Optimal PCR amplification for GC-rich templates using repliQa HiFi ToughMix® and enhancers

Keywords: repliQa HiFi ToughMix, GC-rich, DMSO, Betaine

ABSTRACT

GC-rich DNA templates often require extensive optimization to amplify by PCR. Several compounds have been found to help amplify these GC-rich regions, but they also tend to inhibit the polymerase activity. In this application note we detail a methodology for use of DMSO and betaine with repliQa HiFi ToughMix, demonstrating a fast, inexpensive and efficient method for the PCR amplification of GC-rich templates.

INTRODUCTION

PCR amplification of DNA fragments with high GC-content (>65%) can be difficult. The additional hydrogen bonds between guanine and cytosine (G=C base pair) cause the DNA to be resistant to denaturation, requiring higher melting temperatures. In addition, these un-melted GC-rich DNA regions can form complex secondary structures that prevent primer binding and enzymatic elongation.

One method to overcome the problems associated with the amplification of GC-rich genes is the addition of enhancers like betaine and DMSO[1,2]. Betaine is an amino acid analog that reduces the formation of secondary structures caused by GC-rich regions[2]. DMSO, on the other hand, acts by disrupting inter- and intra-strand reannealing.

In this application note, we present a fast and efficient method to amplify GC-rich gene fragments using repliQa HiFi ToughMix and enhancers. repliQa HiFi ToughMix alone is able to amplify varying levels of GC-content targets (32% – 70% GC-rich), but the addition of betaine and DMSO improves the yield and specificity of high-GC templates (70% to 82.5% GC-rich) without inhibiting the polymerase activity of repliQa HiFi ToughMix.

METHODS

Sample Input

High molecular weight human genomic DNA (11691112001, Roche) was used as the template for the PCR amplifications with a final input of 20 ng per reaction. Primers were designed to amplify specific regions in the human genome known to have high GC-content and associated with genetic diseases. These region-specific targets are between 149 and 281 base pairs.

Target	Primer	Sequence	GC-content of target	Product size (bp)
AF064849	Forward	ATCCCCCACCCCCGCACC	78.5	149
	Reverse	GGGCGCGAGATGGGCTGC		
IGFBP-3	Forward	GCCCCGGTTGCAGGCGTCATG	78.2	261
	Reverse	GCACGGCTGGCCCTCGCTCAG		
L29074:13738	Forward	CGGTGGAGGGCCGCCTCTGA	82.5	263
	Reverse	GCCATTGGAGCCCCGCACTTC		
L29074:13712	Forward	GCTCAGCTCCGTTTCGGTTTCACTTCCGGT	79.8	292
	Reverse	AGCGCCATTGGAGCCCCGCACTTCCA		
FCX	Forward	GCTCAGCTCCGTTTCGGTTTCACTTCCGGT	77.1	281
	Reverse	AGCCCCGCACTTCCACCACCAGCTCCTCCA		

Table 1 Primer sequences of the GC-rich targets.

repliQa HiFi ToughMix

1. The repliQa HiFi master mix was prepared on ice according to Table 2.

Component	Volume for 1 sample (µl)
repliQa HiFi ToughMix (2X)	10
10 µM Primer mix	1
Nuclease-free water	7 – x
Enhancer	x
Template DNA (10 ng/µl)	2
Total	20

GC Enhancer Composition	GCE1 5M Betaine	GCE2 DMSO	GCE3 DMSO + 5M Betaine
DMSO Volume	–	1	1
5M Betaine Volume	4	–	4
Total GC Enhancer Volume	4	1	5

Table 2 **PCR master mix for GC-rich targets.** A master mix was prepared according to the volumes given in the table, scale up for the number of samples with 10% overage.

2. For each reaction, 2 µl DNA sample was added (20 ng total input DNA)
3. Alternatively, 1 µl of DMSO (Millipore Sigma) was added for a final concentration of 5% of DMSO and/or 4 µl of 5M betaine (Millipore Sigma) for a final concentration of 1M to the PCR master mix. The volume of nuclease-free water was adjusted in these cases.
4. The PCR was run according to the cycling program detailed in Table 3.

Step	Temperature	Incubation Time	Cycles
Initial denaturation	98°C	30 sec	1
Denaturation	98°C	10 sec	35
Extend	68°C	1 sec	
Hold	4°C	Hold	1

Table 3 **PCR cycling conditions for GC-rich targets.**

To verify the size of the PCR 1 µl of the DNA was run on a D5000 ScreenTape on the 4200 TapeStation® System (Agilent).

RESULTS

High GC-content regions in our genome play a crucial role in gene regulation, gene expression, genome functionality and disease development, therefore it is important to have an optimal method to study them. In this application note, we chose five GC-rich human targets (GC-content ranging from 77.1% to 82.5%) which did not amplify previously with wild type *Taq* DNA polymerase and traditional buffer systems. repliQa HiFi ToughMix is specially formulated to provide high sensitivity, fast and processive extension of a wide range of targets. First, we tested if these five GC-rich targets can be amplified with repliQa HiFi ToughMix without the use of enhancers.

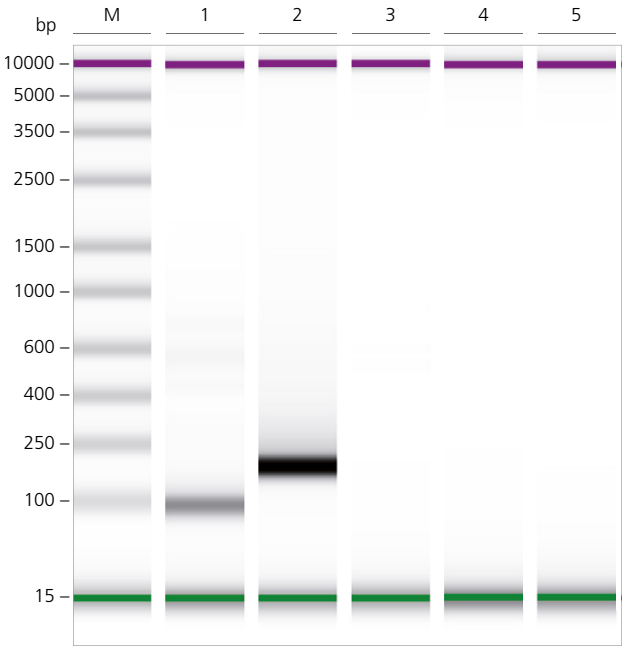

Figure 1 **Amplifying high GC targets with repliQa HiFi ToughMix.**
1: AF064849
2: IGFBP-3
3: L290074:13712
4: L290074:13712
5: FCX

repliQa HiFi ToughMix alone was able to amplify two gene targets AF064849 and IGFBP-3 as visualized by TapeStation electrophoresis (Figure 1). These smaller fragments are 149 and 261 base pairs respectively and the GC-content of both targets was ~78%. The remaining three gene fragments with the highest GC-content (>78.5% GC-rich) and length between 263 and 281 bp were not amplified by repliQa HiFi ToughMix alone. The PCR cycling took only 28 minutes due to the high processivity of repliQa HiFi ToughMix.

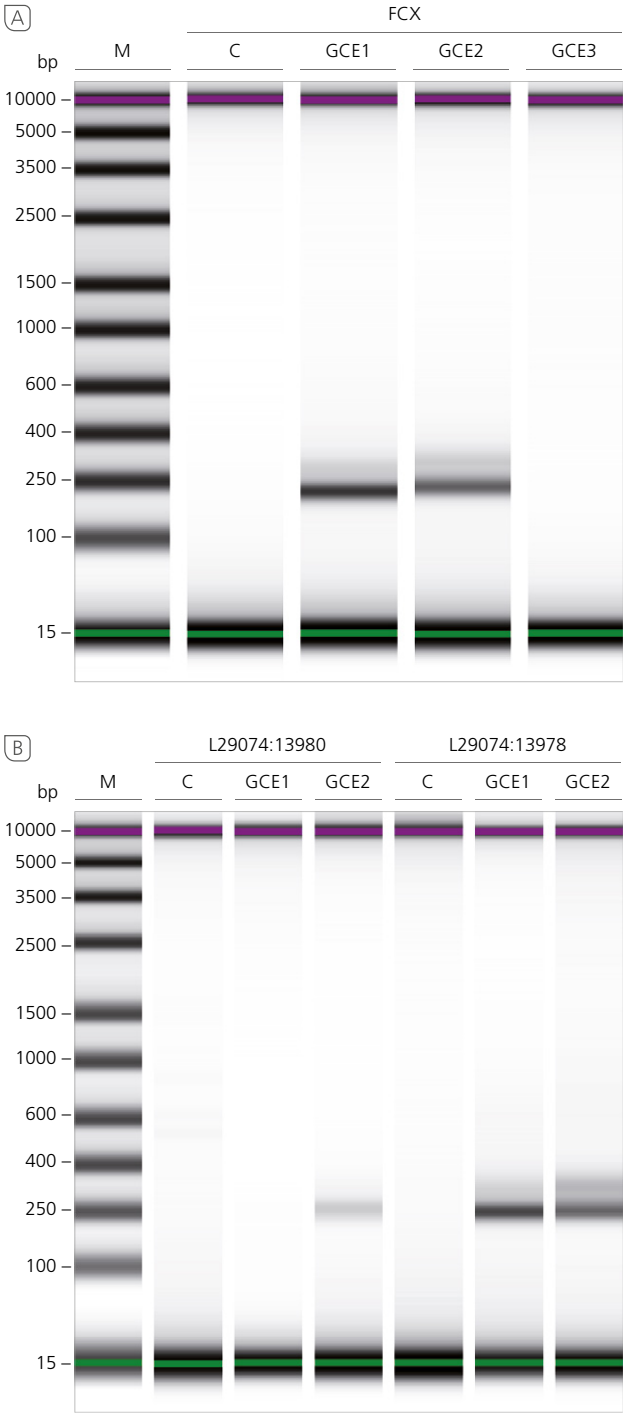

Figure 2 **Using traditional GC enhancers improves specificity and yield of difficult GC-rich targets.** A Amplification of FCX target with 77.1% GC-content. B Amplification of L29074:13980 and L29074:13978 with 82.5% and 79.8% GC-content respectively. **C**: Control, **GCE1**: 1M Betaine, **GCE2**: 5% DMSO, **GCE3**: 1M Betaine + 5% DMSO.

Next, we tested if the enhancers Betaine and DMSO had any effect on the PCR amplification with repliQa HiFi ToughMix. As shown in figure 2A, both 1M Betaine and 5% DMSO greatly improved the amplification for the target FCX with 77.1% of GC-content. However, the addition of betaine and DMSO together in the PCR mix was not effective in amplifying this GC-rich target. Consequently, we stopped testing the mixture of both DMSO and Betaine.

We also tested the effect of 1M Betaine and 5% DMSO with two other gene targets L29074:13980 (GC-content 82.5%) and L29074:13978 (GC-content 79.8%). Similar to FCX target, amplification of L29074:13978 (GC-content 79.8%) target was greatly enhanced by both 1M Betaine and 5% DMSO. But for the target L29074:13980 (GC-content 82.5%), only 5% DMSO was effective in amplifying this target.

CONCLUSIONS

In this application note we demonstrated that DMSO and betaine increased the ability of repliQa HiFi ToughMix to amplify targets with high GC-content (>70%). Both enhancers greatly improved target product specificity and yield during PCR amplification without inhibiting the polymerase activity of repliQa HiFi ToughMix. This PCR method will require testing with both DMSO and betaine to select the best enhancer for any specific GC-rich target. We recommend this inexpensive, fast and efficient PCR method that could be extremely useful when studying high GC-regions that are difficult to amplify in biological and clinical studies.

References

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