



An introduction to real-time PCR and its applications in food safety and environmental testing

QIAGEN

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The needs of food safety testing facilities

The globalized food market calls for more stringent safety and quality testing

- Pathogen detection
 - All major food pathogens, e.g., *Salmonella* spp., *Listeria* spp., *E. coli*
- Genetically modified organism DNA detection
 - DNA introduced into plants for pesticide qualities or herbicide resistance
- Ingredient authentication
 - For halal or kosher certification and to rule out cheap adulterants

Testing requirements

- Rapid — quick release of product to market
- Specific and sensitive — no false positive or false negative results
- Easy to use — straightforward adoption by laboratories

The needs of environmental testing facilities

Public safety demands rapid and accurate safety and quality testing for water, soil, air, etc.

- Microbial pathogen detection, quantification, and genotype identification
 - Bacteria, viruses, fungi, and protozoa must all be identified

Testing requirements

- Rapid — limiting danger to the public
- Specific and sensitive — no false positive or false negative results
- Easy to use — straightforward adoption by laboratories

Advantages of real-time PCR

- Delivers results in approximately 3 hours
- Accurately detects DNA in challenging samples
- Has simple protocols that can be fully automated
- Detects specific targets without false negatives and false positives
- Unaffected by food processing or high contaminant contents

Real-time PCR is highly suitable for sensitive detection of pathogens in samples of food, animal feed, water, soil, and air.

Real-time PCR is highly suitable for the specific DNA detection needed for GMO detection and ingredient authentication.



PCR-based testing with QIAGEN's *mericon* portfolio

An integrated testing system that meets laboratory demands

- Sample preparation and detection assay kits and instruments

Top performance

- Sensitive, target-specific, inhibitor-resistant chemistry

Ease of use

- Streamlined, robust, user-friendly sample preparation and assay protocols

Speed

- Hands-off, automatable sample preparation
- Rapid results with low- and high-throughput solutions

Coverage

- Broad range of sample types
- Success with challenging samples
- Broad coverage of food and environmental pathogens
- Broad range of GMO- and ingredient-specific assays

Standardization

- Streamlined, standardized sample preparation
- Single consistent protocol for all assays

PCR workflow for food safety testing

Processed or Raw Food Sample



Golgi apparatus, Glycoproteins, Microtubules, Mitochondria, Mitochondrial nucleic acids, Vacuoles, Tain, Nucleolus, Polymerases, Ceramides, Chromosomes, Chromatin, mRNA, Cytoplasm, Leucocytes, Sugars, Lipids, Salts, Urea, Carbonic acids, Colofactors, Precursors, Hemoglobins, Erythrocytes, Monocytes, Smooth endoplasmatic reticulum, Macrophages, Thrombocytes, Platelets, Lymphocytes, Basophils, Eosinophils, Neutrophils, Megacaryocytes, Plasma, Clotting factors, Actin, Microfilaments, Serum, Fibrin, Lysosomes, Ezrin, DNA, Hemoglobins, Heptaglobins, Transferrin, Fibrinogen, Serum albumin, rRNA, Salts, Polymerases, DNA, Centrioles, Immunoglobulins, Cytokines, Angiotensins, Chemokines, Bradykines, Plasma membranes, Ribosomes, Actin, Vesicles, Complement components, Nuclei, Rough endoplasmatic reticulum, Nucleoli, Golgi apparatus, Glycoproteins, Microtubules, Mitochondria, Mitochondrial nucleic acids, Vacuoles, Tain, Nucleolus, Polymerases, Ceramides, Chromosomes, Chromatin, mRNA, Cytoplasm, Leucocytes, Sugars, Lipids, Salts, Urea, Carbonic acids, Colofactors, Precursors, Hemoglobins, Erythrocytes, Monocytes, Smooth endoplasmatic reticulum, Macrophages, Thrombocytes, Platelets, Lymphocytes, Basophils, Eosinophils, Neutrophils, Megacaryocytes, Plasma, Clotting factors, Actin, Microfilaments, Serum, Fibrin, Lysosomes, Ezrin, Hemoglobins, Heptaglobins, Transferrin, Fibrinogen, Serum albumin, rRNA, Carrier proteins

mericon Food Sample Preparation



Golgi apparatus, Glycoproteins, Microtubules, Mitochondria, Mitochondrial nucleic acids, Vacuoles, Tain, Nucleolus, Polymerases, Ceramides, Chromosomes, Chromatin, mRNA, Cytoplasm, Leucocytes, Sugars, Lipids, Salts, Urea, Carbonic acids, Colofactors, Precursors, Hemoglobins, Erythrocytes, Monocytes, Smooth endoplasmatic reticulum, Macrophages, Thrombocytes, Platelets, Lymphocytes, Basophils, Eosinophils, Neutrophils, Megacaryocytes, Plasma, Clotting factors, Actin, Microfilaments, Serum, Fibrin, Lysosomes, Ezrin, DNA, Hemoglobins, Heptaglobins, Transferrin, Fibrinogen, Serum albumin, rRNA, Salts, Polymerases, DNA, Centrioles, Immunoglobulins, Cytokines, Angiotensins, Chemokines, Bradykines, Plasma membranes, Ribosomes, Actin, Vesicles, Complement components, Nuclei, Rough endoplasmatic reticulum, Nucleoli, Golgi apparatus, Glycoproteins, Microtubules, Mitochondria, Mitochondrial nucleic acids, Vacuoles, Tain, Nucleolus, Polymerases, Ceramides, Chromosomes, Chromatin, mRNA, Cytoplasm, Leucocytes, Sugars, Lipids, Salts, Urea, Carbonic acids, Colofactors, Precursors, Hemoglobins, Erythrocytes, Monocytes, Smooth endoplasmatic reticulum, Macrophages, Thrombocytes, Platelets, Lymphocytes, Basophils, Eosinophils, Neutrophils, Megacaryocytes, Plasma, Clotting factors, Actin, Microfilaments, Serum, Fibrin, Lysosomes, Ezrin, Hemoglobins, Heptaglobins, Transferrin, Fibrinogen, Serum albumin, rRNA, Carrier proteins

Pure DNA



DNA

mericon Detection

Target detected

Yes ☒

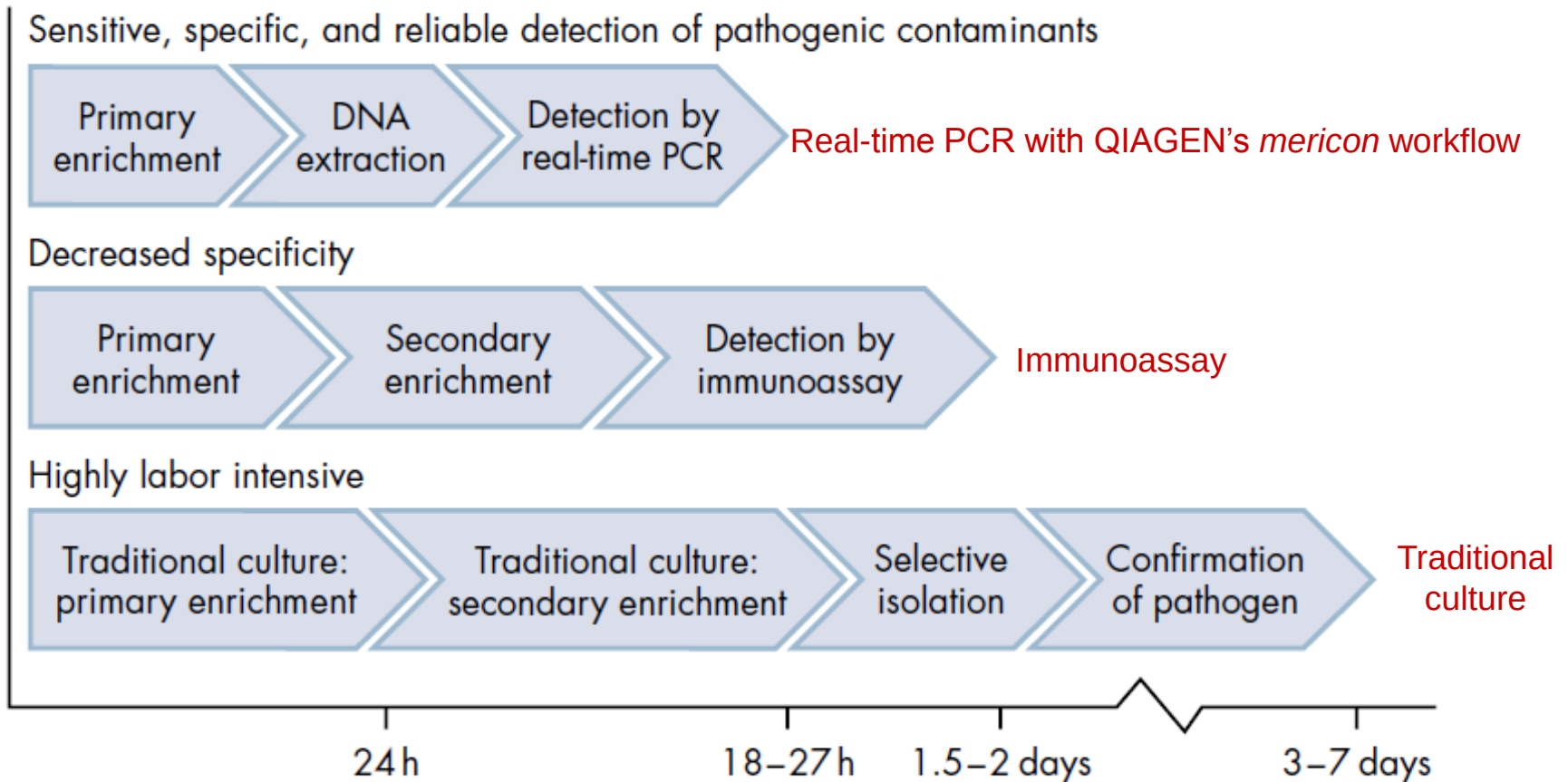
No ☐

Information

Sample Technologies

Assay Technologies

QIAGEN's *mericon* workflow yields rapid, sensitive and highly specific results



Real-time versus traditional PCR

- Real-time PCR allows detection during early stages of amplification
 - Detection during the exponential phase of amplification is very accurate
- Traditional PCR uses agarose gels for detection
 - The results are not very accurate or discriminatory

Agarose gel cannot discriminate between 10 and 50 starting copies of DNA

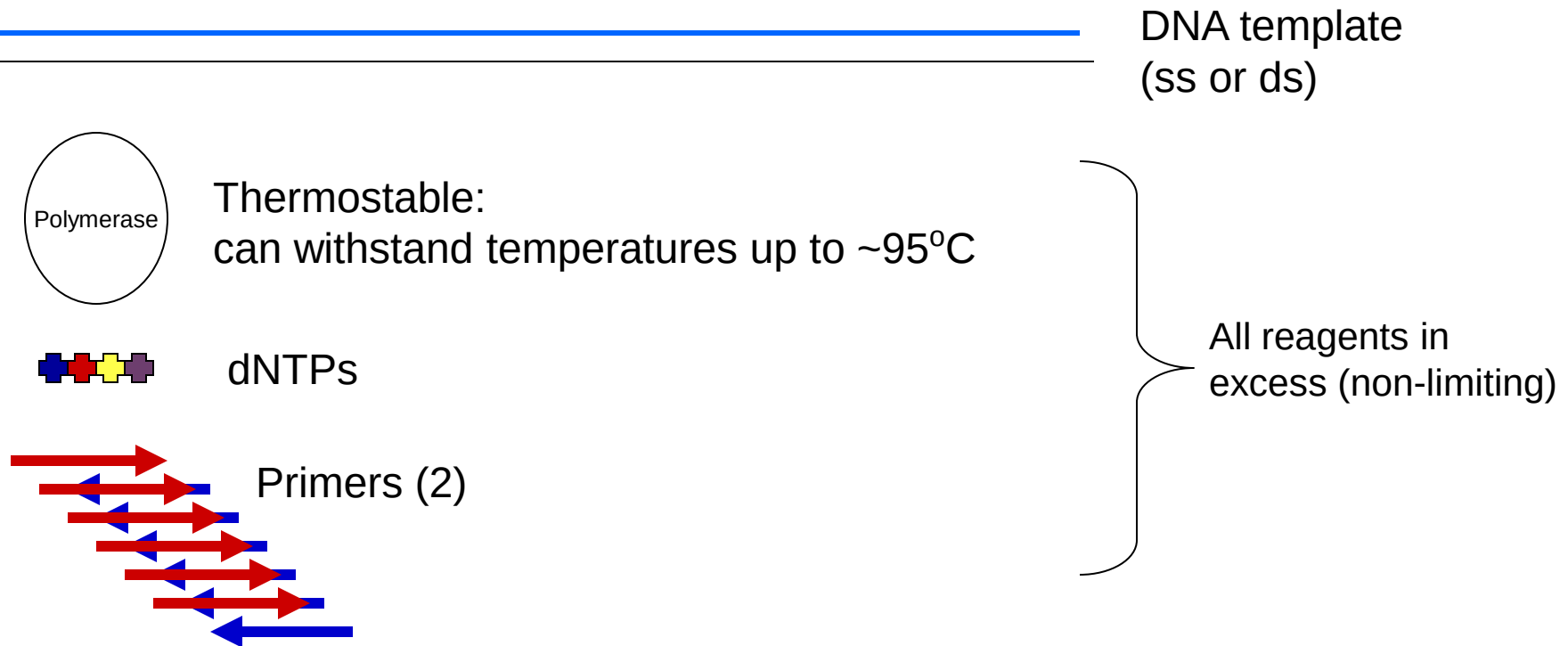


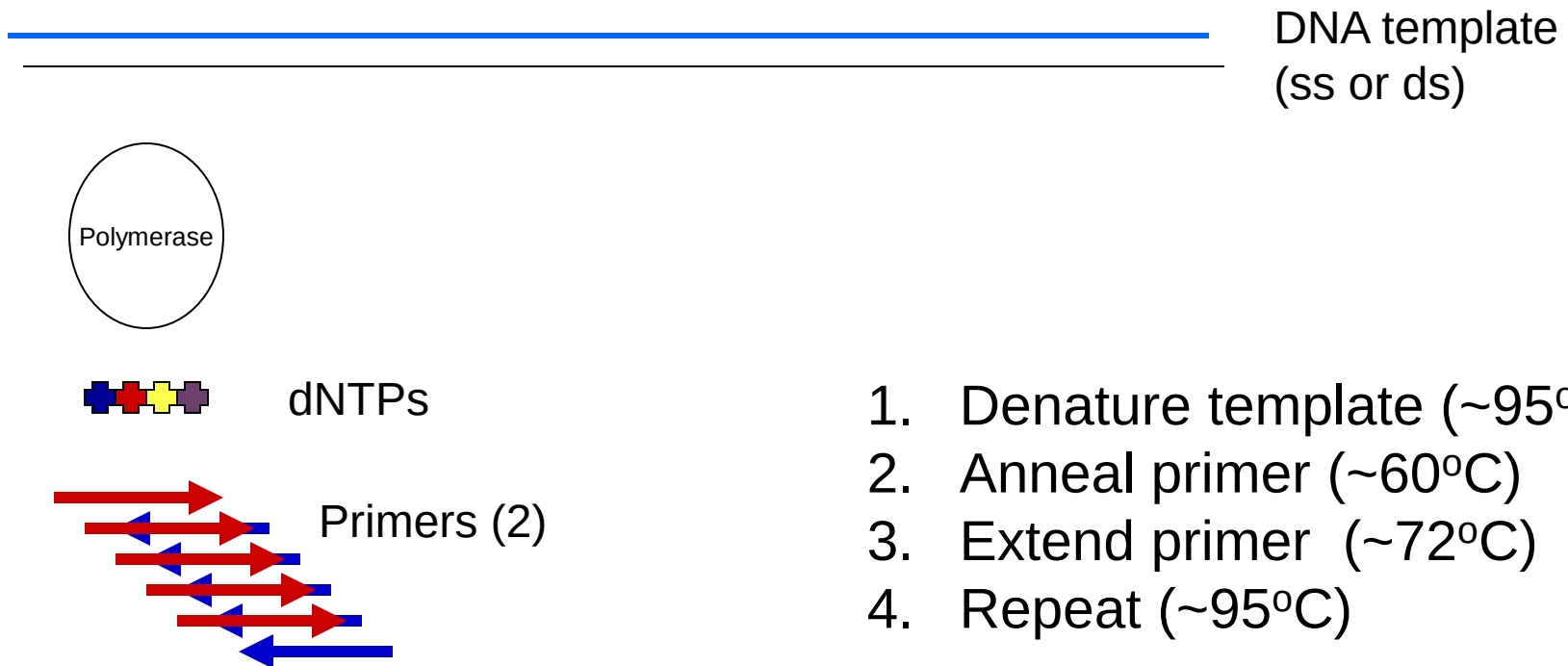
Real-time PCR

- Sequence amplification and real-time detection using sequence-specific probes
- Rapid, sensitive, and specific detection of pathogens in biological samples

PCR = **P**olymerase **C**hain **R**eaction

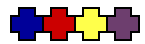
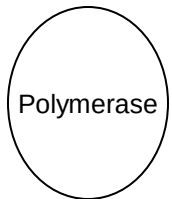
Exponential amplification of DNA in single tube



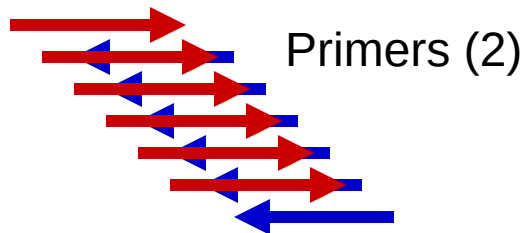


↑
Denature template

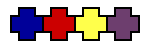
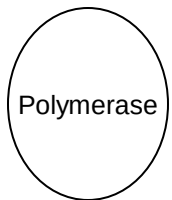
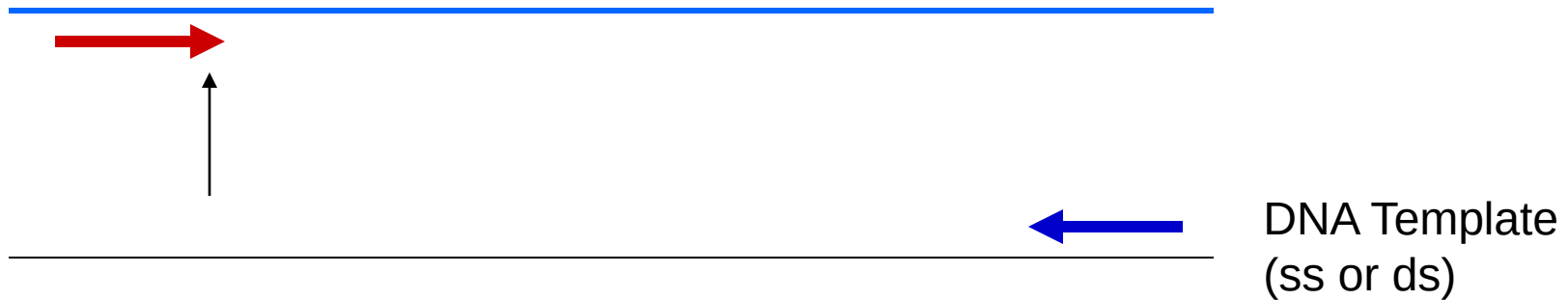
DNA template
(ss or ds)



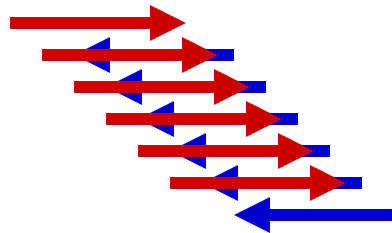
dNTPs



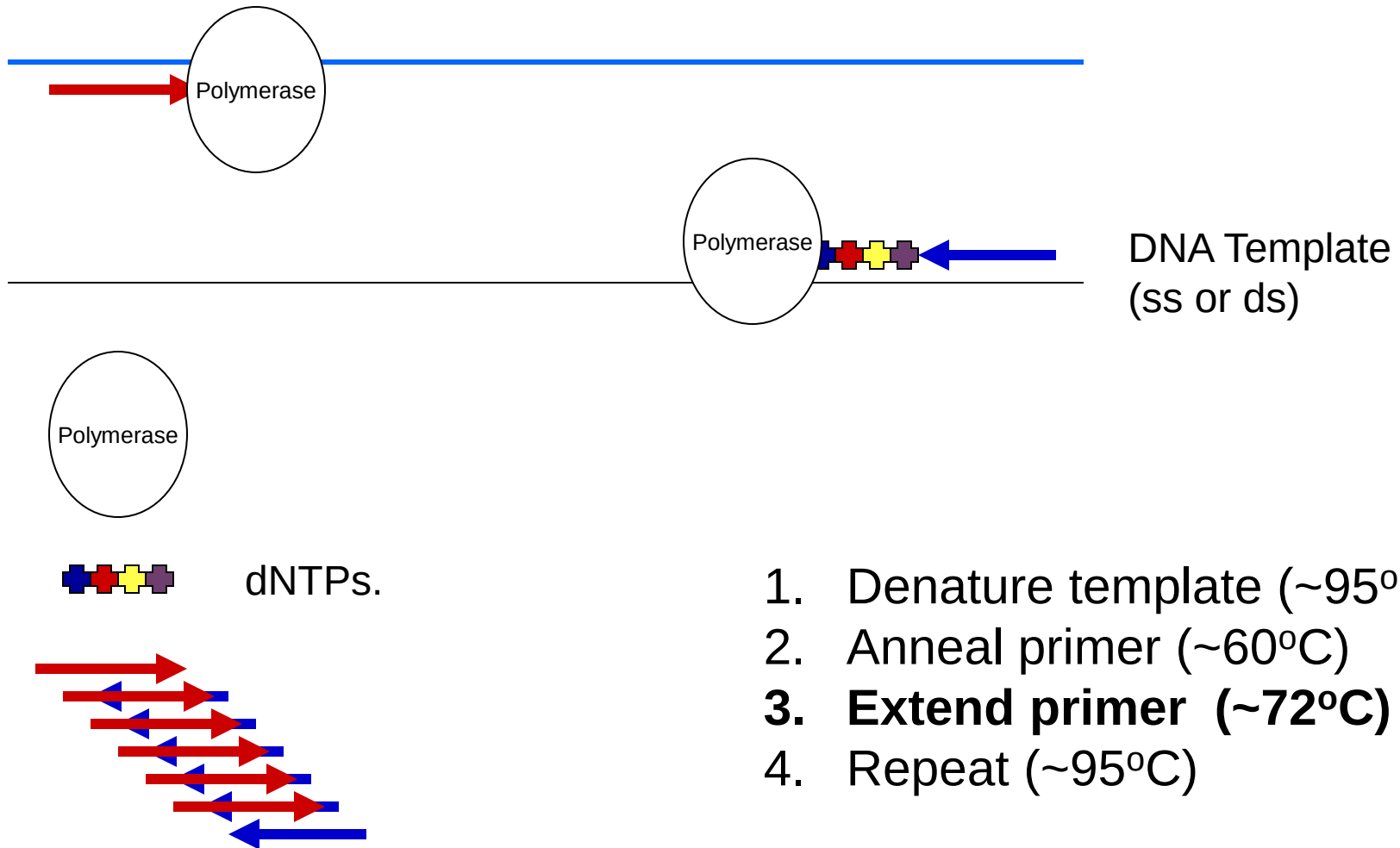
1. **Denature template (~95°C)**
2. Anneal primer (~60°C)
3. Extend primer (~72°C)
4. Repeat (~95°C)

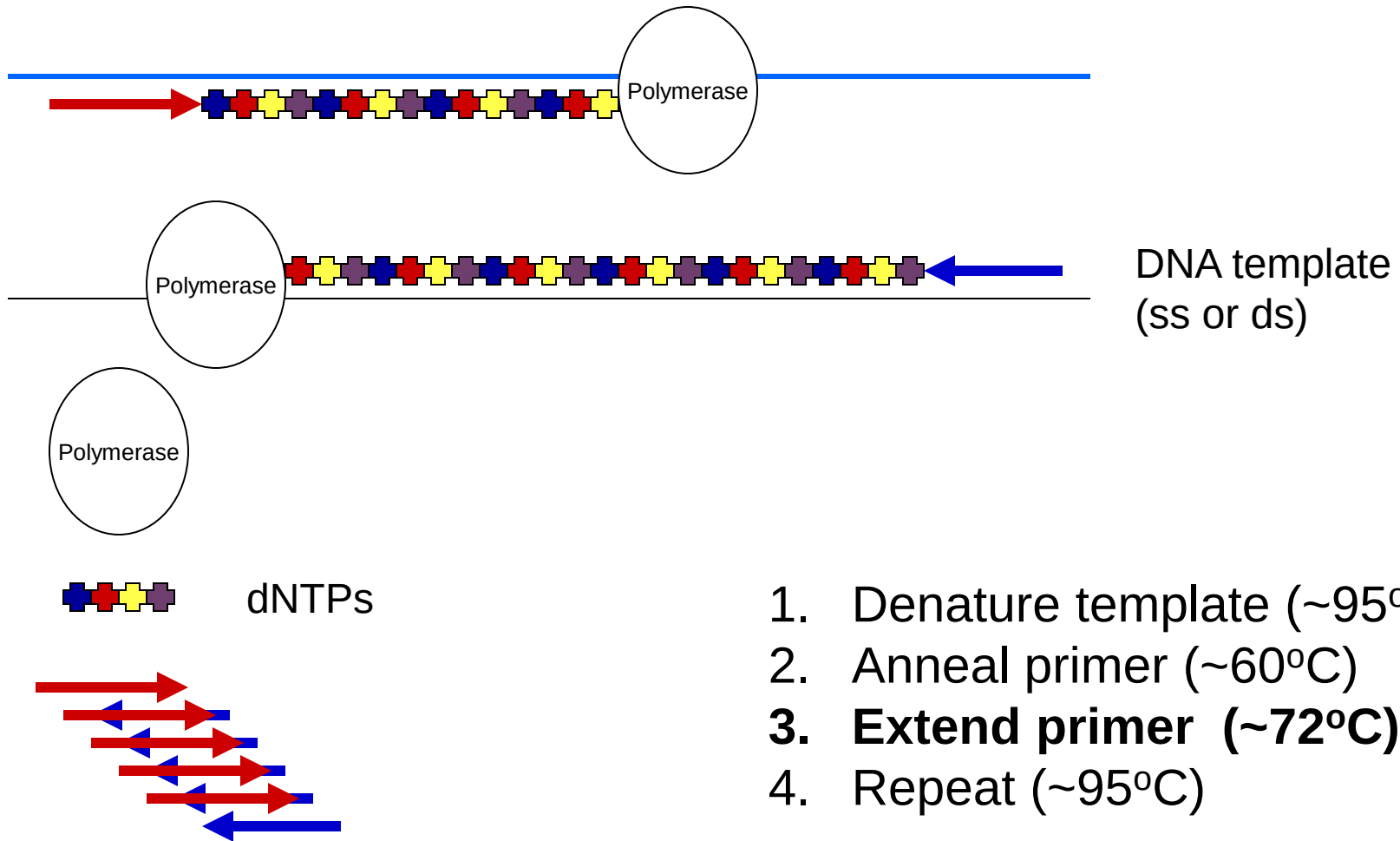


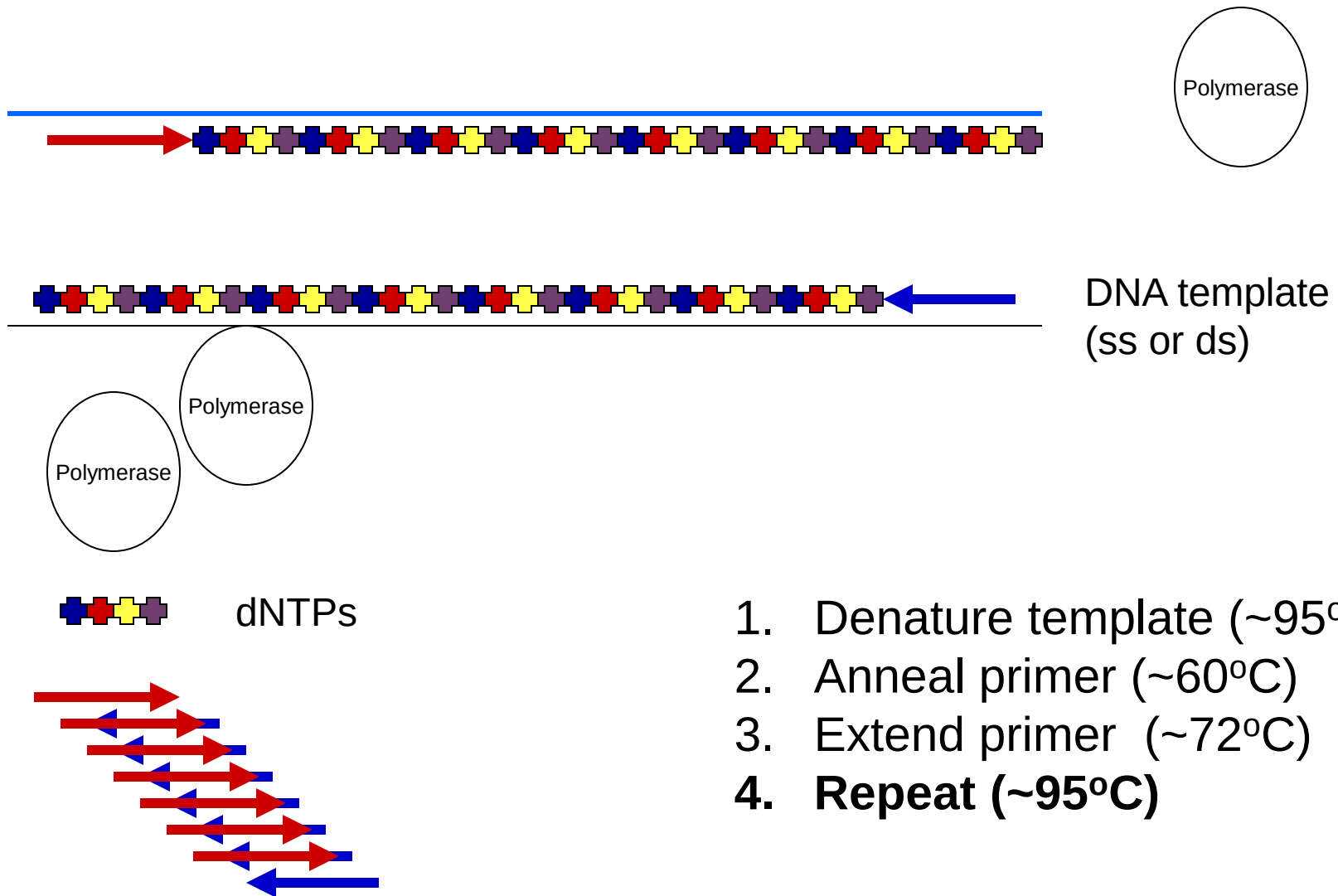
dNTPs

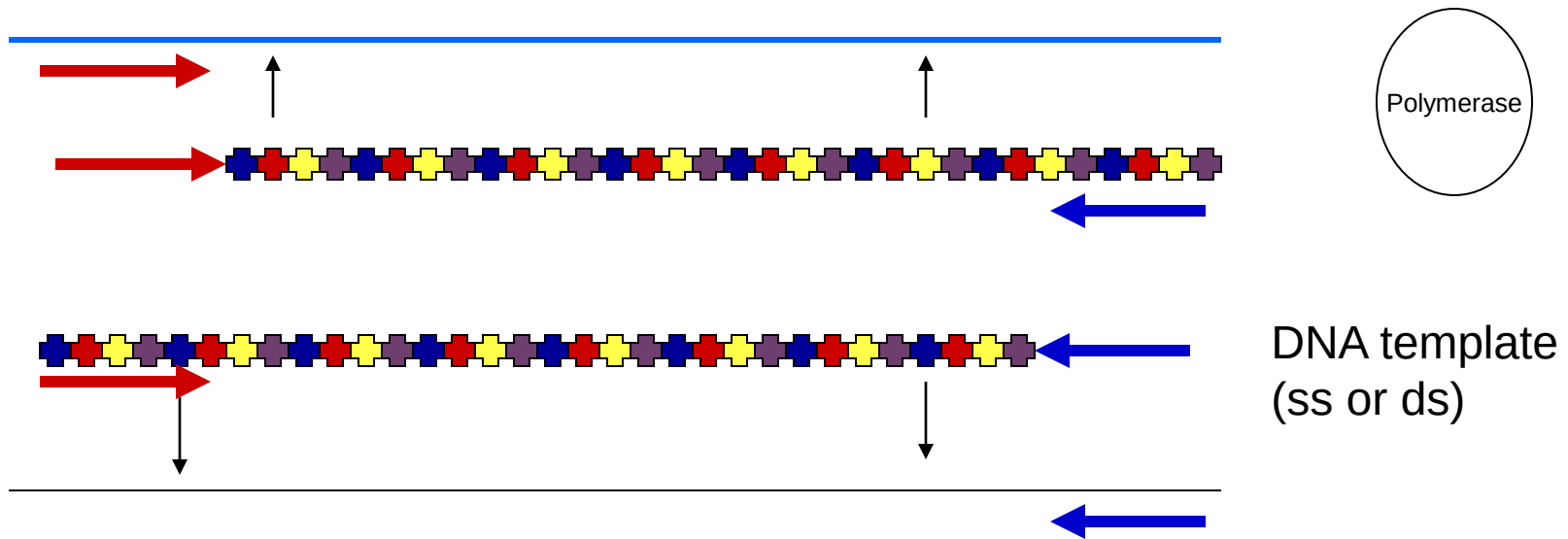


1. Denature template ($\sim 95^{\circ}\text{C}$)
2. **Anneal primer ($\sim 60^{\circ}\text{C}$)**
3. Extend primer ($\sim 72^{\circ}\text{C}$)
4. Repeat ($\sim 95^{\circ}\text{C}$)

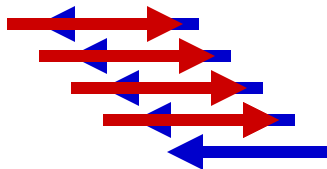








 dNTPs



1. Denature template ($\sim 95^{\circ}\text{C}$)
2. **Anneal primer ($\sim 60^{\circ}\text{C}$)**
3. Extend primer ($\sim 72^{\circ}\text{C}$)
4. Repeat ($\sim 95^{\circ}\text{C}$)

Advantages of real-time PCR

- Real-time PCR is a highly **sensitive and reliable** method for the detection and quantification of nucleic acids (DNA, RNA, and cDNA).
- It is based on **detection of fluorescence** emitted from a reporter molecule **in real time**.
- This detection occurs during the accumulation of the PCR product **with each cycle of amplification**.

This allows monitoring of the **PCR reaction during early exponential phase**, when the first significant increase in the amount of PCR product correlates to the initial amount of target template.

There are many real-time chemistries available.

■ Intercalating dyes:

- ☐ SYBR Green I
- ☐ LC Green
- ☐ EvaGreen
- ☐ SYTO 9

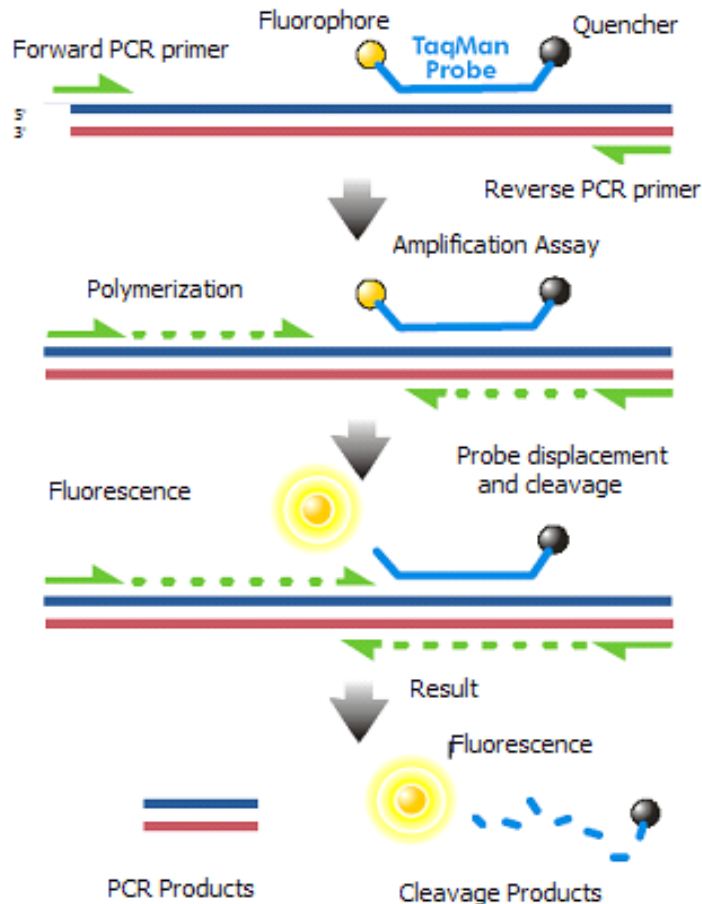
HRM Dyes

■ Probe-based chemistries:

- ☐ TaqMan (Applied Biosystems/LTI)
- ☐ FRET/Hybridization/LightCycler Probes (Roche)
- ☐ Molecular Beacons
- ☐ Linear probes



Hydrolysis-based probe



The fluorescence of the reporter dye is suppressed by the quencher

Primer binding is followed by extension

Probe cleavage by Taq to free the reporter dye so the fluorescence intensity correlates with the initial sample input amounts. Taq has 5' → 3' exonuclease activity

Each amplicon needs a sequence-specific probe

How does a real-time PCR cycler work?

Thermal cycler

Excitation source

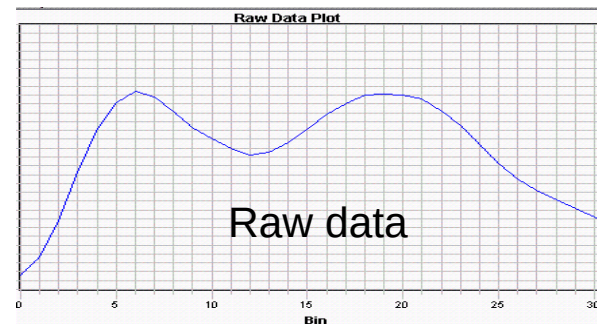
Excitation Sources:

- Halogen Lamp
- Light Emitting Diode (LED)
- Argon Ion Laser

Focusing filters,
lenses, and
mirrors

More focusing
filters, lenses,
and mirrors

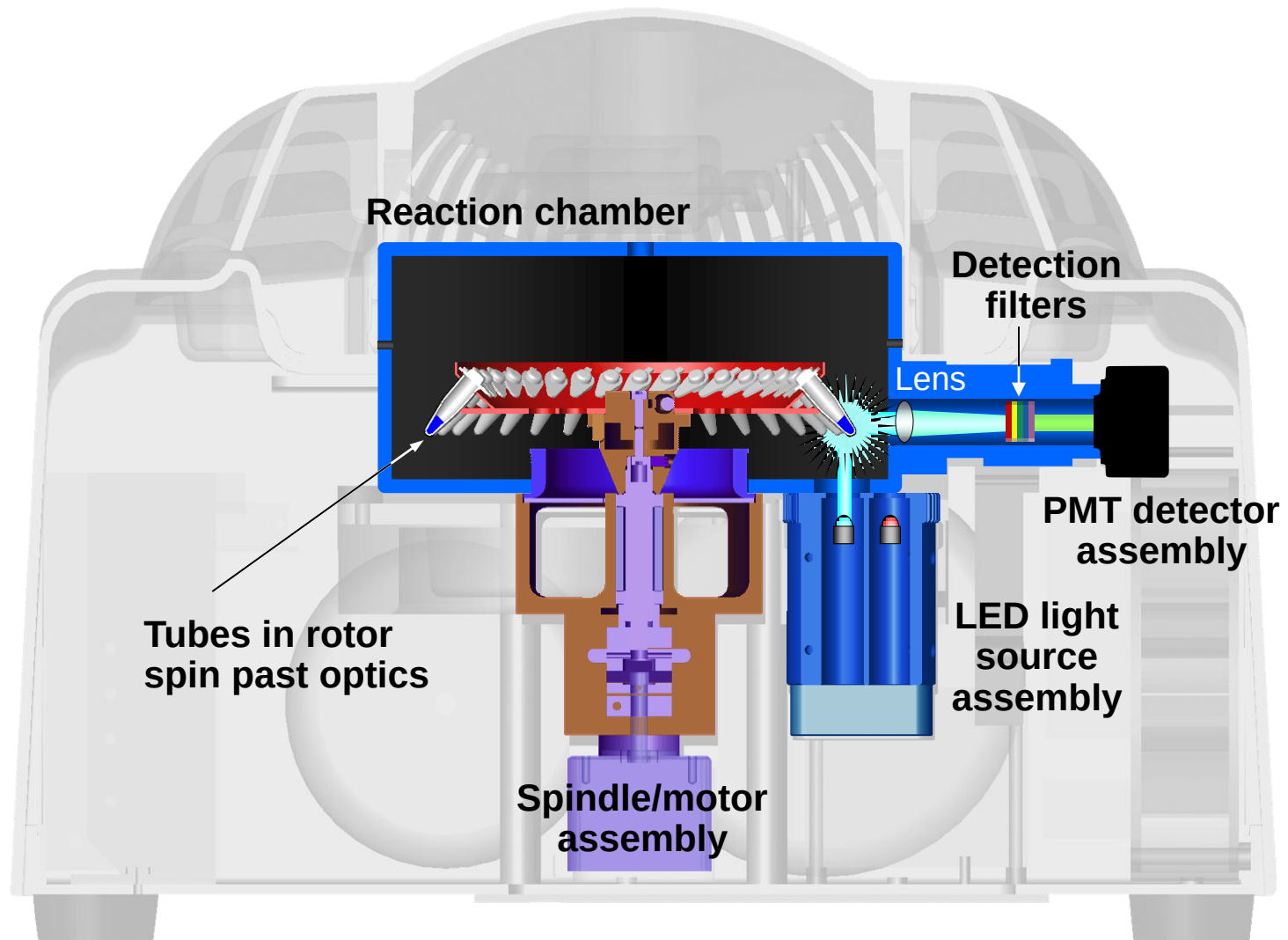
Detection device



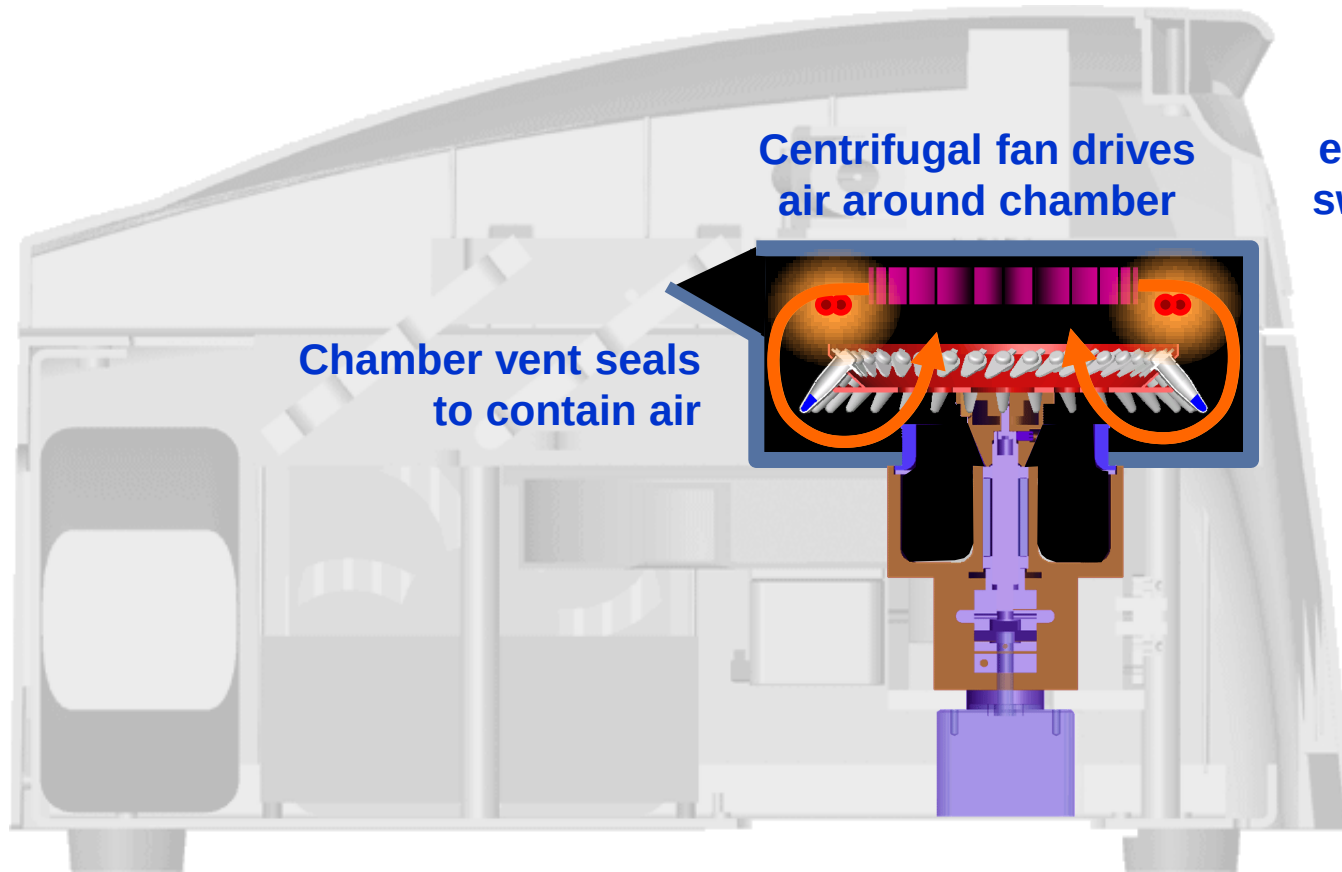
Detection Devices:

- Charge Couple Device (CCD Camera)
- Photomultiplier Tube (PMT) * Rotor-Gene Q
- Photodiode

Rotor-Gene Q — superior rotary optics



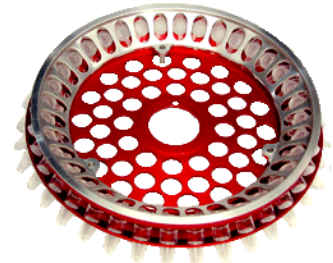
Rapid air based cycling — heating



Centrifugal fan drives
air around chamber

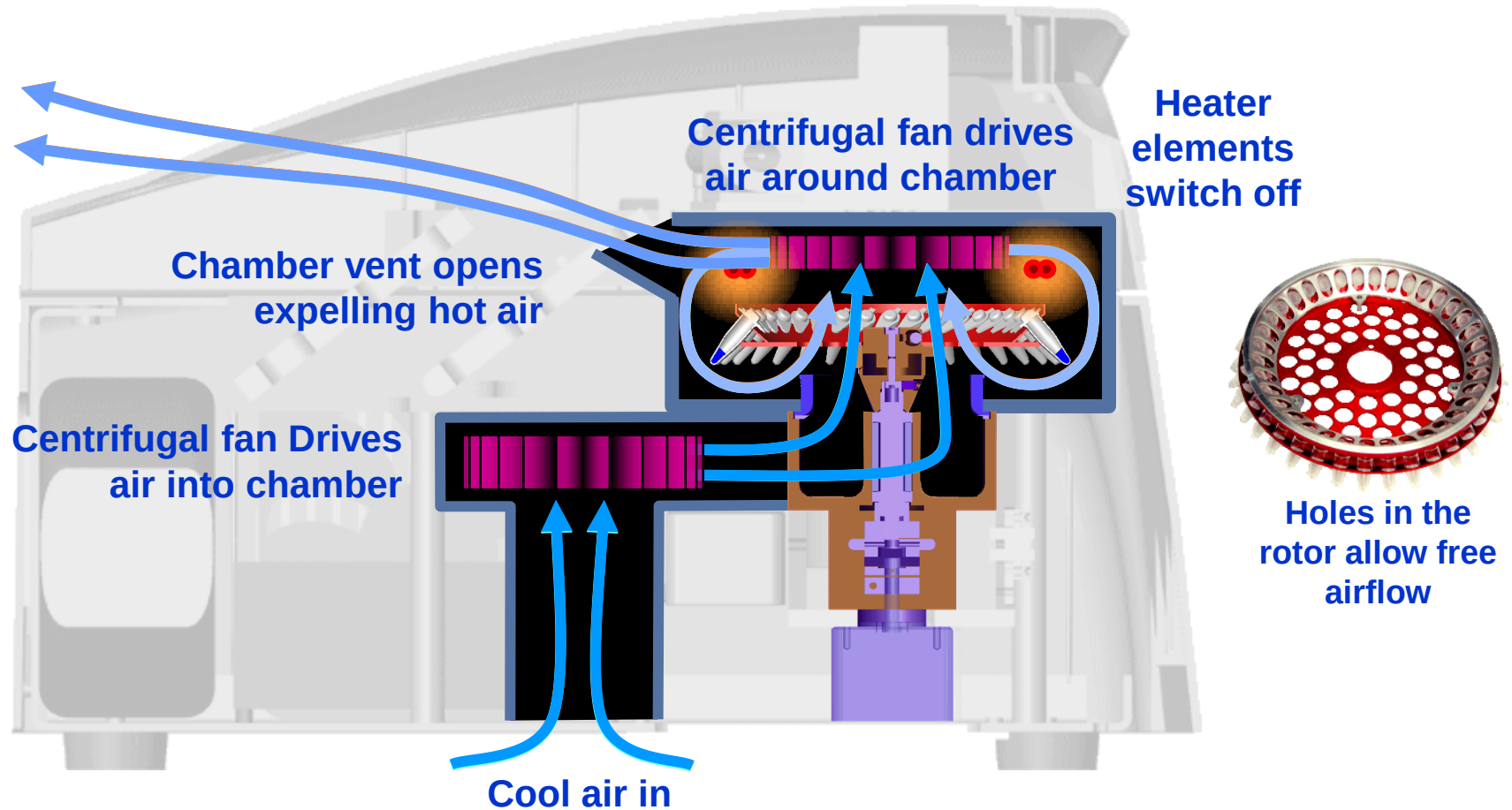
Chamber vent seals
to contain air

Heater
elements
switch on



Holes in the
rotor allow
free airflow

Rapid air based cycling — cooling



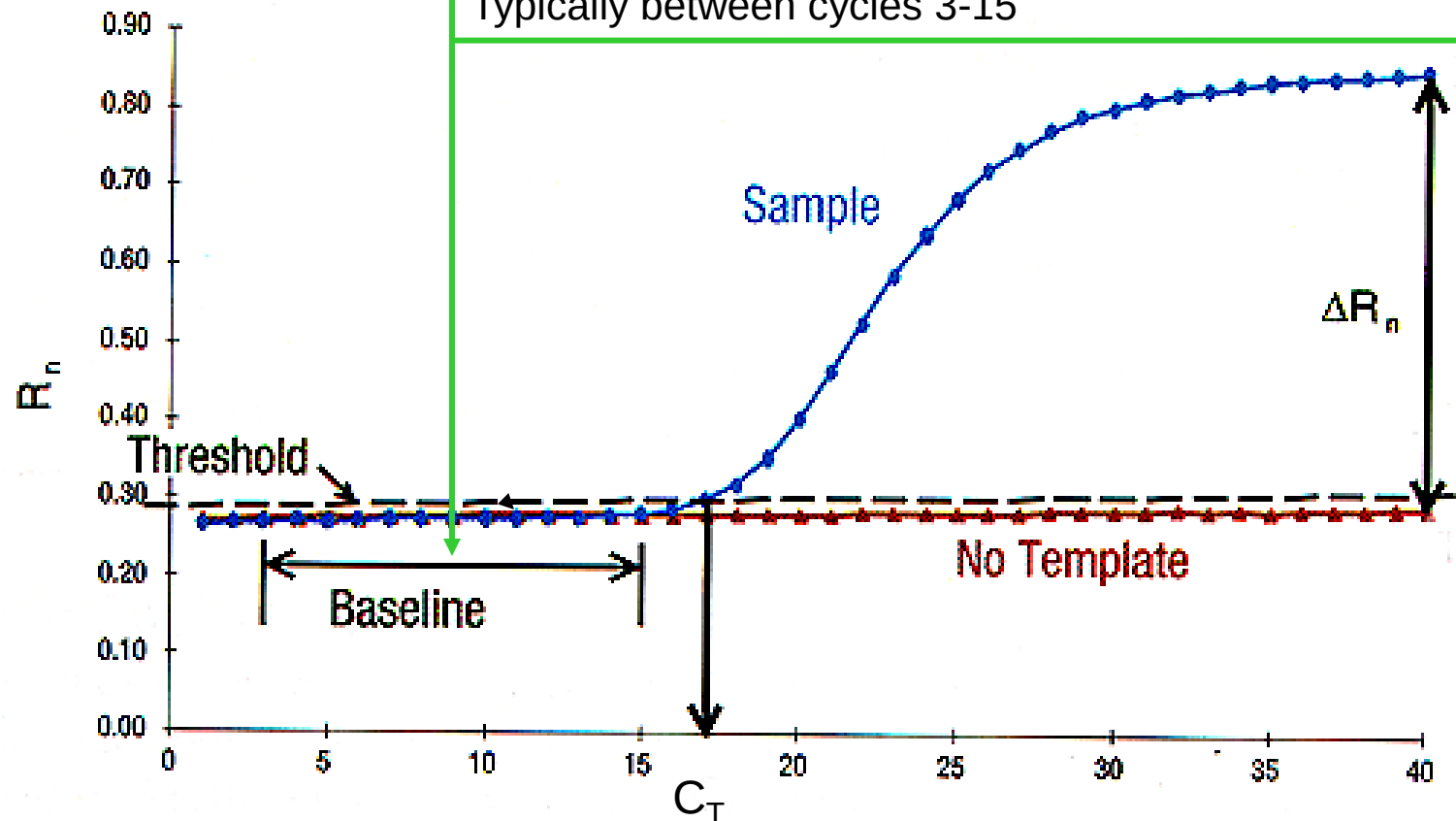
These terms are important to the analysis of real-time PCR data:

- **Baseline**
- **Threshold**
- **C_T**
- **No-Template Control (NTC)**

Let's examine each of these terms a little bit more closely.

Baseline

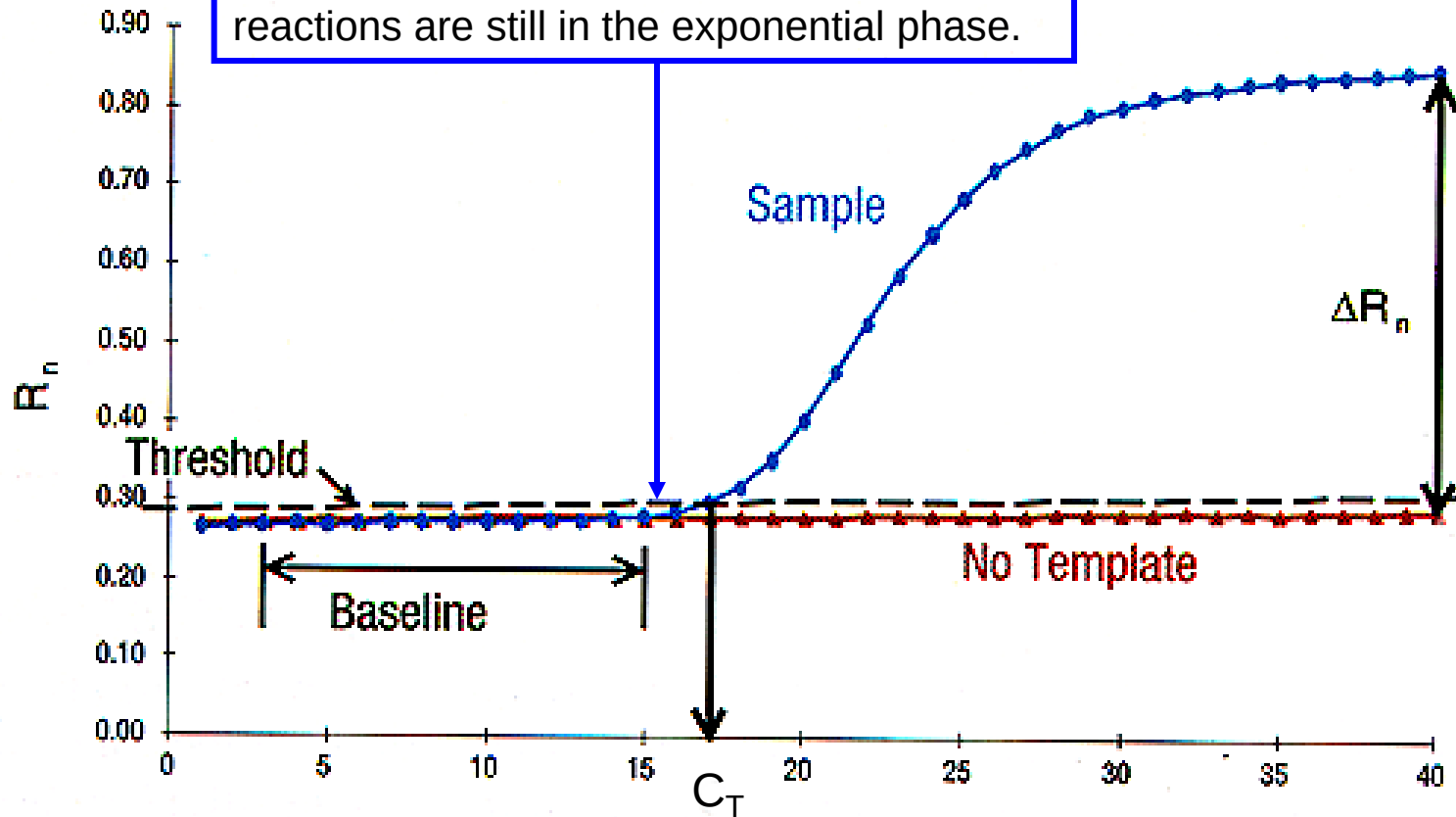
The initial cycles prior to amplification in which there is little change in fluorescent signal.
Typically between cycles 3-15



Threshold

Threshold

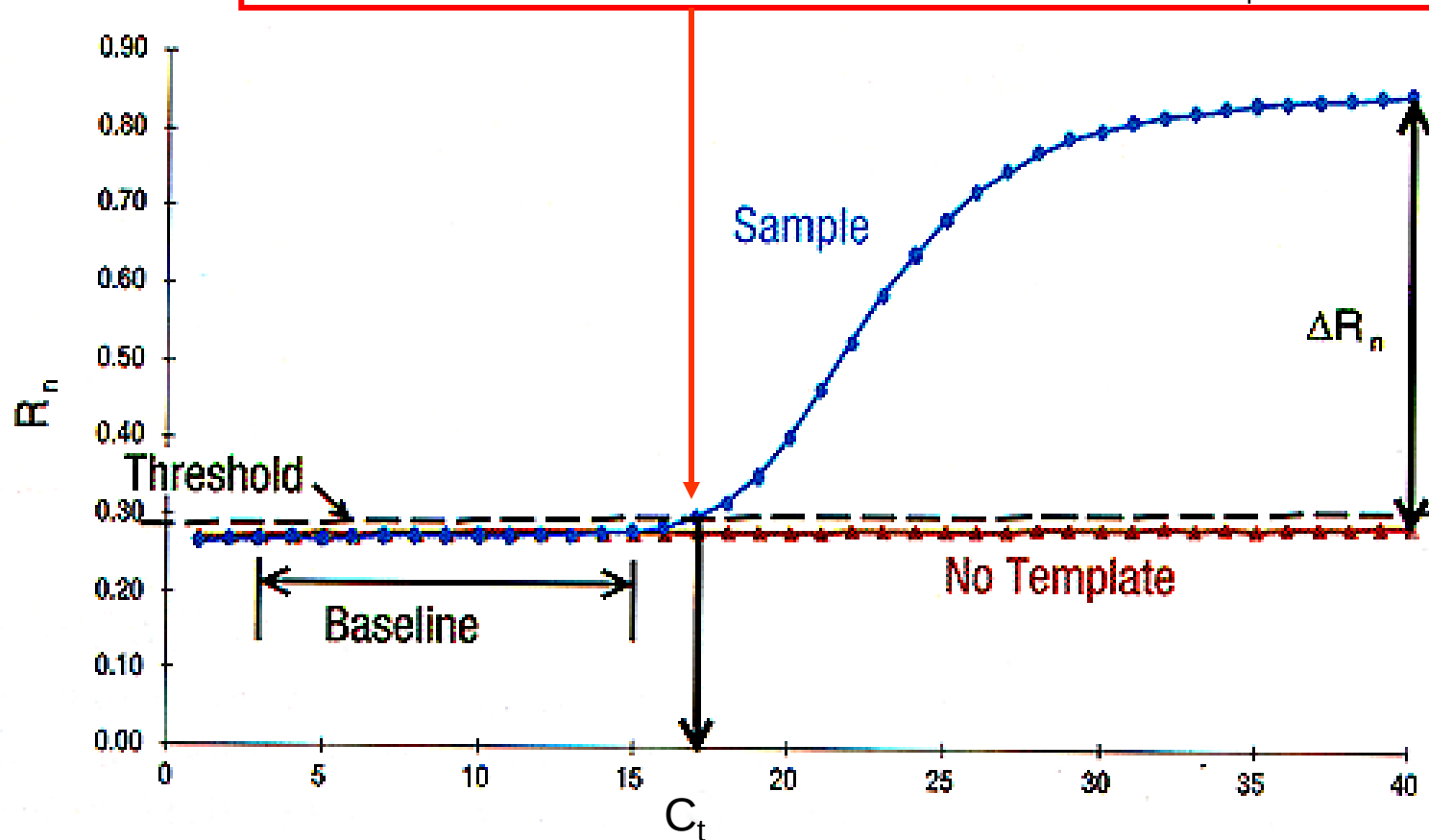
The level at which the amplification fluorescence is above the **baseline**, but the reactions are still in the exponential phase.



Threshold cycle (C_T)

Threshold cycle (C_T)

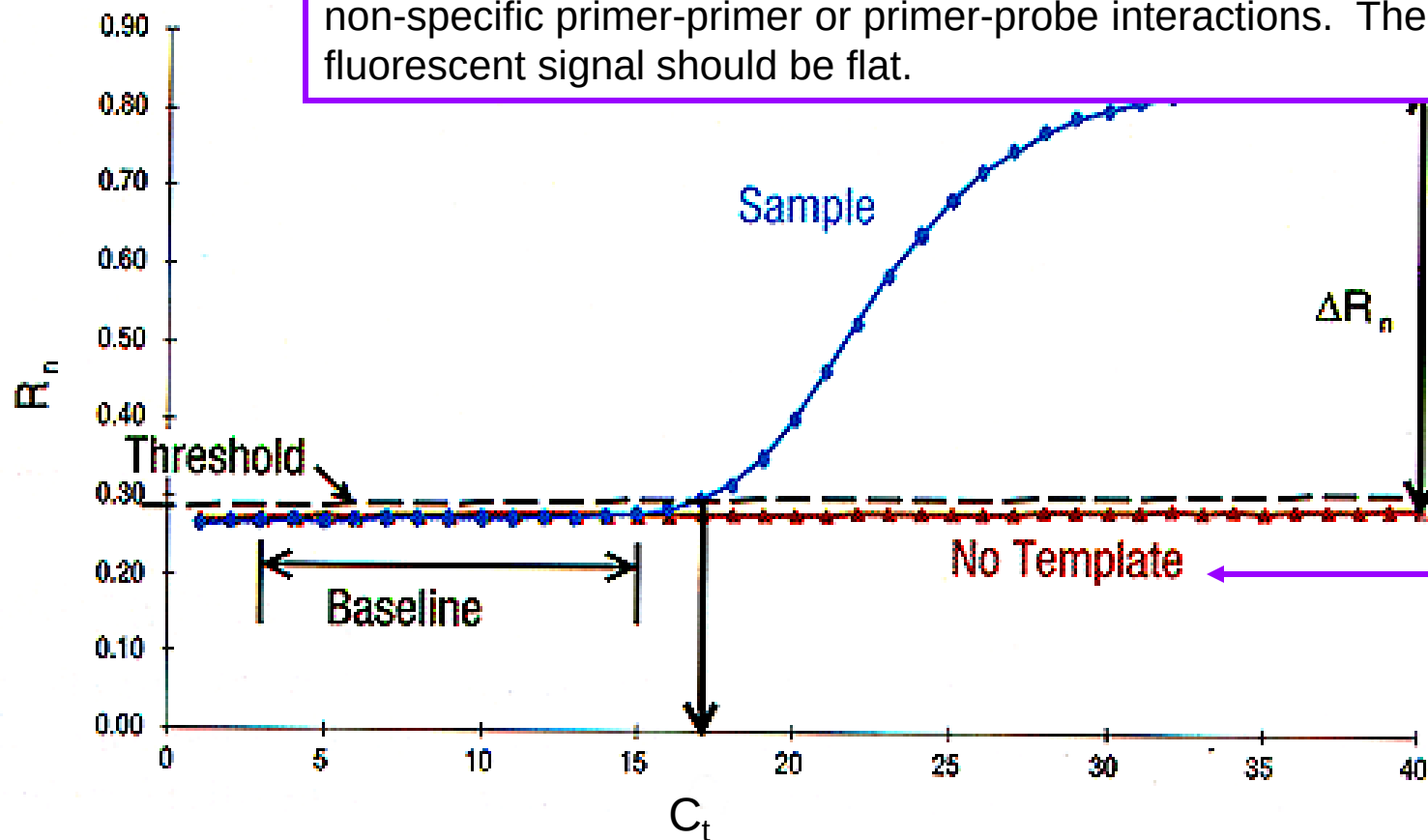
The cycle number at which the amplification plot crosses the threshold line. Roche instruments use the term crossing point (C_p).



No-Template Control (NTC)

No-Template Control (NTC)

There is no nucleic acid template in the reaction. This is used to measure if there is any contamination of reaction components or non-specific primer-primer or primer-probe interactions. The fluorescent signal should be flat.



Critical factors for a successful assay

- **DNA or RNA sample preparation — template quality**

Choose appropriate sample preparation kits or reagents

Inhibitors can compromise your PCR

- **Assay design — specificity, efficiency, chemistry**

Consider your throughput and cost per result

Use thoroughly validated assays

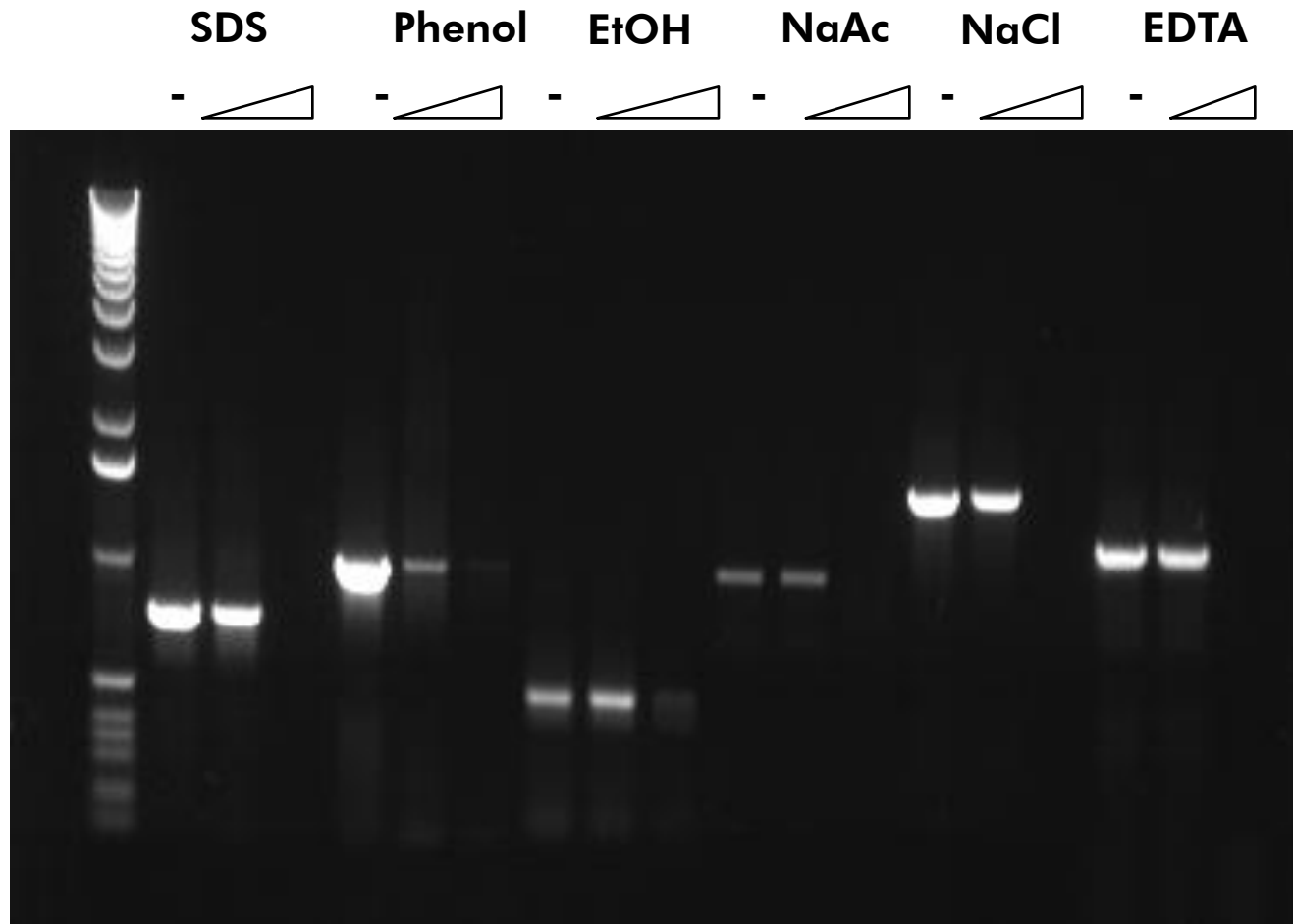
- **Running PCR**

Choose high quality reagents (primer, probe, master mix)

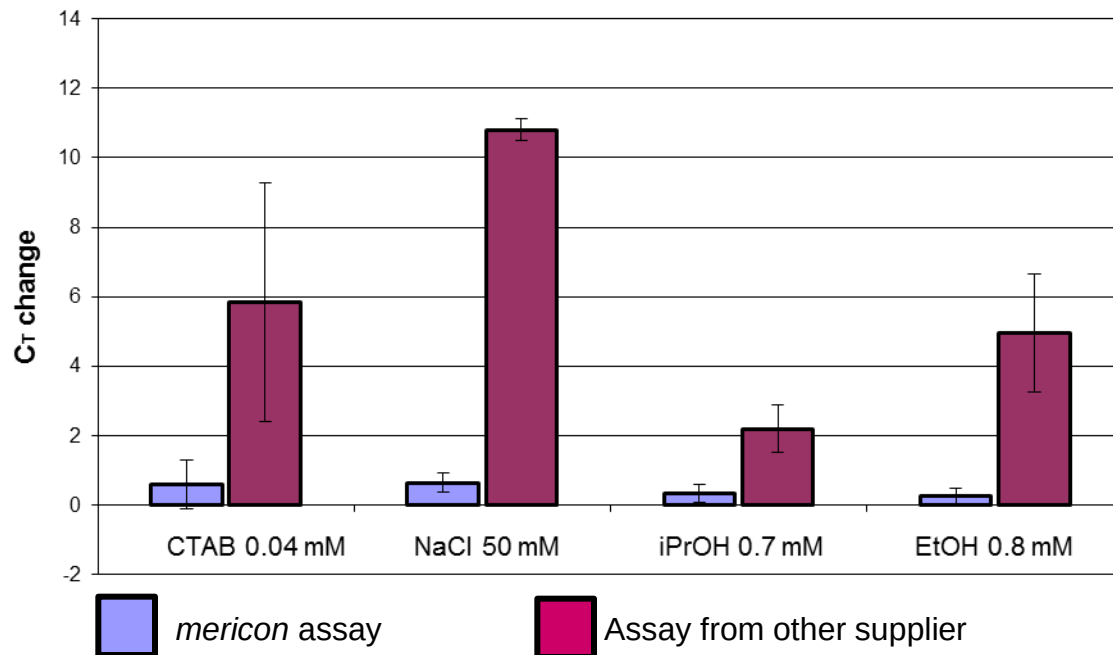
- **Data analysis tool**

Work with user-friendly, streamlined data analysis modules

Increasing amounts of inhibitors can completely inhibit PCR



Inhibitor tolerance of multiplex PCR master mix

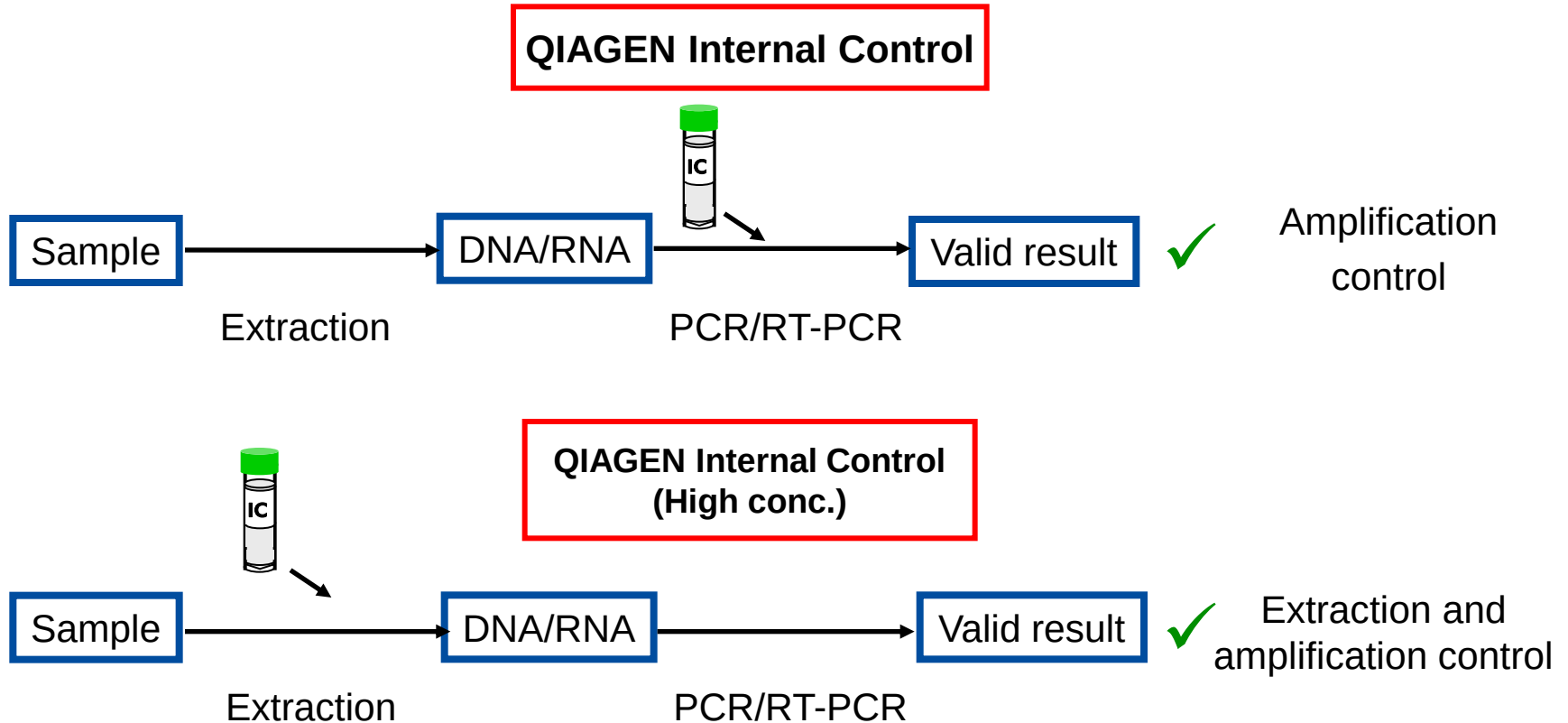


Observed effects in PCR analyses

- Effects on general C_T range
- Quenching of end fluorescence
- Scattering of end fluorescence
- Effects of buffer pH
- Scattering of replicates
- PCR boost

mericon PCR Master Mix

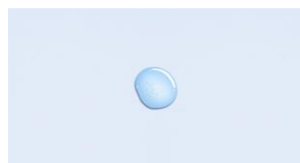
- Stable with most inhibitors
- Less replicate scattering
- No end fluorescence effects



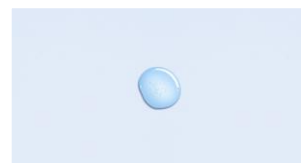
Flexible use — control of extraction and/or amplification



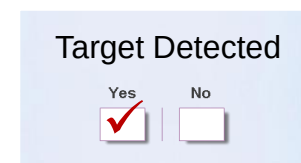
nucleic acids, Vacuoles, Tail, Nucleolus, Polymerases, Ceramides, Chromosomes, Chromatin, mRNA, Cytoplasm, Leucocytes, Sugars, Lipids, Salts, Urea, Carbonic acids, Cofactors, Precursors, Hemoglobins, Erythrocytes, Monocytes, Smooth endoplasmic reticulum, Macrophages, Thrombocytes, Platelets, Lymphocytes, Basophils, Eosinophils, Neutrophils, Megacaryocytes, Plasma, Clotting factors, Actin, Microfilaments, Serum, Fibrin, Lysosomes, Ezrin, **DNA**, Hemoglobins, Heptaglobins, Transferrin, Fibrinogen, Serum albumin, tRNA, Salts, Polymerases, Centrioles, Immunoglobulins, Carrier proteins, Cytokines, Angiotensins, Chemokines, Bradykinins, Plasma membranes, Ribosomes, Actin, Vesicles, Complement components, Nucleol, Rough endoplasmic reticulum, Nucleoli, Golgi apparatus, Glycoproteins, Microtubules, Mitochondria, Mitochondrial nucleic acids, Vacuoles, Tail, Nucleolus, Polymerases, Ceramides, Chromosomes, Chromatin, mRNA, Cytoplasm, Leucocytes, Sugars,



DNA



DNA



Information



Disruption and thermal or enzymatic lysis



Manual or automated purification



Manual or automated setup of PCR



Detection of DNA



Food or environmental testing laboratory



Sample

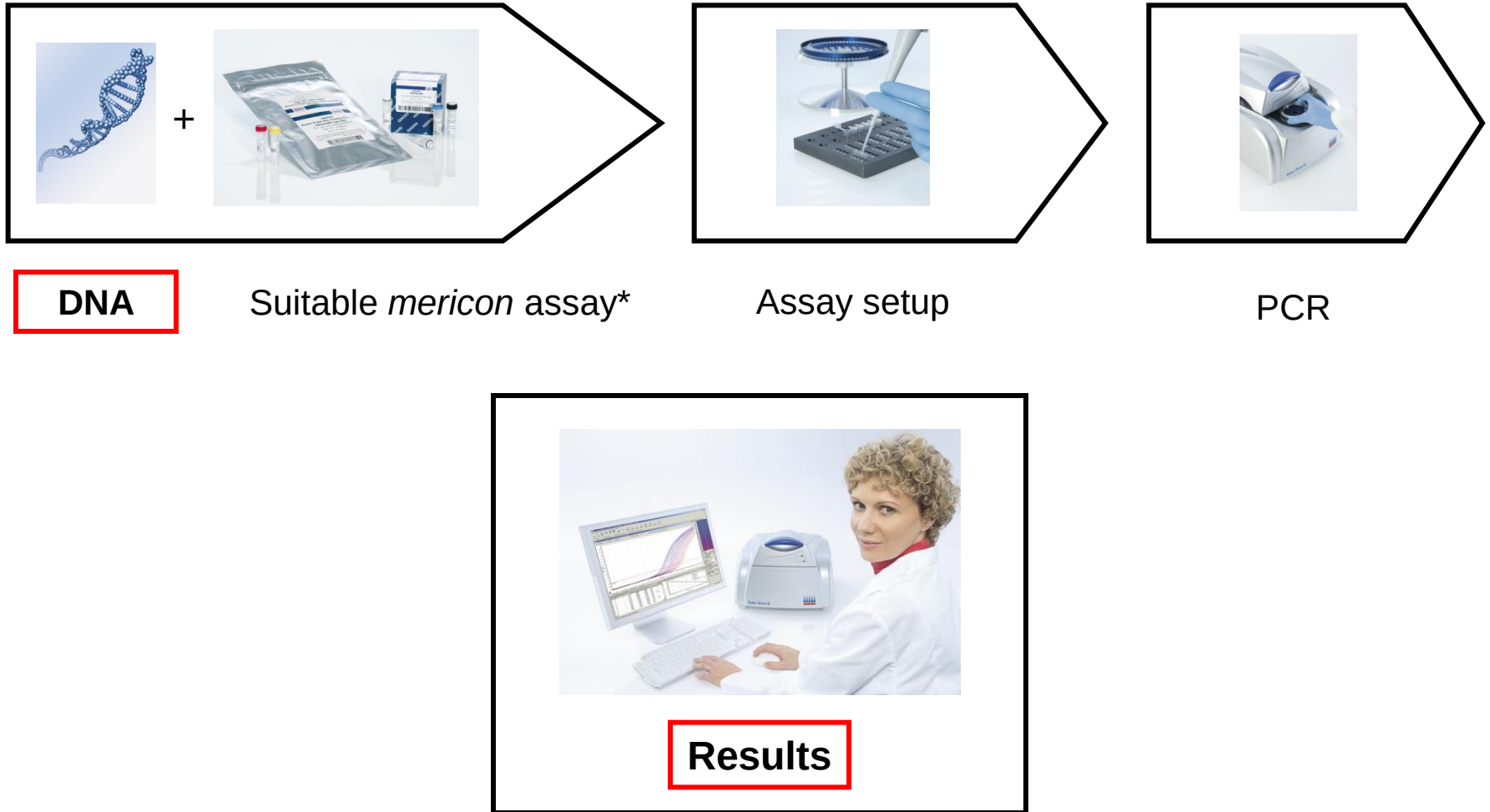


Suitable DNA extraction kit, such as the *mericon* DNA Bacteria or Bacteria Plus Kit, DNeasy *mericon* Food Kit, or QIAamp UCP Pathogen Mini Kit



DNA

QIAGEN workflow — assay setup and detection



* Assays are available for a broad range of specific pathogens, genetically modified organisms, and ingredients. QIAGEN also has kits suitable for lab-developed assays.

Thermal cycling program setup for real-time PCR

Rotor-Gene Q Series Software VIRTUAL MODE - Run 2011-12-15 (1)

File Analysis Run Gain View Window Help

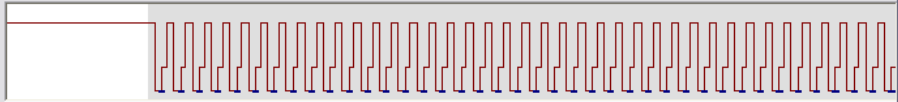
New Open Save Start Pause Stop Help View Settings Progress Profile Temp. Samples Analysis Reports Arrange

Channels

Edit Profile

New Open Save As Help

The run will take approximately 73 minute(s) to complete. The graph below represents the run to be performed :



Click on a cycle below to modify it :

Hold
Cycling

Insert after...
Insert before...
Remove

This cycle repeats 40 time(s).

Click on one of the steps below to modify it, or press + or - to add and remove steps for this cycle.

Timed Step

60°C
15 seconds
Acquiring to Cycling A
on Green, Yellow

☐ Long Range
☐ Touchdown

95°C for 15 secs
60°C for 15 secs
72°C for 10 secs

OK

Page: Page 1

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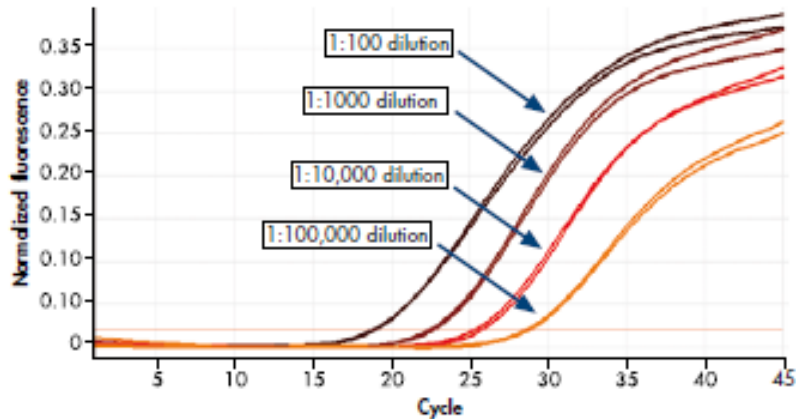
Bank On Bank Off
Named On All On All Off
Edit Samples...

Rotor-Gene Q Series Software 2.0.2

VIRTUAL MODE

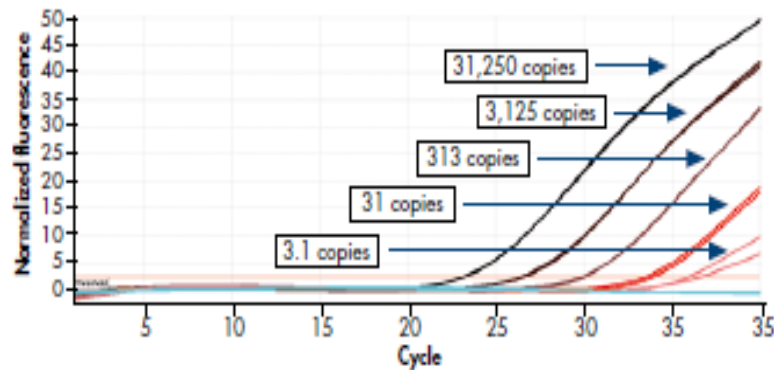
start Home - Global ... 36 Reminders SharePoint - A... Windows E... Microsoft ... Rotor-Gene Q ... Rotor-Gene Q ... 1:42 AM

Real-time PCR results



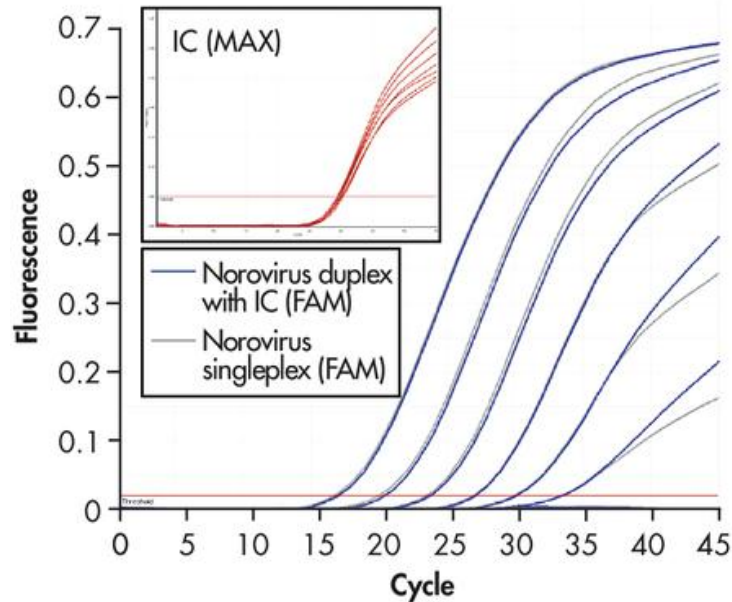
Highly sensitive pathogen detection, even in difficult food matrices such as peanut butter.

Enrichment cultures of salmonella in buffered peptone water were prepared. DNA was extracted from serial dilutions of this enrichment culture. The *mericon* Salmonella spp Kit was used for the assay, and salmonella was reliably detected at a dilution factor of 1:100,000.



Reliable detection of trace amounts of pig DNA.

The *mericon* Pig Kit was used to test for pig DNA in a series of diluted samples. High amounts of DNA do not interfere with the PCR. The assay is sensitive enough to detect fewer than 10 copies of target DNA.



Sensitive detection of Norovirus using the QuantiFast Pathogen RT-PCR +IC Kit on the Rotor-Gene Q.

QIAGEN real-time enzyme kits

Highly sensitive detection of RNA and DNA

- Many citations for water testing applications available

Highly flexible

- Can be used on any cycler

Convenient setup

- No need to optimize reaction and cycling conditions

Examples of pathogen nucleic acids detected with the QuantTect Probe PCR and RT-PCR Kits

Echovirus, Norovirus and somatic coliphages

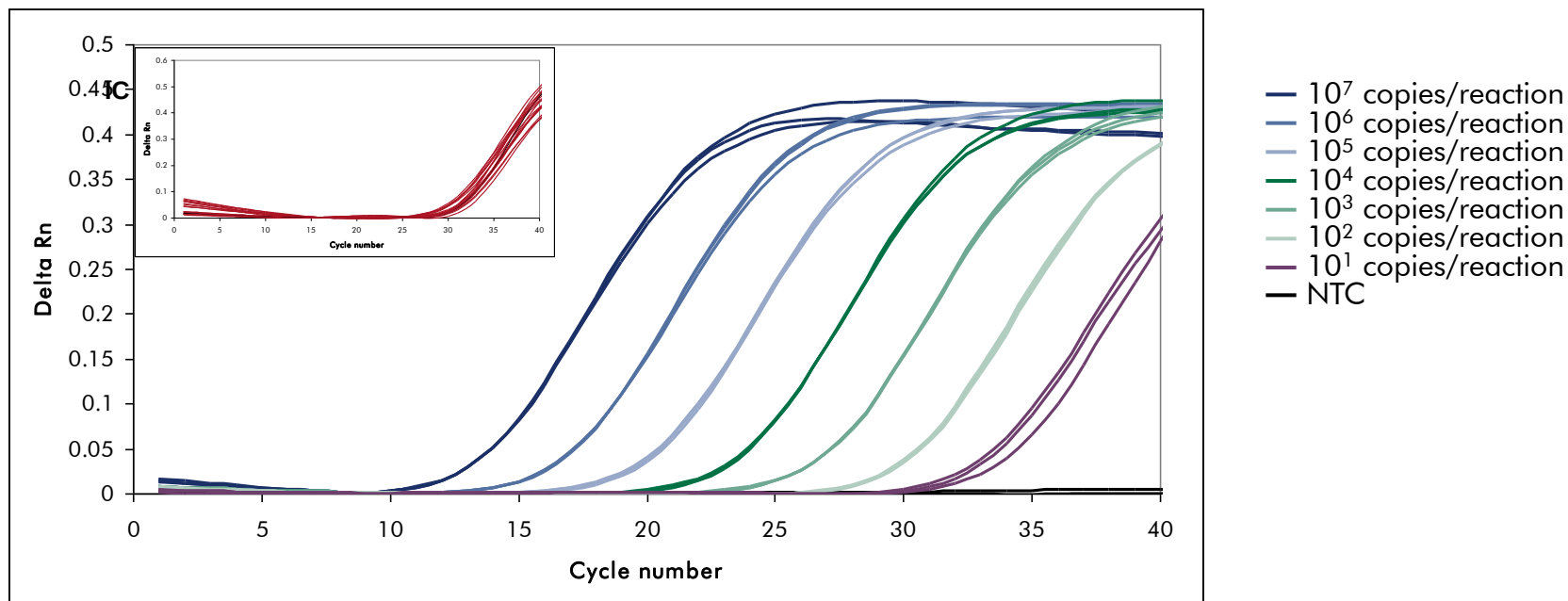
Enterococcus spp., *Clostridium* spp., *Giardia* spp., *Salmonella* spp. and bacteriophages

Legionella spp., *Cryptosporidium* spp.

Legionella pneumophila detection

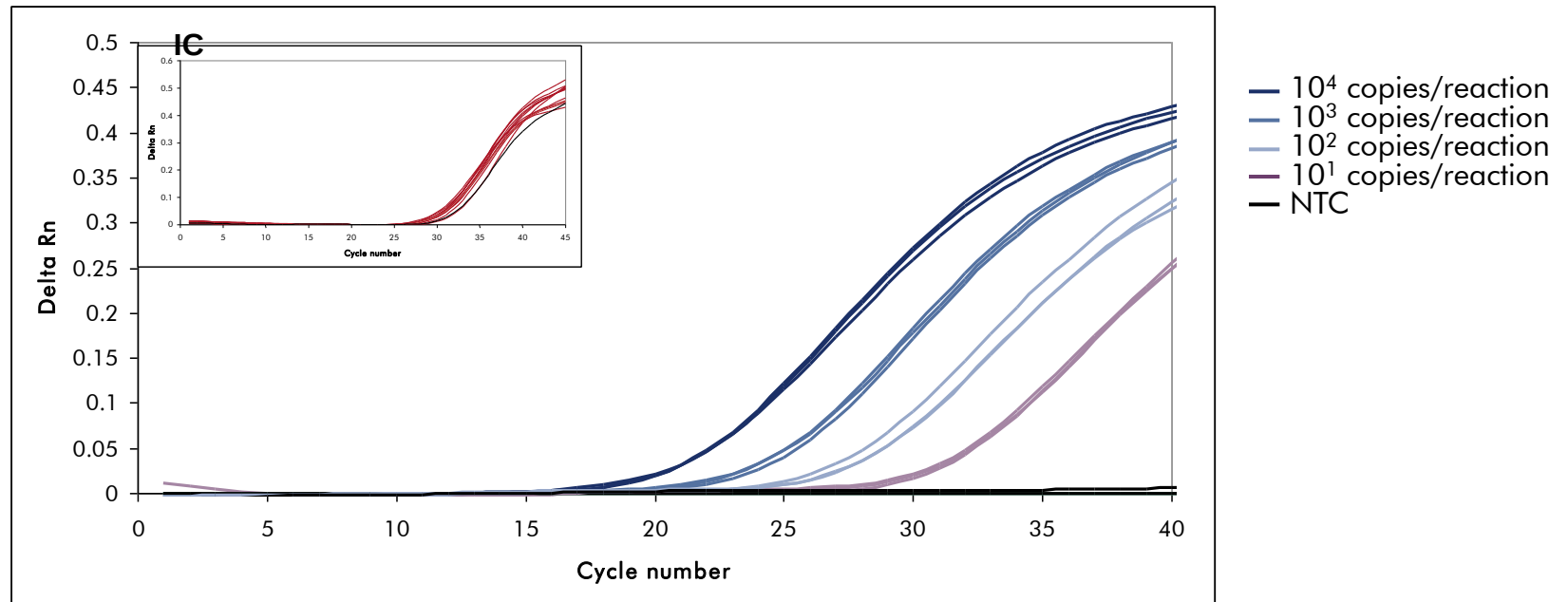
The assay detects DNA of *Legionella pneumophila* over a concentration range from 10 million down to 10 copies per reaction.

- All assay targets gave a signal in the green fluorescent channel
- The internal control (IC) was detected in the yellow fluorescent channel



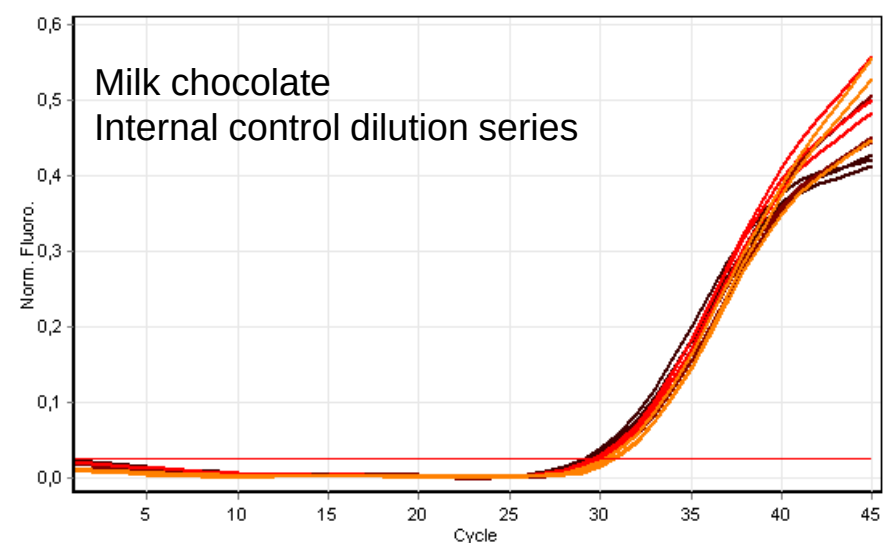
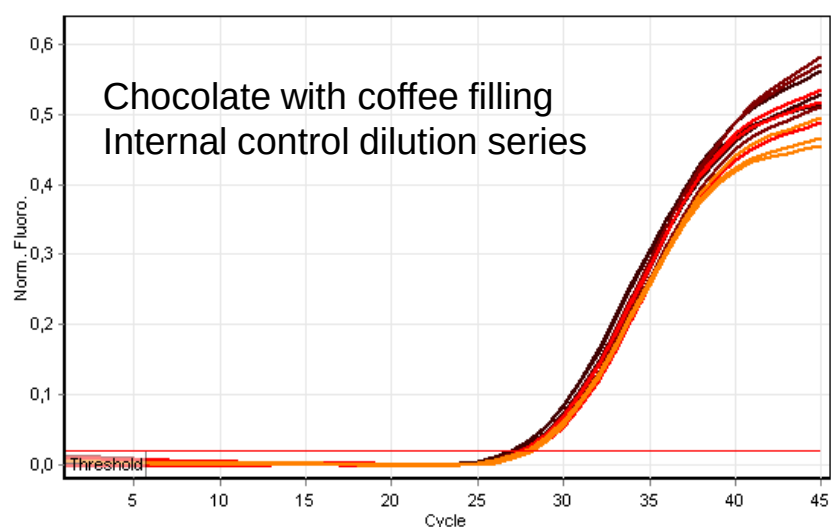
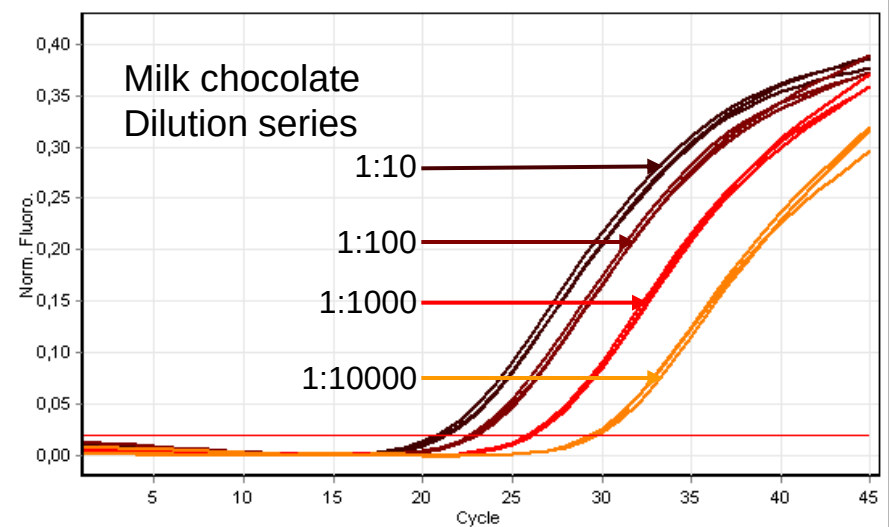
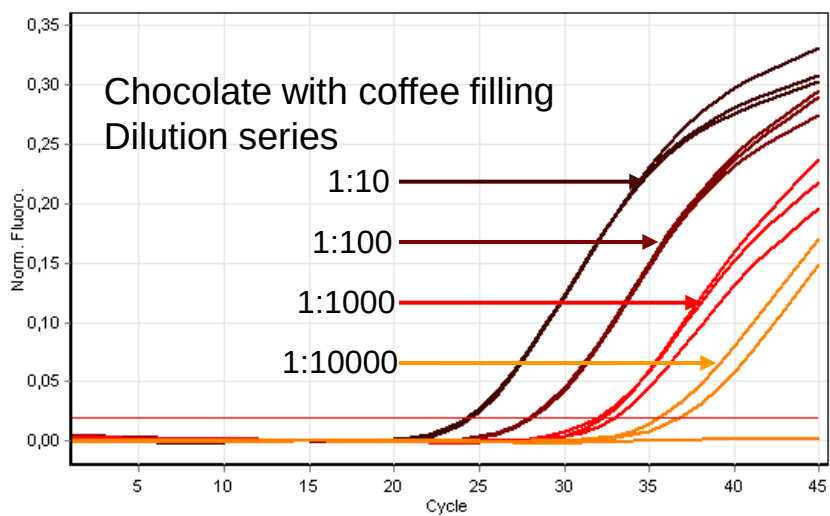
The assay detects DNA of the genus *Salmonella* over a wide concentration range down to 10 copies per reaction.

- All assays targets gave a signal in the green fluorescent channel
- The internal control (IC) was detected in the yellow fluorescent channel



Detection of salmonella in chocolate matrices

The QIA Symphony RGQ Pathogen Protocol





QIAGEN provides

- **Easy-to-use systems** that are fast to learn and implement with basic training in every lab
- **Large choice of validated workflows**, depending on throughput and regulation
- **Access to R&D and application labs** for specific development requests
- **Specific technical support** in English and in local languages
- **Field service engineers**, familiar with routine testing lab requirements
- **Extensive experience** in molecular testing

- Real-time PCR is a rapid, sensitive, and reliable method for the detection of pathogens in food and environmental samples.
- Real-time PCR is highly suitable for the specific DNA detection needed for GMO detection and ingredient authentication.
- QIAGEN is here to support you in your food safety and environmental testing work.



Thank you

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

