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Clinical utility of a novel engineered DNA polymerase for the sensitive and reliable detection of pathogens

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Introduction

Undesired DNA synthesis can occur during PCR due to either mispriming or the formation of primer dimers. These two events, occurring in seconds at ambient temperature, can hinder the sensitivity and specificity of the PCR. Furthermore, DNA polymerases are susceptible to potential inhibitors from clinical specimens and as such, nucleic acids must be purified from the specimen prior to amplification. We addressed these caveats by engineering a novel Taq DNA polymerase based on prior studies of cold-sensitive mutants[1]. The Taq Hotstart DNA Polymerase is inactive at room temperature. Moreover, it displays strong tolerance to common PCR inhibitors from clinical samples.

DNA polymerase mutation through rational design

To create a hot start DNA polymerase that exhibits higher specificity and sensitivity in a PCR setup, we generated a mutant that actives only at the optimal PCR cycling temperatures. For this purpose, a structure elucidation of DNA polymerases was performed resulting in an identification of promising mutation sites based on rational design (Figure 1). To further improve the hot start activity of the polymerase, an aptamer sequence was specifically designed to bind to the enzymatic pocket of the active site and block the activity of the enzyme in a thermally reversible manner (Figure 2).

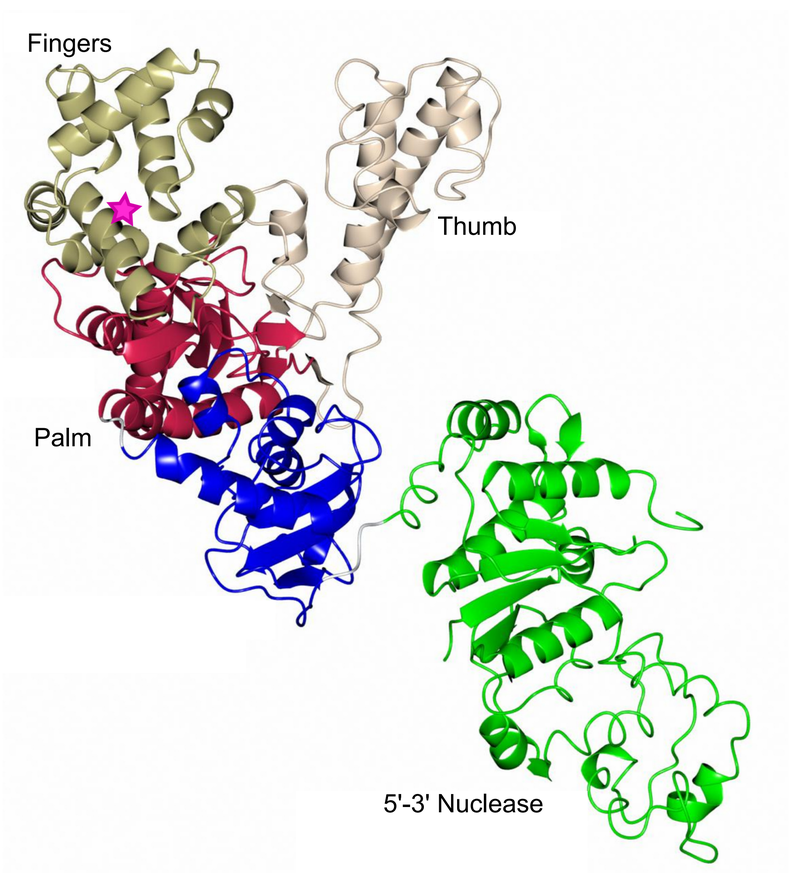

Figure 1. *The mutation in the a helix 28 of Taq Hotstart DNA Polymerase confers inactivity of the enzyme at room temperature.*

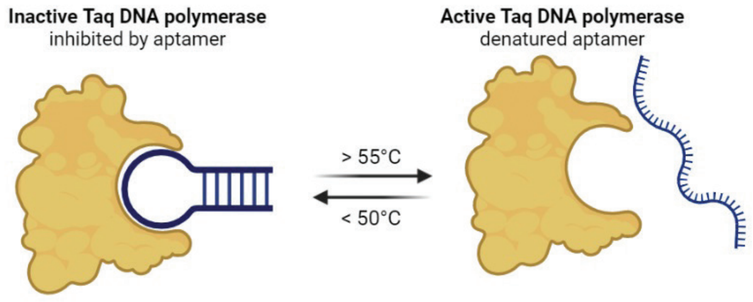

Figure 2. *Enzyme inactivation at lower temperatures is achieved by a tight bond of the aptamer to the polymerase. Temperatures above 55°C results in an activation of the enzyme by dislodging the aptamer from the active site.*

1 Simplify PCR setup

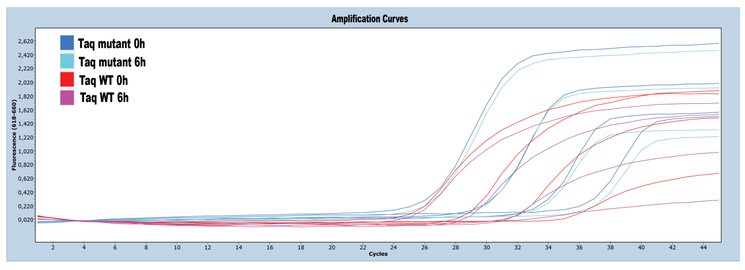

Figure 3. *Taq Hotstart DNA Polymerase shows no unspecific amplification when compared with Taq wild type (WT). As template a DNA serial dilution from 1 to 0.001 ng/µL was used. An incubation of the enzyme with template was performed for 6h at 21°C.*

2 Reduce time to result

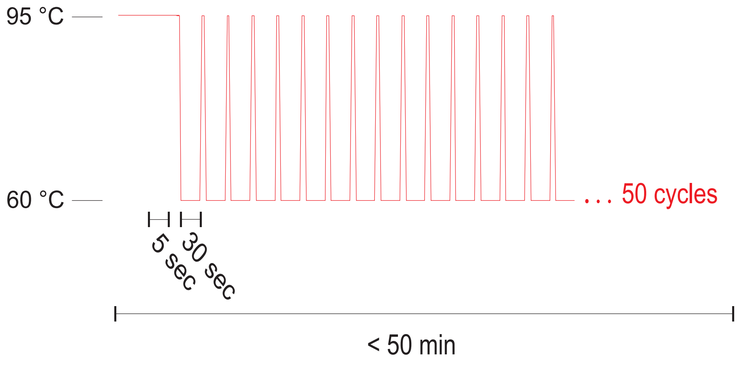

Figure 4. *The PCR run time can be reduced significantly, since no activation step is needed for the engineered Taq DNA polymerase. Fast start PCR protocol is used for multiplex pathogen detection depicted in Figure 5.*

3 High sensitivity and specificity by multiplexing

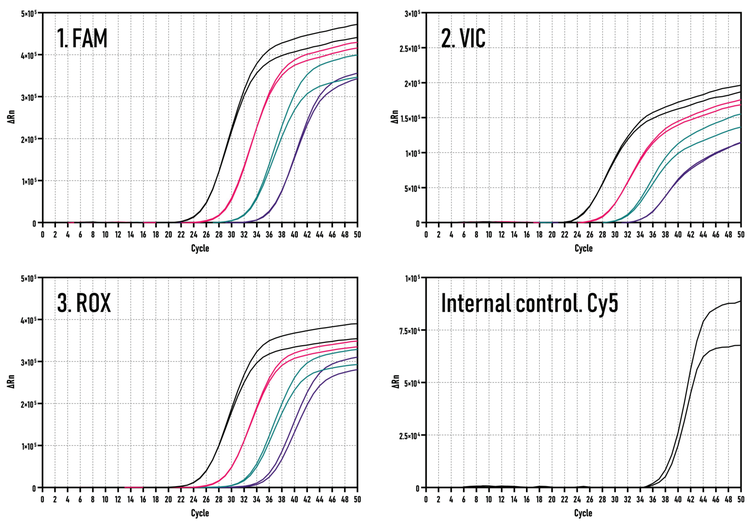

Figure 5. *The engineered Taq DNA polymerase can specifically detect food pathogens in a multiplex assay. The DNA serial dilution spans from 10,000 to 10 copies/ reaction for the targets and 5000 copies/reaction for the internal control. The amplification plots of the duplicates demonstrate the applicability of the engineered enzyme for detecting pathogens.*

4 Amplify directly from blood

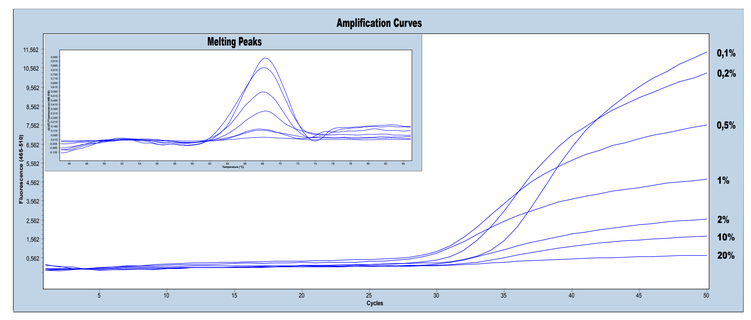

Figure 6. *Targets can be reliably amplified from a variety of sources without a purification step, since the polymerase is exhibiting a strong tolerance to common PCR inhibitors. A detection is possible in the presence of up to 10% blood. Taq Hotstart DNA Polymerase amplified the human coagulation risk factor 5 target from EDTA-supplemented blood.*

References

1. Kermekchiev, M. B., Tzekov, A. & Barnes, W. M. Cold-sensitive mutants of Taq DNA polymerase provide a hot start for PCR. Nucleic Acids Res. 31, 6139–6147 (2003).

2. Dang, Jayasena, DNA inhibitors of Taq DNA polymerase facilitate detection of low copy number targets by PCR. J.Mol.Biol., 1996.

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