Coverage study of GC TEMPase Master Mix I

During the development phase of chimeric DNA polymerases our R&D team found that GC-TEMPase Master Mix I exhibits an excellent coverage. The coverage study of GC TEMPase Master Mix I is shown in figure 1.

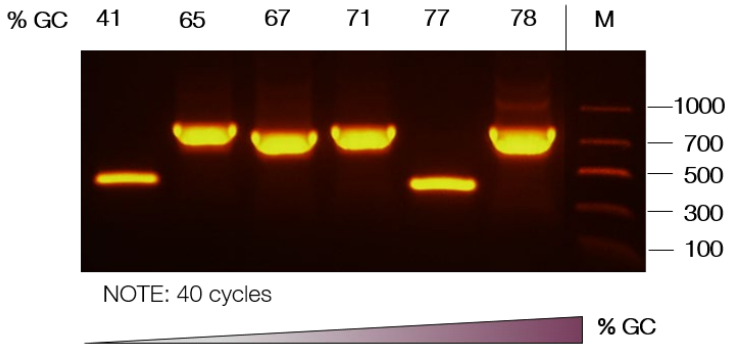

Figure 1. Coverage study GC Master Mix I
Five genes with increasing percentage of GC content were amplified using GC TEMPase Master Mix I. Lane M is Marker (Iqon DNA Mini Ladder). Number of PCR cycles used in this study is 40.

PCR setup for the coverage study:

PCR Reaction Mix

Component	GC TEMPase Master Mix I
Master mix	1x
$MgCl_2$	2 mM
Primers	0,2 µM
Human gDNA	25 ng

PCR Cycler Steps

Step	Ampliqon
Initial heating	95 °C, 15 min.
Denaturation	95 °C, 30 sec.
Annealing	60 °C, 30 sec.
Elongation	72 °C, 30 sec.
No. of cycles	40
Final elongation	72 °C, 1 min.

Targets

Target	GC	Length
DMD17	40.7 %	416 bp
BAIP3	64.6 %	788 bp
CEND	67.2 %	737 bp
KLF14	71.4 %	777 bp
FECH1	76.6 %	381 bp
NM_203423	77.8 %	779 bp

The coverage study shows that GC TEMPase I displays superior performance on a broad range of amplicons with a GC content ranging from 41 – 78 %. The results are in compliance with previous optimization studies obtained during the development of GC TEMPase Master Mix I and II. In the optimization study GC Buffer I supports correct amplification of all the selected targets (GC content ranging from 58 – 77 %). See figure 2.

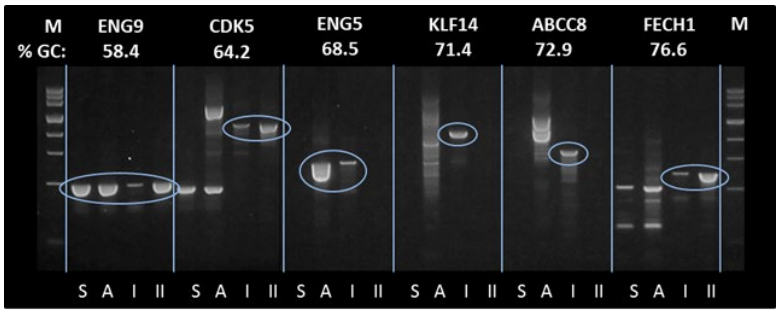

Figure 2. Optimization GC-rich DNA amplification
Six genes with varying percentage of GC contents were amplified using TEMPase with Standard Buffer (lanes S), Ammonium Buffer (lanes A), GC Buffer I (lanes I) and GC Buffer II (lanes II). M. Marker. Correctly amplified products are encircled.

The optimization study also showed that with an increasing percentage of GC content in the expected amplicon, Standard Buffer and Ammonium Buffer fail to give the correct amplification products, while GC Buffer I and GC Buffer II succeed.