

## PCR Protocol Part

### Protocol using SuperHot *Taq* DNA Polymerase

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

- 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.

#### Points to do before starting

- If required, prepare a dNTP-mix containing 10mM of each dNTP (or ready-to-use product M3016 from Genaxxon bioscience). Store this mix in aliquots at -20° C.

#### Procedure

1. Thaw 10X buffer, dNTPs or dNTP-mix, primer solutions, and 25mM MgCl<sub>2</sub> (if required) at RT or on ice. Keep solutions on ice after complete thawing, and mix well before use to avoid local differences in salt concentrations.

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. Prepare a master mix according to Table 1

A master mix typically contains all of the components needed for PCR except the template DNA. Prepare a volume of master mix 10% greater than that required for the total number of PCR assays to be performed. A negative control (without template DNA) should always be included in every experiment. The optimal MgCl<sub>2</sub> concentration should be determined empirically but in most cases a concentration of 2.5mM, as provided in 10X E-buffer complete, will produce satisfactory results. Keep the master mix on ice.

**Note:** The Mg<sup>2+</sup> concentration provided by the supplied PCR-buffer will produce satisfactory results in most cases. However, in some cases, reactions may be improved by increasing the final Mg<sup>2+</sup> concentration according to Table 2.

**Note:** A negative control (PCR without template DNA) should be included in every experiment.

**Note:** It is recommended that the PCR tubes are kept on ice until they are placed in the thermal cycler.

Table 1: PCR reaction components (50µL PCR reaction)

Components	Quantities
Template DNA	< 10ng plasmid DNA < 500ng genomic DNA
Nucleotides	1µL (10mM) each dATP, dCTP, dGTP, dTTP 1µL of 10mM ready-to-use dNTP mix.
10X amplification buffer	5µL
25mM MgCl <sub>2</sub> *	5µL (if no complete buffer is used)
primer 1:	0.1 - 0.5µM (5 - 25 pmol absolute)
primer 2:	0.1 - 0.5µM (5 - 25 pmol absolute)
sterile, bidistilled water	up to 50µL
SuperHotStart <i>Taq</i> DNA Polymerase	0.25 - 0.5µL (1.25 - 2.5 units)

\* if Buffer E complete is used, normally no MgCl<sub>2</sub> has to be added

Table 2: Final MgCl<sub>2</sub> concentration in a 50µL reaction

Final MgCl <sub>2</sub> conc. in reaction (mM) complete buffer E	2.5	3.0	3.5	4.0	4.5	5.0	5.5
Additional volume of 25mM MgCl <sub>2</sub> per 50µL reaction (µL)	0	1	2	3	4	5	6
Final MgCl <sub>2</sub> conc. in reaction (mM) incomplete buffer E	1.5	2.0	2.5	3.0	3.5	4.0	4.5
Additional volume of 25mM MgCl <sub>2</sub> per 50µL reaction (µL)	3	4	5	6	7	8	9

**Note:** The optimal Mg<sup>2+</sup> concentration should be determined empirically but in most cases a concentration of 2.5mM, as provided in the 10X complete buffer E will produce satisfactory results.

3. **Mix the master mix thoroughly**, and dispense appropriate volumes into PCR tubes.  
 Mix gently, for example, by pipetting the master mix up and down. It is recommended that PCR tubes are kept on ice before placing in the thermal cycler.
4. **Add template DNA** (<1µg/reaction) to the individual tubes containing the master mix.  
 For RT-PCR, add an aliquot from the reverse transcriptase reaction. The volume added should not exceed 10% of final PCR volume.
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 - 100µL mineral oil.
6. **Program the thermal cycler** according to the manufacturer's instructions.  
 A typical PCR cycling program is outlined in Table 3. 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 3: PCR conditions (Thermal cycler)**

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

\* It is very important to have at least a 10 minutes activation step at 95 °C. Otherwise the polymerase will not work fully! We recommend to program a 15 minute activation step to get full recovery of the polymerase activity.

**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**

<b>Length:</b>	18-30 nucleotides
<b>GC-Content:</b>	40-60%
<b>Tm:</b>	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.
<b>Sequence:</b>	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	1000bp - 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	