The Influence of Mismatches on Polymerase Activity

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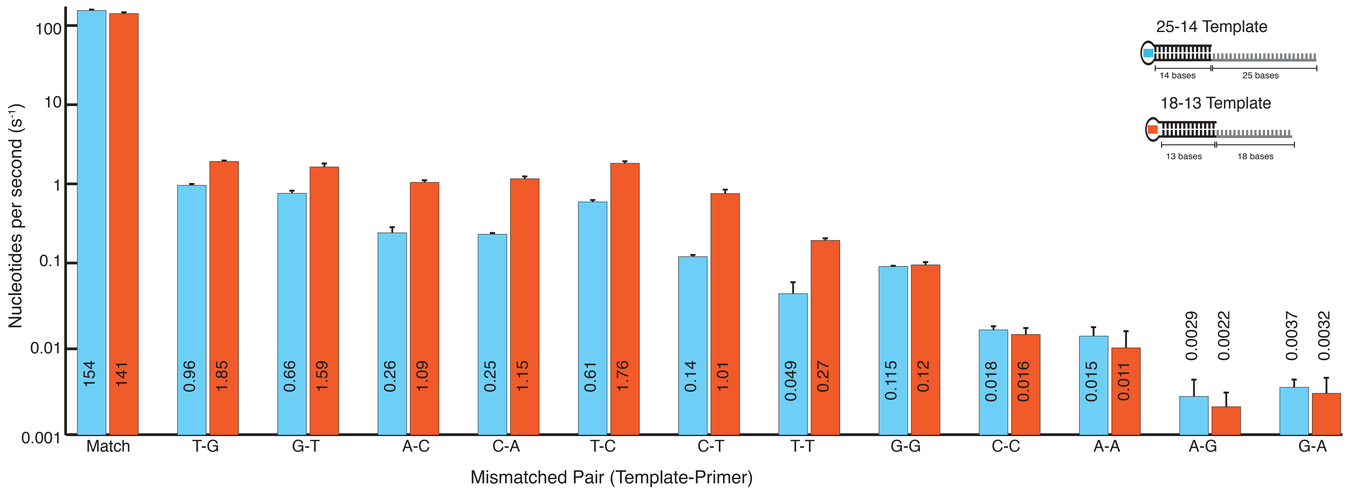
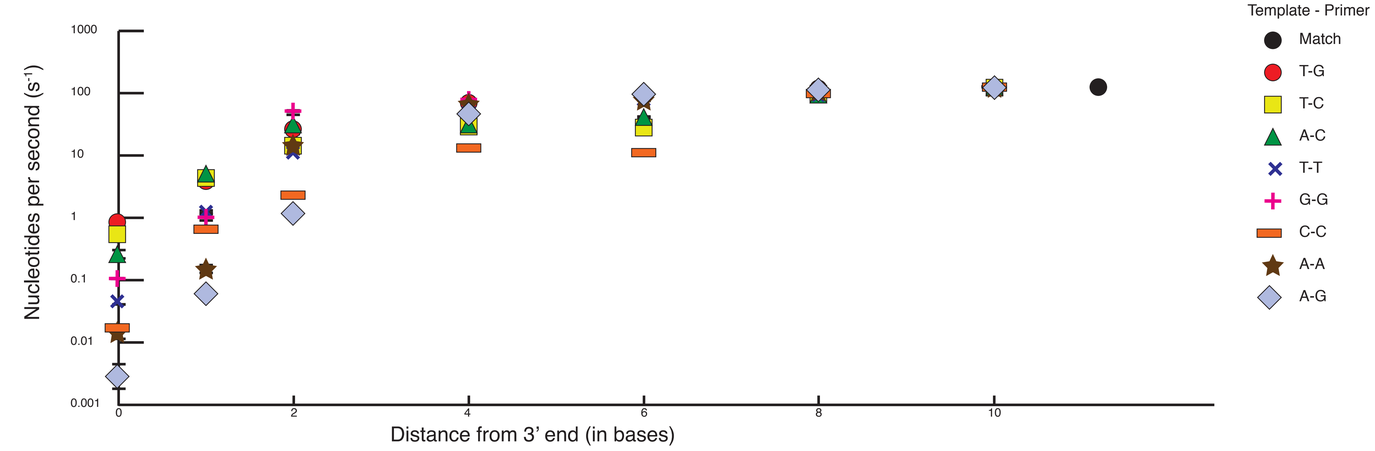
Introduction

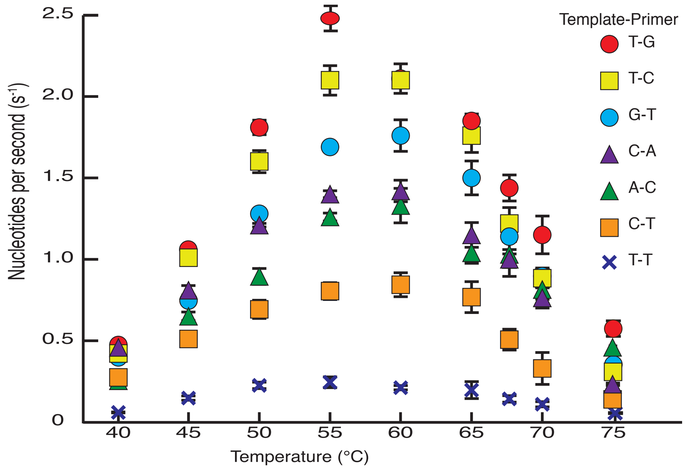
Understanding how base pair mismatches influence nucleotide incorporation is critical for the optimization of Allele-specific PCR assays. This study utilizes a simple, fluorescence based stopped-flow assay to characterize the kinetic effects of base pair mismatch type, position, and temperature on DNA polymerase activity under PCR applicable conditions in quantitative terms.

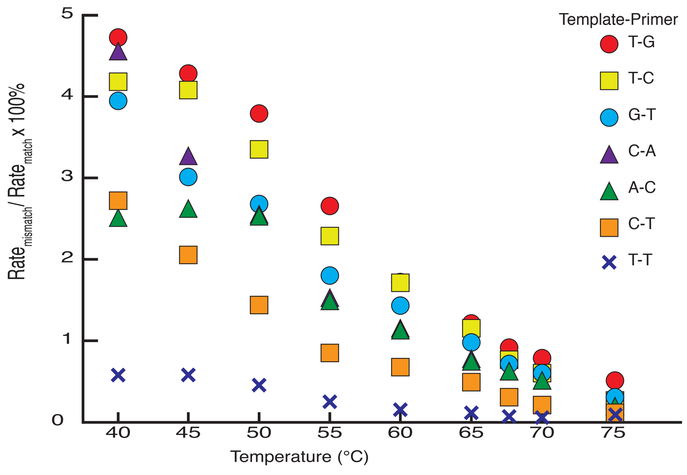
Methods

- The study was performed on a temperature controlled stopped-flow instrument (Biologic, inc)™ and monitored using a noncovalent dsDNA dye [1]. Reactions were performed at 65°C and contained 50 mM Tris pH 8.3, 3 mM Mg^{2+}, 500 µg/mL BSA, 100 nM template, 200 µM dNTPs, 1x EvaGreen® dye, and 5-20 nM Klentaq1.
- Reactions were either monitored to exhaustion or normalized using synthetic analogs of fully extended templates. The signal was scaled between zero and the average number of extendable bases per polymerase molecule and the initial rate was taken to correspond to the rate of nucleotide incorporation per polymerase (s^{-1}). Rates measured varied within 5% between approaches.
- Mismatched templates were based on 2 perfectly matching self-annealing hairpins. The first ("25-14") had a 25bp extension region, 14bp stem, and 6bp loop while the second ("18-13") had an 18bp extension region, 13bp stem, and 6bp loop. All 12 possible 3'-end mismatches were studied using each template set. A subset of hairpins was studied from 40-75 °C. The 25-14 template was also used to study 8 mismatches at positions 1, 2, 4, 6, 8, and 10 bases from the 3'-end.









Results

Mismatches were inhibitory as far as 8 bases from the 3'-end. At 65 °C, extension rates for 3'-mismatches ranged from 1.85 (T-G mismatch with the G on the primer) to 0.0022 s^{-1} (A-G mismatch) compared to 141-154 s^{-1} for perfectly matched templates. 3' mismatches were shown to reduce the temperature for optimal polymerase activity from 68 °C to 55-60 °C. At 55 °C, rates for mismatched templates increased by as much as 80% while the rate for a perfect match equivalent was reduced by 40%.

Discussion

By studying extension in isolation the effects of base pair mismatches can easily be assessed. The influence of mismatch type and position varied predictably across template sets. Rates decreased dramatically with mismatches 0-4 bases from the 3'-end. Rates for 3' mismatches vary several orders of magnitude (10^0 to 10^{-3}) highlighting the importance of primer design and target selection in allele-specific PCR.

Optimal temperature for polymerase activity decreased with introducing mismatches at the 3'-end. This may be due to an increase in klentaq binding, which has been shown to be optimal between 40-50 °C [2]. Differences in optimal temperature for matched and mismatched oligonucleotides suggest utilizing higher annealing and extension temperatures may improve allele-specific PCR specificity. Furthermore, preparation of PCR reactions at decreased temperatures and/or the use of hot-start polymerases and methods may substantially improve Allele-specific PCR assays.

References: [1] Montgomery JL, et al. Anal Biochem, 441(2): 133-9, 2013 [2] Data K, et al. Nucleic acids 31.19 (2003): 5590-5597. **Contact:** nick.rejali@utah.edu