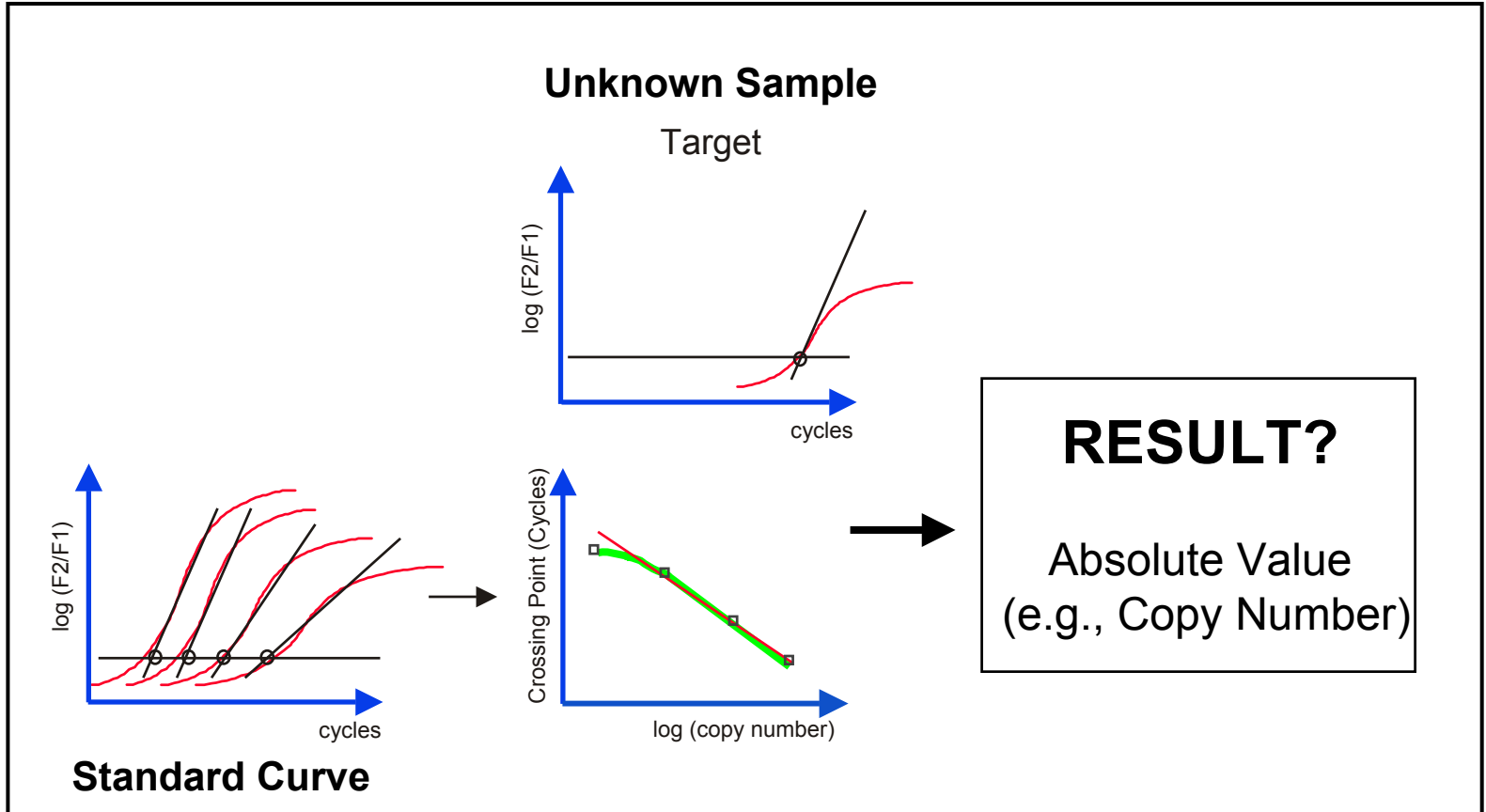
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## Data depending Phenomenon: PCR Efficiency



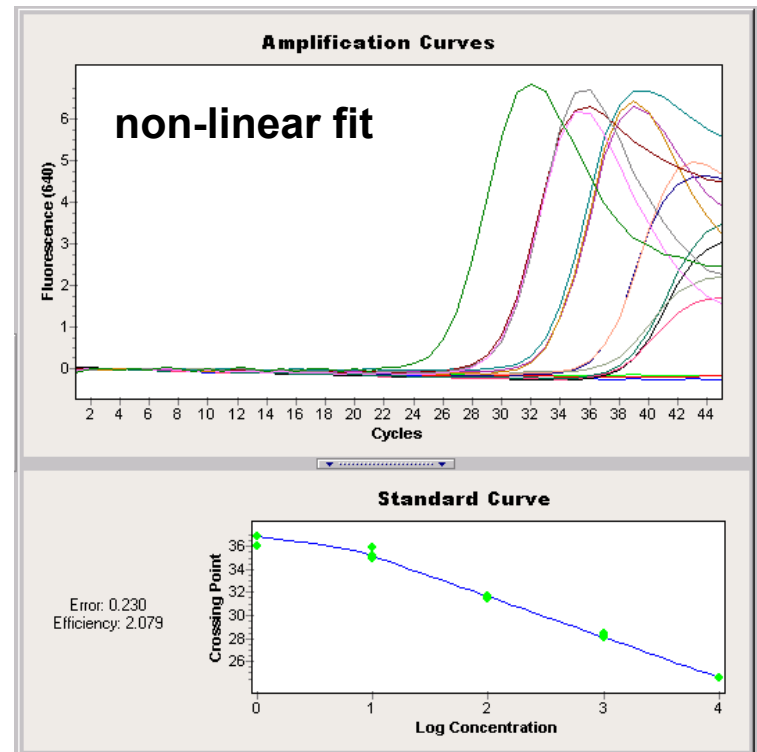
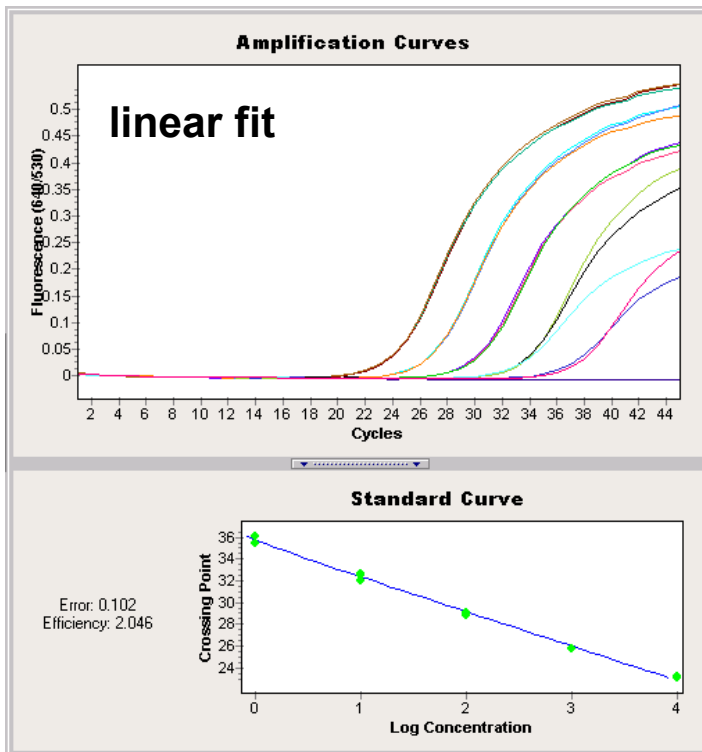
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## Standard Curves: Curve Fit Depending on Data



- standards in the same experiment, or
- import of a previously saved standard curve object

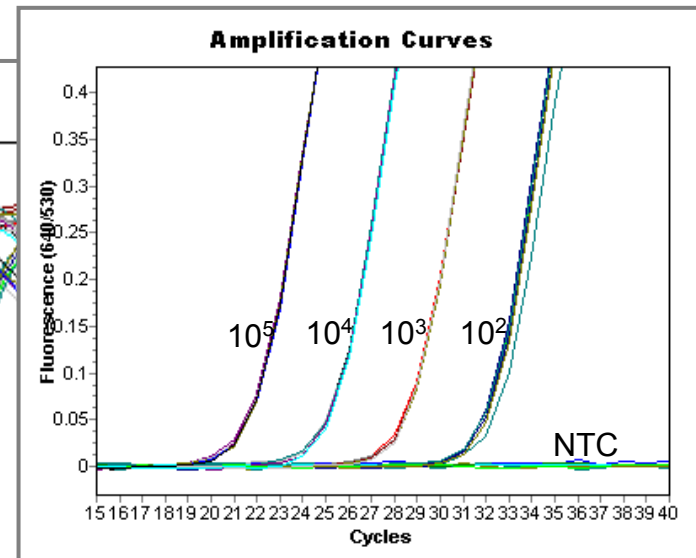
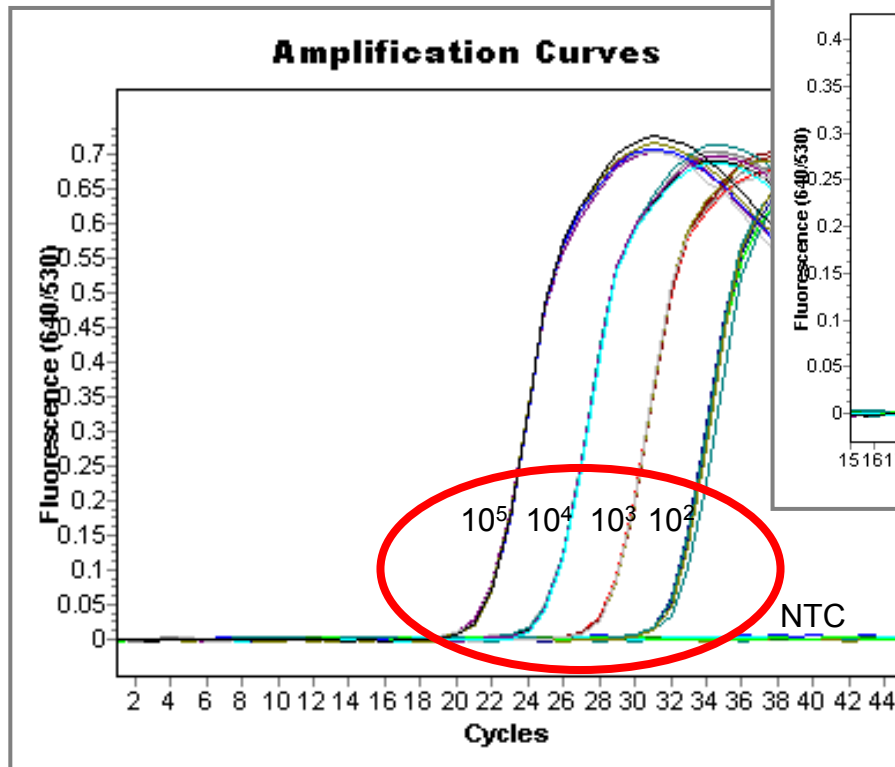
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## Reproducibility of LightCycler PCR



Data show replicates (4x) of plasmid DNA dilutions containing 10<sup>5</sup>-10<sup>2</sup> copies; statistics: all replicates with standard derivation below 0,04; Amplification with HybProbe Probes (LC Red 640); NTC: no template control

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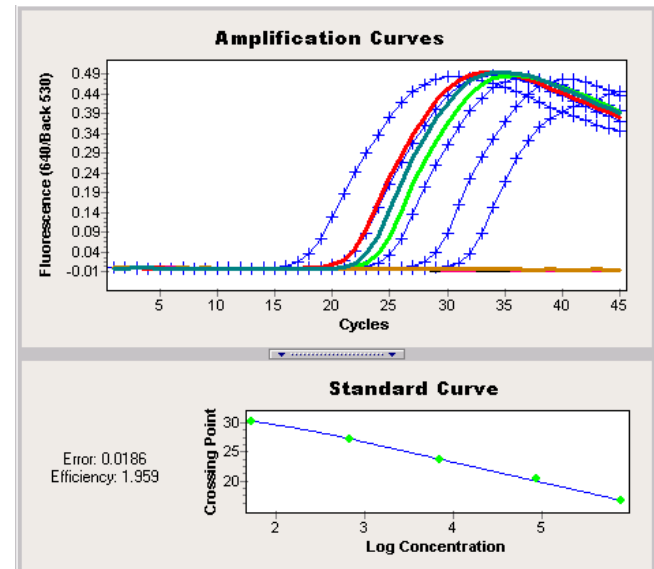
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## Example: Identification of False Negatives with Internal Control

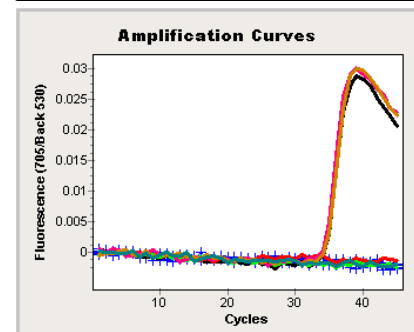
### Detection of Target (640)

Samples				Results			Calls		
Include	Color	Pos	Name	CP	Conc (copies/rxn)	Standard	Combined	Target	Control
<input checked="" type="checkbox"/>			1 no template control				Success	Negative	Success
<input checked="" type="checkbox"/>	Blue		2 Standard 1	16.69	8.31E5	7.70E5	Success	Positive	Success
<input checked="" type="checkbox"/>	Blue		3 Standard 2	20.33	7.17E4	8.50E4	Success	Positive	Success
<input checked="" type="checkbox"/>	Blue		4 Standard 3	23.64	7.76E3	7.00E3	Success	Positive	Success
<input checked="" type="checkbox"/>	Blue		5 Standard 4	27.30	6.64E2	6.70E2	Success	Positive	Success
<input checked="" type="checkbox"/>	Blue		6 Standard 5	30.26	5.30E1	5.30E1	Success	Positive	Success
<input checked="" type="checkbox"/>	Black		7 Negative Control				Success	Negative	Success
<input checked="" type="checkbox"/>	Red		8 Sample 1	20.21	7.78E4		Positive	Positive	Success
<input checked="" type="checkbox"/>	Green		9 Sample 2	22.60	1.56E4		Positive	Positive	Success
<input checked="" type="checkbox"/>	Pink		10 Sample 3				Negative	Negative	Success
<input checked="" type="checkbox"/>	Orange		11 Sample 4				Negative	Negative	Success
<input checked="" type="checkbox"/>	Teal		12 Sample 5	21.59	3.07E4		Positive	Positive	Success



### Detection of Internal Control (705)

Samples				Results			Calls		
Include	Color	Pos	Name	CP	Conc (copies/rxn)	Standard	Combined	Target	Control
<input checked="" type="checkbox"/>			1 no template control				Success	Negative	Success
<input checked="" type="checkbox"/>	Blue		2 Standard 1				Negative	Negative	Success
<input checked="" type="checkbox"/>	Blue		3 Standard 2				Negative	Negative	Success
<input checked="" type="checkbox"/>	Blue		4 Standard 3				Negative	Negative	Success
<input checked="" type="checkbox"/>	Blue		5 Standard 4				Negative	Negative	Success
<input checked="" type="checkbox"/>	Blue		6 Standard 5				Negative	Negative	Success
<input checked="" type="checkbox"/>	Black		7 Negative Control	32.25			Success	Positive	Success
<input checked="" type="checkbox"/>	Red		8 Sample 1				Success	Negative	Success
<input checked="" type="checkbox"/>	Green		9 Sample 2				Success	Negative	Success
<input checked="" type="checkbox"/>	Pink		10 Sample 3	32.10			Success	Positive	Success
<input checked="" type="checkbox"/>	Orange		11 Sample 4	32.33			Success	Positive	Success
<input checked="" type="checkbox"/>	Teal		12 Sample 5				Success	Negative	Success



## Relative Quantification of Gene Expression

- **Goal: Normalized expression level of a target RNA**

$$\longrightarrow \frac{\text{Amount of target RNA in a sample}}{\text{Amount of housekeeping RNA in a sample}}$$

Note: Same approach can be used to determine gene amplification relative to a single copy gene, or to measure the amount of a target nucleic acid per e.g. cell equivalent.

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## Errors, Generated by Differences in PCR Efficiency

**Errors of quantification relative to a theoretical PCR efficiency of 2.00**

Detection Cycle (n)	10	15	20	25	30	35
PCR efficiency (E)						
2.00	-	-	-	-	-	-
1.97	16%	25%	35%	46%	57%	70%
1.95	29%	46%	66%	88%	113%	142%
1.90	67%	116%	179%	260%	365%	500%
1.80	187%	385%	722%	1290%	2260%	3900%
1.70	408%	1045%	2480%	5710%	13.000%	29.500%
1.60	830%	2740%	8570%	26.400%	80.700%	246.400%

Error calculation:  $(2^n/E^n - 1) \times 100$

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## Relative Quantification: Monocolor / Dual Color

- Automatic determination of target gene vs. reference gene ratio
  - normalized to a calibrator
  - corrected for different PCR efficiencies of target and reference gene
- No need to include standard dilutions in each analysis run (one calibrator only)
- Flexible/multiple target - reference groupings possible
- With or without efficiency correction (use of in run or external standard curves)

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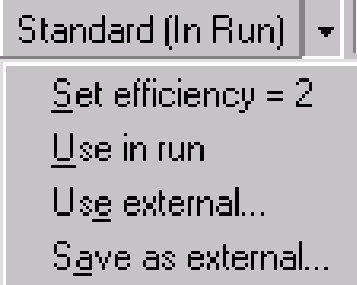
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## Relative Quantification: Standard Curves - Efficiency Correction

Options:



- **without** efficiency correction → Set efficiency = 2
- **with** efficiency correction →
  - Use in run: Standard curve generated from standards in the current experiment
  - Use external: imported standard curve object
    - delivered with Roche Kits
    - customer specific (save as external)

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## Relative Quantification: Analysis

- Flexible pairing of target and reference samples possible

Relative Quantification - Monocolor

Group 1 | Summary

Target | Reference | Pairing | Results

Add Set | Delete Set | Clear Sets | Correction Factor | Multiplication Factor | View Chart | Group ▾

Result Sets

- Result Set 1
- Result Set 2**
- Result Set 3

Samples

Targets			References		
Position	Name	Cp	Position	Name	Cp
4	Target Sample 1	28.23	10	Reference Sample 1	27.19
5	Target Sample 2	27.81	11	Reference Sample 2	25.91
6	Target Sample 3		12	Reference Sample 3	27.49

Calibrator Targets			Calibrator References		
Position	Name	Cp	Position	Name	Cp
2, 3	Target Calibrator	27.12	8, 9	Reference Calibrator	28.31

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## Relative Quantification: Analysis - Result

- Determination of target gene vs. reference gene ratio normalized to a calibrator

Relative Quantification - Monocolor						
Group 1   Summary						
Target   Reference   Pairing   Results						
Group ▾						
Set	Sample Type	Pos	Sample Name	Cp Median	Concentration Ratio	Normalized Ratio
	Target Calibrator	2, 3	Target Calibrator	27.12	2.29	1.00
	Reference Calibrator	8, 9	Reference Calibrator	28.31		
Result Set 1	Target Unknown	4	Target Sample 1	28.23	0.49	0.21
	Reference Unknown	10	Reference Sample 1	27.19		
Result Set 2	Target Unknown	5	Target Sample 2	27.81	0.27	0.12
	Reference Unknown	11	Reference Sample 2	25.91		
Result Set 3	Target Unknown	6	Target Sample 3		0	0
	Reference Unknown	12	Reference Sample 3	27.49		

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## Comparison of Different Efficiency Adjustments

	without efficiency correction	Efficiency correction with linear fit function	Efficiency correction with non-linear fit function
<b>Adrenal Gland RNA</b>			
40 ng	1.03	1.18	1.41
8 ng	2.21	1.79	1.01
1.6 ng	6.00	4.17	1.17
Mean value	3.08	2.38	1.21
Standard deviation	2.5967	1.5799	0.2173
Coefficient of variation	<b>84.3%</b>	<b>66.4%</b>	<b>18.0 %</b>

**Test System:** Total RNA from - human adrenal gland tissue (sample)  
- HeLa cell line (calibrator)


**Duplicate measurements** for each data point

**Analysis with Relative Quantification SW:** calibrator-normalized target/housekeeping ratios



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| Coming Soon:

- LightCycler Multiplex Master HybProbe
- LightCycler Probe Design Software 2.0

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## Summary of Product Features

- **Speed**
  - PCR (20µl capillaries): up to 30 min
  - PCR (100µl capillaries): up to 60 min
- **Reaction volume**

20 µl and 100 µl capillaries are available
- **Flexibility**

System supports different assay formats like SYBR Green I, Hybridization Probes, Hydrolysis Probe, or Single Label Probe
- **Real Time and Online Monitoring**

Data are collected at each cycle and displayed immediately
- **Quantification**

Accurate quantification in the log linear phase of the reaction
- **Melting Curve Analysis**

For easy product identification and for mutation detection (SNPs)
- **Multicolor Detection**

For multiplex PCR and for SNP detection
- **Software 4.0**

Supports *automated* analysis and interpretation of real time quantification and melting curve data

# Roche Applied Science PCR Workflow System



Thank You for Your Attention

