Enzyme Engineering: Directed Evolution and Rational Design for Enhanced DNA Polymerases and their Applications

Audrey Crousilles[1], Christoph Albrecht[2], Maja Studencka-Turski[2], Ramon Kranaster[2], Laura Vaahtoranta[1], Giuseppina Sannino[2], Kevin Eboigbodin[1]

[1] Oy Medix Biochemica Ab, Klovinpellontie 3, FI-02180 Espoo, Finland

[2] myPOLS Biotec GmbH, Byk-Gulden-Strasse 2, 78467 Konstanz, Germany

Medix Biochemica

10th Gene Quantification Event 2023
20 - 24 March 2023
Freising-Weihenstephan, Germany

Introduction

Nucleic acids amplification is an essential method in molecular diagnostics, genetic manipulations, and analysis. Accordingly, DNA polymerases are the critical enzymes enabling efficient and accurate DNA amplification, yet many desired functions are not readily available in natural polymerases. Thus, new or improved functions are engineered for specific applications in biotechnology. Directed evolution of enzymes[1,2] has proven successful to obtain DNA polymerases with new or improved properties.

We have effectively employed protein engineering techniques such as directed evolution, rational design, and enzyme homology alignment to develop novel DNA polymerases with unique features. Our polymerase-engineering efforts focus on generating innovative DNA polymerases that can, for example, recognise mismatching nucleotides, or have increased reverse transcriptase activity. In this poster, we aim to highlight these recent approaches that led us to the development of new state-of-the-art DNA polymerases for diagnostics and genetic analysis.

Approach 1:

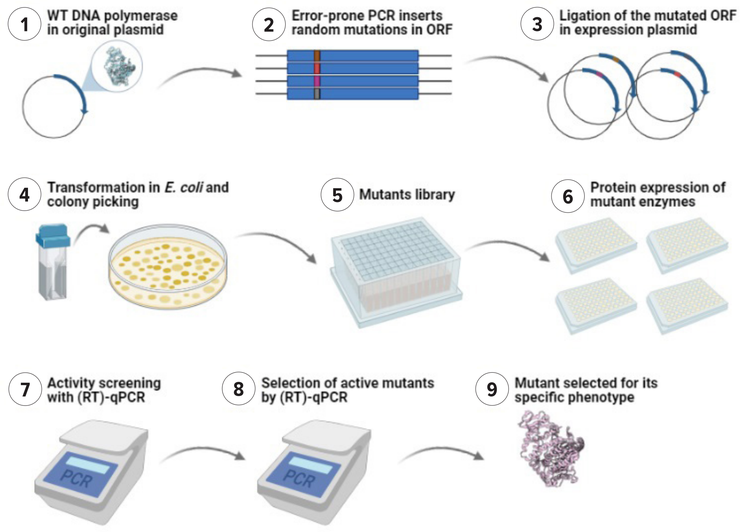
Directed evolution and homology alignment, study case of Isotherm3G

The typical workflow for directed evolution of DNA polymerases starts with the introduction of mutations throughout a selected gene resulting in a library of mutants. The enzyme library is screened for expression levels and activity required to finally select the mutant with the best phenotype (see Figure 1)[3,4]. With this process, we have successfully generated a Taq DNA polymerase with reverse transcriptase activity that is stable at room temperature for several hours. Furthermore, the mutations found to increase the reverse transcription activity of the Taq polymerase were transferred onto a different polymerase using homology alignment. For this, a Bst-derived scaffold was chosen. The resulting polymerase displays both strand displacement and reverse transcription activity. Consequently, it is able to amplify directly either or both DNA and RNA templates in isothermal amplification methods such as LAMP (see Figure 2).

Approach 2:

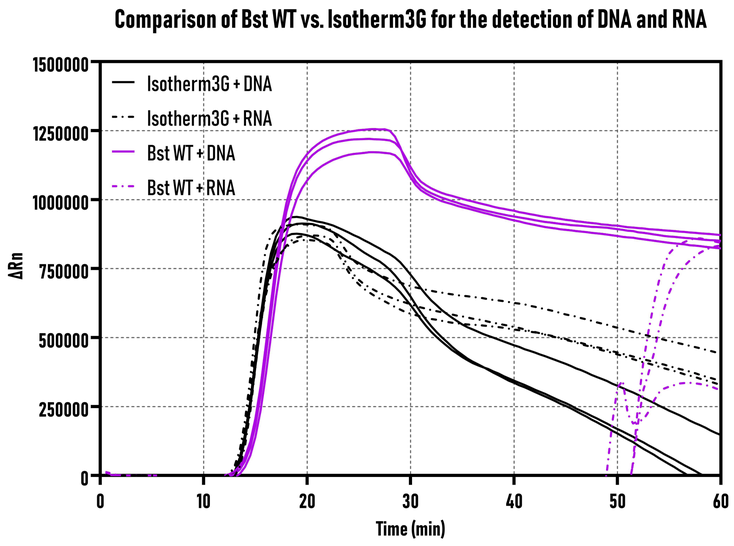
Rational design and mutations combination, study case of HiDi® DNA polymerase

An alternative approach which is complementary to direct evolution is based on structure elucidation of DNA polymerases revealing the structure-function relationship with their DNA substrate (PDB-code 3KTQ). A rational study of structural data identified five basic amino acids that bind with the primer (see Figure 3). A saturation mutagenesis at these five positions revealed that in several cases, substitutions to hydrophobic amino acids resulted in an increased mismatch extension selectivity, in agreement with previous findings[5–7]. The final enzyme, developed from this work, called HiDi® DNA polymerase, exhibits increased mismatch selectivity and demonstrates its suitability for SNP detection (see Figure 4).



Directed evolution of DNA polymerases supposes the introduction of random mutations in the dedicated ORF, followed by ligation, transformation and colony picking of the mutants. The mutants library can then be generated, and spilt up for protein expression. Mutant enzymes are screened for their activity and further selected to find the best phenotype.

Figure 1. *Process workflow for the directed evolution from a WT DNA polymerase to a mutant with the desired phenotype.*



Comparison of the isothermal amplification directly from DNA or RNA for the Isotherm3G against *Geobacillus stearothermophilus* isothermal DNA polymerase (Bst WT). Isotherm3G is able to amplify DNA in a direct LAMP assay from over 1000 genomic units of HeLa DNA; and in a direct RT-LAMP assay using 10^6 copies/ µL of RNA as template, it is evident that the reverse transcriptase activity of Isotherm3G is greatly increased in comparison to Bst WT.

Figure 2. *Isothermal amplification plots from SARS-CoV-2 RNA and HeLa genomic DNA template.*

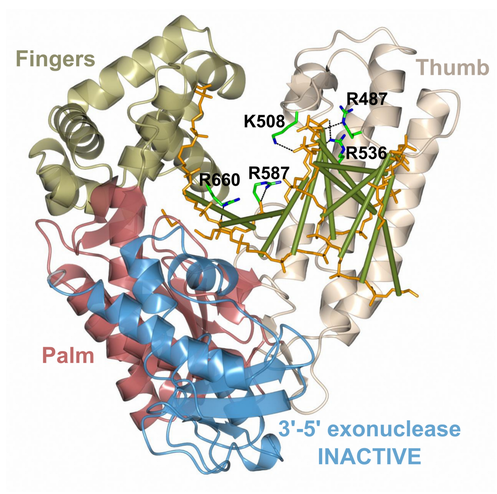
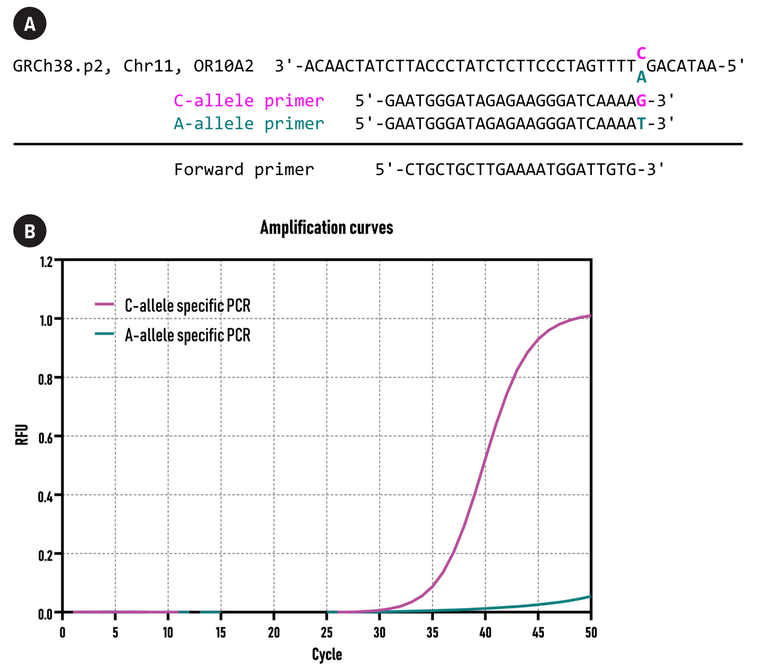


Figure 3. *Structural data of the Klenow fragment of Taq DNA polymerase bound to a primer/template DNA complex. PDB code: 3KTQ.*



A) Primer design for the C-allele and A-allele specificity.
B) Amplification curves distinctly show specific amplification from the C-allele primer.
C) Melting curves analysis demonstrate a single peak from a single amplified product.
D) Agarose gel electrophoresis corroborates the specificity of the results obtained.

Figure 4. *Allele specific-PCR results with HiDi DNA polymerase showing increased mismatch selectivity.*

Conclusion

The use of either directed evolution or rational design proved to be very successful in generating novel DNA polymerases with new functions for biotechnological applications. The recent achievements in the field of DNA polymerase engineering pave the way for the development of polymerases capable of amplifying unnatural biopolymer or even performing DNA amplification without the need of a thermal cycler. Tailored engineered DNA polymerases already underpin many biotechnological applications and will continue to attract interest for the industry.

References

1. Turner, N. J. Directed evolution drives the next generation of biocatalysts. Nat Chem. Biol. 5, 567–573 (2009).

2. Tracewell, C. & Arnold, F. Directed enzyme evolution: climbing fitness peaks one amino acid at a time. Curr. Opin. Chem. Biol. 13, 3–9 (2009).

3. Brakmann, S. Directed evolution as a tool for understanding and optimizing nucleic acid polymerase function. Cell. Mol. Life Sci. 62, 2634–2646 (2005).

4. Holmberg, R. C., Henry, A. A. & Romesberg, F. E. Directed evolution of novel polymerases. Biomol. Eng. 22, 39–49 (2005).

5. Drum, M., Kranaster, R., Ewald, C., Blasczyk, R. & Marx, A. Variants of a Thermus aquaticus DNA polymerase with increased selectivity for applications in allele- and methylation-specific amplification. PLoS One 9, (2014).

6. Rudinger, N. Z., Kranaster, R. & Marx, A. Hydrophobic Amino Acid and Single-Atom Substitutions Increase DNA Polymerase Selectivity. Chem. Biol. 14, 185–194 (2007).

7. Summerer, D., Rudinger, N. Z., Detmer, I. & Marx, A. Enhanced fidelity in mismatch extension by DNA polymerase through directed combinatorial enzyme design. Angew. Chemie - Int. Ed. 44, 4712–4715 (2005).

myPOLS Biotec GmbH
Byk-Gulden-Str. 2 // 10, 78467 Konstanz, Germany

Medix Biochemica Group
Klovinpellontie 3, FI-02180 Espoo, Finland
mdx@medixbiochemica.com • www.medixbiochemica.com