



Speed Matters -  
Fast ways from template to result

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# Overview

## Agenda

- Fast PCR
  - The Challenges
  - The Solutions
  - Application Data
  
- From cells to results
  - The principle
  - Proof Data
  
- Summary



## Benefits of Fast PCR

### Advantages

- Faster generation of results
- Increased sample throughput
- No need for investment in additional instrumentation
- Efficient sharing of cyclers
- Flexible planning of experiments



# Strategies to achieve fast PCR cycling

## Dedicated instrument

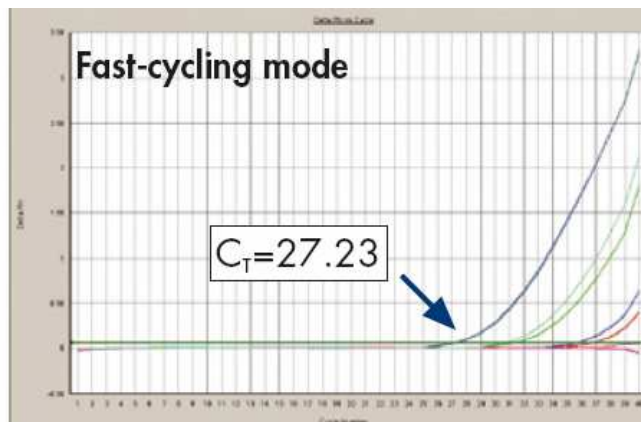
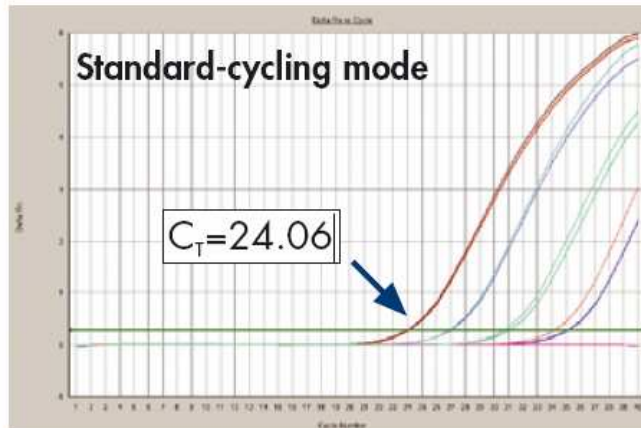
- Rapid ramping rates
- Short detection time

## Fast protocol

- Reduced time for all cycling steps
- Combination of primer annealing and extension
- Shortened RT step in one-step RT-PCR

## Kit chemistry

- Dedicated for fast-cycling conditions
  - Reduced DNA polymerase activation time
  - Rapid primer and probe hybridization



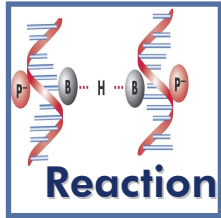
## Loss of performance

- Higher  $C_T$  values
- Reduced sensitivity
- Lower amplification efficiency

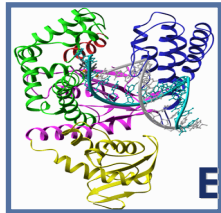
Faster quantitative real-time PCR protocols may lose sensitivity and show increased variability  
 Hilscher *et al.* NAR, 2005, 33, e183



# Key Elements for accelerated PCR



**The  
Reaction Buffer**



**The  
Enzyme**

## Step 1

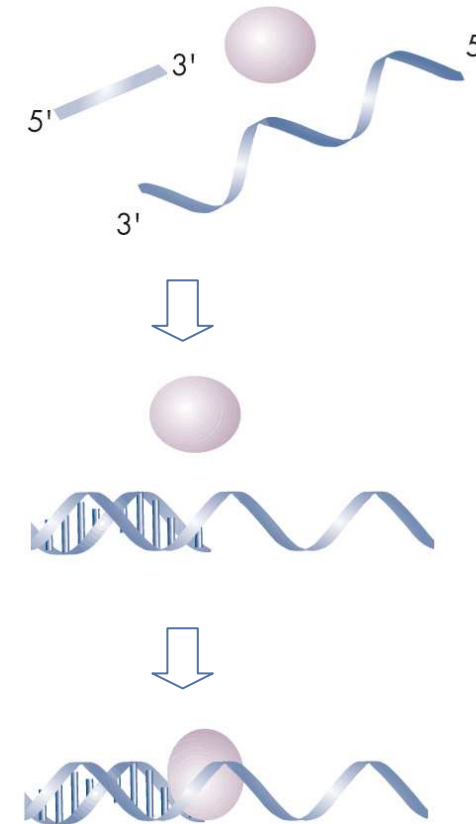
Hybridization of primer to single stranded template (binary complex)

## Step 2

Assembly of polymerase and primer-template complex (ternary complex)

## Step 3

Extension



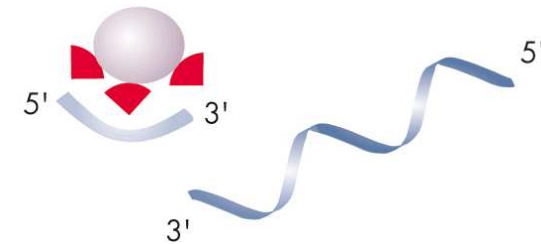
 Taq DNA polymerase  
  Template DNA  
  Primer



# QIAGEN's Fast Cycling – 2-step procedure

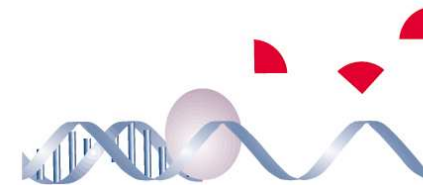
## Step 1




Hybridization of primer / polymerase complex to single stranded template (ternary complex)



## Step 2

Extension



 Q-Bond molecule     *Taq* DNA polymerase     Template DNA     Primer

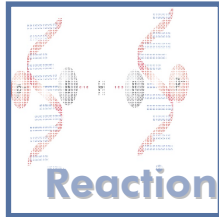


## Q-Bond makes the difference

### Features of Q-Bond

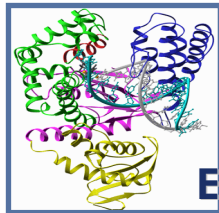
- Novel, non-proteinaceous buffer additive
  - Patent-pending technology
  - Increases binding affinity of DNA polymerase to short, single-stranded DNA
  - Turns 3-step process of
    - (1) template denaturation,
    - (2) primer annealing, and
    - (3) DNA polymerase binding in standard-cycling PCRinto a faster 2-step process
  - Decreases significantly time required for primer annealing and subsequent elongation
- Perfect completion of QIAGEN's unique PCR buffer system containing a balanced combination of KCl and NH<sub>4</sub>Cl promoting fast and specific primer annealing

# Key elements for accelerated PCR



## The Reaction Buffer

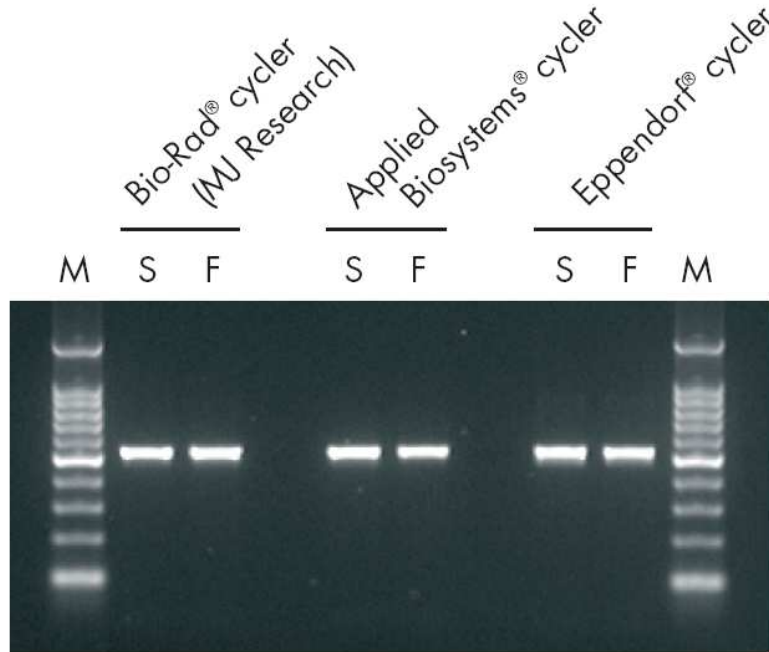
- Specialized reaction buffer chemistry



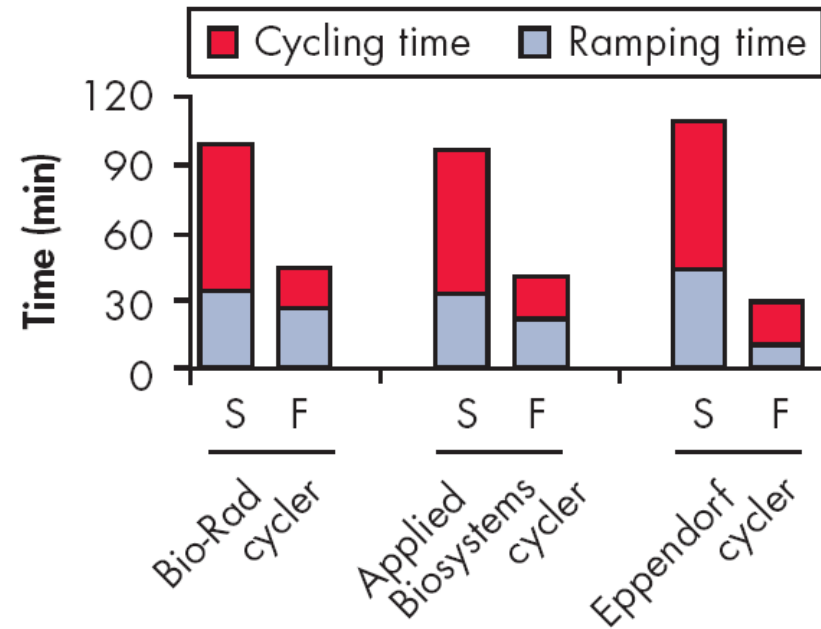
## The Enzyme

- HotStarTaq Plus DNA Polymerase
- Unique chemical modification of recombinant *Taq* DNA
  - Polymerase becomes rapidly active by initial 5 minutes heat incubation step
  - Robust reactivation independent from PCR environment (pH, salts)

# Fast cycling versus standard cycling



S = HotStarTaq *Plus* / Standard cycler  
 F = QIAGEN Fast Cycling PCR Kit / Fast cycler

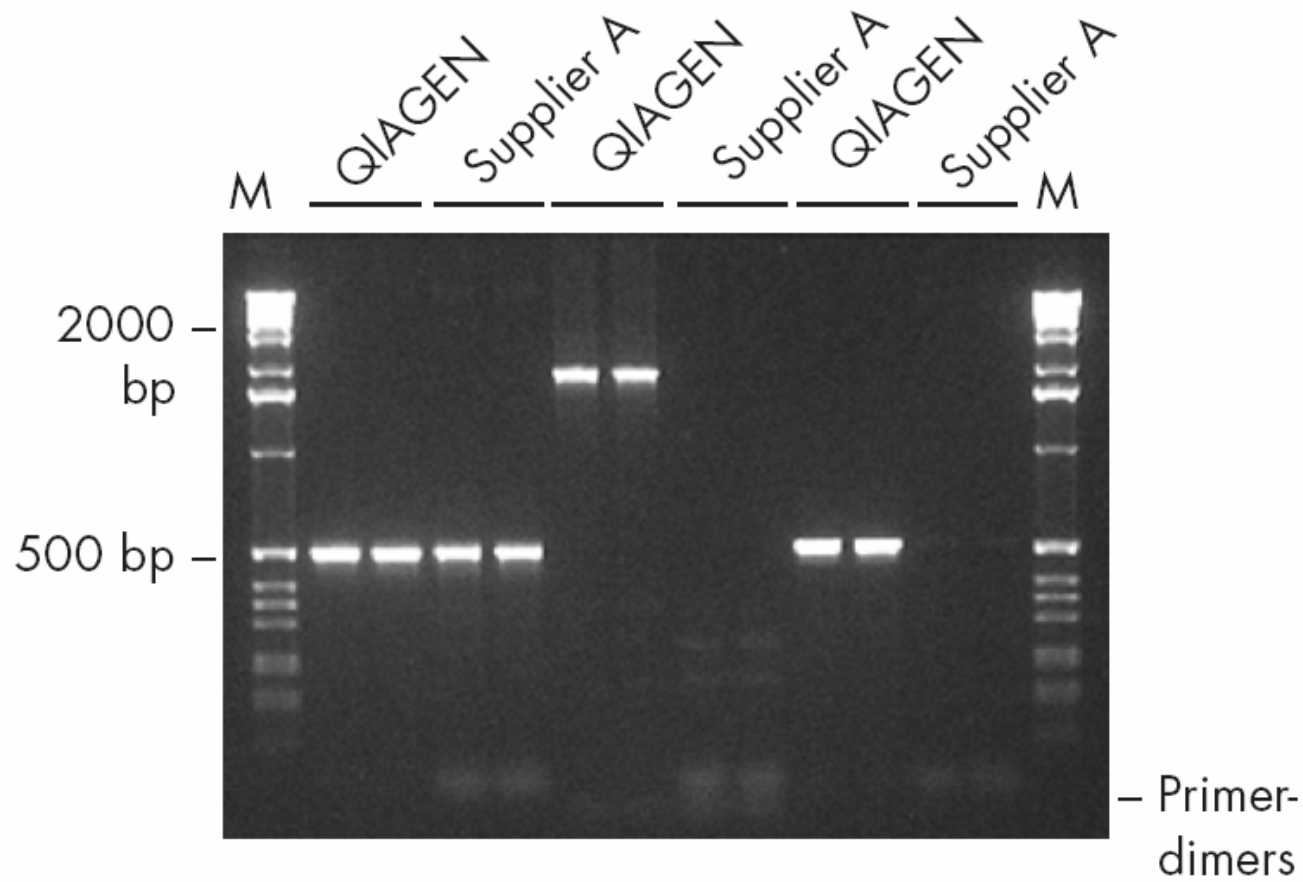


### Fast Cycling

Activation step:	<b>95°C</b>	<b>5 min</b>
Denaturation step:	<b>96°C</b>	<b>5 sec</b>
Annealing step:	<b>60°C</b>	<b>5 sec</b>
Extension step:	<b>68°C</b>	<b>3 sec / 100 bp</b>

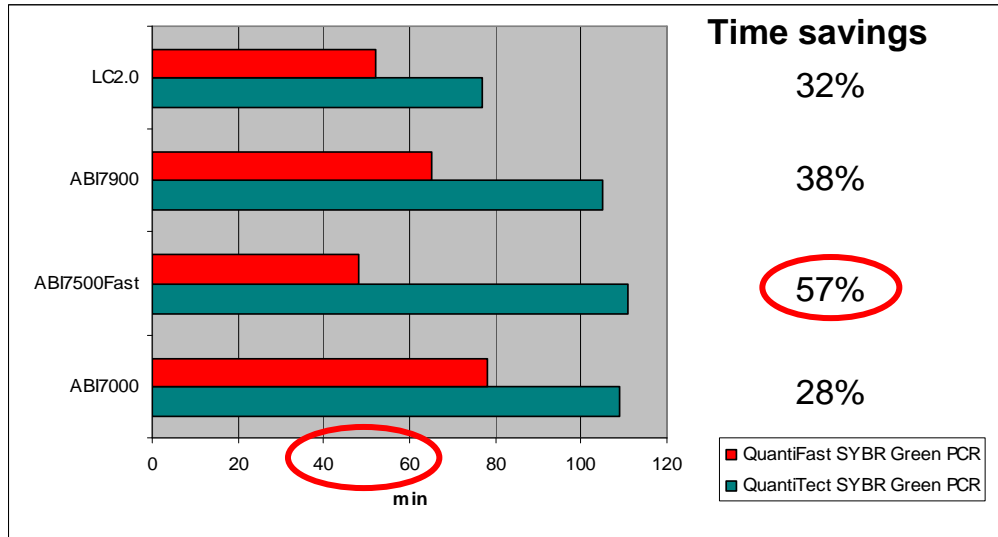


# Not all fast cycling kits are created equal





# Significant time savings in real-time PCR



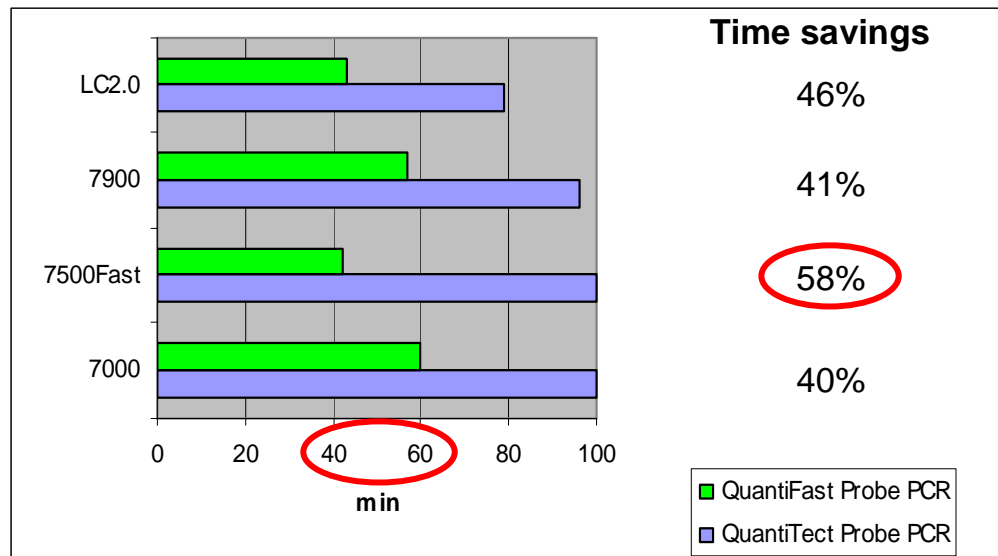
## SYBR Green detection

2-step

40 cycles

### Fast Cycling

Activation: 95°C - 5 min  
 Denaturation: 95°C - 10 sec  
 Annealing/  
 Extension: 60°C - 30 sec



## Probe detection

2-step

40 cycles

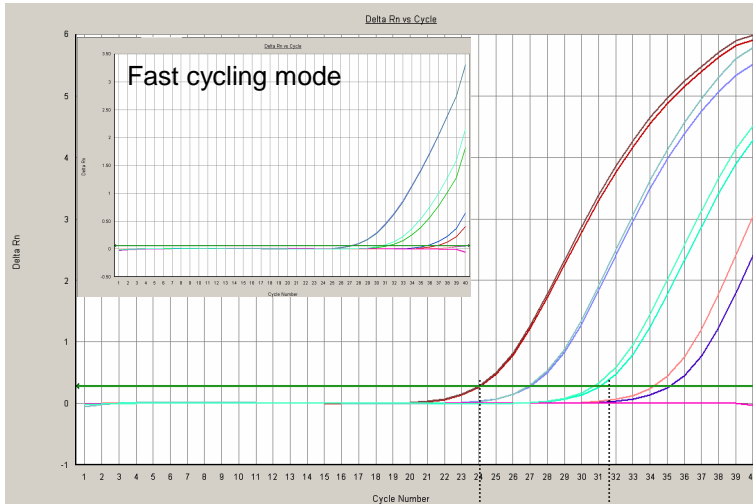
### Fast Cycling

Activation: 95°C - 3 min  
 Denaturation: 95°C - 3 sec  
 Annealing/  
 Extension: 60°C - 30 sec



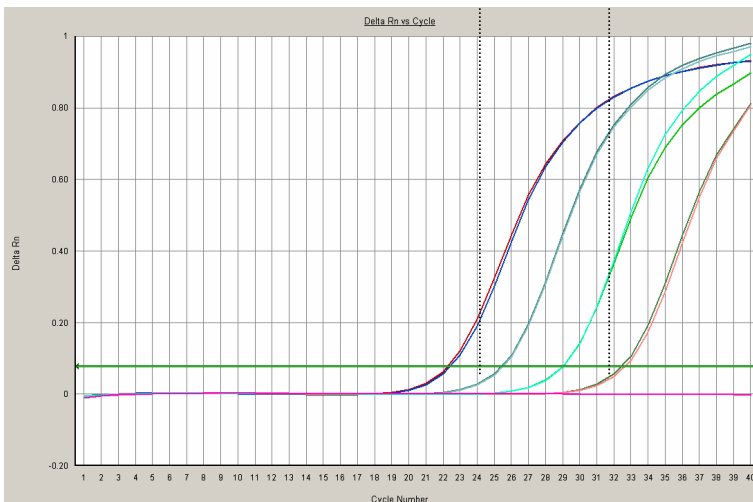
# Faster results without compromising sensitivity

Supplier A, standard cycling mode



cDNA	$C_T$
10 ng	24.06
1 ng	26.97
0.1 ng	30.88
10 pg	34.36
NTC	45.00

QuantiFast SYBR Green PCR Kit, fast cycling mode

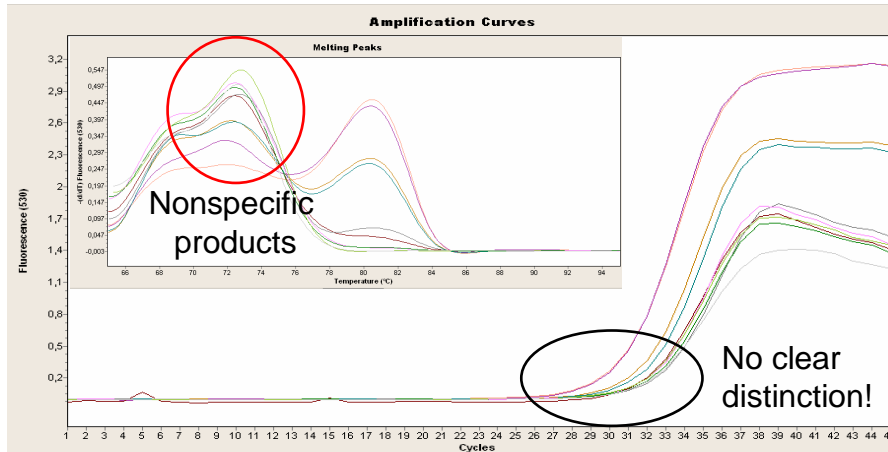


cDNA	$C_T$
10 ng	22.25
1 ng	25.41
0.1 ng	28.87
10 pg	32.41
NTC	45.00

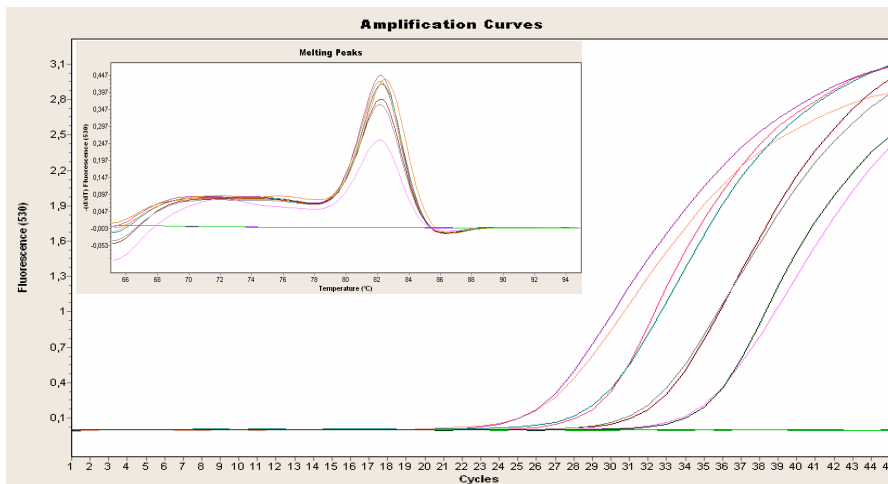
Gene: *MYC* (proto-oncogene)  
 Template: cDNA from human leukocytes  
 Primers: QuantiTect Primer Assay  
 Cycler: ABI7500 Fast System

# Superior specificity in one-step RT-PCR

## Supplier R, fast cycling protocol



## QuantiFast SYBR Green RT-PCR Kit

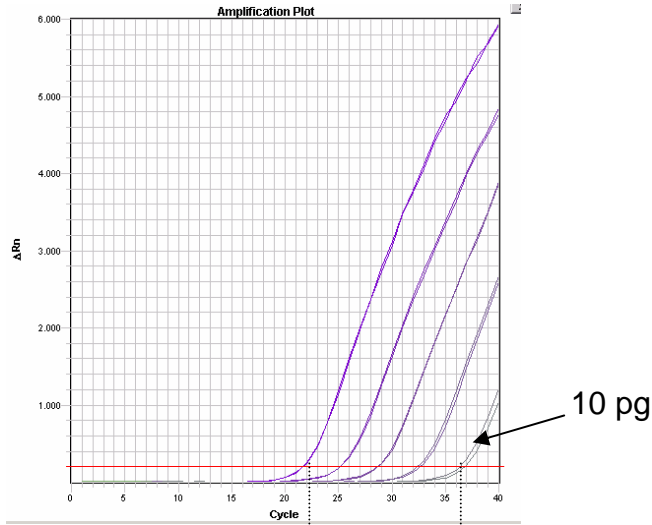


Gene: *BCL2* (apoptosis gene)  
 Template: RNA from HeLa cells  
 Primers: QuantiTect Primer Assay  
 Cycler: LC2.0



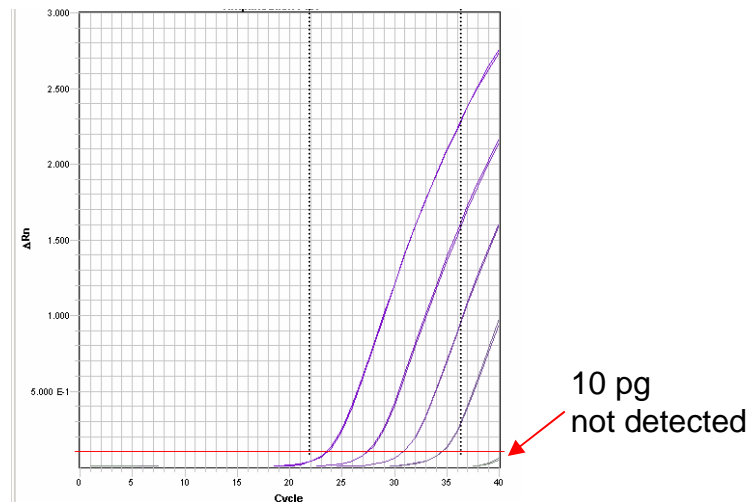
# Uncompromised sensitivity in probe based detection

## QuantiFast Probe PCR Kit



cDNA	C <sub>T</sub>
100 ng	21.66
10 ng	25.17
1 ng	28.69
0.1 ng	32.55
10 pg	36.60
NTC	40.00

## Supplier A, standard cycling mode

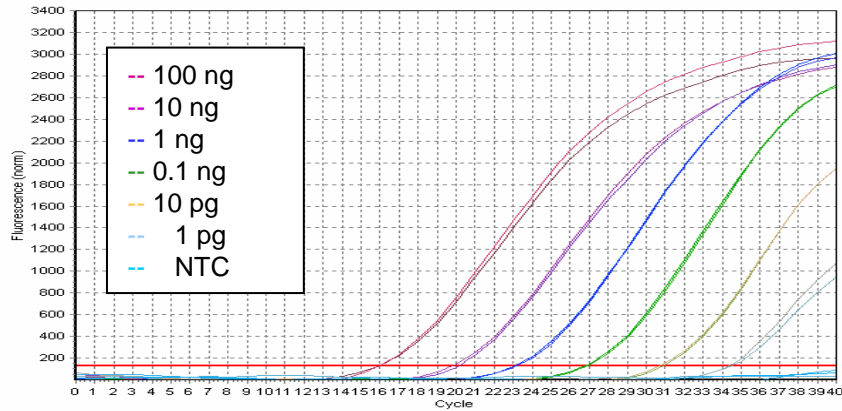


cDNA	C <sub>T</sub>
100 ng	23.60
10 ng	27.43
1 ng	30.89
0.1 ng	34.54
10 pg	--
NTC	40.00

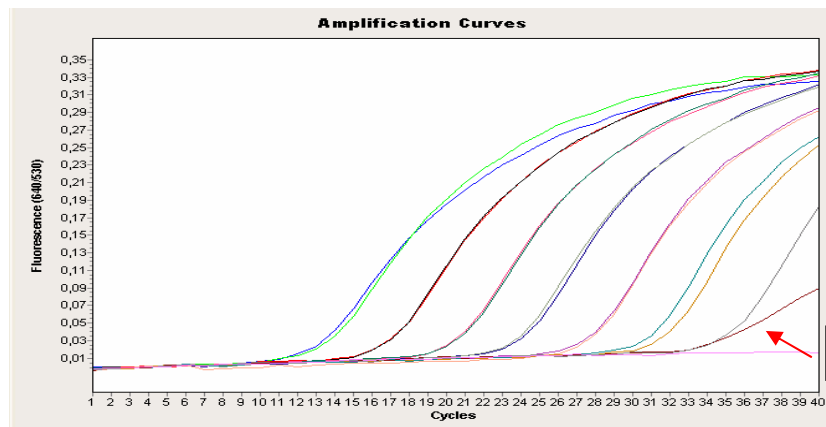
Gene: *IL1RN* (Interleukin receptor antagonist)  
 Template: cDNA from human leukocytes  
 Primer/probes: TaqMan Gene Expression Assay  
 Cycler: ABI7900



# Reliable detection independent of cyclor and probe



Gene: *Ubiquitin* (a regulatory protein)  
Template: cDNA from human leukocytes  
Primer/Probes: **Primer Express designed TaqMan Assay**  
Cycler: **Mastercycler ep *realplex***



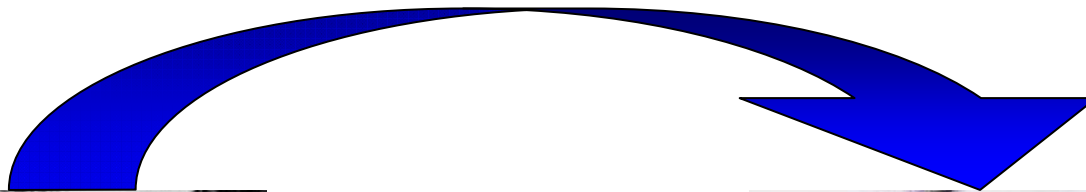
Gene: *B2M* ( $\beta$ 2-Microglobulin)  
Template: human leukocyte cDNA  
Primer/probes: **FRET Probes**  
Cycler: **LC2.0**



## New fast cycling chemistry

For all end-point and qPCR applications

- Significant time savings
- No need to invest into dedicated fast cycling instrument
- Successful amplification without further optimization
- Versatile use on any real-time cycler
- No loss in sensitivity and specificity when switching to fast cycling





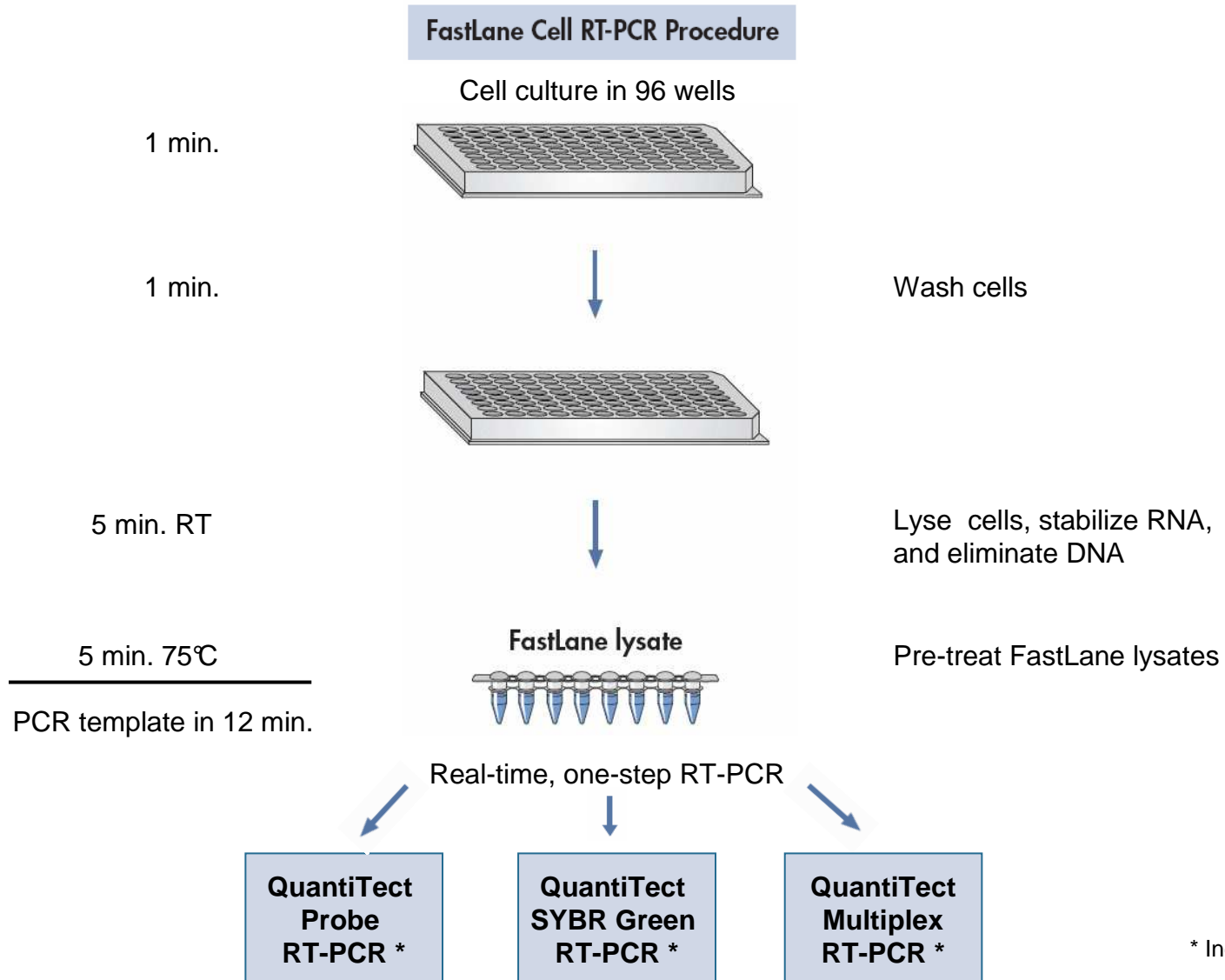
# Overview

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# FastLane Cell RT-PCR - Speed up & Simplify the Workflow

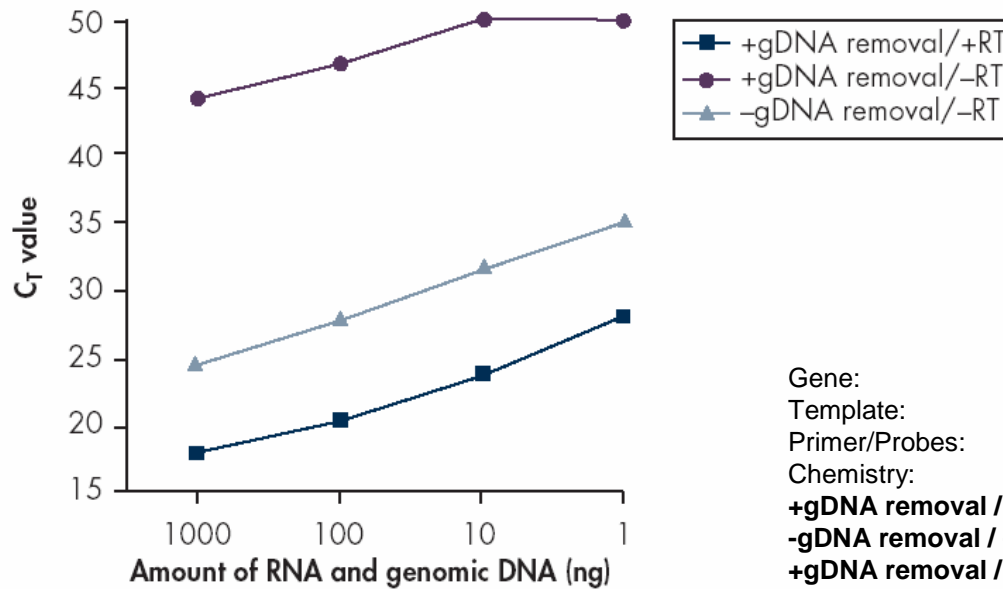


\* Included in FastLane Cell RT-PCR kits



# Effective gDNA Elimination

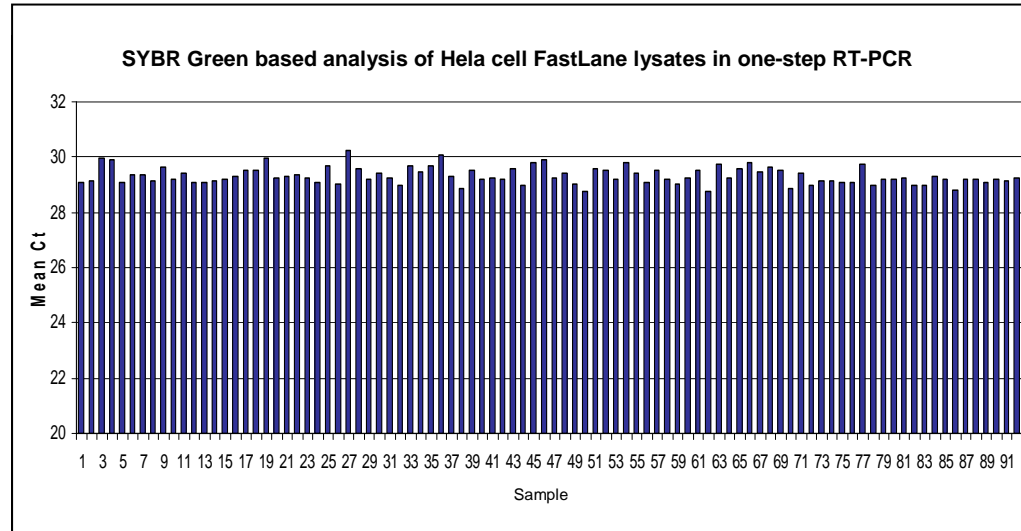
## Efficient Removal of Genomic DNA from RNA Samples



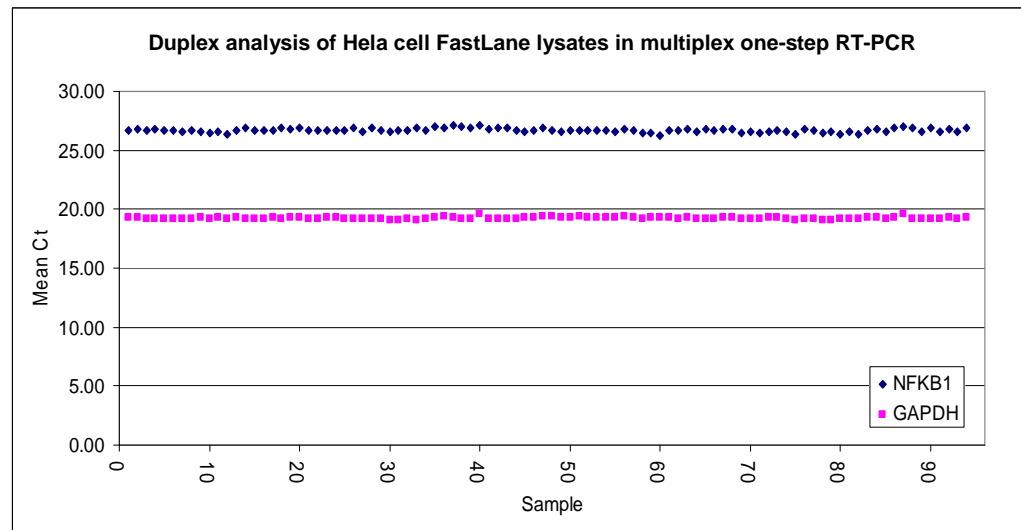
Gene: *GAPDH* (a housekeeping gene)  
Template: 1:1 mixture of RNA and genomic DNA  
Primer/Probes: Probe based Assay detecting RNA and DNA  
Chemistry: QuantiTect Probe PCR Kit  
**+gDNA removal / +RT:** gDNA removal followed by reverse transcription  
**-gDNA removal / -RT:** no gDNA removal and no reverse transcription  
**+gDNA removal / -RT:** gDNA removal and no reverse transcription



# FastLane Cell RT-PCR – Reproducibility in all Formats



Genes: *INPP5D* (a cell proliferation regulator)  
Template: Hela cell culture  
Primers: QuantiTect Primer Assay  
Technology: FastLane SYBR Green  
**96 sample CV: *INPP5D* – 1,02%**



Genes: *GAPDH* (a housekeeping gene)  
*NFKB1* (transcription regulator)  
Template: Hela cell culture  
Primer/Probes: Primer Express designed TaqMan Assays  
Technology: FastLane Multiplex  
**96 sample CV: *GAPDH* – 0,62%  
*NFKB1* – 0,43%**



## FastLane Technology – from Sample Direct to Result

- No RNA purification – significantly saving time
- Just 3 steps from cells to real-time RT-PCR
- RNA detection only due to unique gDNA Wipeout Buffer
- Sensitive detection of low-copy targets using QuantiTect Chemistry included:
  - QuantiTect SYBR Green
  - QuantiTect Probe
  - QuantiTect Multiplex ROX or No ROX



## Summary - Fast ways from sample to results

### QuantiFast Chemistry

- Significant time savings with uncompromised performance
- Universal use on any cyclers

### FastLane Technology

- Integrated gDNA removal
- Fast and simple workflow from cells to results