Expression Analysis

ExiLERATE LNA™ qPCR System for mRNA and lncRNA

Sensitive and accurate quantification of mRNA and lncRNA transcripts is crucial in many areas of research. With this in mind, Exiqon has developed the ExiLERATE LNA™ qPCR System which sets new standards for mRNA and lncRNA qPCR, and guarantees the robust detection of any mRNA or lncRNA. In this technical note we describe how Exiqon's LNA™ technology enhances the power of qPCR assays, and demonstrate the superior performance of the ExiLERATE LNA™ qPCR System when compared with NCBI Primer-BLAST and other commercially available qPCR systems.

Key features

- Robust detection of any mRNA or long non-coding RNA
- Exceptional sensitivity – performance surpasses all major competitors
- Enhanced by LNA™ – superior accuracy
- Greater flexibility in primer positioning – easily detect isoforms, SNPs or splice-variants
- Thermostable RT – efficient amplification of high GC content sequences
- Fast and easy – get results in under 3 hours

Setting new standards for mRNA and lncRNA qPCR

The ExiLERATE LNA™ qPCR System is a two-step process. Firstly, Universal cDNA synthesis is performed using a unique thermostable RT enzyme which enables efficient amplification even of high GC content sequences. Secondly, Real-time PCR amplification is performed using two LNA™-enhanced qPCR primers for exceptional sensitivity and specificity (Figure 1).

The whole workflow is completed in less than 3 hours, ensuring fast results (Figure 2). Enhanced by LNA™, the performance of the ExiLERATE LNA™ qPCR System surpasses other commercially available qPCR systems, and sets new standards for mRNA and lncRNA qPCR.

LNA™ enhances the performance of ExiLERATE LNA™ qPCR

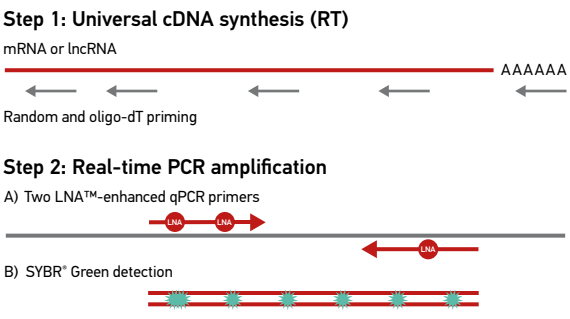


Figure 1. Schematic outline of the ExiLERATE LNA™ qPCR System. Reverse transcription with a unique thermostable RT ensures a high yield of full length cDNA. Two gene-specific LNA™-enhanced forward and reverse primers enable superior sensitivity and specificity.

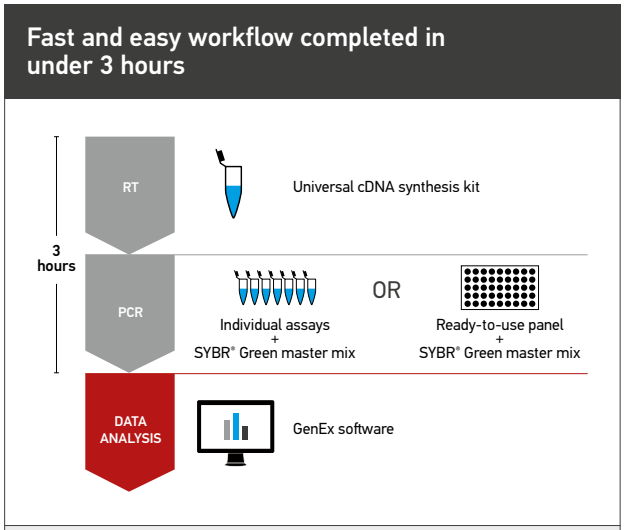


Figure 2. Results are generated in under 3 hours using the ExiLERATE LNA™ qPCR System. The simple protocol reduces variation and errors, and saves time. Data can be analyzed using the powerful Exiqon GenEx Software.

Success guaranteed

All reagents including cDNA synthesis kit, LNA™ qPCR primers and SYBR® Green Master mix have been developed by Exiqon for optimal performance (Figure 3). The high standards achieved mean that with the ExiLERATE LNA™ qPCR System, success is guaranteed.

LNA™ enhances qPCR performance

Primers containing LNA™ display higher binding affinity, which dramatically increases the sensitivity of the qPCR assay. In addition, LNA™ enables primers to be placed anywhere within the transcript, and increases specificity for detection of single nucleotide differences. Read more about how LNA™ takes mRNA and lncRNA qPCR to the next level on "The Power of LNA™" page.

Advanced LNA™ primer design

Exiqon has developed a completely novel approach for optimal design of LNA™ qPCR primers which incorporates more than 45 different design parameters. The advanced primer design was developed based on Exiqon's >20 years' experience with LNA™ design as well as thousands of melting temperature experiments and empirical optimizations. The design parameters include:

- T_m normalization
- Minimizing self-hybridization and cross-hybridization
- Avoiding primer-dimer formation
- Intelligent positioning of LNA™
- Specificity check through analysis of potential off-target hits in the transcriptome
- Adjustment of forward and reverse primers to work together

ExiLERATE LNA™ qPCR primers for any transcript (mRNA or lncRNA including novel transcripts) may be ordered easily online using sequence, accession or keyword. Assays are intron-spanning by default to better avoid any problems arising from genomic DNA contamination. Advanced options enable you to select specific regions of the transcript to target, anchor primers or target introns, splice variants, or SNPs. Alternatively, assays common to all transcript variants may be selected.

High yields of full length cDNA

The ExiLERATE LNA™ cDNA synthesis kit is based on a modified M-MuLV RT, allowing reverse transcription at high temperatures and with no RNase H activity. This ensures a high yield of full length cDNA, even from challenging transcripts such as lncRNAs which can be GC-rich with strong secondary structures.

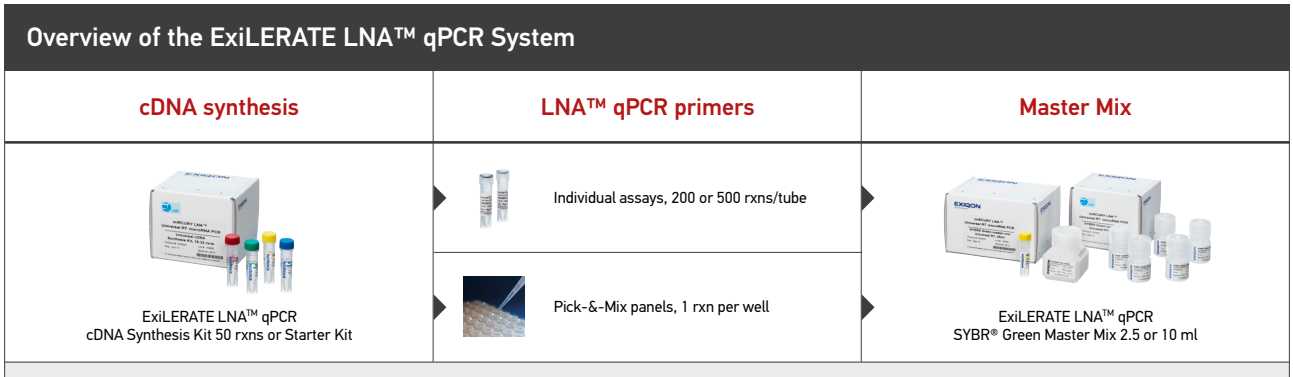
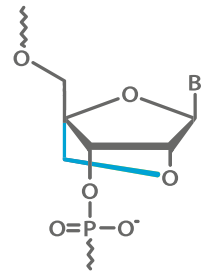


Figure 3. A complete set of optimized ExiLERATE LNA™ qPCR reagents are available from Exiqon. Individual assays for any RNA transcript as well as a range of validated control primer sets are available from exiqon.com/LNA-qPCR.

The Power of LNA™

Locked Nucleic Acids (LNA™) are a class of high-affinity RNA analogs in which the ribose ring is "locked" in the ideal conformation for hybridization with complementary targets. As well as increased binding affinity, LNA™ offers a number of other properties extremely beneficial for mRNA and lncRNA qPCR, summarized in the table below.

The structure of Locked Nucleic Acid (LNA™)



The ribose ring is connected by a methylene bridge (blue) between the 2'-O and 4'-C atoms thus "locking" the ribose ring in the ideal conformation for Watson-Crick base-pairing. When incorporated into a DNA primer, LNA™ increases the stability of the resulting duplex.

Exceptional sensitivity

Primers containing LNA™ exhibit unprecedented thermal stability. For each LNA™ incorporated, the melting temperature (T_m) increases by 2-8 °C, thereby increasing binding affinity and enhancing the sensitivity of the qPCR assay.

T_m normalization

By varying the number of LNA™ incorporated, the primer T_m (binding affinity) can be adjusted so that all qPCR primers have the optimal T_m for the specified qPCR cycling conditions, irrespective of the target GC content. T_m normalization is especially important for AT-rich transcripts, where it is challenging to design DNA primers with sufficient binding affinity.

Flexible primer positioning

T_m normalization also enables optimal primers to be designed to precisely amplify any region of a transcript. This is useful to detect specific isoforms or SNPs, or to avoid certain regions of the transcript targeted by RNA silencing e.g. siRNA or LNA™ GapmeRs.

Superior specificity

Intelligent placement of LNA™ within the primers can increase the difference in T_m between perfect match and mismatch targets by up to 8 °C, enabling better discrimination between closely related sequences such as SNPs. Differences as small as one nucleotide can be readily detected.

What does LNA™ do for you?

LNA™ properties	Result
Higher affinity	• Best in class sensitivity • Less sample input is needed • More transcripts can be detected, even mRNA and lncRNA expressed at low levels
T_m normalization	• Optimal detection of all transcripts under the same conditions, regardless of variation in target GC content • Flexible and accurate primer positioning anywhere within the target sequence • Detection of specific isoforms, splice variants or SNPs • Avoid regions of the transcript targeted by siRNA or LNA™ GapmeRs
Higher specificity	• Accurate discrimination between closely related sequences differing by as little as one nucleotide e.g. SNPs.

Summary of the main beneficial properties of LNA™ for mRNA and lncRNA qPCR.

SYBR® Green optimized for specific amplification

The ExiLERATE LNA™ qPCR SYBR® Green master mix is a high-performance PCR master mix specifically designed for Exiqon's ExiLERATE LNA™ qPCR system. The Master Mix is optimized to eliminate non-specific amplification. A useful feature of SYBR® Green is that it enables data quality control. The specificity of amplification and absence of genomic DNA contamination can be evaluated by melting curve analysis, which confirms that a single amplicon of the correct T_m has been amplified.

Superior performance compared to NCBI Primer-BLAST

ExiLERATE LNA™ qPCR primer sets are qualified using very strict performance criteria. We performed a wet-lab validation of over 100 assays designed for mRNA and lncRNA transcripts using NCBI Primer-BLAST, alongside ExiLERATE LNA™ qPCR assays. Overall, 40% of qPCR assays designed using NCBI Primer-BLAST failed to meet key performance criteria, and NCBI Primer-BLAST failed to design any assay for some of the transcripts (Figure 4). In contrast, the performance of all ExiLERATE LNA™ qPCR primer sets is guaranteed.

ExiLERATE LNA™ qPCR System is the only platform with full linearity

A linear relationship between the amount of RNA and the signal obtained is essential for accurate quantification.

40% of NCBI Primer-BLAST qPCR assays failed on key performance criteria

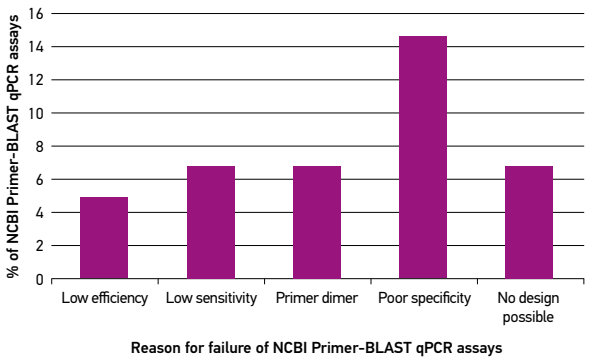


Figure 4. Overall 40 % of qPCR assays designed using NCBI Primer-BLAST failed to meet different key performance criteria. More than 100 RNA transcripts were analyzed using qPCR assays designed using NCBI Primer-BLAST. Assay performance was assessed using a range of key criteria including PCR efficiency, sensitivity and linearity (assessed by dilution series from 10 to 500 ng total RNA), specificity and primer dimer formation (assessed by melting curve analysis). The total RNA sample used for analysis was previously analysed by Next Generation Sequencing, to confirm the presence of the transcripts of interest.

Unrivalled linearity of ExiLERATE LNA™ qPCR down to 0.1 ng

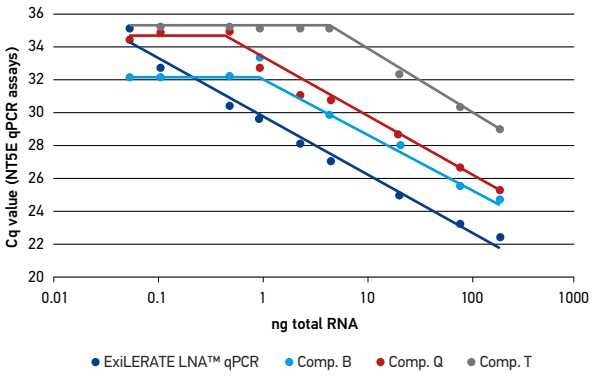


Figure 5. The ExiLERATE LNA™ qPCR System is the only platform to demonstrate full linearity down to 0.1 ng total RNA, ensuring accurate detection of transcripts at any expression level. Linearity was assessed for ten qPCR assays selected at random from the over 100 assays described in Figure 4. A dilution series from 250 to 0.05 ng total RNA input was performed using the ExiLERATE LNA™ qPCR System as well as three commercially available qPCR platforms (referred to as Competitors B, Q and T). Results for the NT5E assays are shown.

A dilution series of total RNA was used to assess the linearity and sensitivity of each qPCR platform. Sensitivity is defined by the limit of detection – the last point that it is still within the linear range. The ExiLERATE LNA™ qPCR System is the only platform to demonstrate full linearity down to 0.1 ng total RNA, with exceptional sensitivity exceeding all major competitors (Figure 5). Linearity even at low amounts of RNA is essential for robust detection of rare transcripts.

ExiLERATE LNA™ qPCR performance surpasses competitors

ExiLERATE LNA™ qPCR shows significantly lower Cq values, demonstrating superior performance compared to the three major competitors (Figure 6). Using just 1 ng total RNA input, ExiLERATE LNA™ qPCR assays detected all of the ten randomly selected mRNA transcripts within the linear range of the assays, surpassing the competitor qPCR platforms (Figure 7). This shows that the ExiLERATE LNA™ qPCR System is capable of accurately detecting even rare or low abundance transcripts.

The enhanced sensitivity of LNA™ qPCR primers, combined with the ExiLERATE LNA™ qPCR reagents optimized to eliminate any background signal, results in signal-to-noise ratios exceeding those of the competitor qPCR assays (Figure 8). A high signal-to-noise ratio reflects the larger dynamic range of the ExiLERATE LNA™ qPCR System, enabling accurate quantification of transcripts at any expression level from low to high.

Superior detection of any mRNA or lncRNA using ExiLERATE LNA™ qPCR

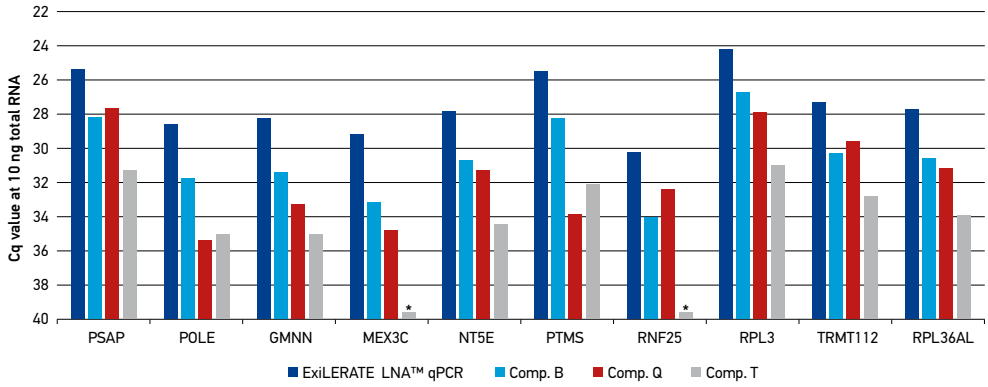


Figure 6. ExiLERATE LNA™ qPCR shows significantly lower Cq values than three commercially available qPCR platforms (referred to as Competitors B, Q and T). Ten mRNA transcripts were selected at random from the over 100 assays described in Figure 4. Average Cq is displayed using 10 ng total RNA (scale of y axis is inverted). * Competitor assays failed to detect the target within the linear range of the assay.

100% detection at low RNA amounts with ExiLERATE LNA™ qPCR

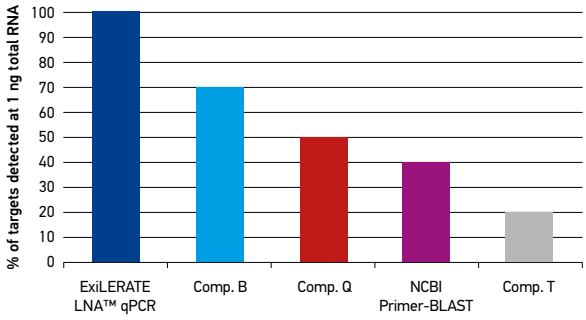


Figure 7. ExiLERATE LNA™ qPCR assays detected 100% of the mRNA transcripts within the linear range of the assays using just 1 ng total RNA. The performance of ExiLERATE LNA™ qPCR was superior to three commercially available qPCR platforms (referred to as Competitors B, Q and T) as well as qPCR assays designed using NCBI Primer-BLAST. Ten mRNA transcripts shown in Figure 6 were analyzed. NCBI Primer-BLAST qPCR primers were used together with ExiLERATE LNA™ cDNA synthesis kit and ExiLERATE LNA™ SYBR® Green master mix.

Superior signal-to-noise ratio with ExiLERATE LNA™qPCR

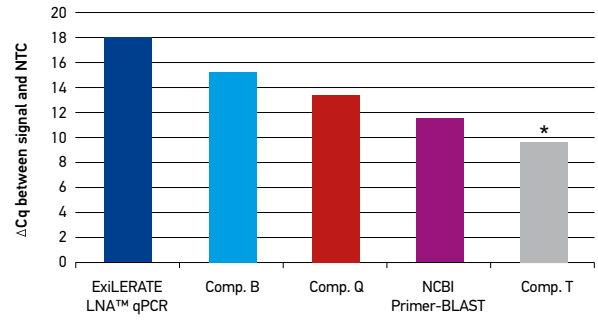


Figure 8. ExiLERATE LNA™ qPCR showed superior signal-to-noise ratio when compared with three commercially available qPCR platforms (referred to as Competitors B, Q and T) as well as qPCR assays designed using NCBI Primer-BLAST. Average ΔCq between signal and NTC (no template control) displayed using 100 ng total RNA. Ten mRNA transcripts shown in Figure 6 were analyzed. *Competitor T failed to detect 2 assays.

Conclusions

The sensitivity of the ExiLERATE LNA™ qPCR System exceeds all major competitors, enabling accurate quantification of any mRNA or long non-coding RNA transcript. LNA™-enhanced qPCR primers offer exceptional sensitivity and specificity, as well as the flexibility to position primers to amplify specific splice variants, isoforms or SNPs. With optimized LNA™ primers and an easy workflow, results are obtained in under 3 hours, and success is guaranteed.

Did you know? The ExiLERATE LNA™ qPCR System is ideal for lncRNA

- High temperature cDNA synthesis ensures full length cDNA synthesis, even from challenging transcripts with GC-rich sequences or strong secondary structures
- Unrivalled sensitivity for accurate detection of rare transcripts (lncRNA expression levels are generally at least one order of magnitude below mRNA)
- LNA™ qPCR assays easily designed for specific splice variants etc.
- LNA™ qPCR assays optimized to avoid off-target hits on the opposite strand

Fully integrated with Exiqon's RNA products and services

The ExiLERATE LNA™ qPCR System is fully compatible with Exiqon's products and services for:

RNA Isolation:

Exiqon's range of miRCURY RNA Isolation Kits for Cell & Plant, Tissue and FFPE are ideal for RNA isolation prior to qPCR analysis using the ExiLERATE LNA™ qPCR System.

Next Generation Sequencing:

The ExiLERATE LNA™ qPCR System is ideal for validation of NGS results obtained using Exiqon's mRNA or whole transcriptome NGS Services.

Exiqon also offers XploreRNA – a unique online service for RNA NGS data analysis which makes it possible to select candidate genes for validation and order ExiLERATE LNA™ qPCR assays with just one click.

RNA Silencing using Antisense LNA™ GapmeRs:

The flexible primer positioning possible with the ExiLERATE LNA™ qPCR System makes it easy to design qPCR assays avoiding regions targeted by LNA™ GapmeRs, to validate gene knockdown in your RNA silencing experiments. Validated ExiLERATE LNA™ qPCR primer sets are also available to detect mRNA and lncRNAs targeted by LNA™ GapmeR positive controls.

ExiLERATE LNA™ qPCR for mRNA and lncRNA

Highest sensitivity, success guaranteed.
Order now at:
exiqon.com/LNA-qPCR

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