Improved PCR Assays with 5'-TINA modified Oligonucleotides

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TINA (Twisted Intercalating Nucleic Acid) modified oligonucleotides improve the efficiency of classical end-point PCR and real-time PCR. TINA is an intercalator, developed by QuantiBact A/S which can stabilise Watson-Crick antiparallel duplex DNA. TINA has been proven to increase the melting temperature (Tm), efficiency and sensitivity of antiparallel duplex formations in hybridisations assays. Eurofins MWG Operon has been selected by QuantiBact A/S as the preferred supplier for TINA modified oligonucleotides.

Introduction

Duplex hybridisation based techniques, especially PCR assays, are widely used in basic research, molecular diagnostics and biotechnology. The sensitivity and specificity of PCR assays rely on the hybridisation properties of primers and probes. The recently developed TINA molecule (Twisted Intercalating Nucleic Acid) (see Fig.1 & 2) enhances the thermal stability and kinetics of an oligonucleotide duplex while leaving the ability to discriminate matching and mismatching oligonucleotides intact.

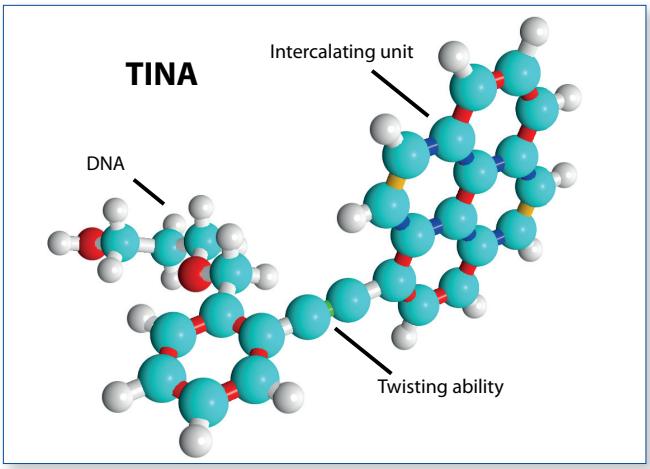

Fig.1 Model of TINA molecule

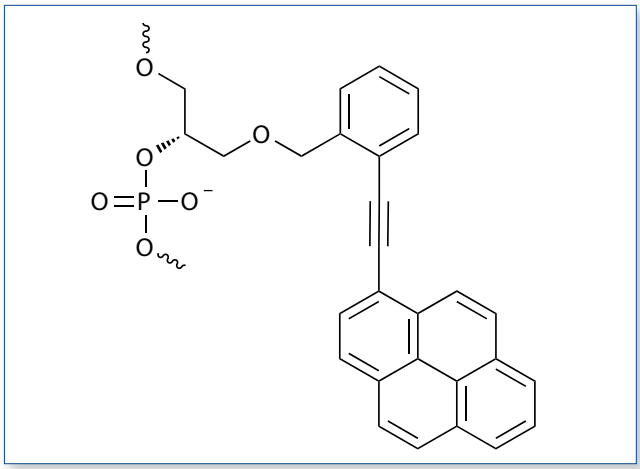

Fig.2 Structure of TINA molecule

Benefits of TINA modified Primers in PCR

TINA modified primers have been shown to increase the annealing temperature (Ta) of classical end-point PCR, as well as real-time PCR, and have been shown to decrease cycle of quantification (Cq) in real-time PCR. In PCR, the positive effect of an increased annealing temperature (Ta) is two-fold:

An increase in Ta reduces the general probability of a PCR primer to anneal unspecifically. The increase in specific primer annealing can be utilised to increase the overall specificity of a given assay. Alternatively by "relaxing" the stringency of the primer annealing, increased sensitivity can be achieved without compromising specificity compared to an identical assay without TINA.

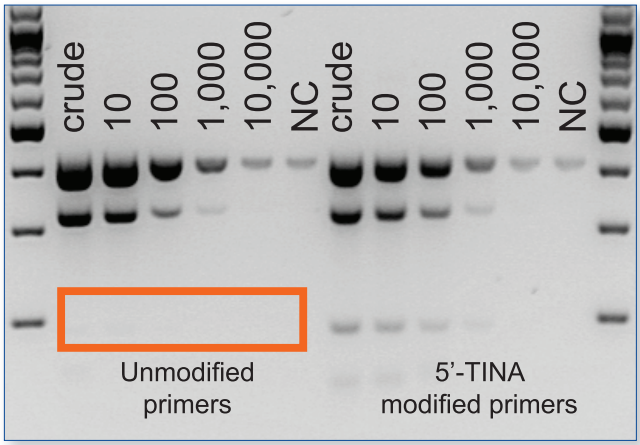

Fig.3 Amplification of an octaplex end-point PCR by unmodified primers and 5'-TINA modified primers. The orange box highlights the lack of amplicons for the gene with unmodified primers.*

Improved Assay Multiplexing Capacity

TINA modified primers reduce the necessary primer concentration by 30-50% and increase the optimal annealing temperature by 3 to 8 °C. Both effects reduce the complexity of a multiplex assay and improve assay specifications.

Improved Assay Robustness

TINA modified primers improve the robustness of the PCR and result in better tolerance of "uncleaned" DNA preparations. They withstand the presence of exonucleases and tolerate high background genomic DNA (see Fig.3).

Easy Assay Design

TINA modified primers generally improve any real-time as well as classical end-point PCR. TINA modified primers are easily and reproducibly synthesised by simply adding the TINA molecule to the 5' terminal position of any PCR primer during conventional oligo synthesis. No special primer design is needed and there is no need to re-design existing primers or change the buffer system or DNA polymerase (see Fig.4 & 5).

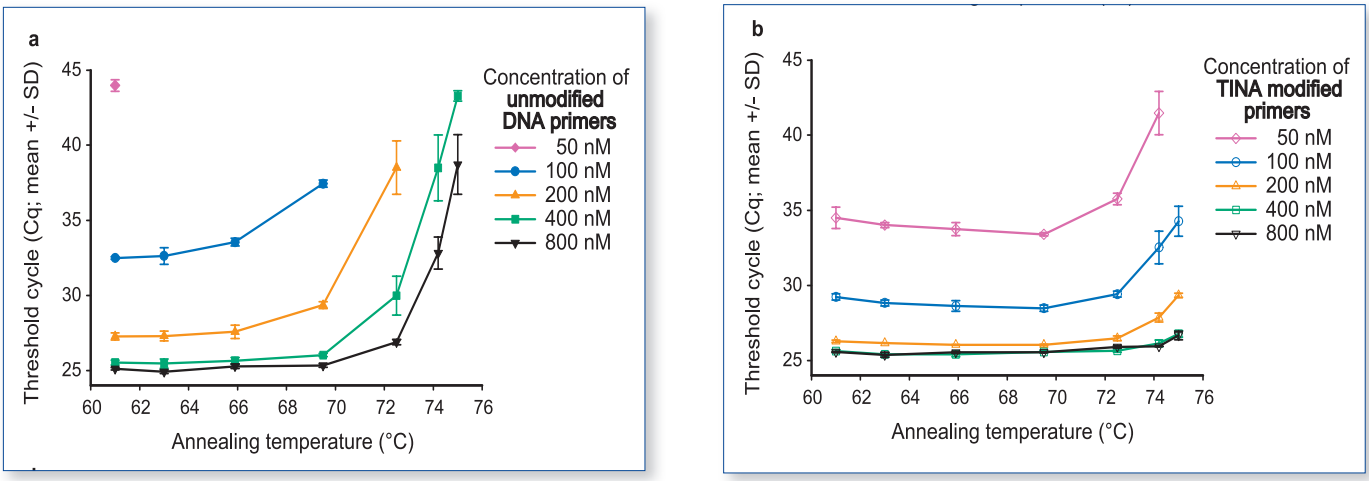

Fig.4,5 Comparison of unmodified and 5'-TINA modified primer concentrations on a temperature gradient. Left figure shows unmodified DNA primer concentrations from 50 nM to 800 nM. Right figure shows 5'-TINA modified primer concentrations from 50 nM to 800 nM*

Conclusion

If you are designing multiplex assays or working with clinical samples, the TINA technology significantly reduces the number of primers in the reaction and allows you to test less purified sample DNA. This leads to improved efficacy, increased robustness and shorter PCR time. You can also expect a stable performance over a wide range of primer concentrations and annealing temperatures and lower quantification cycle (Cq) of end-point and real-time PCR.

Contact

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*Schneider Uv, Mikkelsen Nd, Lindqvist A, Okkels Lm, Jøhnk N And Lisby G (2012) Improved Efficiency And Robustness In Qpcr And Multiplex End-Point Pcr By Twisted Intercalating Nucleic Acid Modified Primers. Plos One 7(6): E38451. Doi: 10.1371/Journal.Pone.0038451