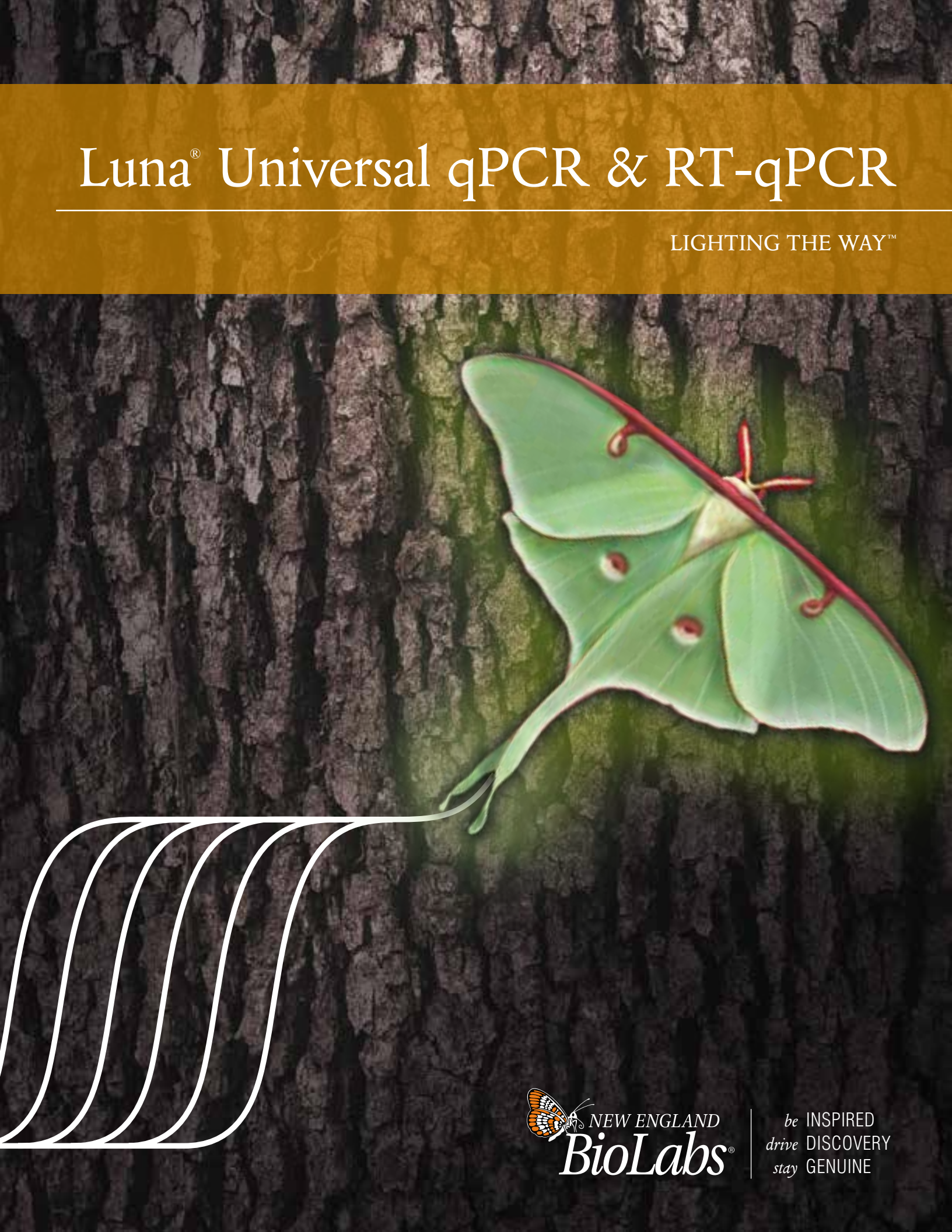
Luna® Universal qPCR & RT-qPCR

LIGHTING THE WAY™



NEW ENGLAND BioLabs®

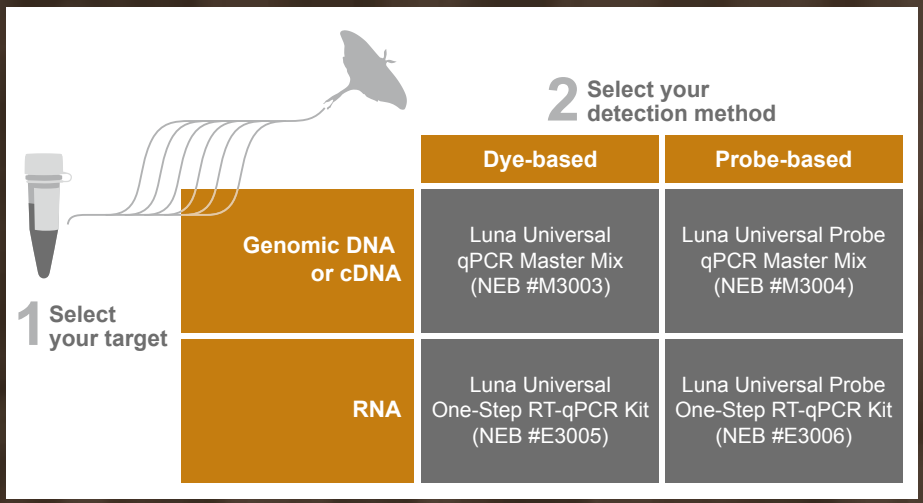
be INSPIRED
drive DISCOVERY
stay GENUINE

Lighting the way

Fluorescence-based quantitative real-time qPCR (qPCR) is the "gold standard" for the detection and quantitation of nucleic acids due to its sensitivity and specificity. Luna products from NEB® are optimized for qPCR or RT-qPCR, and are available for either intercalating dye or probe-based detection methods. All Luna products provide robust performance on diverse sample sources and target types.

Each Hot Start *Taq*-based Luna qPCR master mix has been formulated with a unique passive reference dye that is compatible across a wide variety of instrument platforms, including those that require a ROX reference signal. This means that no additional components are required to ensure machine compatibility. The mixes also contain dUTP, enabling carryover prevention when reactions are treated with NEB's Antarctic Thermolabile UDG (NEB #M0372). A blue visible dye assists in tracking the reagents when pipetting into clear, multi-welled PCR plates. These features, combined with rapid, sensitive and precise real-time qPCR performance, make Luna the universal choice for all your qPCR experiments.

Find the right Luna product for your application



Experience best-in-class performance

- All Luna products have undergone rigorous testing to optimize specificity, sensitivity, accuracy and reproducibility
- Products perform consistently across a wide variety of sample sources
- A comprehensive evaluation of commercially-available qPCR and RT-qPCR reagents demonstrates superior performance of Luna products

Optimize your RT-qPCR with Luna WarmStart® Reverse Transcriptase

- Novel, thermostable reverse transcriptase (RT) improves performance
- WarmStart RT paired with Hot Start *Taq* increases reaction specificity and robustness

Make a simpler choice

- One product per application simplifies selection
- Convenient master mix formats and user-friendly protocols simplify reaction setup
- Non-interfering, visible tracking dye helps to eliminate pipetting errors

Visit **LUNAqPCR.de** (in Germany & Austria) or **LUNAqPCR.fr** (in France) to request a **free sample** and learn more in our videos.

We tested plates and plates of reactions so you don't have to

Evaluating qPCR results: capturing performance as "dots in boxes"

NEB has developed a method to better evaluate the large amount of qPCR data generated in an experiment. The output of this analysis is known as a dot plot, and captures the key features of a successful, high-quality qPCR experiment as a single point. This method of analysis allows many targets and conditions to be compared in a single graph.

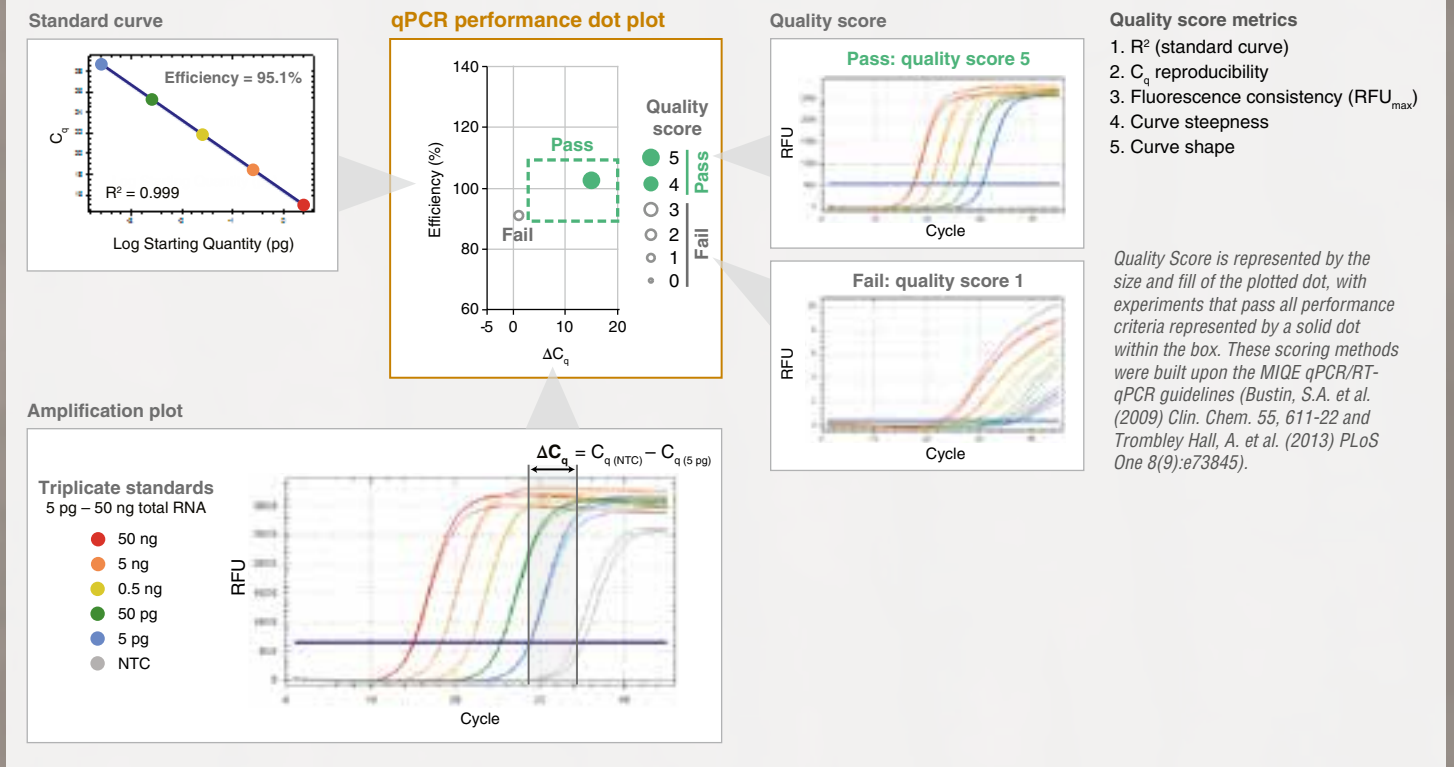
For each experiment, triplicate reactions are set up across a five-log range of input template concentrations (Amplification plot, bottom-left). Three non-template control (NTC) reactions are also included, for a total of 18 reactions per condition/target. Efficiency (%) is calculated (Standard plot, top-left) and is plotted against ΔC_q (dot plot, top-center), which is the difference between the average C_q of the NTC and the lowest input. This parameter captures both detection of the lowest input and non-template amplification.

Acceptable performance criteria are defined as an Efficiency of 90–110% and a ΔC_q of ≥ 3 (green box – pass).

Other performance criteria are captured using a 5-point quality score (Quality score metrics, top-right). Included are:

1. Linearity of amplification, as indicated by the R^2 standard curve
2. Reproducibility, as indicated by the consistency of triplicate C_q values for each input concentration
3. Fluorescence consistency, as indicated by similar endpoint fluorescence (RFU_{max})
4. Curve steepness
5. Sigmoid curve shape

Breaking it down: how we translate qPCR data into "dots in boxes"



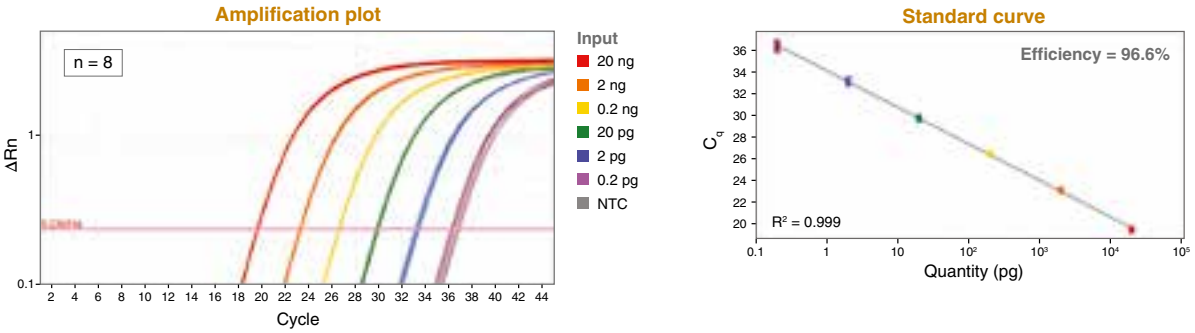
Quality Score is represented by the size and fill of the plotted dot, with experiments that pass all performance criteria represented by a solid dot within the box. These scoring methods were built upon the MIQE qPCR/RT-qPCR guidelines (Bustin, S.A. et al. (2009) Clin. Chem. 55, 611-22 and Trombley Hall, A. et al. (2013) PLoS One 8(9):e73845).

Visit **LUNAqPCR.de** or **LUNAqPCR.fr** for a video explanation of our "dots in boxes" evaluation.

Experience best-in-class performa

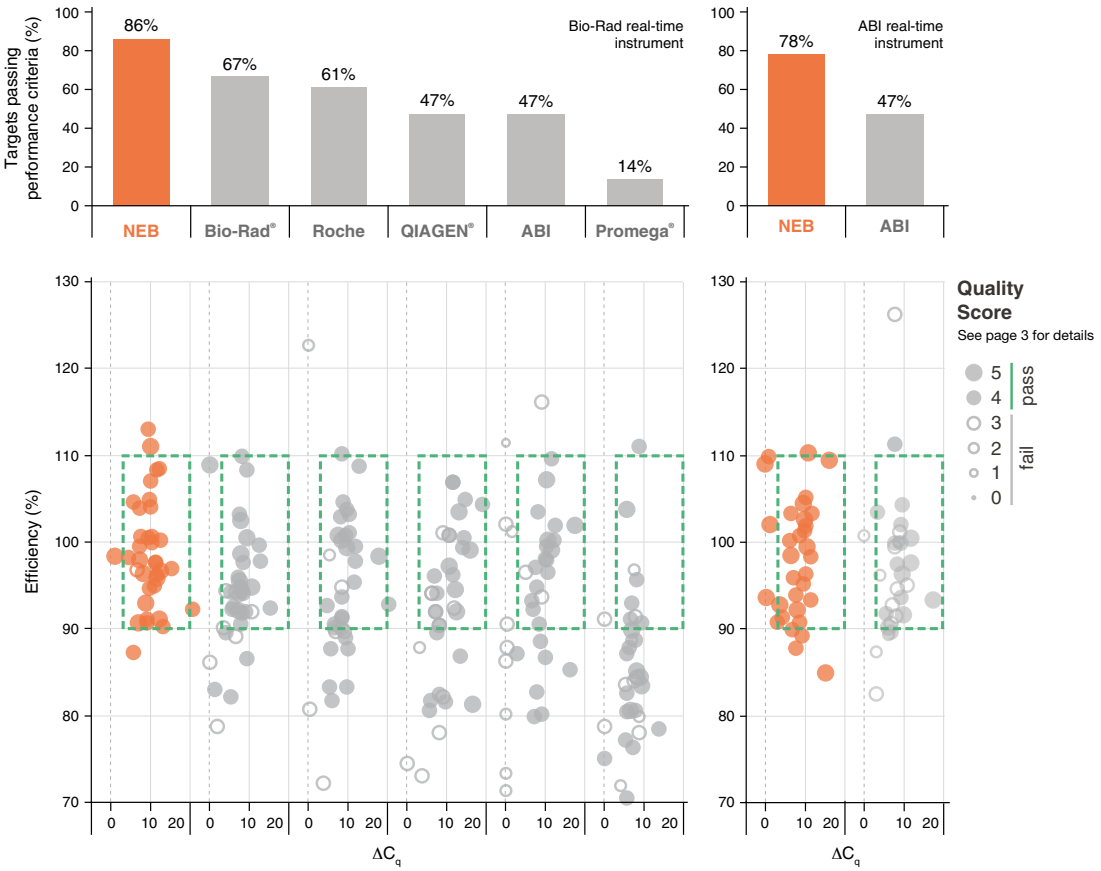
All NEB products undergo rigorous testing to ensure optimal performance, and Luna is no exception. We took into consideration numerous important traits when evaluating qPCR, including specificity, sensitivity, accuracy and reproducibility, to develop best-in-class qPCR reagents. Furthermore, we did a comprehensive evaluation of commercially-available qPCR and RT-qPCR reagents, and developed a method of analysis that allows you to quickly compare and evaluate the performance of these products. We wanted to be sure that Luna products will perform to your expectations for all your targets.

Luna products offer exceptional sensitivity, reproducibility and qPCR performance



qPCR targeting human GAPDH was performed using the Luna Universal Probe qPCR Master Mix over a 6-log range of input template concentrations (20 ng – 0.2 pg Jurkat-derived cDNA) with 8 replicates at each concentration. cDNA was generated from Jurkat total RNA using the NEB Protoscript® II First Strand cDNA Synthesis Kit (NEB #E6560). NTC = non-template control

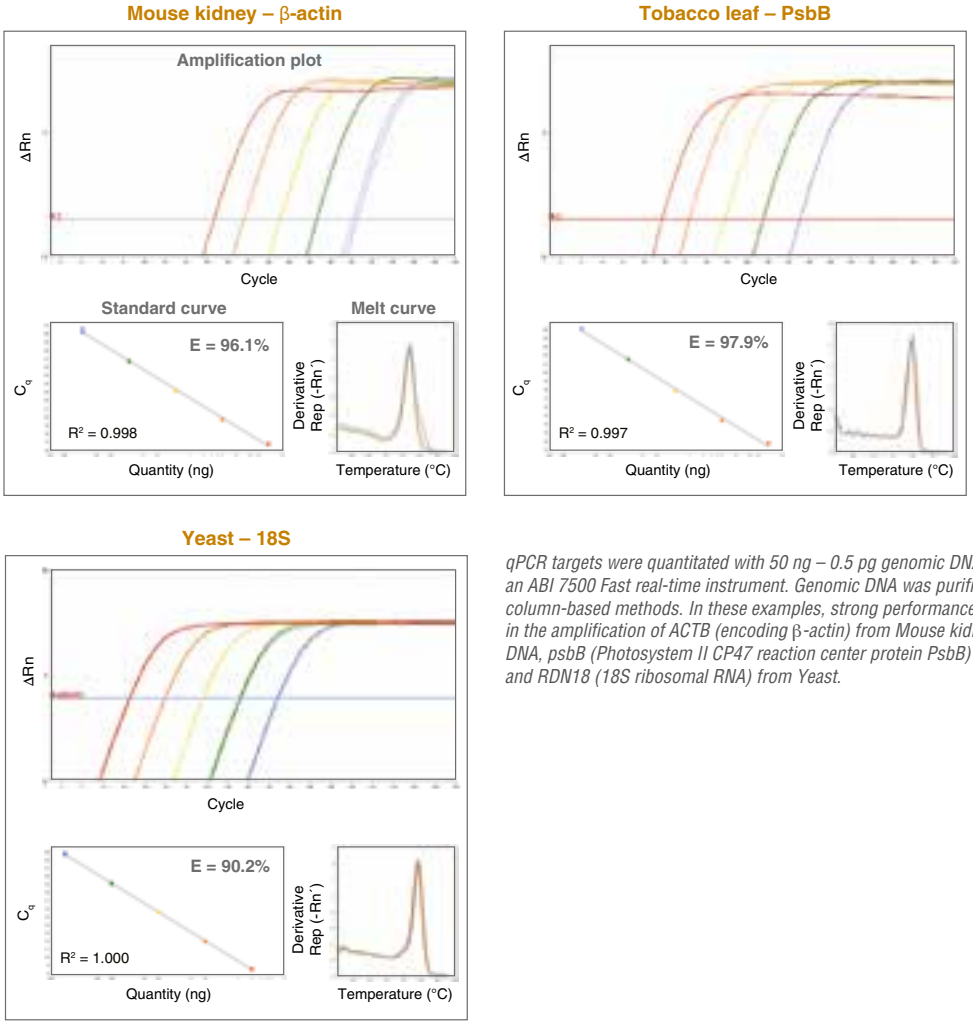
Evaluation of commercially-available dye-based qPCR reagents demonstrates the robustness and specificity of Luna



qPCR reagents from NEB and other manufacturers were tested across 16–18 qPCR targets varying in abundance, length and %GC, using either Jurkat genomic DNA or Jurkat-derived cDNA as input (10 genomic DNA targets and 8 cDNA targets on a Bio-Rad real-time instrument, 9 genomic and 7 cDNA targets on an ABI instrument). For each testing condition, data was collected by 2 users and according to manufacturer's specifications. Results were evaluated for efficiency, low input detection and lack of non-template amplification (where ΔC_q = average C_q of lowest input – average C_q of non-template control). In addition, consistency, reproducibility and overall curve quality were assessed (Quality Score). Bar graph indicates % of targets that met acceptable performance criteria (indicated by green box on dot plot and Quality Score > 3). Results for NEB and other major manufacturers are shown: Bio-Rad, SsoAdvanced™ Universal SYBR® Green Supermix; Roche, FastStart™ SYBR Green Master; QIAGEN, QuantiTect® SYBR Green PCR Kit; ABI, PowerUP™ SYBR Green Master Mix; Promega, GoTaq® qPCR Master Mix. NEB's Luna Universal qPCR Master Mix outperformed all other reagents tested.

nce for your qPCR & RT-qPCR

Luna products provide sensitive, accurate detection & quantitation across a wide variety of genomic DNA sources



qPCR targets were quantitated with 50 ng – 0.5 pg genomic DNA as input using an ABI 7500 Fast real-time instrument. Genomic DNA was purified by typical column-based methods. In these examples, strong performance can be observed in the amplification of ACTB (encoding β-actin) from Mouse kidney genomic DNA, psbB (Photosystem II CP47 reaction center protein PsbB) from Tobacco, and RDN18 (18S ribosomal RNA) from Yeast.

Probe- versus dye-based detection methods

Which should I choose for my qPCR?

qPCR is typically measured in one of two ways: either an intercalating dye that fluoresces more strongly upon binding to double-stranded DNA, or a fluorescently-labeled "probe" oligonucleotide that anneals to a specific sequence in the PCR amplicon.

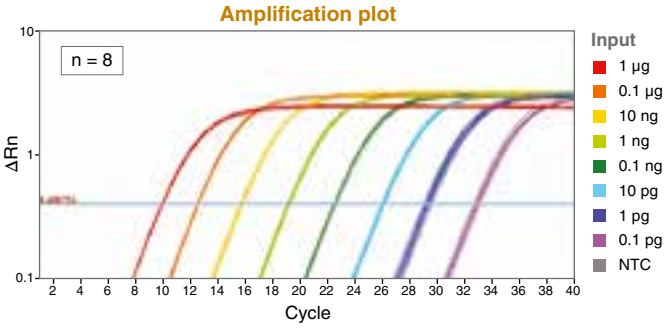
Dye-based detection requires only the addition of PCR primers, making it a cost-effective qPCR option. However, the intercalating dye will detect any dsDNA produced in the reaction. Therefore, off-target and non-template amplification (NTC) can be observed for some primer sets, resulting in inaccurate quantitation. Denaturation (melt) curves performed after the PCR can be used to distinguish between correct and nonspecific products. Additionally, only a single amplicon can be measured in a dye-based qPCR with no ability to perform multiplex reactions.

Probe-based detection requires designing and obtaining a sequence-specific fluorescently-labeled probe oligonucleotide in addition to typical PCR primers. This increases assay costs, but probe-based qPCR experiments benefit from extreme specificity and are unlikely to result in inaccurate quantification due to NTC amplification. Multiplex reactions are possible with probes, as different amplicons can be designed with unique fluorophores according to the optical capabilities of the qPCR instrument.

Optimize your RT-qPCR with unique WarmStart technology

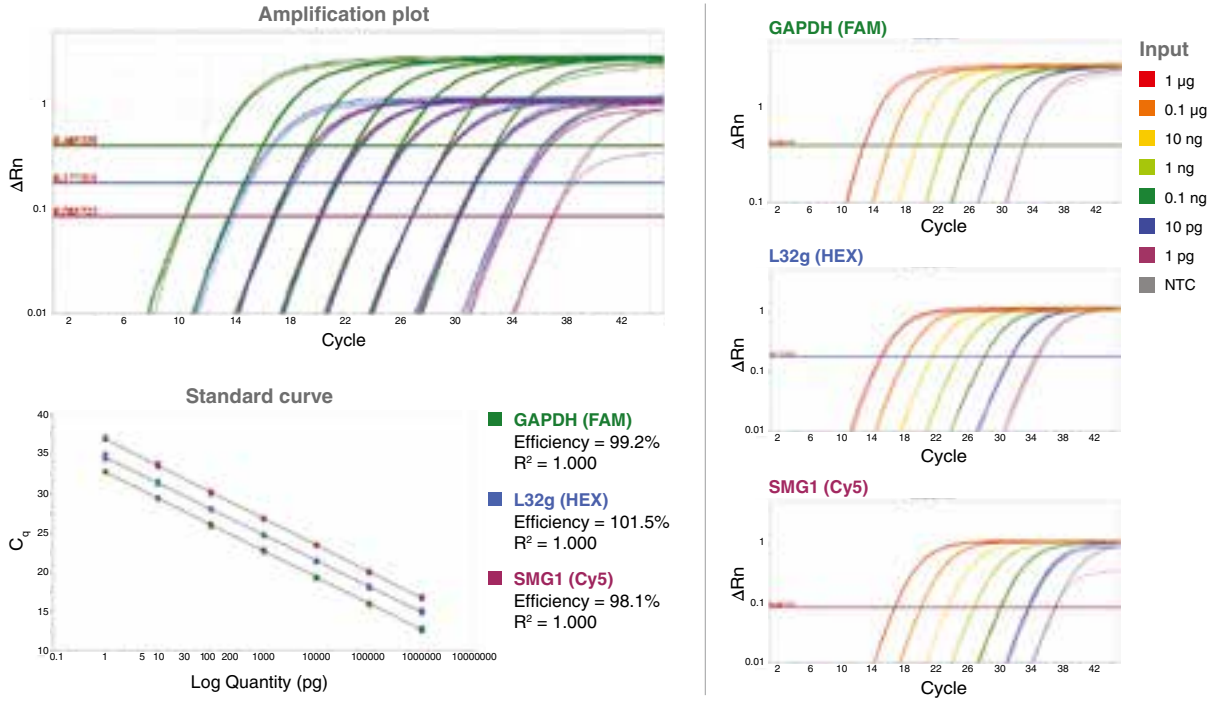
The Luna RT-qPCR kits contain a novel, *in silico*-designed reverse transcriptase (RT) engineered for improved performance. Both the WarmStart Luna Reverse Transcriptase and Hot Start *Taq* DNA Polymerase, included in these kits, utilize a temperature-sensitive, reversible aptamer, which inhibits activity below 45°C. This enables room temperature reaction setup and prevents undesired non-specific activity. Furthermore, the WarmStart RT has increased thermostability, improving performance at higher reaction temperatures.

Luna RT-qPCR products offer exceptional sensitivity, reproducibility and performance



RT-qPCR targeting human GAPDH was performed using the Luna Universal One-Step RT-qPCR Kit over an 8-log range of input template concentrations (1 µg – 0.1 pg Jurkat total RNA) with 8 replicates at each concentration. Reaction setup and cycling conditions followed recommended protocols, including a 10-minute RT step at 55°C for the thermostable Luna WarmStart Reverse Transcriptase. NTC = non-template control

Luna RT-qPCR products offer robust performance in multiplex applications



Multiplex RT-qPCR targeting human GAPDH, ribosomal protein L32g and PI3-Kinase-Related Kinase SMG1 was performed using the Luna Universal Probe One-Step RT-qPCR Kit over a 7-log range of input template concentrations (1 µg – 1 pg Jurkat total RNA) with 4 replicates at each concentration. Amplification plots are shown both overlayed (left) and for each multiplex target (right). To account for copy number differences, 0.4 µM primer was used for lower-copy target (SMG1) and 0.2 µM primer for higher-copy targets (L32g and GAPDH). Luna maintains superior efficiency, reproducibility, sensitivity and performance in multiplex RT-qPCR. NTC = non-template control

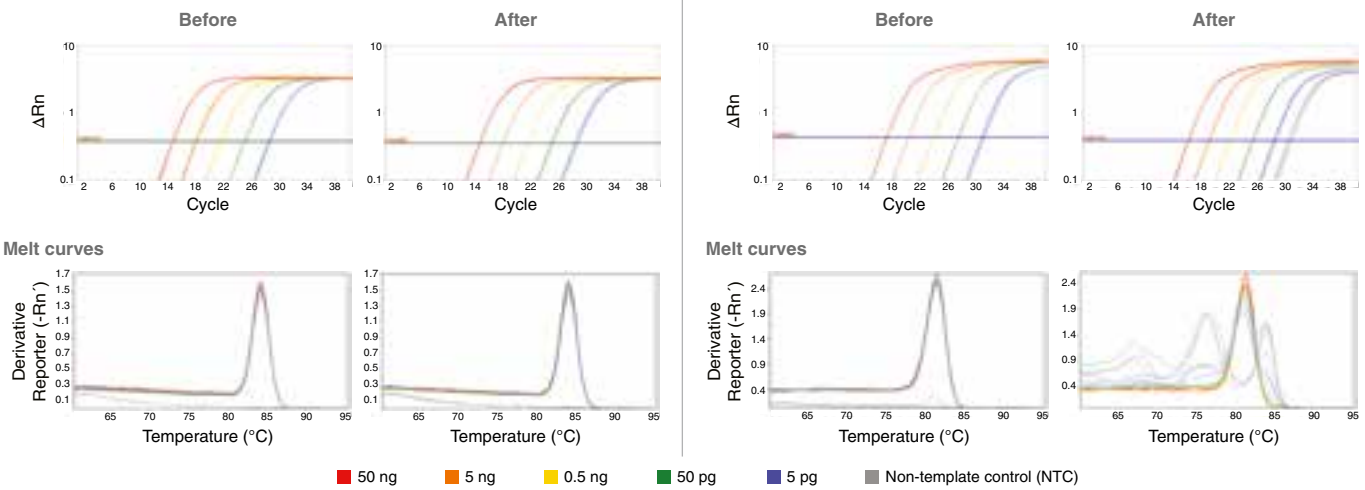
What is WarmStart Luna Reverse Transcriptase?

"WarmStart" is the term we use to describe a mesophilic enzyme that is inactive at room temperature, and becomes active when the reaction is warmed above approximately 40°C. This feature enables flexible reaction setup and improves reaction specificity and thermostability.

WarmStart Luna Reverse Transcriptase prevents spurious amplification resulting from room-temperature pre-incubation

NEB Luna Universal One-Step RT-qPCR Kit
(featuring Luna WarmStart Reverse Transcriptase)

ABI Power SYBR® Green 1-Step Kit
(featuring ArrayScript™ UP Reverse Transcriptase)

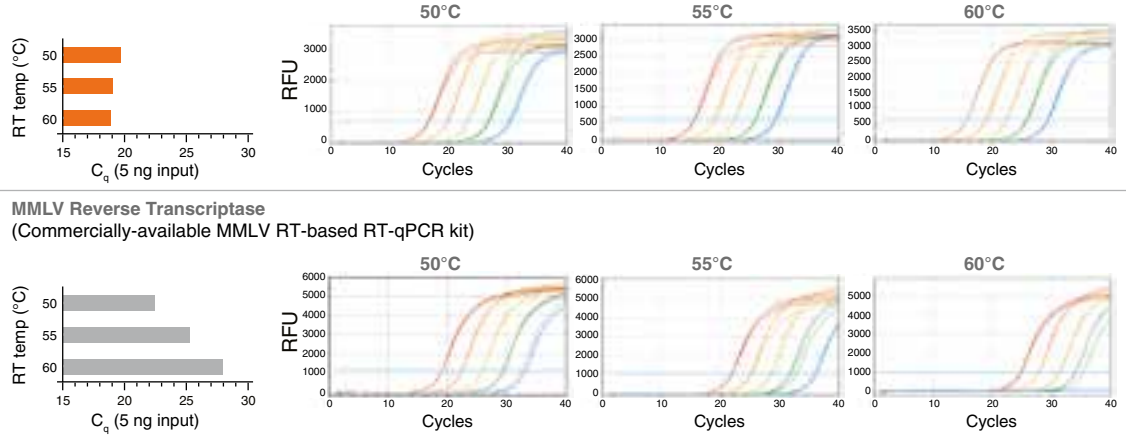


RT-qPCR targeting human ribosomal protein L32 was performed before and after a 24-hour incubation at room temperature, with triplicate reactions for a 5-log range of input human (Jurkat) total RNA and a non-template control. The Luna Universal One-Step RT-qPCR Kit featuring Luna WarmStart Reverse Transcriptase exhibited robust performance and no detectible non-template amplification, either with or without a 24 hour, 25°C pre-incubation, while the ABI 1-Step Kit, featuring a non-WarmStart reverse transcriptase, exhibited significant non-template amplification.

The increased thermostability of WarmStart Luna Reverse Transcriptase improves performance at higher reaction temperatures

A **Luna WarmStart Reverse Transcriptase**
(NEB Luna Universal One-Step RT-qPCR Kit)

B **MMLV Reverse Transcriptase**
(Commercially-available MMLV RT-based RT-qPCR kit)



RT-qPCR experiments targeting human ribosomal protein L32 RNA were performed in triplicate over a 5-log range of input human (Jurkat) total RNA (5 pg – 50 ng) using an initial 10 min RT step performed at 50°C – 60°C, as indicated.

A. Luna WarmStart Reverse Transcriptase (recommended incubation temperature: 55°C) exhibited rapid Cq values (bar graph) and robust RT-qPCR performance (amplification plots) at each temperature, indicating that efficient reverse transcription was not perturbed by reaction temperature alterations.

B. In contrast, a commercially available MMLV (recommended incubation temperature: 50°C) exhibited delayed (increased) Cq values, poorer performance, and loss of low-input detection at elevated temperatures, consistent with loss of RT activity.

Available products include:

Luna Universal qPCR Master Mix

Rapid, sensitive, and precise dye-based qPCR detection and quantitation of target DNA and cDNA sequences

NEB #M3003S/L 200/500 rxns

Luna Universal Probe qPCR Master Mix

Rapid, sensitive, and precise probe-based qPCR detection and quantitation of target DNA and cDNA sequences

NEB #M3004S/L 200/500 rxns

Luna Universal One-Step RT-qPCR Kit

Includes everything you need for rapid, sensitive, and precise dye-based qPCR detection and quantitation of RNA targets

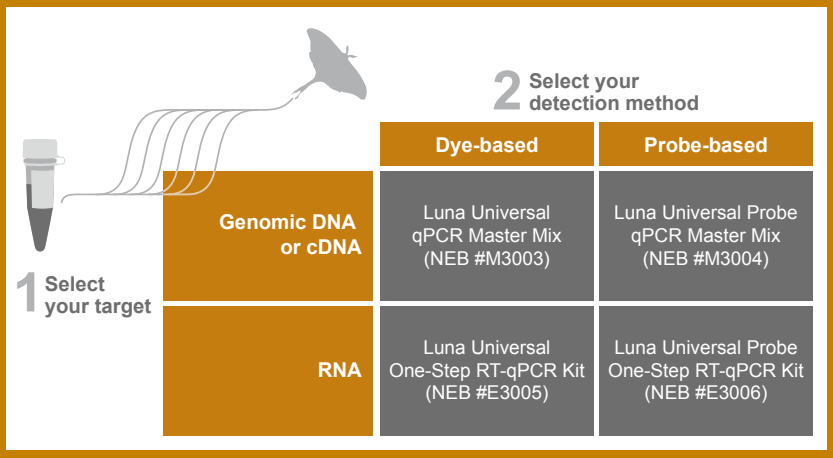
NEB #E3005S/L 200/500 rxns

Luna Universal Probe One-Step RT-qPCR Kit

Includes everything you need for rapid, sensitive, and precise probe-based qPCR detection and quantitation of RNA targets

NEB #E3006S/L 200/500 rxns

Find the right Luna product for your application



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