GRS Taq 2x Mastermix

#GE02.0100

(for research only)

Product: GRS Taq DNA polymerase is a recombinant thermostable enzyme (94kDa monomer) with identical characteristics as native Taq regarding activity, specificity, thermostability, and performance in PCR. GRS Taq possesses 5´→3´ polymerase activity as well as weak 5´→3´ exonuclease activity. The enzyme has no 3´→5´ exonuclease activity (thus no proofreading). GRS Taq is suitable for amplification of targets up to 5kb and is capable of incorporating modified nucleotides (e.g. fluorescent labeled nucleotides). GRS Taq produces 3´ A-overhangs allowing TA-cloning.

Source: Purified from *E.coli* harbouring the DNA polymerase gene from *Thermus aquaticus* YT-1.

Quantity: GRS Taq DNA polymerase is supplied as a convenient 2x mastermix containing all required components except specific primers. GE02.0100 comprises of 1,25ml 2x mastermix, which is enough for 100 reactions of 25µl (or 125 reactions of 20µl). Final concentration of $MgCl_2$ will be 2mM.

Applications: Routine PCR

Properties:
Amplicon size: up to 5kb.
Extension Rate 1kb/min
Hotstart: No
A-overhang: Yes

QC: Functionally tested in PCR. Bacterial DNA free (tested by PCR). Absence of endonucleases, exonucleases, and ribonucleases was confirmed by appropriate assays.

Storage: -20ºC for at least 1 year.

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Basic Protocol

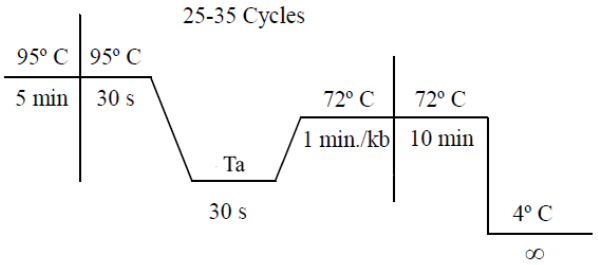
Optimal PCR cycling conditions (incubation times and temperatures) depend on DNA target (GC-content, size, quantity, purity, etc) and specific primers and need to be determined case by case. We suggest to start with the following basic protocol and optimize annealing temperature, incubation times and cycling number.

Mix for each PCR reaction, starting with the greatest volume (usually water) and ending with Taq:

Component	Volume	Final Conc.
GRS Taq Mastermix (2x)	12.5 µl	1X
Forward primer (5 pmol/ µl)	1.5 µl	0.3 µM
Reverse primer (5 pmol/ µl)	1.5 µl	0.3 µM
Template DNA	0.25 - 10 µl	2-300 ng*
PCR –grade water	up to 25 µl	

*) In case of plasmid DNA use 1-3 ng, in case of gDNA add up to 100-300ng
For smaller/larger reaction volumes, scale it down/up proportionally.

Set-up PCR amplification as follows:



After an initial cycle of 5 min at 95ºC (complete denaturation of template DNA including removal of all secondary DNA structures such as hairpins), cycle 25-35 times for 30 seconds at 95ºC, 30 seconds at Ta and 1 min (per kb of target DNA) at 72ºC for extension. Set the annealing temperature (Ta) as the melting temperature (Tm) of the primer with the lowest Tm. After amplification, include a final extension step of 10 min at 72ºC to ensure that all amplicons are fully extended and include 3´-A-overhang. Analyze PCR products by DNA Agarose gel electrophoresis.

Optimization

Annealing Temperature (Ta)
Optimizing the annealing temperature is crucial, as a too low temperature might result in non-specific amplification whereas a too high temperature results in no amplification. The melting temperature (Tm) is defined as the temperature in which 50% of the primer and its complementary sequence of the target DNA are present as duplex DNA. By increasing the temperature above the melting temperature, this percentage decreases, however, primers will still anneal (up to a certain point) and initiate extension. PCR can therefore be performed at temperatures of several degrees higher than Tm and it is therefore recommended to optimize the Ta by performing a temperature gradient.

$MgCl_2$
GRS Taq Mastermix contains 4mM $MgCl_2$ (final concentration is 2mM). If desired one could optimize the final $MgCl_2$-concentration (normally between 1,5 and 3mM) in steps of 0.25mM by adding the required amount of 25mM MgCl2 (not provided).

Incubation times
Denaturation and annealing steps may require less time depending on the thermocycler apparatus, reaction volume and PCR tube (varies with the efficiency of heat-transfer). It might be worthwhile to optimize (reduce) times to as low as 10-15 seconds for both denaturation and annealing steps, which will greatly reduce overall PCR time.

GC-rich targets
If having troubles amplifying GC-rich targets, one could try adding 5-10% DMSO, up to 10% glycerol, or 1-2% formamide (or any combination of these solvents) to the reaction mixture. Note that the optimal Ta will decrease approximately 5ºC in 10% DMSO.