High-fidelity amplification of GC-rich DNA is a challenging application due to the low processivity and lack of robustness of most proofreading DNA polymerases.

The novel KAPA HiFi DNA Polymerase and its proprietary buffer system are ideally suited for the high-fidelity amplification of DNA with a GC content >65%. The unique properties of this enzyme include high processivity and increased tolerance to DNA melting agents, which enables robust amplification of GC-rich DNA with extremely low error rates.

Introduction

High-fidelity amplification of short GC-rich DNA fragments is important for several applications, including genetic profiling, single-molecule sequencing, and cloning. The amplification of GC-rich DNA with proofreading enzymes is traditionally difficult due to the low processivity and lack of robustness of these polymerases. Stable secondary structures in GC-rich templates are not only resistant to melting, but cause DNA polymerases to stall, resulting in incomplete amplification products. Furthermore, GC-rich regions often contain secondary primer annealing sites from which spurious fragments are produced. GC-rich PCR typically results in low yields of the target fragment, ladders of non-specific products, amplicons of the incorrect length, primer-dimers, and/or complete reaction failure. Many methods and additives have been developed to facilitate template denaturation and reaction specificity. Nevertheless, high-fidelity amplification of GC-rich amplicons with wild-type proofreading DNA polymerases remains unreliable.

Consistent and reproducible high-fidelity amplification of GC-rich targets is achievable with the highly processive KAPA HiFi DNA Polymerase. The enzyme is supplied with a proprietary reaction buffer designed specifically for GC-rich PCR. In addition to higher success rates, KAPA HiFi DNA Polymerase is capable of amplifying GC-rich DNA with error rates 100X lower than that of wild-type *Taq*, and up to 10X lower than that of other proofreading DNA polymerases—in total reaction times up to 50% shorter than those typically required for high-fidelity PCR.

KAPA HiFi is also available in an antibody-mediated HotStart formulation, which may yield improved results with some primer-template combinations.

Results

To demonstrate the success of KAPA HiFi in GC-rich PCR, sixteen amplicons (275 – 900 bp) with a GC content ranging from 65.5 – 85% were amplified from human genomic DNA. Results are summarized in Figure 1 and Table 1.

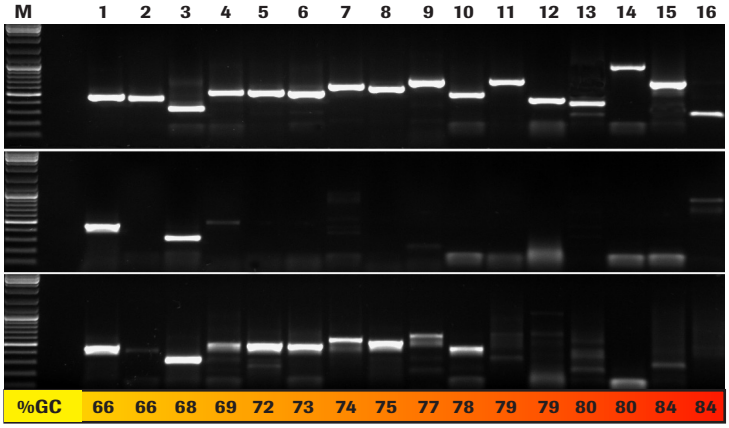

Figure 1. Amplification of GC-rich DNA fragments. Amplification of different types of GC-rich DNA fragments using KAPA HiFi HotStart (top panel), a competitor engineered proofreading DNA polymerase containing a dsDNA-binding domain (hot start formulation, middle panel) and wild-type Pfu (bottom panel). Amplicon GC content increases from left (yellow) to right (red). KAPA HiFi HotStart reactions were performed as described on the next page. Competitor reactions were performed in according to manufacturers' instructions and were performed in GC Buffer (engineered competitor), or contained DMSO at a final concentration of 5% (Pfu). All reactions contained 25 ng human genomic DNA as template. Cycling was performed using an Eppendorf Mastercycler epgradient S. Lanes marked M contain DNA ladder.

Table 1. High-fidelity GC-rich PCR success rates and cycling times of KAPA HiFi DNA Polymerase and competitor enzymes

Enzyme system	Success rate	Published error rate*	Cycling time
KAPA HiFi	100%	2.8×10^{-7}	40 min
Competitor 1 (engineered)	12.5%	4.4×10^{-7}	40 min
Competitor 2 (wild-type Pfu)	56%	2.2×10^{-6}	1 h 10

*Errors per nt incorporated.

Reaction conditions and cycling parameters

The reaction conditions and cycling parameters given in Tables 2 and 3 are recommended as a starting point for high-fidelity GC-rich PCR using KAPA HiFi DNA Polymerase. The GC Buffer should be used the first approach for amplicons with a GC content between 65 and 80%. For amplicons with a very high (>80%) GC content, or that are recalcitrant to amplification, the GC Buffer may be combined with 1X KAPA Enhancer 1, a proprietary betaine-like additive (available on request). Alternatively, PCR-grade betaine may be added to KAPA HiFi reactions to a final concentration of 1 M, to resolve amplicons with a high GC content, or to improve the yield of a target fragment or specificity of amplification.

Table 2. KAPA HiFi reaction setup for high-fidelity GC-rich PCR

Reaction component*	Final conc.	Per 25 µL reaction
PCR grade water	–	Up to 25.0 µL
5X KAPA HiFi GC Buffer	1X	5.0 µL
10 mM dNTP Mix	0.3 mM each dNTP	0.75 µL
Forward primer (10 µM)	0.3 µM	0.75 µL
Reverse primer (10 µM)	0.3 µM	0.75 µL
Template DNA	10 – 50 ng	–
KAPA HiFi DNA Polymerase (1 U/µL)	0.5 U/reaction	0.5 µL

*5.0 µL of a 5X KAPA Enhancer 1 solution or a 5 M PCR-grade betaine solution may be added to improve yields and/or specificity.

Table 3. KAPA HiFi cycling parameters for high-fidelity GC-rich PCR

Cycling step	Temperature and time	
Initial denaturation	2 – 5 min at 95°C	1
Denaturation	20 sec at 98°C	x25 – 35 cycles
Annealing	15 sec at 65°C	
Extension	15 sec/kb at 72°C	
Final extension	1 – 5 min	1

Tips for successful high-fidelity GC-rich PCR

- Do not use more than 0.5 U KAPA HiFi DNA Polymerase per 25 µL reaction.
- KAPA HiFi GC Buffer is a uniquely formulated buffer with a much higher salt concentration than the reaction buffers used with most other DNA polymerases. Denaturation at 98°C for 20 sec in each cycle is essential to ensure full denaturation of DNA in the presence of high salt.
- It is important to fully denature GC-rich targets. Although KAPA HiFi HotStart only needs 30 sec for activation, an initial denaturation of at least 5 min for complex, genomic templates and 2 min for less complex DNA is recommended.
- Because of the unique formulation of KAPA HiFi GC Buffer, optimal annealing temperatures are typically higher than those used for the same primer pairs in other PCR buffers. Determine the optimal annealing temperature for a specific primer pair in an annealing temperature gradient PCR. Alternatively, start with an annealing temperature of 65°C. The annealing temperature may be increased (up to 75°C) to improve specificity or decreased (but not lower than 60°C) to improve yields.
- Two-step cycling profiles with a combined annealing/extension 30 – 60 sec/kb at 68 – 75°C is recommended for long primers, primers with an actual optimal annealing temperature ≥68°C or recalcitrant amplicons.
- To improve yields, the extension time in each cycle may be increased to 30 – 60 sec/kb. An annealing time >15 sec per cycle is not recommended.
- The volumes of all components may be scaled down for smaller reactions. However, do not use less than 10 ng complex, genomic DNA in a 10 µL reaction.
- For less complex targets (e.g., plasmid or lambda DNA), use 1 – 5 ng DNA per 25 µL reaction.
- For high-fidelity amplification it is important to use good quality DNA, i.e., DNA that is not degraded, sheared, or damaged. Always dilute DNA (and primers) in TE or 10 mM Tris-HCl, pH 8.0 – 8.5 and not in water. Use the lowest number of cycles required to give a sufficient yield of the target fragment for analysis or downstream use.
- KAPA HiFi GC Buffer contains Mg_2+ at a 1X concentration of 2 mM. Results may be improved by adding an additional 0.5 mM $MgCl_2$ to reactions (e.g., 0.5 µL 25 mM $MgCl_2$ per 25 µL reaction).
- For more information on reaction setup, cycling parameters, and optimization, please consult the **KAPA HiFi or KAPA HiFi HotStart Technical Data Sheet**, or the KAPA HiFi FAQs, which are available at **sequencing.roche.com**.

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