Absolute quantification of miRNAs with high accuracy and precision using digital PCR

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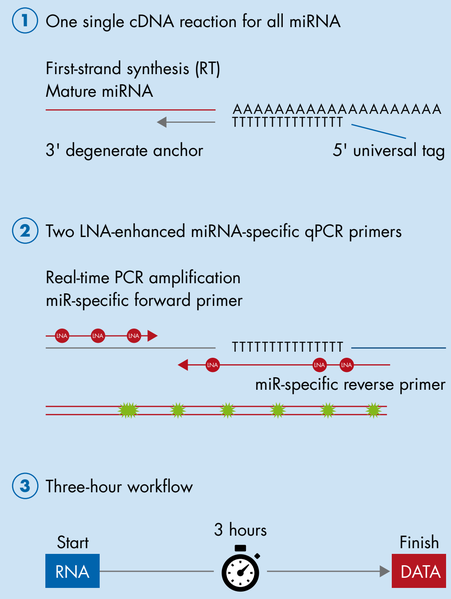
Absolute miRNA quantification with high sensitivity and precision

microRNAs (miRNAs) are short non-coding RNAs shown to post-transcriptionally regulate gene expression. Since miRNAs are present in tissues as well as body fluids, miRNAs show high potential as non-invasive diagnostic and prognostic biomarkers.

Deregulated miRNA expression has been identified to significantly contribute to pathogenesis, progression and prognosis of serious human diseases such as various types of cancers. miRNA expression in malignant cells is often attributed to alterations in genomic miRNA copy numbers and gene locations. In the past decades, research into miRNA involvement in cancer has made tangible progress.

However, robust miRNA quantification is not always easy, especially when analyzing samples with a high inhibitory burden or low amount of nucleic acid. Digital PCR using the QIAcuity® Digital PCR System allows you to overcome these hurdles and to detect and quantify miRNAs with high sensitivity and precision without the need for pre-amplification. Using dedicated miRCURY® LNA® miRNA PCR Assays that contain LNA modifications increases specificity allowing for a highly accurate miRNA quantification.



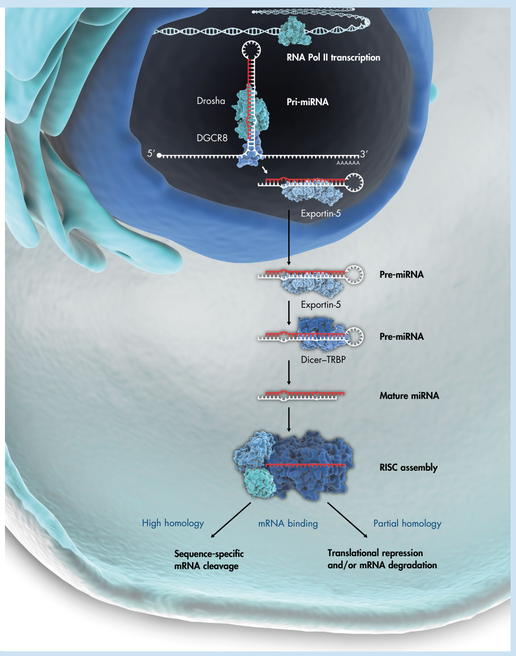
miRCURY LNA miRNA PCR System workflow.

miRNAs are important regulators of gene expression

miRNAs have emerged as key regulators of biological processes in animals. As small non-coding RNAs, they serve as post-transcriptional regulators via either translational repression or mRNA degradation. Dysregulation of miRNAs is associated with many human diseases, particularly cancer.

miRNA characteristics

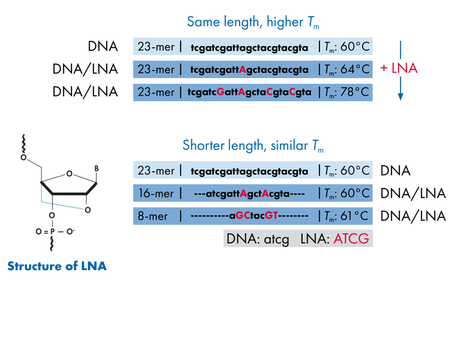
- Small non-coding RNAs
- Involved in a wide range of developmental processes
- Pivotal role in various diseases such as cancer
- Biomarkers for diagnosis and prognosis
- Easily accessible and measurable through minimally invasive procedures. High sensitivity allows disease detection before the appearance of clinical symptoms



Canonical pathway of miRNA biogenesis and miRNA characteristics. DNA sequences encoding for miRNAs are transcribed into pri-miRNAs and further processed to pre-miRNAs which are exported into the cytoplasm for the last step of miRNA maturation. miRNAs are associated with various diseases and are being explored as biomarkers for diagnosis and prognosis of diseases.

miRCURY LNA miRNA PCR assays enable miRNA quantification with unmatched specificity and sensitivity

LNA modifications in both the forward and reverse PCR amplification primers make it possible to design assays that can distinguish between miRNA sequences that differ by only one nucleotide.



Structure of LNA

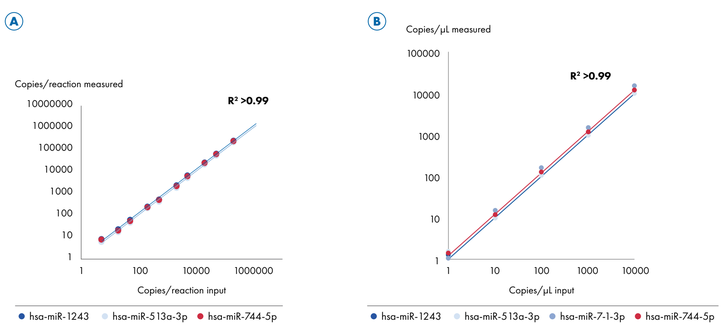
LNA: Locked nucleic acid assays

- LNA is an RNA analog in which the sugar ring is locked in the ideal Watson-Crick conformation
- LNAs increase the strength of base pairing and binding affinity compared to conventional DNA and RNA oligos
- The power of LNA includes:
  - Increased T_m (2–8°C per base) allows shorter, non-overlapping primer design
  - Improved mismatch discrimination enables single-nucleotide differentiation
  - Higher sensitivity and specificity

LNA modifications and its effects on base pairing and binding characteristics.

Broad dynamic range allows quantification of low- and high-expressed miRNAs

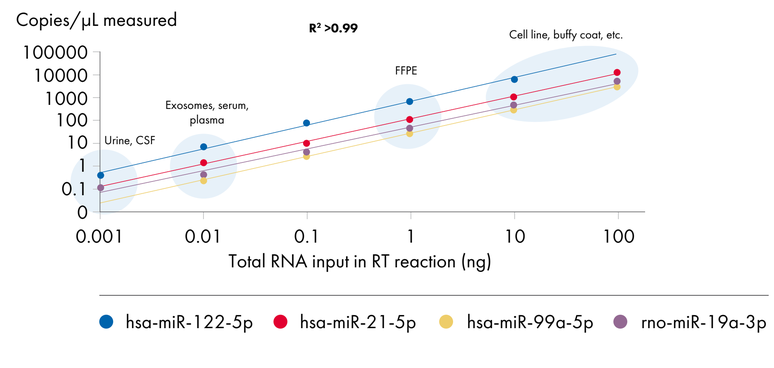
The QIAcuity dPCR System enables miRNA quantification of up to 200,000 copies per reaction with high accuracy and precision. Precise thresholding is important for absolute quantification of miRNAs.



QIAcuity dPCR dynamic range on 26k and 8.5k nanoplates. A Serial dilutions of synthetic cDNAs were performed from 5 copies/reaction to 200,000 copies per reaction on 26k nanoplates and **B** from 12 copies/reaction up to 120,000 copies/reaction on 8.5k nanoplates. **C** 1D-scatterplot of 3 human miRNAs with a high signal-to-noise ratio. miRCURY LNA miRNA PCR assays together with the QIAcuity EG MM were used for miRNA quantification.

Reliable miRNA detection from a wide range of sample types

Outstanding sensitivity allows for quantification of low-abundance miRNAs without pre-amplification even from low-miRNA-containing samples such as urine and CSF.



Excellent results with a broad range of total RNA input into the RT reaction. 1 pg – 100 ng of total RNA was used as input into the RT reaction. Data from serial dilutions of AM6000 total RNA are shown. All miRNA assays exhibited linear readout with R^2 >0.99.

Conclusion

- miRNAs can be reliably detected and quantified on the QIAcuity dPCR System using miRCURY LNA miRNA PCR assays without the need for references or standard curves
- Pre-amplification of low-expressed miRNAs is not needed due to unprecedented sensitivity
- LNA-enhanced assays provide increased specificity
- QIAcuity dPCR System provides increased tolerance towards inhibitors, allowing quantification of miRNA in samples with higher inhibitor loads

Reference:

Bondensgaard K, Petersen M, Singh SK, Rajwanshi VK, Kumar R, Wengel J, Jacobsen JP. Structural studies of LNA:RNA duplexes by NMR: conformations and implications for RNase H activity. Chemistry. 2000;6(15):2687-95.

Sample to Insight