Philisa® Thermal Cycler Technical Note

Streck Philisa Thermal Cycler Run Time and Yield Comparison

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Overview:
The purpose of this study was to compare the Philisa® Thermal Cycler against a competitor's thermal cycler with respect to total run time and amplification yield. Optimization efforts were performed on each thermal cycler prior to comparison. The results demonstrate that the Philisa Thermal Cycler provides equivalent or higher yields in approximately half the run time required by the competitor's instrument.

Methods:
In studies 1 and 3, a 402 bp region within enterobacteria phage lambda DNA (New England Biolabs) was amplified using primers (F) 5'-TGGCGGCAAATGAGCAGAAA-3' and (R) 5'-GGTCATGGTGTATTTGCCCTT-3'. In study 2, a 213 bp region of the GAPDH gene in human genomic DNA (Promega) was targeted using primers (F) 5'- CTTCATACCCTCACGTATTCCC-3' and (R) 5'- GGTTACCATATACCCAAGGGAG-3'. In studies 1 and 2, the Philisa Thermal Cycler was paired with KOD Hot-Start polymerase, while the competitor instrument utilized the recommended Phusion Hot-Start II polymerase. In study 3, Platinum Taq was used in both thermal cyclers using a standard reaction mixture and protocol. Three or four replicates were run in each study. In all studies, 10 µl of each PCR product was electrophoresed on a 1% agarose gel and stained with ethidium bromide along with a 100 bp reference ladder.

Study 1 — Comparison of Run Time and Yield Using Lambda Phage DNA

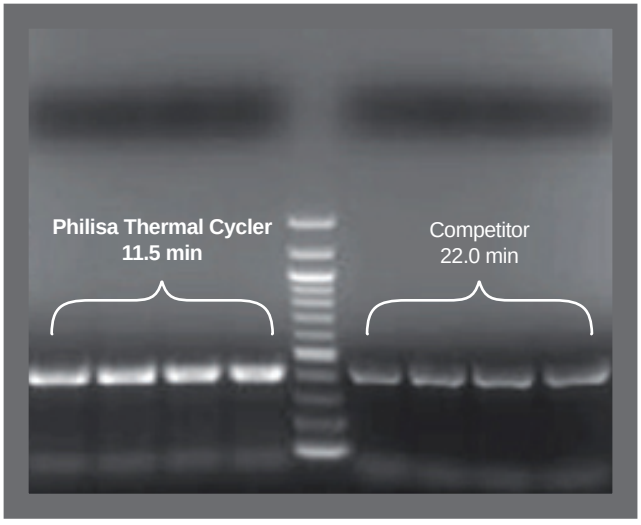
Objective:
Compare run time and yield of a 402 bp amplicon from lambda phage DNA using the Philisa Thermal Cycler with KOD Hot-Start, and the competitor instrument with Phusion Hot-Start II.

Optimized reaction mix & protocol:

	Philisa	Competitor
Reaction Mix:		
Mg^{++}	4 mM $MgSO_4$	3 mM $MgCl_2$
dNTPs (each)	0.2 mM	0.2 mM
Primers	0.5 µM each	0.5 µM each
Buffer	1 x KOD	1 x Phusion HF
Polymerase	0.4U KOD Hot-Start	0.4U Phusion Hot-Start II
Lambda Phage DNA	16 pg	16 pg
Total Volume	20 µl	20 µl
Protocol:		
Hot-Start	95°C for 30 s	98°C for 30 s
30 Cycles of	95°C for 5 s 60°C for 5 s 72°C for 5 s	98°C for 3 s 60°C for 3 s 72°C for 3 s
Final Extension	72°C for 10 s	72°C for 10 s

Results:
Results demonstrate that the Philisa Thermal Cycler obtained higher yield in under 12 minutes while the competitor's thermal cycler required 22 minutes.

Yield and Amplification Time Comparison



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Study 2 Comparison of Run Time and Yield Using Human Genomic DNA

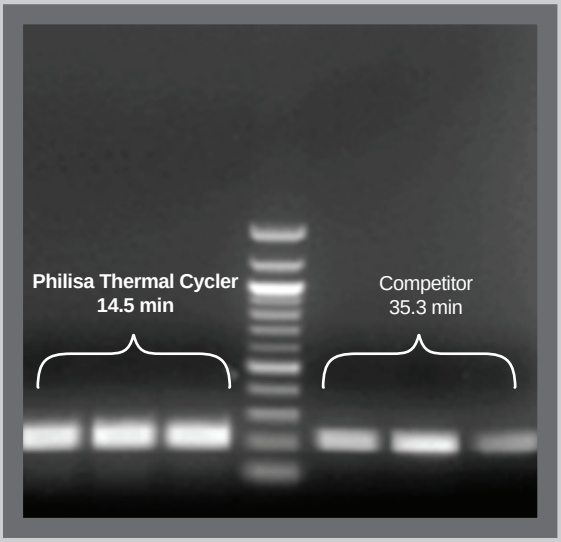
Objective:
Compare run time and yield of a 213 bp amplicon from human genomic DNA using the Philisa Thermal Cycler with KOD Hot-Start, and the competitor's instrument with Phusion Hot-Start II.

Optimized reaction mix & protocol:

	Philisa	Competitor
Reaction Mix:		
Mg^{++}	3 mM $MgSO_4$	1.5 mM $MgCl_2$
dNTPs (each)	0.2 mM	0.2 mM
Primers	0.4 µM each	0.4 µM each
Buffer	1 x KOD	1 x Phusion HF
Polymerase	0.4U KOD Hot-Start	0.4U Phusion Hot-Start II
Human Genomic DNA	80 ng	80 ng
Total Volume	20 µl	20 µl
Protocol:		
Hot-Start	95°C for 30 s	98°C for 30 s
40 Cycles of	95°C for 6 s 55°C for 5 s 74°C for 2.5 s	98°C for 5 s 55°C for 5 s 72°C for 5 s
Final Extension	74°C for 5 s	72°C for 10 s

Results:
Results demonstrate that the Philisa Thermal Cycler obtained consistently high yield in all three samples with a run time under 15 minutes. The competitor's thermal cycler required a run time of 35 minutes and showed some inconsistency in well-to-well yield.

Yield and Amplification Time Comparison



Study 3 Comparison of Run Time and Yield Using Platinum® Taq

Objective:
Compare run time and yield of a 402 bp amplicon from lambda phage DNA using a fixed Platinum Taq reaction mixture and protocol for both the Philisa Thermal Cycler and the competitor instrument.

Identical reaction mix and protocol for both Philisa Thermal Cycler and the competitive thermal cycler (all samples aliquoted from a single master mix):

Reaction Mix:	
$MgCl_2$	3.5 mM
dNTPs (each)	0.2 mM
Primers	0.5 µM each
Buffer	1 x Taq
Polymerase	0.75 U Platinum Taq
Lambda Phage DNA	16 pg
Total Volume	20 µl
Protocol:	
Hot-Start	95°C for 60 s
30 Cycles of	95°C for 5 s 60°C for 5 s 72°C for 7 s
Final Extension	72°C for 10 s

Results:
Results demonstrate that the Philisa Thermal Cycler and the competitor's thermal cycler produced equivalent yields when the same enzyme and reaction mixture was used. The Philisa Thermal Cycler completed the run in approximately half the time of the competitor's instrument.

Yield and Amplification Time Comparison