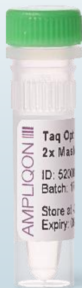
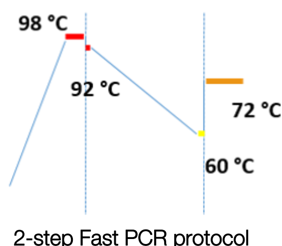


AMPLIQON
PCR ENZYMES & REAGENTS

Fast PCR protocol:

Additional reduction of PCR run time – three approaches



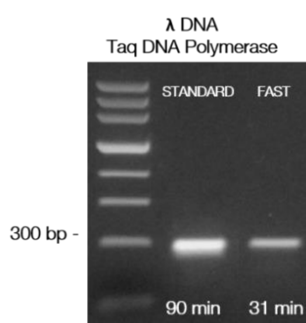
2-step Fast PCR protocol

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.	
Extension*	60 °C	2 sec.	30
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



Experimental setup – Amplification of λ DNA using Taq DNA Polymerase

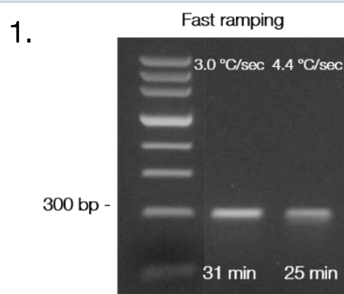
Reaction mix*	
Ammonium buffer	1x
dNTP mix	0,2 mM each
$MgCl_2$	1,5 mM
Primers	0,2 µM
λ DNA	1 ng
Taq DNA polymerase	0.5 – 1U

* 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.

Fast ramping

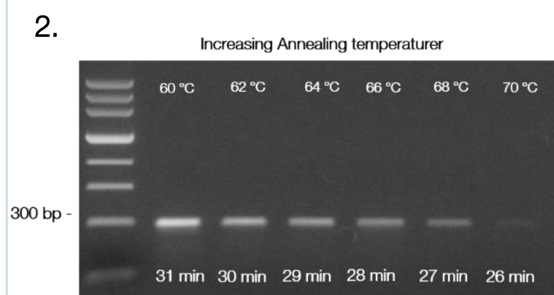


1. Applying fast ramping technology

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.

Increasing Annealing temperaturer

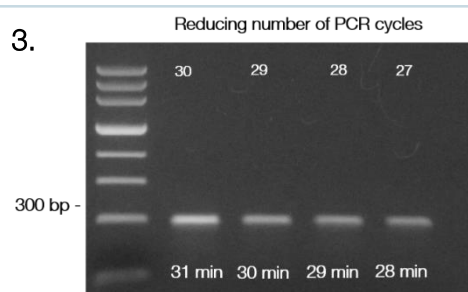


2. Optimization of annealing temperature

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.

Reducing number of PCR cycles



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.

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