A Highly Discriminating Taq polymerase Ideal for allele-specific PCR (AS-PCR), SNP detection and CRISPR/Cas gene editing

HiDi® 2x PCR Master Mix Cat no. #9101S, #9101M

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Medix Biochemica

AMP 2022 Annual Meeting & Expo
November 1-5, 2022
Phoenix, Arizona USA

Introduction

HiDi® stands for High Discrimination of nucleotides mismatches at the 3'-terminus of PCR primers. The HiDi® 2X PCR master mix is a SYBR Green-compatible ready to use mix that contains a highly selective recombinant DNA polymerase suitable for allele-specific PCR (AS-PCR)[1], primer extension or methylation-specific PCR. HiDi® is similarly effective for checking the quality of genome editing techniques such as CRISPR/Cas or TALEN-based approaches[2,3]. The enzyme uses a hot-start aptamer-based technology while the optimized master mix chemistry promotes high sensitivity and robust polymerase processivity. Temperatures above 50°-55°C allows the aptamer's secondary structure to melt and will set-free the DNA polymerase. HiDi® efficiently discriminates transversion mutations as well as transition mutations in a more delayed fashion. For probe-based assays, the HiDi® Taq 2X PCR master mix is also available (Cat no. #4200S or #4200M).

Methods

HiDi® 2x PCR Master Mix and three competitors were used to amplify a 100 bp-long fragment of actin gene (Genbank NM_001101.5). A 10-fold dilution series of human genomic DNA was amplified using SYBR Green chemistry over 40 cycles as per Table 1. Matching versus 3'-mismatching reverse primers were designed according to Figure 1. Reaction setup and final primer concentrations were applied according to manufacturer recommendations.

Step	Temperature	Time	Cycles
1	95°C	2 min	1
2	95°C	15 sec	40
3	60°C	30 sec	
4	72°C	30 sec	

Table 1. *Thermal cycling conditions. Step 1 of initial denaturation was extended to 10 min for competitor C.*

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NM_001101.5:85-1212   3'-GTCTTTGCGGATGTCCACGTCACACTTCATGATGGAGT-5'
          WT primer           5'-GGATGTCCACGTCACACTTC-3'
          A-mismatch primer   5'-GGATGTCCACGTCACACTTA-3'
          T-mismatch primer   5'-GGATGTCCACGTCACACTTT-3'
          ------------------------------------------------
          Forward primer      5'-CACTCTTCCAGCCTTCCTTC-3'
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Figure 1. *Primer design. The mismatching reverse primers exhibit either a transversion (A-mismatch primer) or transition (T-mismatch primer) mutation vs. the matching WT primer. All primers have a Tm of ~63°C. WT stands for Wild Type.*

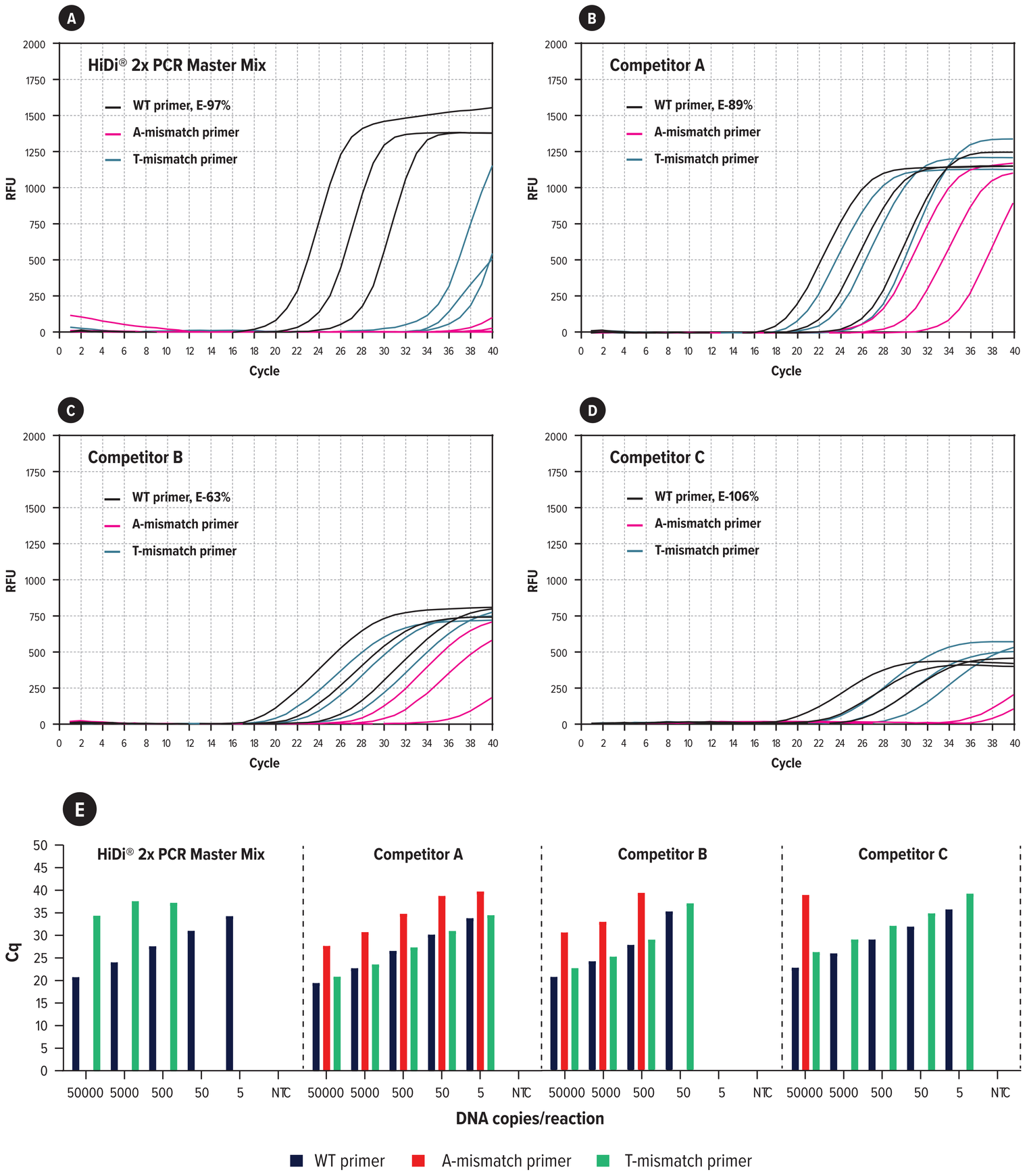

Figure 2. *Amplification curves and Cq values plots.*
A) *HiDi® 2x PCR Master Mix discriminates primers with a 3'-end mismatch.*
B) *Competitor A does not discriminate either of the mismatches.*
C) *Competitor B performances result in poor discrimination.*
D) *Competitor C performances result in poorer discrimination and signals.*
E) *The Cq values for HiDi® 2x PCR Master Mix and competitors across DNA copies demonstrates the high discriminative power of HiDi® in presence of a transversion or transition mutation.*

Note: *All three amplification curves presented for WT, A-mismatch and G-mismatch primers correspond to the 50000, 5000 and 500 DNA copy numbers in the samples.*

Results

Firstly, HiDi® 2x PCR Master Mix outperformed and was the most efficient for discriminating against a transversion mutation (A-mismatch primer) in comparison with the WT primer as shown in panels A and E of Figure 2. The product of the T-mismatch primer is significantly delayed and hence demonstrates good discrimination. Furthermore, the competitors' products yielded lower fluorescence signals and result in poor discrimination of either the A-mismatch or T-mismatch primer as shown in panels B, C, D and E of Figure 2.

Secondly, the efficiency of HiDi® 2x PCR Master Mix after amplification with the matching WT primer stands at 97% and proves to be superior and over the three other products tested for three major suppliers.

Conclusions

- HiDi® 2x PCR Master Mix displays superior capacity for discriminating against a single mutated nucleotide. It is a product of choice for mutation detection using AS-PCR and for sensitive genome editing techniques.
- HiDi® 2x PCR Master Mix demonstrates superior performance with SYBR Green chemistry compared to three mixes from leading suppliers.
- HiDi® 2x PCR Master Mix is an equally efficient mix for highly sensitive PCR amplification.

References:

1. Miotto, O. et al. Emergence of artemisinin-resistant Plasmodium falciparum with kelch13 C580Y mutations on the island of New Guinea. PLoS Pathog. 16, 1–21 (2020).
2. Morisaka, H. et al. CRISPR-Cas3 induces broad and unidirectional genome editing in human cells. Nat. Commun. 10, (2019).
3. Sakurai, T. et al. Bindel-PCR: a novel and convenient method for identifying CRISPR/Cas9-induced biallelic mutants through modified PCR using Thermus aquaticus DNA polymerase. Sci. Rep. 9, 1–14 (2019).

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