GENAXXON bioscience

SuperHot Mastermix (2X)

with chemically modified Taq DNA-Polymerase

PCR Protocol Part

Protocol using SuperHot Mastermix (2X)

This protocol serves as a guideline for PCR amplification. Optimal reaction conditions such as incubation times, temperatures, and amount of template DNA may vary and must be determined individually.

Important notes before getting started

- SuperHot Mastermix provides a final concentration of 1.5mM MgCl2 which will produce satisfactory results in most cases. However, if a higher Mg2+ concentration is required, the Genaxxon bioscience SuperHot Mastermix is shipped with additional 25mM MgCl2.
- Set up all reaction mixtures in an area separate from that used for DNA preparation or PCR product analysis.
- Use disposable tips containing hydrophobic filters to minimize cross-contamination.

Procedure

1. **Thaw primer solutions**
   Keep on ice after complete thawing, and mix well before use.

   Optional: Prepare a primer mix of an appropriate concentration using sterile, bidest water. This is recommended if several amplification reactions using the same primer pair are to be performed. The final volume of diluted primer mix plus the template DNA, added at step 4, should not exceed 12.5µL per reaction.
2. **Thaw SuperHot Mastermix (2X)** at RT or on ice.
   Keep the solutions on ice after complete thawing. It is very important to mix the SuperHot Mastermix well before use to avoid local differences in salt concentration. The Genaxxon bioscience SuperHot Mastermix is provided as a 2X concentrated (i.e. a 12.5µL volume of SuperHot Mastermix is required for PCR reactions with a final volume of 25µL). For volumes smaller than 50µL, the 1:1 ratio of SuperHot Mastermix to diluted primer mix, template DNA and water should be maintained. A negative control (PCR without template DNA) should be included in every experiment. It is recommended that the PCR tubes are kept on ice until they are placed in the thermal cycler.
3. **Distribute the appropriate volume of diluted primer mix into the PCR tubes containing the SuperHot Mastermix.**
4. **Add template DNA** (<1µg/reaction) to the individual PCR tubes.
   For RT-PCR, add an aliquot from the reverse transcriptase reaction. The volume added should not exceed 10% of final PCR volume.

Table 1: PCR reaction components using SuperHot Mastermix (2X) (**25µL PCR reaction**)

Components	Quantities
SuperHot Mastermix	12.5µL
Diluted primer mix	
primer 1:	variable volume: 0.1 - 0.5µM (5 – 25 pmol absolute)
primer 2:	variable volume: 0.1 - 0.5µM (5 – 25 pmol absolute)
Template DNA	variable volume: < 5ng plasmid DNA variable volume: < 250ng genomic DNA
sterile, bidestilled water	up to 25µL

5. **When using a thermal cycler with a heated lid**, do not use mineral oil. Proceed directly to step 6. Otherwise, overlay with approximately 50µL mineral oil.
6. **Program the thermal cycler** according to the manufacturer's instructions.
   A typical PCR cycling program is outlined in Table 2. For maximum yield and specificity, temperatures and cycling times should be optimized for each new target or primer pair.
7. **Place PCR tubes in the thermal cycler and start program.**

Table 2: PCR conditions (**Thermal cycler**)

Step	time	temperature	comments
Initial denaturation:	15 min.	95°C	
3-step cycling			
Denaturation:	0.5 - 1 min.	95°C	
Annealing:	0.5 - 1 min.	50 - 68°C	Approximately 5°C below Tm of primers.
Extension:	0.5 - 1 min.	72°C	For PCR products longer than 1kb, use an extension time of approximately 1min./kb DNA.
Number of Cycles	25 - 35		
Final extension	5 min.	72°C	

Note: After amplification, samples can be stored at 4°C overnight, or -20°C for long term storage.

Table 4: Recommendations for Standard PCR-Primers

Length:	18-30 nucleotides
GC-Content:	40-60%
Tm:	Design primer pairs with similar Tm values.
	Optimal annealing temperature may be above OR below the estimated Tm. As a starting point, use an annealing temperature of 3°C to 5°C below Tm of the primer with the lower Tm-Value.
Sequence:	Avoid complementarities of two or more bases at the 3' ends of primer pairs.
	Avoid runs of 3 or more Gs or Cs at the 3' end.
	Avoid a 3'-end T.
	Avoid complementary sequences within primer and between primer pairs.

Table 5: Migration Chart of some Gel Tracking Dyes

Dye in agarose gel	0.5%-1.5%	2.0%-3.0%	CAS-number	Cat-No. Genaxxon
Xylene cyanol	10000bp - 4000bp	750bp - 200bp	2650-17-1	M3312
Cresol Red	2000bp - 1000bp	200bp - 125bp	62625-29-0	M3371
Bromophenol blue	500bp - 400bp	150bp - 50bp	115-39-9	M3092
Orange G	<100bp	<20bp	1936-15-8	M3180
Tartrazine	<20bp	<20bp	1934-21-0	