PCR Mix

PCR Master Mix uses the latest developments in polymerase technology and buffer chemistry to enhance PCR speed, yield and specificity. The enzyme and buffer system allow for superior PCR performance on complex templates. Reactions can be directly loaded onto agarose gels without additional loading buffer.

PCR Master Mix is a robust mix for all your everyday PCR applications including genotyping, screening and library construction.

PCR Master Mix can perform consistently well on a broad range of templates (including both GC and AT rich).

PCR Master Mix has an error rate of approximately 1 error per 2.0×10^5 nucleotides incorporated. PCR products generated with 2x PCR Master Mix are A-tailed and may be cloned into TA cloning vectors.

High-throughput screening has resulted in a buffer system that allows efficient amplification from GC-rich and AT-rich templates, under fast and standard cycling conditions.

Components

2x PCR Master Mix	1 x 1000µl

To be provided by user: 0.2ml reaction tubes, PCR grade dH_2O, oligonucleotide primers, and DNA templates.

For genotyping of worms, use with the NemaMetrix Worm Lysis Kit.

Shipping and storage

On arrival the kit should be stored at -20°C. Avoid prolonged exposure to light. If stored correctly the kit will retain full activity for 12 months. The kit can be stored at 4°C for 1 month. The kit can go through 30 freeze/thaw cycles with no loss of activity.

Limitations of product use

The product may be used only for in vitro research purposes.

Important considerations

PCR Master Mix: The 2x mix contains HS Taq DNA Polymerase, 6mM $MgCl_2$, 2mM dNTPs, enhancers, stabilizers and a red dye for tracking during agarose electrophoresis. It is not recommended to add further PCR enhancers or $MgCl_2$ to the reaction. The buffer composition has been optimised to maximise PCR success rates.

Template: For eukaryotic DNA use between 5ng and 500ng per reaction, for cDNA use below 100ng per reaction.

Primers: Primers should have a predicted melting temperature of around 60°C, using default Primer 3 settings (http://frodo.wi.mit.edu/primer3/). The final primer concentration in the reaction should be between 0.2µM and 0.6µM.

Annealing: We recommend performing a temperature gradient to experimentally determine the optimal annealing temperature. Alternatively, we recommend a 55°C annealing temperature then increase in 2°C increments if non-specific products are present.

Extension: Optimal extension is achieved at 72°C. The optimal extension time is dependent on amplicon length and complexity of template. 15 seconds per kilobase (kb) is recommended for amplification from eukaryotic DNA for amplicons between 1kb and 6kb. For shorter amplicons a 1 second extension is sufficient.

Agarose gel electrophoresis dye migration: The 2x mix contains a red dye for tracking during agarose gel electrophoresis. In a 2% agarose TAE gel the dye migrates at a rate equivalent to 350bp of DNA. In a 1% agarose TAE gel the dye migration rate is equivalent to 600bp of DNA.

Protocol

Reaction Setup - Set up your PCR mix as indicated in the table below.

Reagent	Amount	Final Concentration
2x PCR Master Mix	10ul	1x
Forward primer (10µM)	0.8µl	400nM
Reverse primer (10µM)	0.8µl	400nM
Template DNA	2ul of diluted sample from Worm Lysis Kit (or other amount depending on template source)	May vary
PCR grade water	Up to 20ul final volume	

Cycling conditions.

Cycles	Temperature	Time	Notes
1	95°C	1 min	Initial denaturation
40	95°C 55°C to 65°C 72°C	15 seconds 15 seconds 1 to 90 seconds	Denaturation Anneal Extension (15 sec/kb)