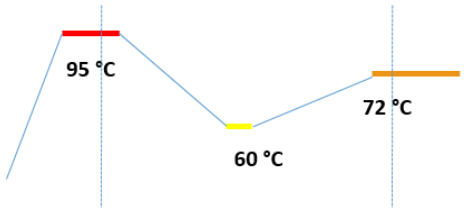
Fast PCR protocol:

All Apex Taq DNA Polymerases and Taq master mixes

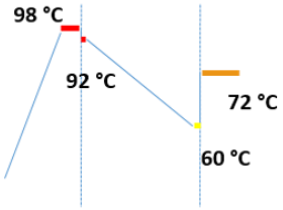
90 min



3-step Standard PCR protocol

SAVE 1 HOUR

31 min



2-step Fast PCR protocol

PCR program for 3-step Standard PCR – 90 min total

Cycler step	Temperature	Duration	Cycles
Initial heating	95 °C	3 min.	1
Denaturation	95 °C	30 sec.	30
Annealing*	60 °C	30 sec.	
Extension	72 °C	30 sec.	
Final extension	72 °C	5 min.	1

** the annealing temperature depends on the primer set*

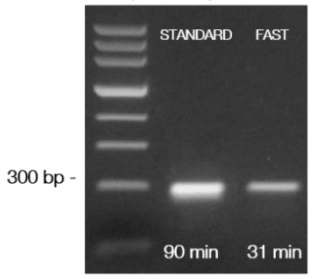
Save time - just by changing PCR cycler settings!

PCR program for 2-step Fast PCR – 31 min total

Cycler step	Temperature	Duration	Cycles
Initial heating	98 °C	40 sec.	1
Denaturation	92 °C	2 sec.	30
Extension*	60 °C	2 sec.	
Final extension	72 °C	20 sec.	1

** the extension temperature depends on the primer set. For fast PCR choose highest possible Tm values*

λ DNA
Taq DNA Polymerase



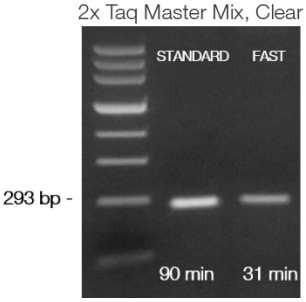
Amplification of λ DNA using Taq DNA Polymerase – Experimental setup

Reaction mix*	
Ammonium buffer	1x
dNTP mix	0,2 mM each
$MgCl_2$	1,5 mM
Primers	0,2 µM
λ DNA	1 ng
Taq DNA polymerase	0.5 – 1U

** H_2O up to a total volume of 25 µl*

ID	Primer sequence (5'-3')	Length
LAM300-F	ACGGATAGAAACTGCCGGTCAGGACA	300 bp
LAM300-R	GTTATCGAAATCAGCCACAGGGC	

Genomic DNA
2x Taq Master Mix, Clear



Amplification of gDNA using 2x Taq OptiMix CLEAR - Experimental setup

Reaction mix*	
Taq OptiMix	1x
Primers	0,2 µM
gDNA	20 ng

** H_2O up to a total volume of 25 µl*

ID	Primer sequence (5'-3')	Length
ENG9-F	AATGGCTGTGACTTGGGACCCCTG	293 bp
ENG9-R	GCACCAACCAGGCTGGTCCTGATA	

Please require our Application note for Fast PCR: "**Additional reduction of PCR run time – three approaches**"

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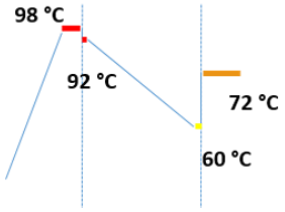
Fast PCR protocol:

Additional reduction of PCR run time – three approaches

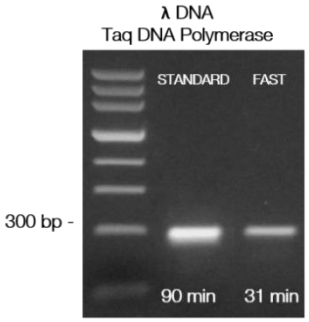


2-step Fast PCR protocol

PCR program for 2-step Fast PCR – 31min total

Cycler step	Temperature	Duration	Cycles
Initial heating	98 °C	40 sec.	1
Denaturation	92 °C	2 sec.	30
Extension*	60 °C	2 sec.	
Final extension	72 °C	20 sec.	1

** the extension temperature depends on the primer set. For fast PCR choose highest possible Tm values*

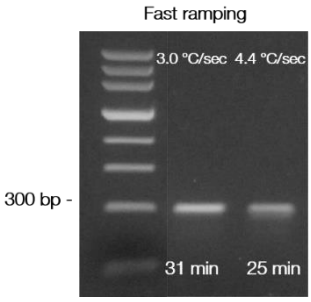


Experimental setup – Amplification of λ DNA using Taq DNA Polymerase

Reaction mix*	
Ammonium buffer	1x
dNTP mix	0,2 mM each
$MgCl_2$	1,5 mM
Primers	0,2 µM
λ DNA	1 ng
Taq DNA polymerase	0.5 – 1U

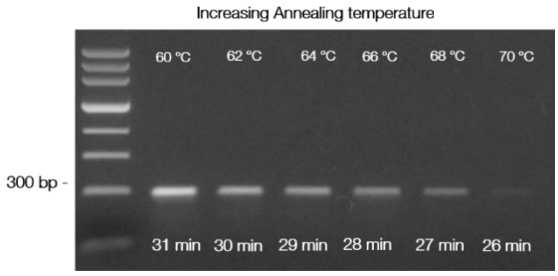
** H_2O up to a total volume of 25 µl*

ID	Primer sequence (5'-3')	Length
LAM300-F	ACGGATAGAAACTGCCGGTCAGGACA	300 bp
LAM300-R	GTTATCGAAATCAGCCACAGGGC	



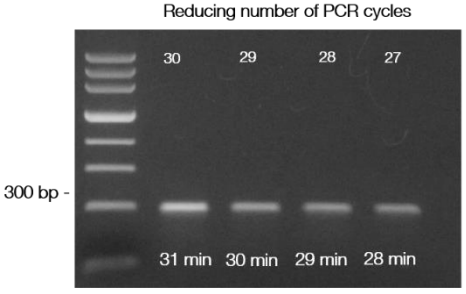
1. Applying fast ramping technology

Applying the Fast PCR protocol on a fast ramping PCR instrument with a ramping time of 4.4 °C/sec, results in reduction of 6 minutes, compared when the PCR protocol was applied on a standard PCR instrument with a ramping time of 3.0 °C/sec



2. Optimization of annealing temperature

Reducing the temperature difference between the annealing steps and denaturation steps results in shortened ramping time. By increasing annealing temperature in incidents of 2 °C starting at 60 °C, the PCR run time of the Fast PCR protocol was shortened by up to 5 minutes. PCR products with acceptable yield are obtained at up to 66 °C.



3. Reduce the number of PCR cycles

Fast PCR protocol gives fine amplification results using 30, 29, 28 and 27 cycles. Using 27 cycles instead of 30, reduces PCR run time by 3 minutes, ending up with a run time of 28 min.

Tips for successful Fast PCR setup

- Without the need for fast ramping PCR cyclers and specialized DNA Polymerases

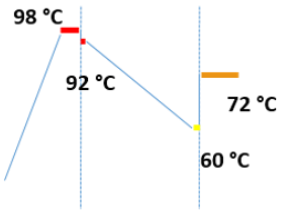
- Start up with our recommended 2-step Fast PCR protocol: 98 °C, 40 sec; then 30 cycles of 92 °C, 2 sec. and 60 °C, 2 sec. and then 72 °C, 20 sec.
- Optimize the Annealing/Extension temperature by performing a temperature gradient.
- Primers must have the highest possible T_m values in the range 58 – 72 °C.*
- At low starting target number, it is recommended to perform 5 – 10 additional cycles.
- If PCR product/amplicon is > 300 bp it may be necessary to increase the hold time of the Annealing/Extension step.
- Sufficient denaturation of for example GC rich templates and other difficult DNA templates is crucial – increase to 95 °C and increase hold time.
- If using TEMPase Hot Start DNA Polymerase the initial denaturation should be 15 min.
- Primer and template quality is important for successful fast PCR protocols.

* *Primers with highest T_m values supports highest annealing temperature → leading to reduced PCR run time, due to shortened ramping time between the Annealing/Extension and Denaturation steps.*

Recommended protocol for 2-step Fast PCR

– Valid for all variants of VWR Taq DNA Polymerases and Taq Master mixes

31 min



2-step Fast PCR protocol

PCR program for 2-step Fast PCR – 31min total

Cycler step	Temperature	Duration	Cycles
Initial heating	98 °C	40 sec.	1
Denaturation	92 °C	2 sec.	30
Extension*	60 °C	2 sec.	
Final extension	72 °C	20 sec.	1

* the extension temperature depends on the primer set. For fast PCR choose highest possible Tm values.

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