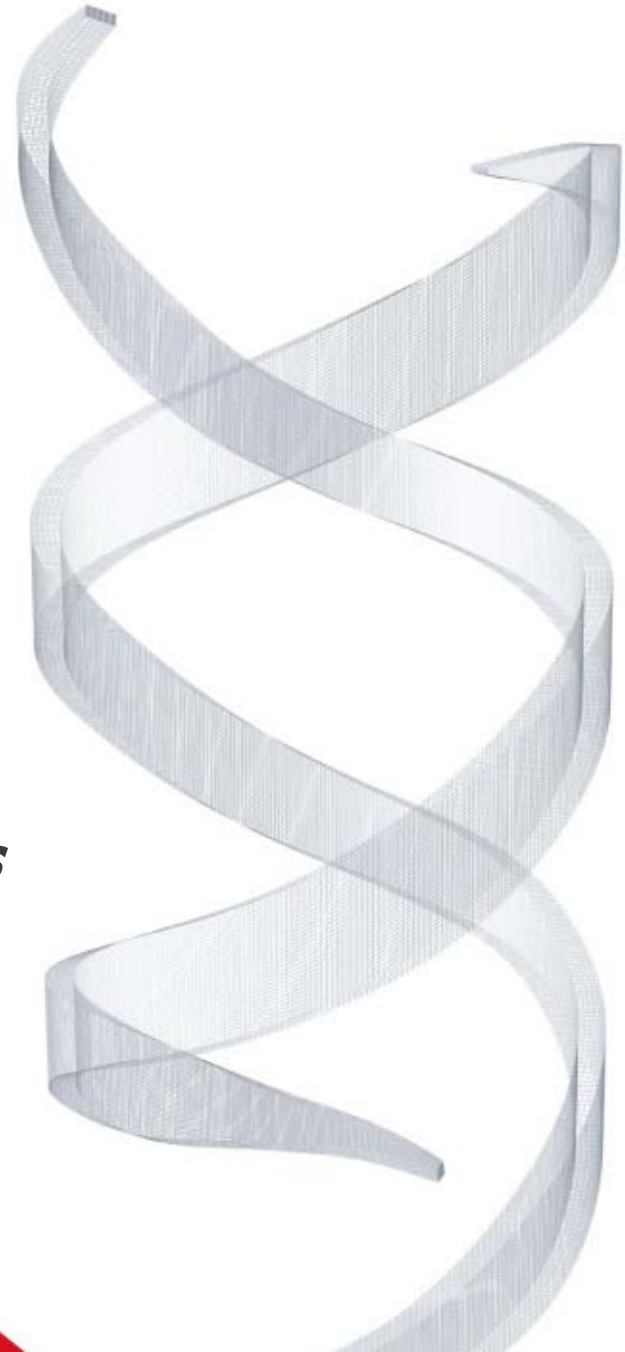
invitrogen™
accelerate

Improving cDNA yield using SuperScript™ III



Debra Ann Nickson
Senior Product Manager-Genomics
qPCR Symposium – Leipzig
March 2005

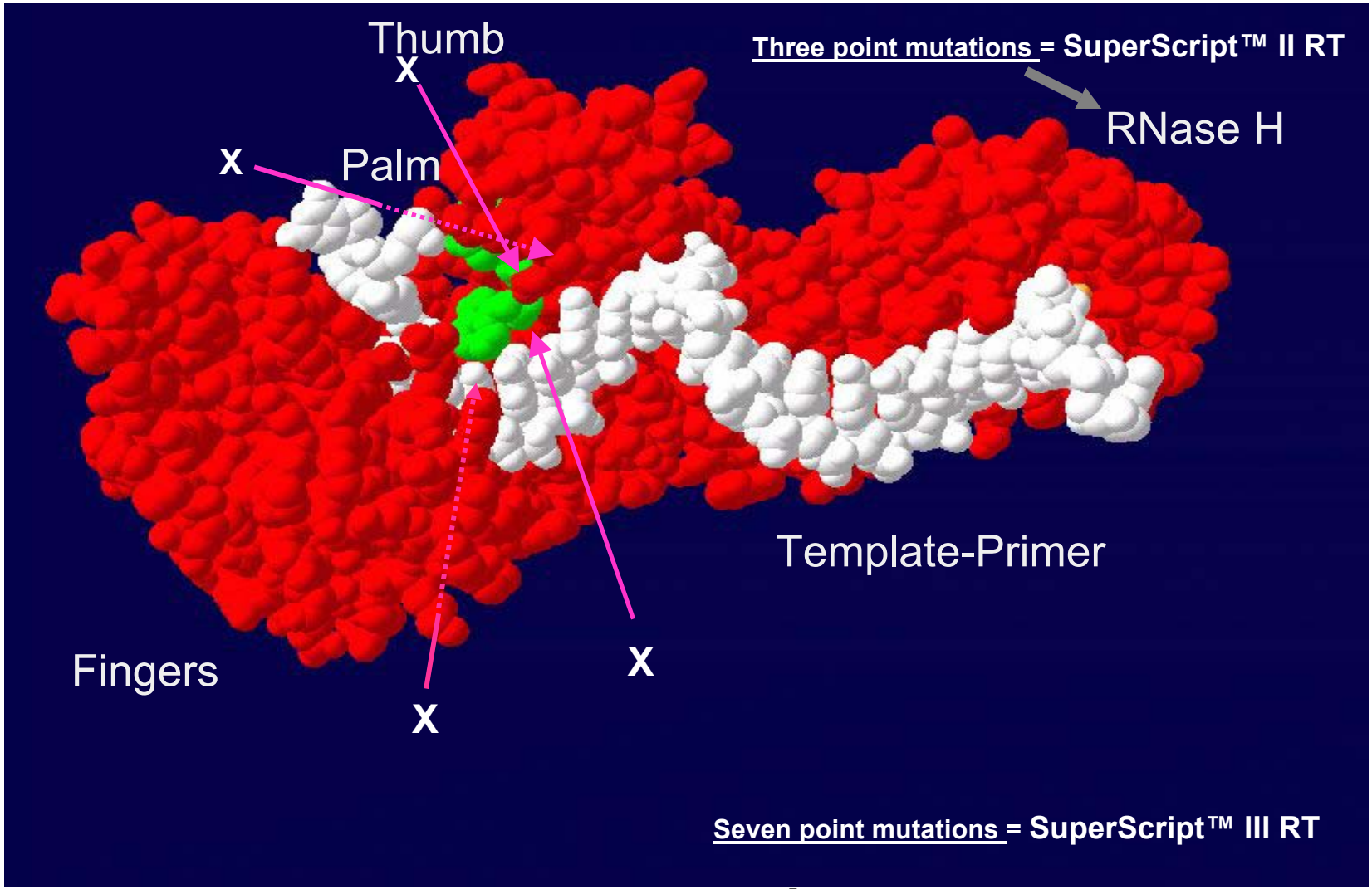
Overview

- Researchers needs for qRT-PCR
- Properties of SuperScript™ III reverse transcriptase
- RNA preparation
- Efficiencies of cDNA synthesis measured in two-step qRT-PCR
- Choosing the best qRT-PCR product for your application
  - One step vs two step
- Summary

Researchers needs for real-time qRT-PCR

- Dynamic range
  - Broad linear range routinely over 6-7 orders of magnitude
- Sensitivity
  - Low limit of detection (a few copies, sub-picogram levels of DNA/RNA)
- Specificity
  - Quantify only the target of choice
- Multiplexing
  - Detect multiple genes in one tube, commonly for amplifying gene of interest and endogenous reference gene simultaneously
- Convenience
  - Closed tube assay format, mastermix configuration, amenable to high throughput applications
- Flexibility
  - Compatible with various detection platforms and instrument platforms

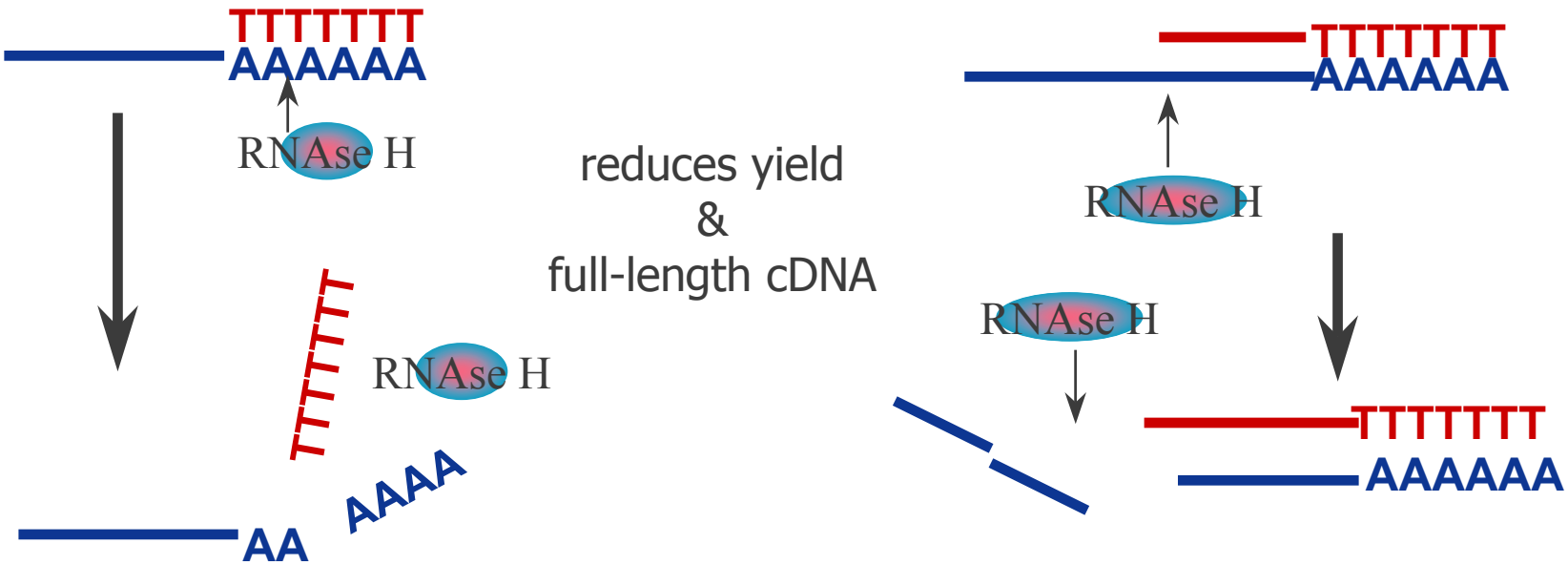
SuperScript™ III RT Engineering



Fidelity: MMLV, SuperScript™ II/III, AMV: ~ $5X10^{-5}$ error rate (LacZ assay)

SuperScript™ III – the new gold standard

RNaseH activity

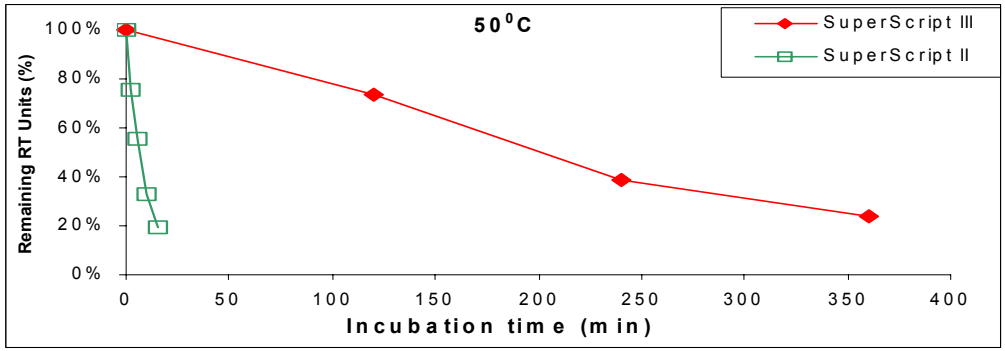


	MML-V	SuperScript™ III
Total cDNA	381ng	725ng
Full length cDNA	181ng	355ng
Polymerase activity(units/mg)	330,000	410,000

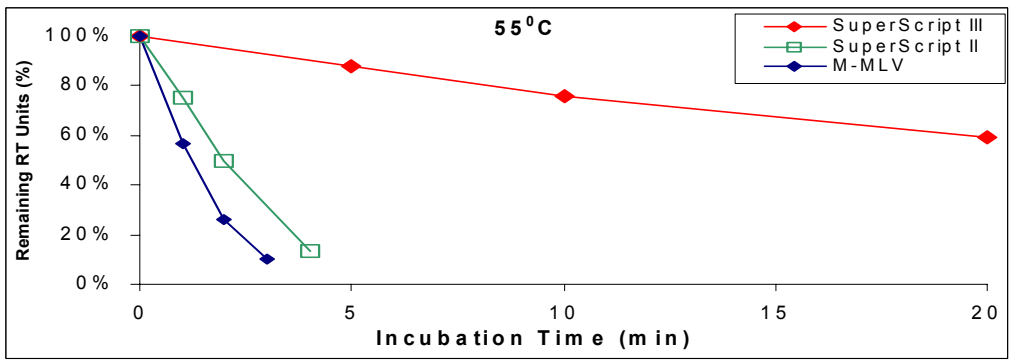
Relative results using 2 μg of a 7.5kb RNA (40% which is poly A+)

SuperScript™ III Thermostability

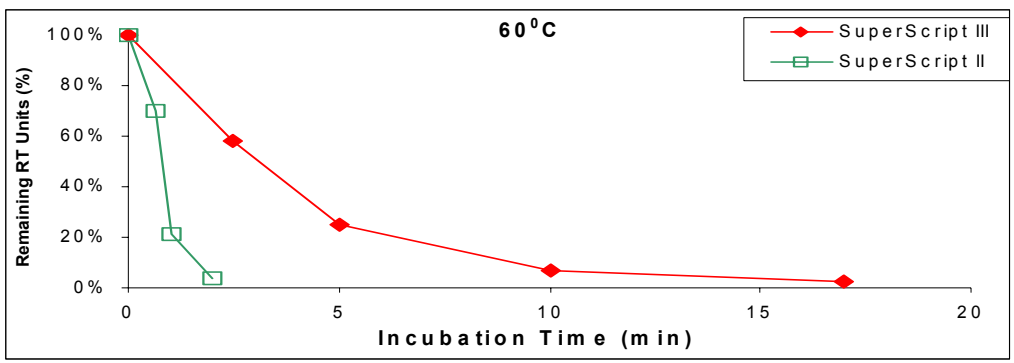
50ºC



55ºC

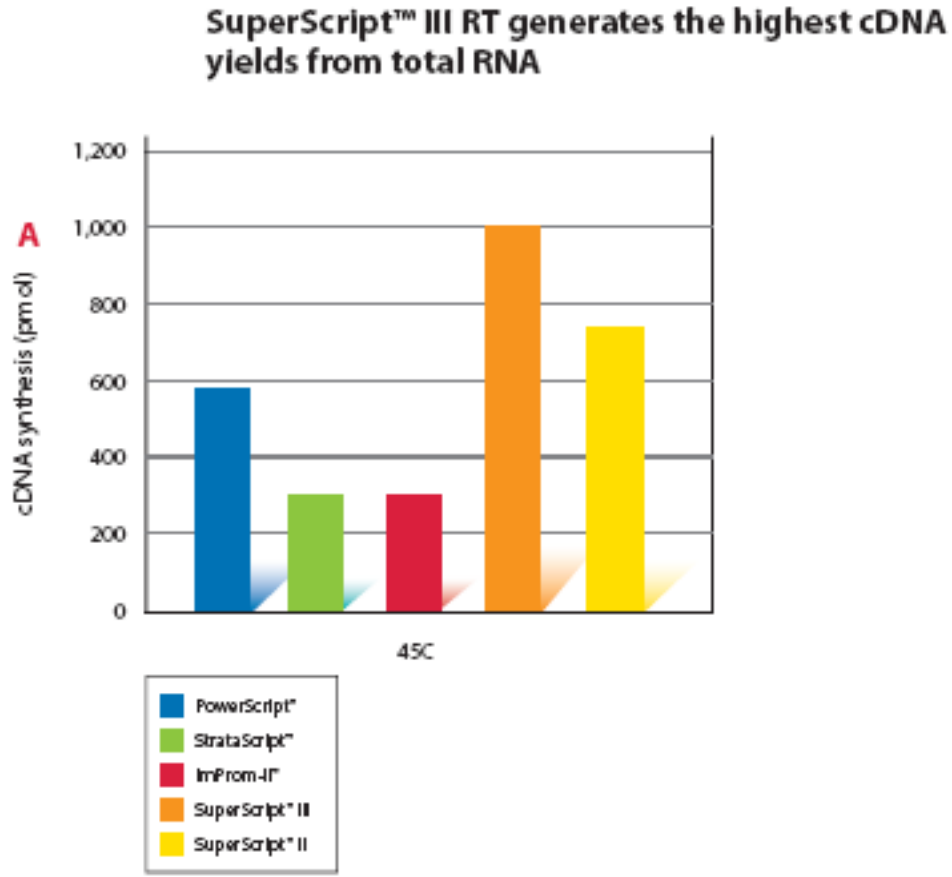


60ºC

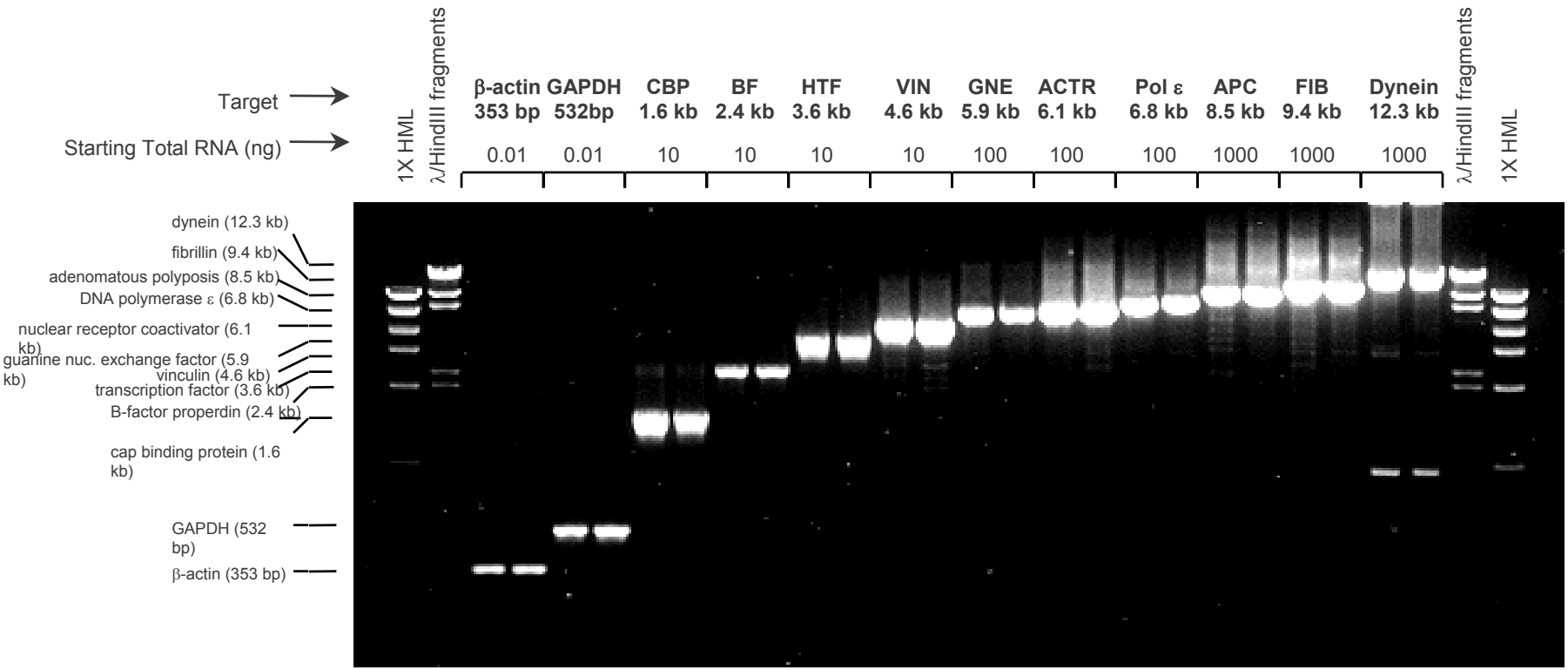


Reverse Transcriptase	Half-life (min) at 50°C
SuperScript™ III	220
SuperScript™ II	6.1
MMLV	3.5

SuperScript™ III high cDNA yields



SuperScript™ III Target Size



A word on template preparation

RNA Template Preparation:

- Ready to use solutions TriZOL®
- Silica-based technology PureLink™ Micro-to-Midi™ Total RNA Kit
- High-Throughput Isolation PureLink™ 96 Total RNA Purification Kit

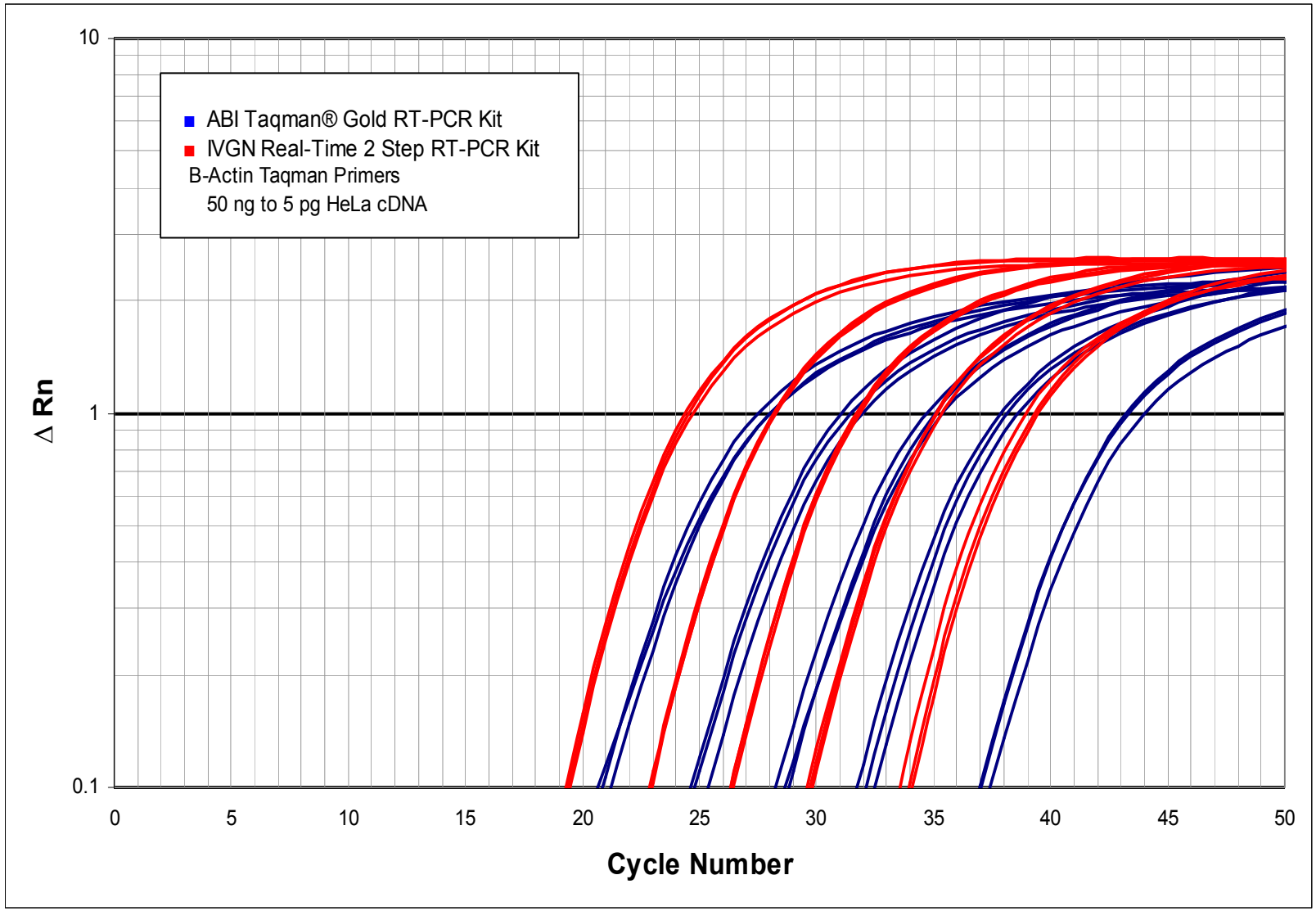
Eliminate genomic DNA contamination

- RNA should be treated with DNase I Amplification grade
- DNase I is then heat-inactivated by adding EDTA followed by incubation at 65ºC for 10 min.

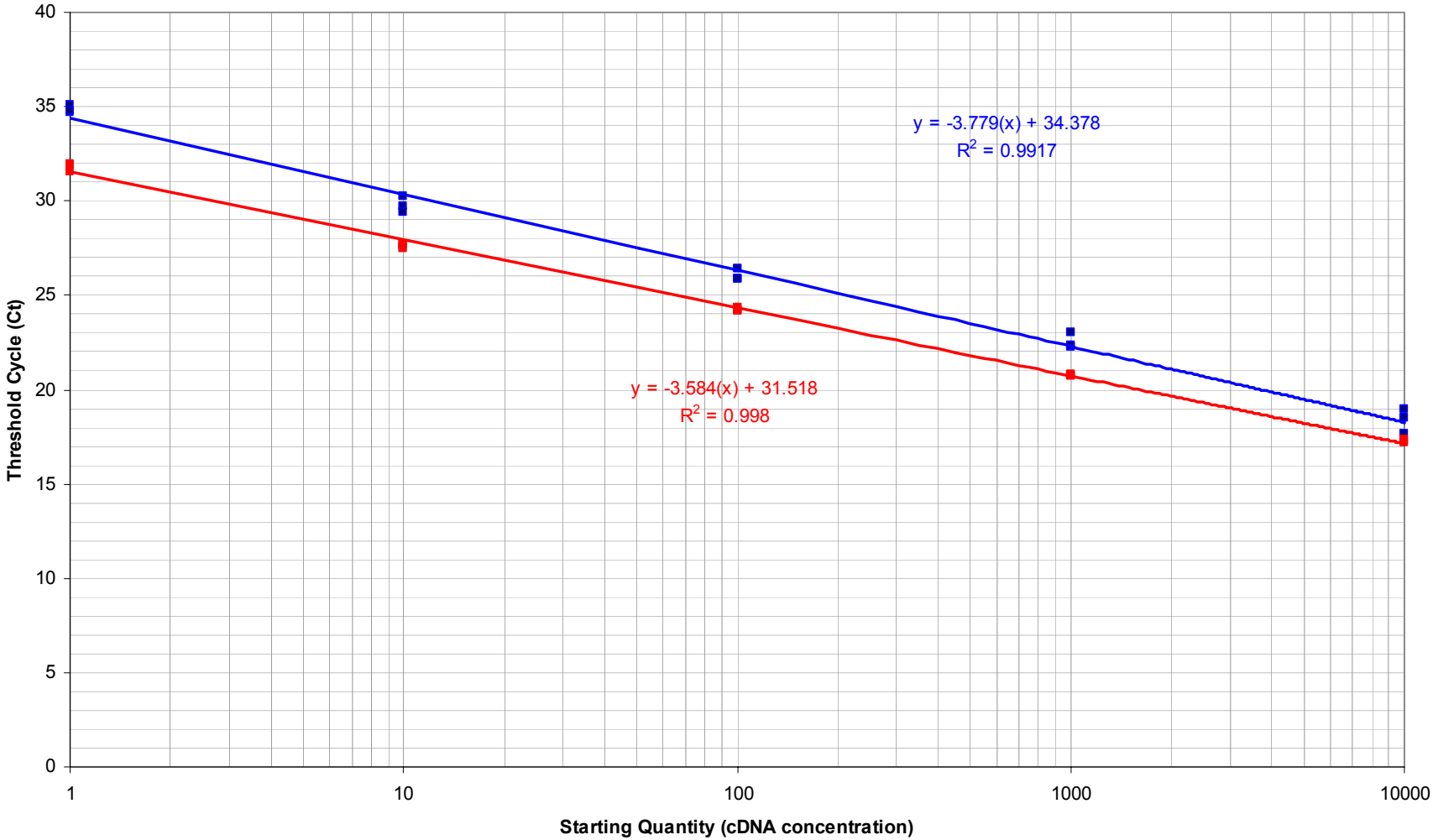
All RNA used should have an A_{260}/A_{280} ratio higher than 1.95

Efficiencies of cDNA synthesis measured in two-step qRT-PCR

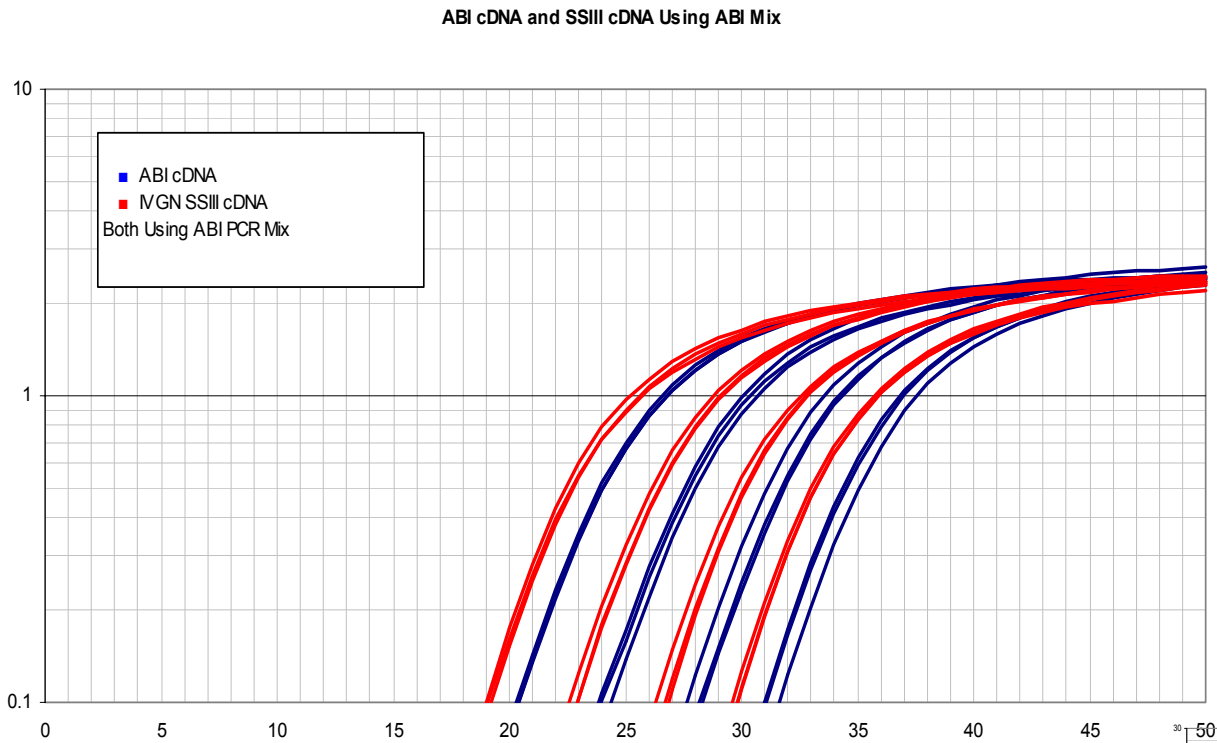
Comparison of two step RT-PCR Kits

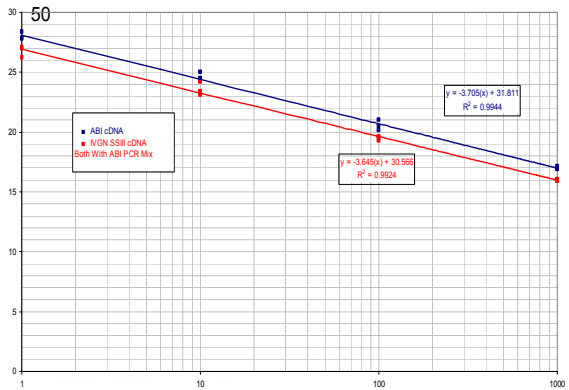


Comparison of two step RT-PCR Kits

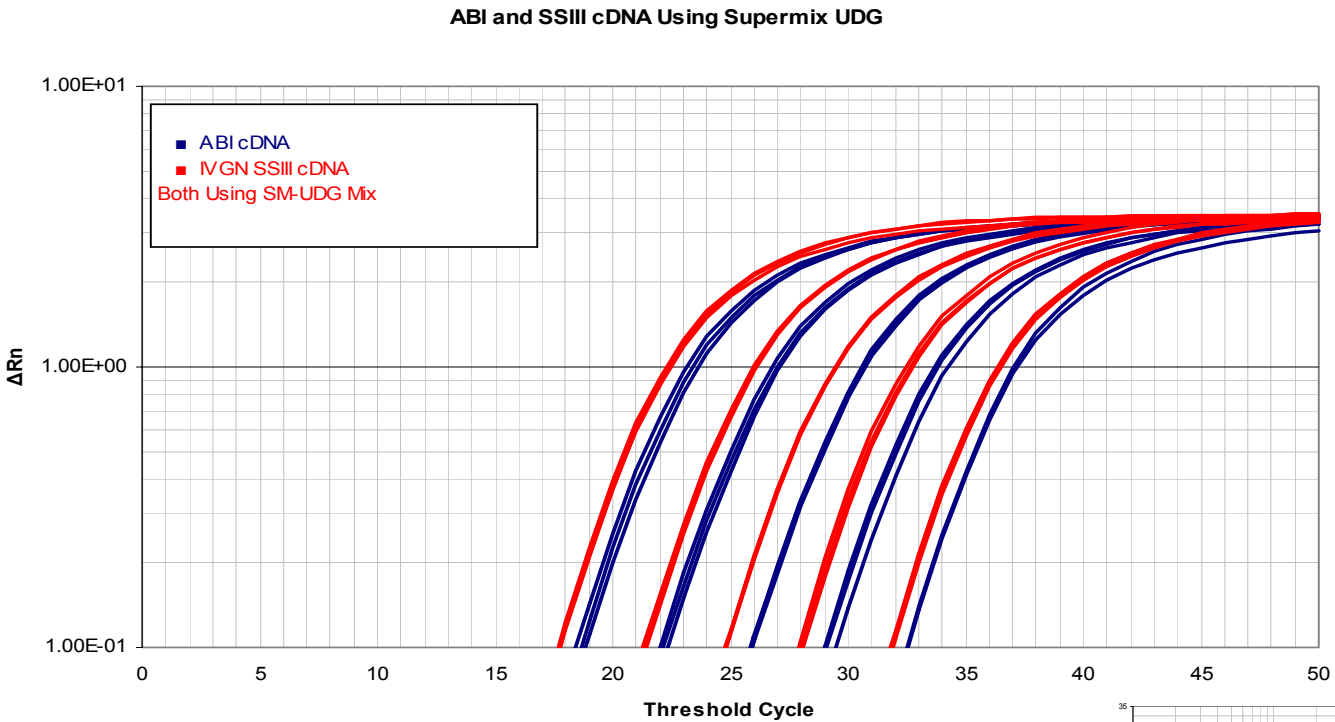


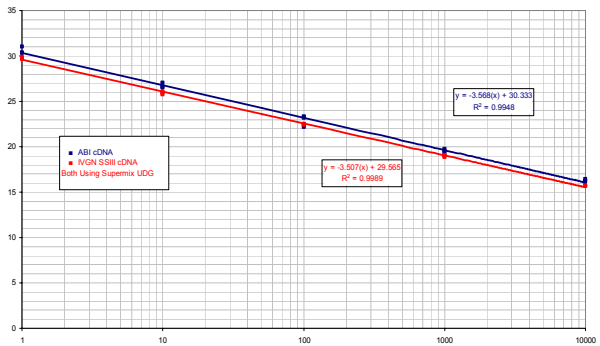
Comparison of cDNA sensitivity competitive audit





Comparison of cDNA sensitivity competitive audit





The best qRT-PCR product for your application

High performance, real-time quantitative RT-PCR requires high performance enzymes and systems

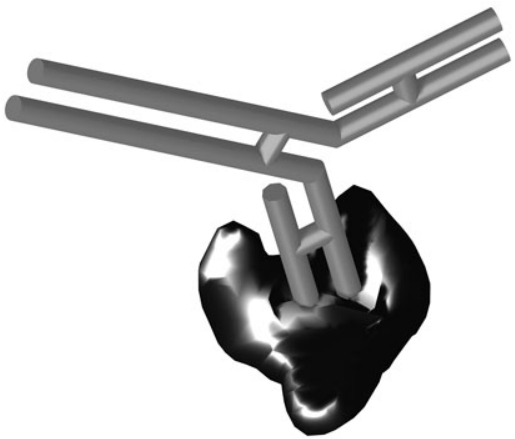
- **SuperScript™ III Reverse Transcriptase** with increased thermostability and half-life for higher cDNA yields and better specificity with gene-specific primers
- **Platinum® *Taq* DNA Polymerase** with antibody-mediated hot-start technology for higher PCR specificity and yield with room temperature setup for faster, more convenient setup, without performance loss
- Easy-to-use qPCR supermixes, one-step and two-step qRT-PCR systems

Automatic Hot Start

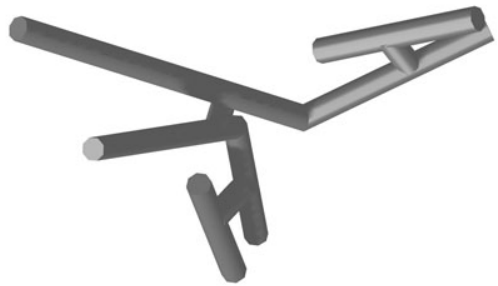
PCR Assembly

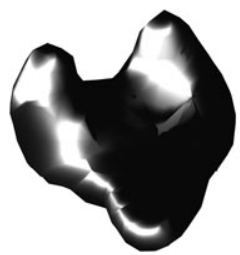
Initial Template Denaturation

Temperature Cycling



96°C, 30 s - 2 min

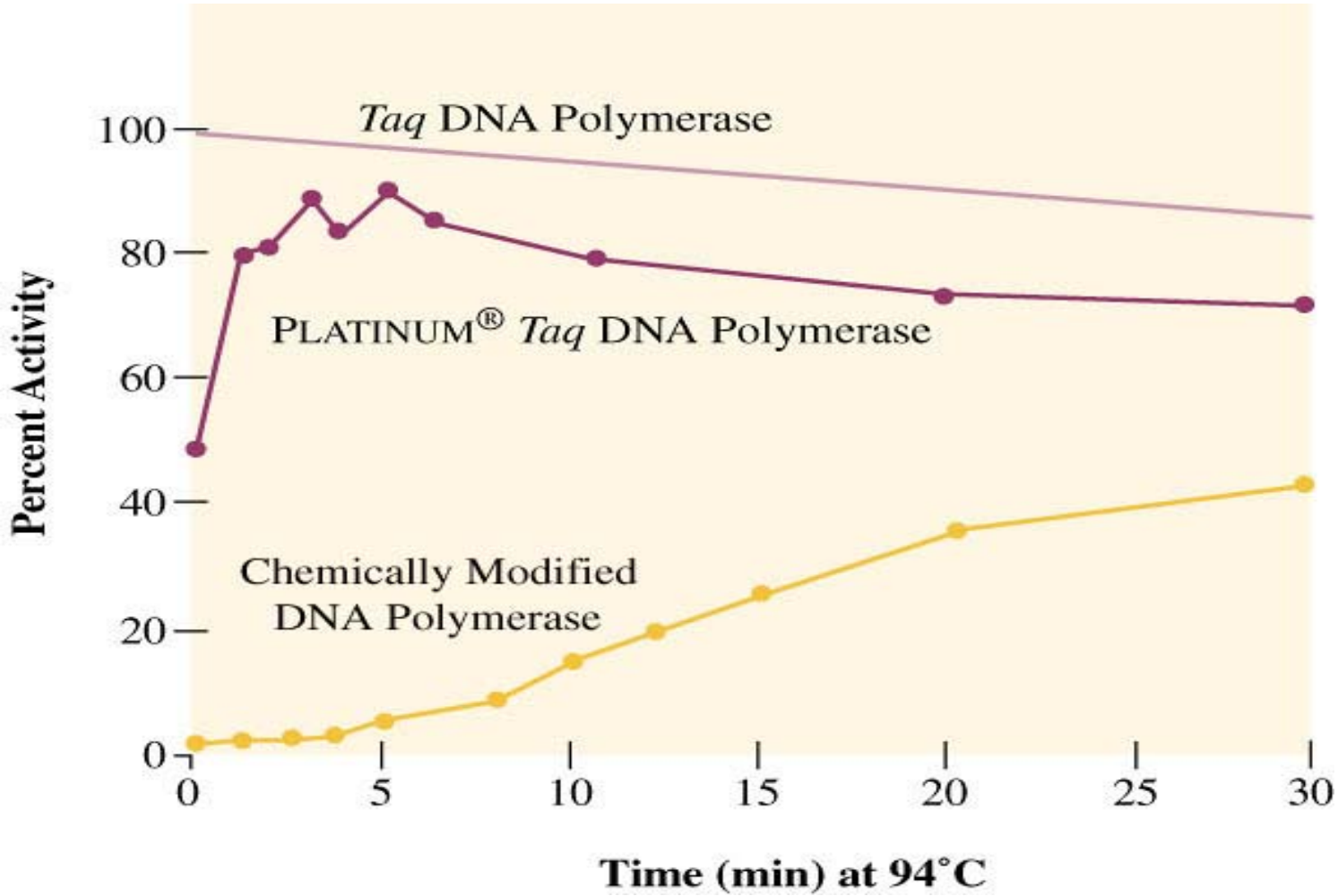




Inactive *Taq* DNA Polymerase

Fully Active *Taq* DNA Polymerase

Reactivation of Platinum® Taq activity



Platinum® *Taq* is activated quicker and has more enzyme activity during the PCR

Choice of qRT-PCR assay formats

Two-Step RT-PCR

- Separate conditions for cDNA synthesis & PCR
- Flexible choice of primer
  - Typically oligo(dT) and/or random hexamers are used
- Ideal for quantification of multiple genes from a limited number of RNA samples

One-Step RT-PCR

- Highly defined conditions to support RT and *Taq*
- Requires gene specific primer
- Higher throughput (many RNA samples)
- Can use all the RNA from a small sample
- Ideal for quantification of 1 or 2 messages from a large number of RNA samples

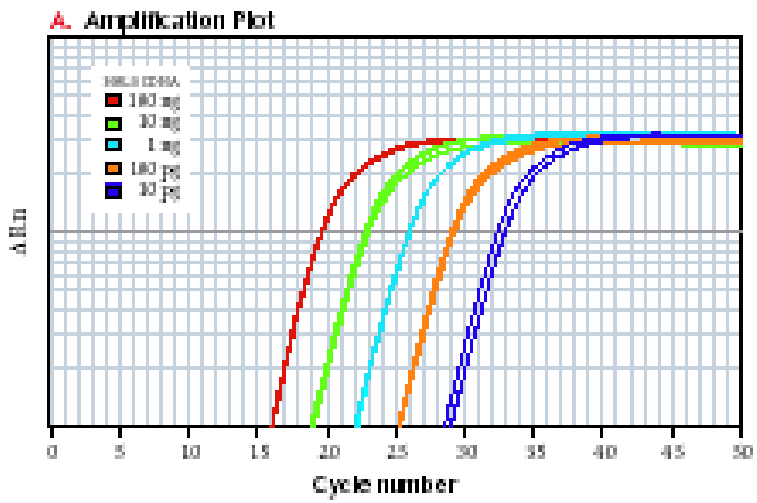
Flexible two step qRT-PCR kits

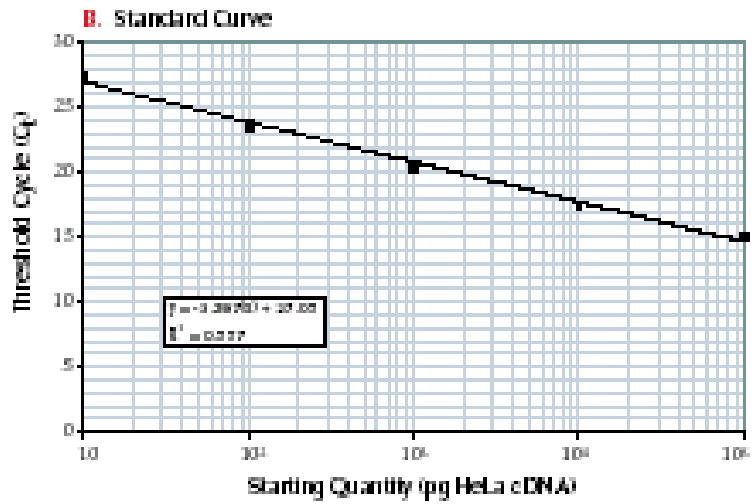
SuperScript™ III Platinum® Two-Step qRT-PCR Kit
For LUX™ and probe based detection systems
SuperScript™ III Platinum® Two-Step qRT-PCR Kit with SYBR® Green
For detection based on SYBR® Green

Kit Components:
SuperScript™ III Enzyme Mix
2x RT Reaction Mix
E. coli RNase H
DEPC-treated water
50mM $MgCl_2$
20x BSA 5 mg/ml
ROX Reference Dye
Platinum® Quantitative PCR SuperMix-UDG
or
Platinum® SYBR® Green qPCR SuperMix-UDG

SuperScript™ III Platinum® Two-Step qRT-PCR Kit with SYBR® Green

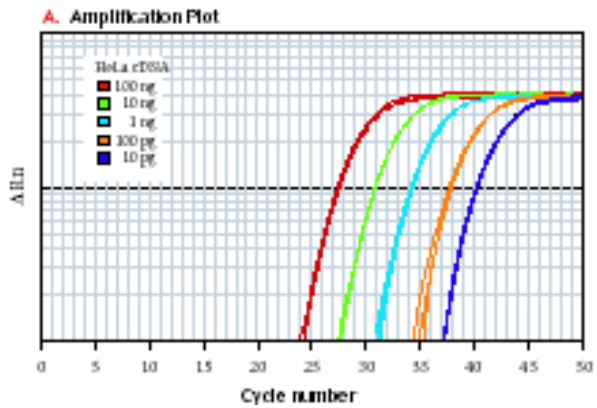
provides easy and convenient detection with SYBR® Green I dye

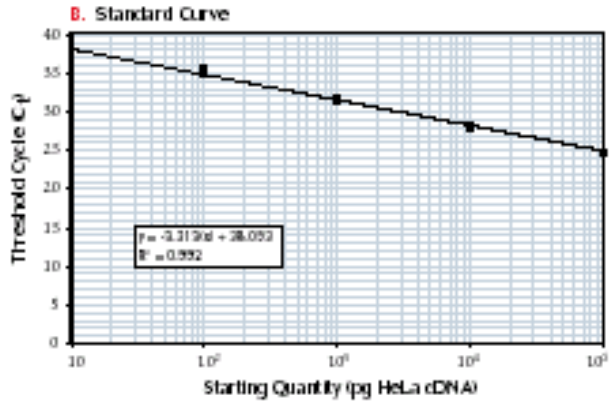




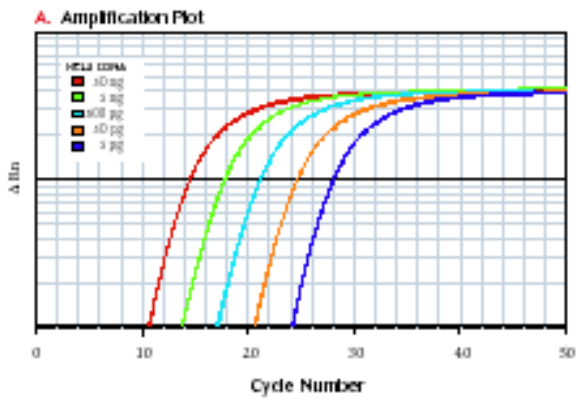
SuperScript™ III Platinum® Two-Step qRT-PCR Kit

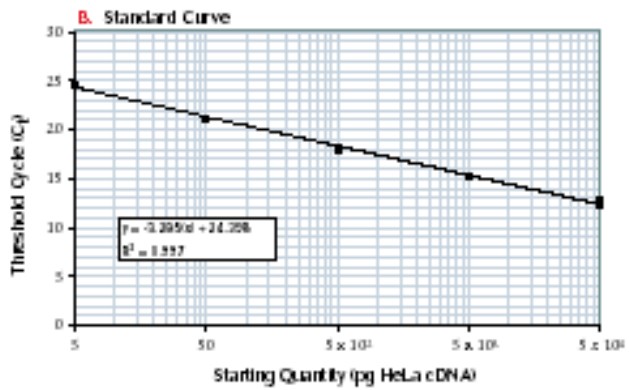
Provides specific detection with LUX™ Fluorogenic Primers





Provides sensitive detection with TaqMan® Probes

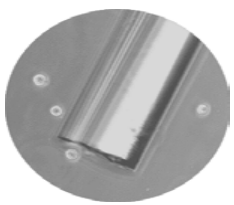


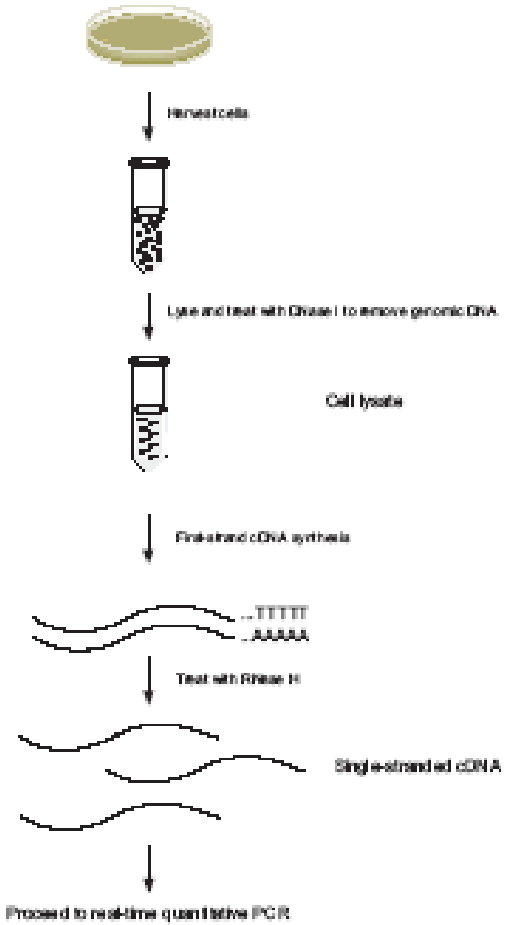


Quantitative RT-PCR from cell lysates

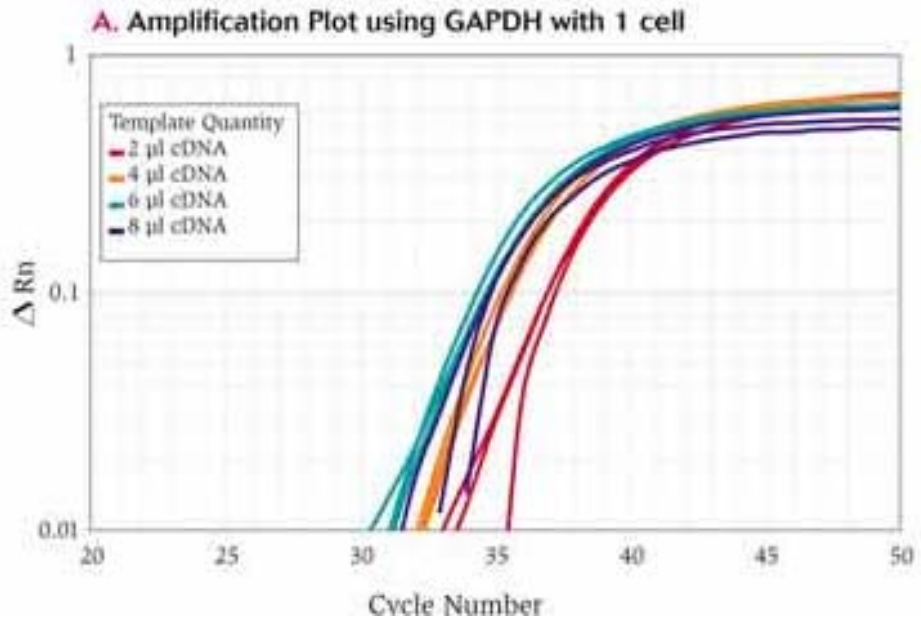
SuperScript™ III Platinum® CellsDirect Two-Step qRT-PCR Kit

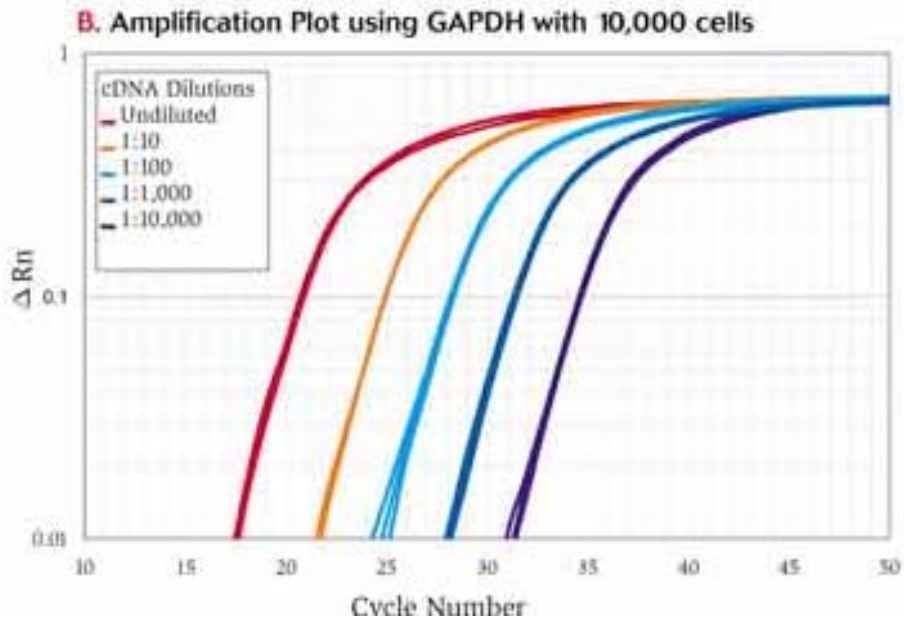
SuperScript™ III Platinum® CellsDirect Two-Step qRT-PCR Kit with SYBR® Green





Single Cell Detection





Comparison of Standard and HTP method

Standard Method

RNA+Primer+dNTP

↓

65 degrees for 5’

↓

Place on ice for > 1’

↓

Add 10x buffer, MgCl2, DTT, and RnaseOut

↓

Mix Gently

↓

42 degrees for 2’

↓

Add 1ul SSIII/reaction

↓

25 degrees 10’
42 degrees 50’
85 degrees 5’

↓

Add 1ul Rnase H/reaction
37 degrees 20’

HTP Protocol/MasterMix format

Combine components for first-strand synthesis:
Enzyme Mix, Reaction Mix, RNA Template

↓

25 degrees 10’
42 degrees 50’
70 degrees 20’

↓

Add 1ul **Rnase H**/reaction
37 degrees 20’

↓

ready to use cDNA

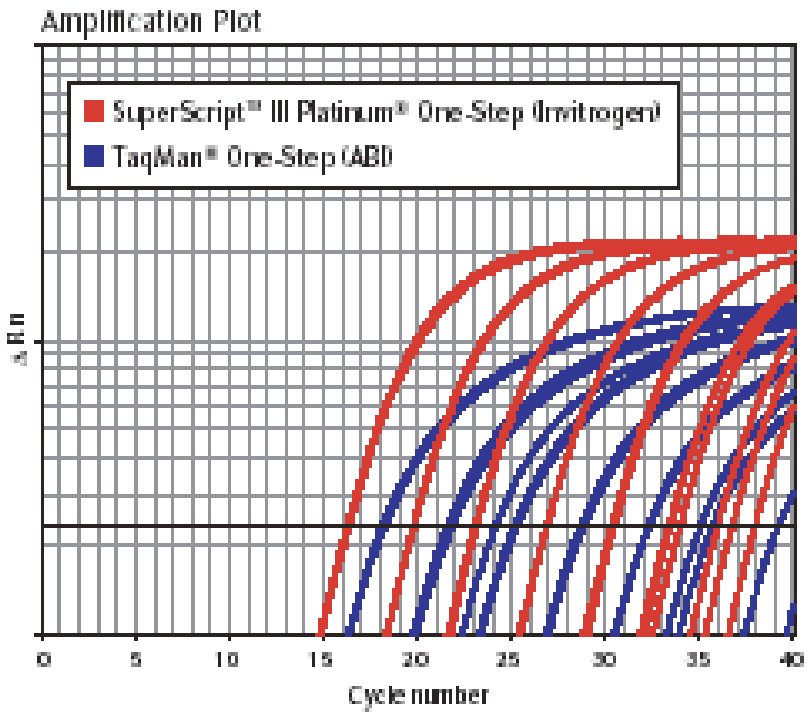
Sensitive one step qRT-PCR kits

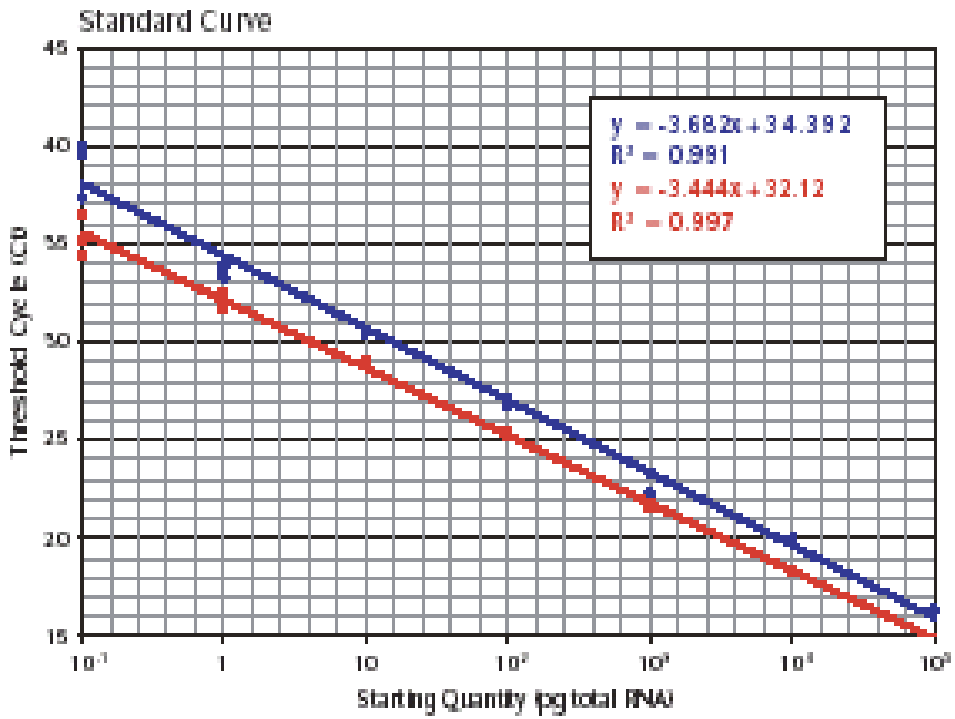
SuperScript™ III Platinum® One-Step qRT-PCR Kit
For LUX™ and probe based detection systems

SuperScript™ III Platinum® One-Step qRT-PCR Kit with SYBR® Green
For detection based on SYBR® Green

Kit Components:
One-Step Enzyme Mix
2X Reaction Mix
ROX Reference Dye
20X Bovine Serum Albumin

Superior sensitivity with dual-labelled probes



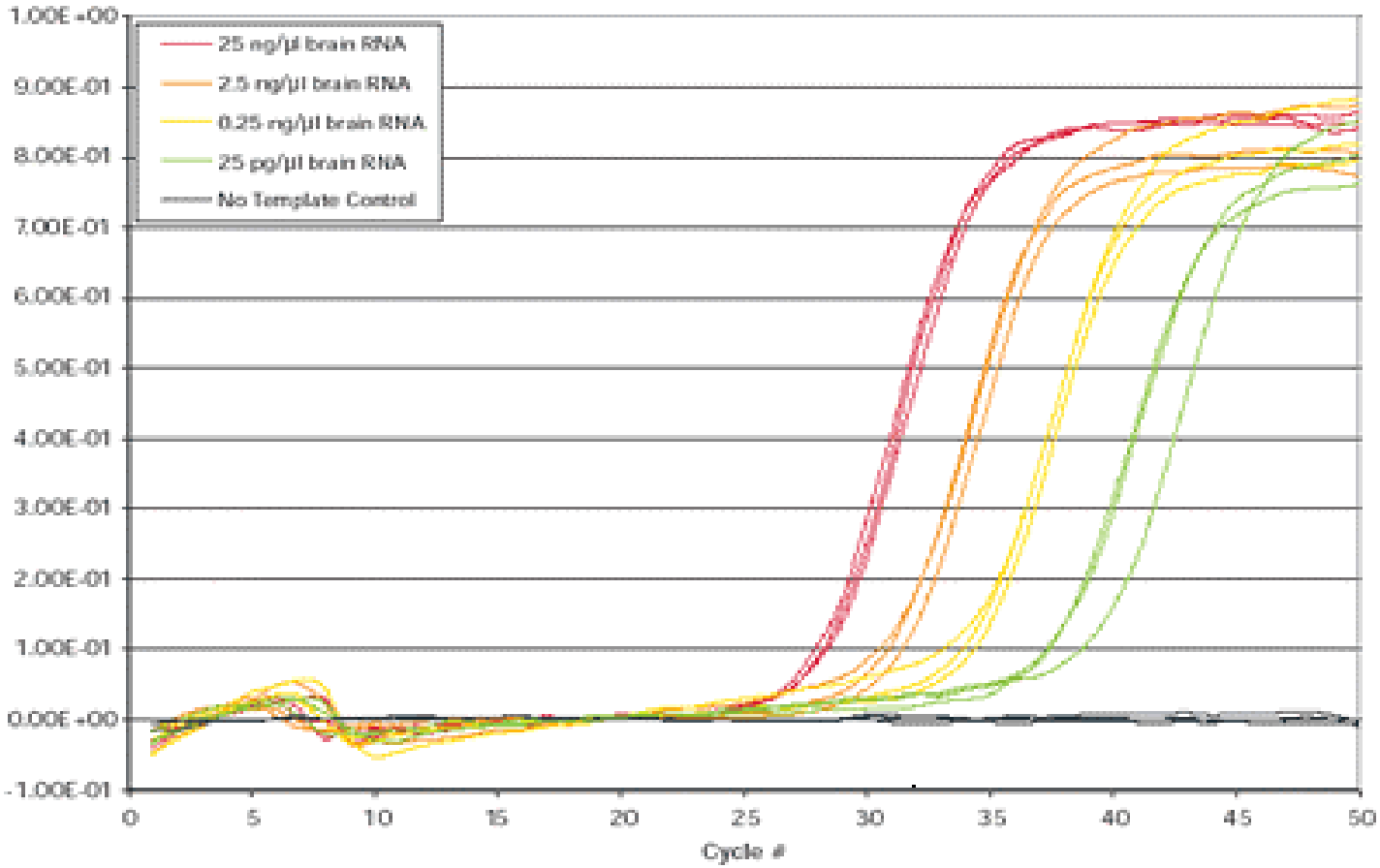


Ultra Sensitive one step qRT-PCR kits

RNA UltraSense™ One-Step Quantitative RT-PCR System

Kit Components:
RNA UltraSense™ Enzyme Mix
RNA UltraSense™ 5 x Reaction Mix
ROX Reference Dye
20X Bovine Serum Albumin

Ultra Sensitive one step qRT-PCR kit



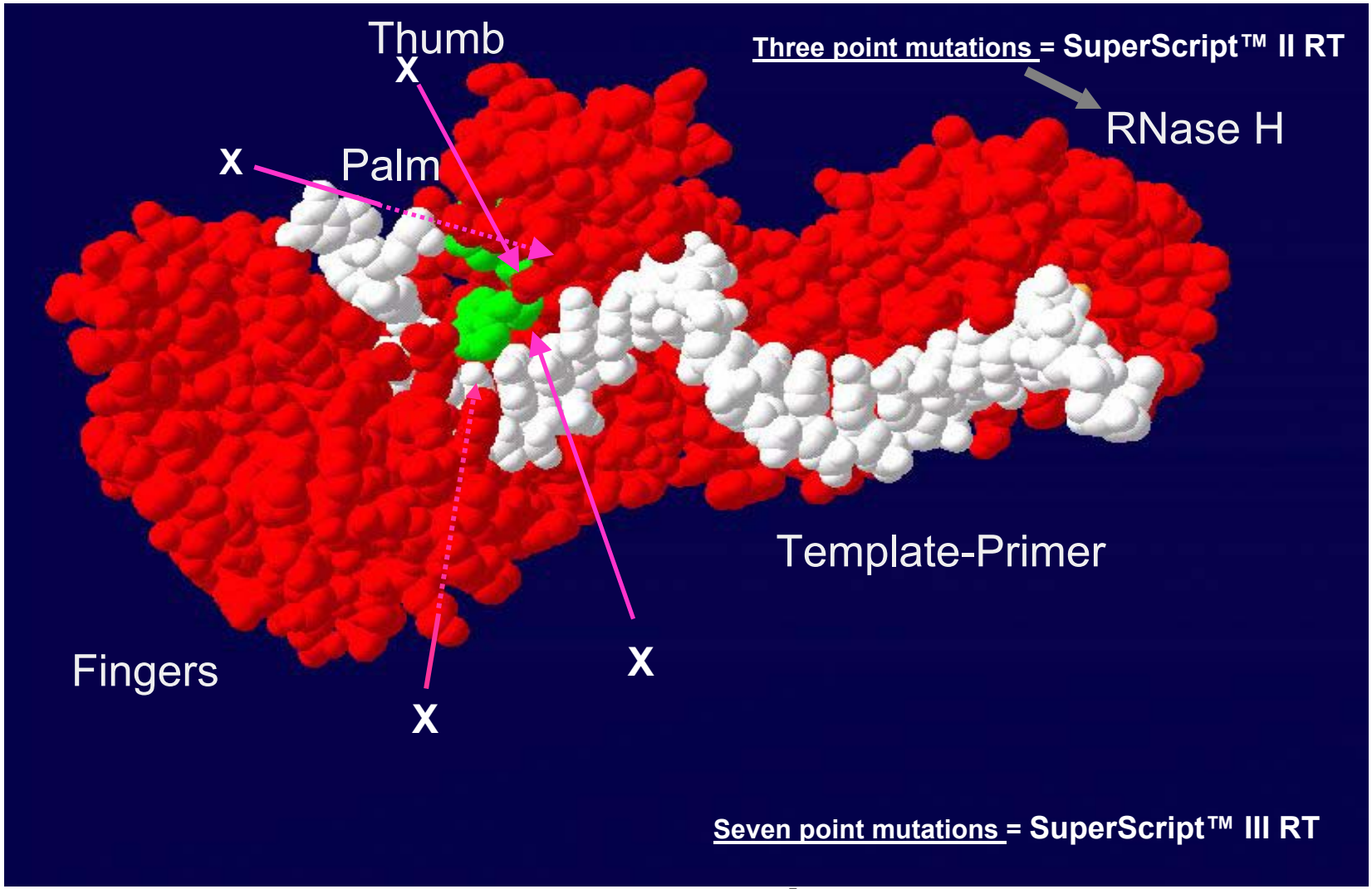
Two step kits product summary

- **Characteristics**
  - High performance and complete kit for 2-step real-time RT-PCR, for highly sensitive and specific detection and quanitation of RNA in gene expression profiling studies
- **Applications**
  - 2-step real-time RT-PCR, using fluorogenic detection (TaqMan, LUX™) or SYBR® Green detection
  - Real-time quantitative RT-PCR results directly from cells
- **Ideal for**
  - Researchers doing 2-step qRT-PCR that want best performing reagents for reproducibility and sensitivity
  - Researchers currently buying separate reagents to do 2-step qRT-PCR
  - Researchers that want high-through-put compatible cDNA synthesis protocols

One step kits product summary

- **Characteristics**
  - Easy to use; reduce contamination and human error
  - Optimized enzyme blend of SuperScript™III RT and Platinum® *Taq* DNA Polymerase
  - RNA UltraSense™ 2.5 more concentrated than other supermixes
- **Applications**
  - One-step real-time RT-PCR, using fluorogenic detection (TaqMan, LUX™) or SYBR® Green detection
- **Ideal for**
  - Researchers that want best performing reagents for reproducibility and sensitivity
  - Researchers that need the highest sensitivity amplification of ultra low-abundance RNA
  - Researchers currently buying separate reagents to do 2-step qRT-PCR
  - Researchers that want rapid, streamlined protocols

SuperScript™ III RT Engineering



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