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Optimised Pathogen Detection by Real-Time PCR Assays with 5'-TINA modified Oligonucleotides

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In many diagnostic applications for pathogen detection, assays are still in use that involve growing cultures to screen samples for the presence of pathogenic microorganisms. However, such culture-based methods for pathogen detection could be replaced by other methods. Real-Time PCR is a faster and more specific method that discriminates between microorganisms. The design of Real-Time PCR could be challenging if DNA sequence differences between species are diminutive or this discriminating DNA regions are AT rich. In such cases, oligos modified with 5'-TINA could be useful.

Introduction

In certain countries assessments of used buildings prior to bargain includes detection of specific human pathogens including mold species. The cultivation of these species in microbial laboratories is time-consuming. Detection of the microorganisms by Real-Time PCR with DNA extracted from filter swabs could solve this problem. We compared the performance off an assay with non-modified oligos and with 5'-TINA modified oligos for the detection of Streptomyces spp.. Identical oligo sequences and identical PCR conditions were examined by comparising Cp / Ct values and the overall increase of fluorescence signal.

Material and Method

TINA PCR primers were specifically designed for Streptomyces spp. DNA. The melting temperature of all primers was about 60° C calculated by the Primer Express® Software (Life Technologies). Oligos were synthesised non – modified and 5'-TINA modified, respectively. Real-Time PCR was performed with the non – modified oligos (0.5 µM) and the 5'-TINA oligos (0.5 µM, 0.375 µM and 0.25 µM) using ABI Taqman SybrGreen Universal Mastermix® under standard conditions (60°C annealing and elongation) in a two-step protocol on a Roche LC 480 II instrument with an input of 50 to 500,000 copies target DNA.

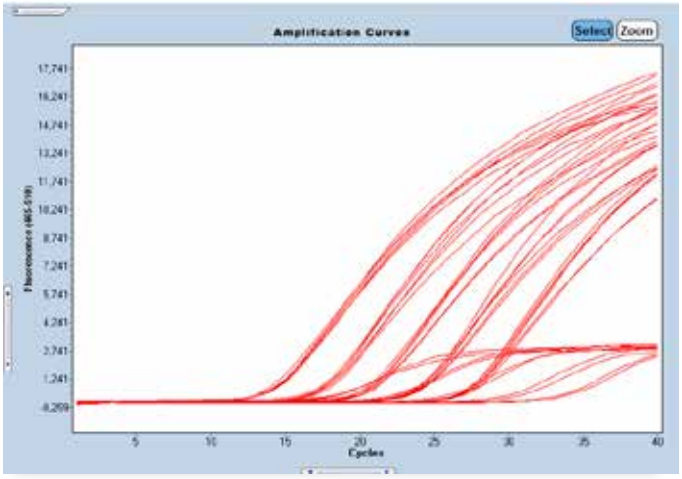

Fig.1 Model comparison of amplification curves with TINA modified oligos (high fluorescence) and non modified oligos (low fluorescence), detection with SybrGreen 500.000 to 50 target copies.

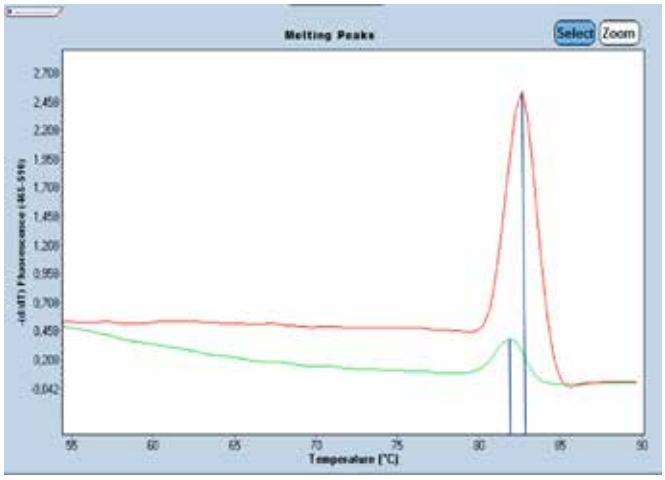

Fig.2 MeltCurve analyis after PCR with 500 copies template DNA with non modified oligos (green) and TINA modified oligos (red): sharper peak and higher fluorescence values with modified oligos; slight increase of temperature maximum at TINA modified oligos.

Results

Compared to PCR with non – modified oligos, TINA enhances the efficiency of the PCR reactions. Amplification curves with TINA modified oligos showed higher fluorescence signals (detected with SybrGreen®) compared to the PCR with non - modified oligos by an input of 50 to 50.000.000 target copies (see figure 1). In addition, we saw 3 to 5 Cp / Ct differences between the assay with non modified oligos and TINA modified oligos at identical template input (see figure 1 & 3).

Based on the higher fluorescence signal generated during the PCR, the melting curve peaks from reactions with TINA modified oligos are higher and sharper compared to those from non – modified oligos. Additionally a slight switch in the maximum melting temparature (melt max) was observed (see figure 2).

Titration experiments showed similar Cp / Ct values with an input of 0.5 µM, 0.375 µM and 0.25 µM TINA modified oligos. The different concentrations showed no influence on the melting curve analysis (figures 3 & 4).

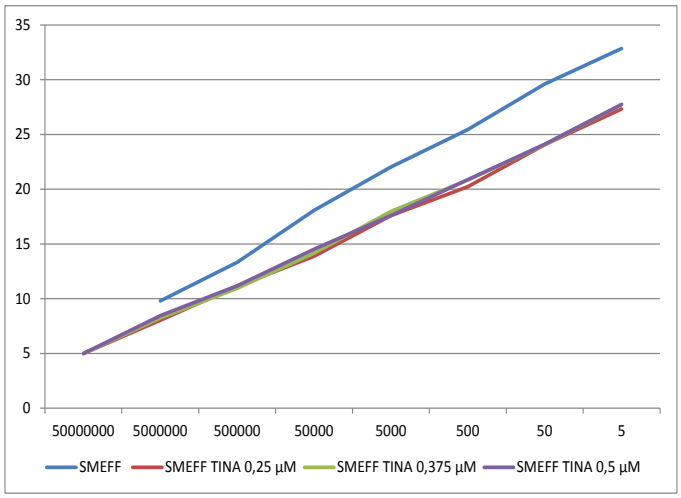

Fig.3 Comparison of Cp / Ct values after Real-Time PCR with non – modified oligos (0.5 µM) and TINA modified oligos (0.25 to 0.5 µM)

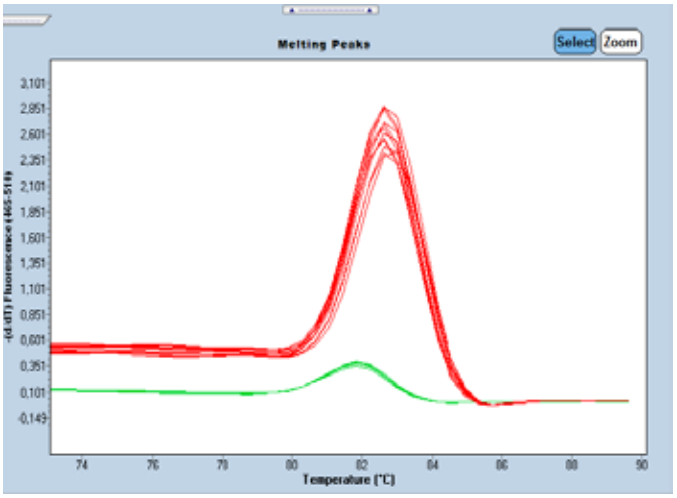

Fig.4 MeltCurve analysis after PCR with 500 copies template DNA with non modified oligos, 0.5µm (green) and TINA modified oligos 0.25 µm, 0.375 µm and 0.5 µm (red).

Conclusion

The TINA molecule can help increase the sensitivity and specificity of „difficult" assays. The assay for detecting Streptomyces spp. DNA is more sensitive with TINA modified oligos compared to the assay with non modified oligos (Difference of about 3 to 5 Cp / Ct). Furthermore sharper peaks and higher fluorescence values can be detected in melting curve analysis with TINA modified oligos.

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