

PCR Protocol Part

Protocol using Taq-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.

Things 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. For convenience, the Mastermix (M3014) already contains a premixed solution containing HotStart Taq-Polymerase, PCR buffer, and dNTPs.

Procedure

1. **Thaw 10X buffer, dNTPs or dNTP-mix, primer solutions, and 25mM MgCl₂** (if required) at RT or on ice. Keep the solutions on ice after complete thawing. Mix well before use to avoid localized differences in salt concentration.
2. **Prepare a master mix according to Table 1.**
 The 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 MgCl₂ 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 Mg²⁺ 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 Mg²⁺ concentration according to Table 2.

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 ₂ * | 3µL (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, bidestilled water | up to 50µL |
| Taq-Polymerase | 0.25 - 0.5µL (1.25 - 2.5 units) |

* if Buffer S complete is used, normally no MgCl₂ has to be added

Table 2: Final MgCl₂ concentration in a 50µL reaction

| | | | | | | | |
|------------------------------------------------------------------------------|-----|-----|-----|-----|-----|-----|-----|
| Final MgCl ₂ conc. in reaction (mM) complete buffer S | 1.5 | 2.0 | 2.5 | 3.0 | 3.5 | 4.0 | 4.5 |
| Additional volume of 25mM MgCl ₂ per 50µL reaction (µL) | 0 | 1 | 2 | 3 | 4 | 5 | 6 |
| Final MgCl ₂ conc. in reaction (mM) incomplete buffer S | 1.5 | 2.0 | 2.5 | 3.0 | 3.5 | 4.0 | 4.5 |
| Additional volume of 25mM MgCl ₂ per 50µL reaction (µL) | 3 | 4 | 5 | 6 | 7 | 8 | 9 |

Note: The optimal Mg²⁺ concentration should be determined empirically but in most cases a concentration of 1.5mM, as provided in the 10X complete buffer S 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.

Table 3: PCR conditions (Thermal cycler)

| Step | time | temperature | comments |
|-----------------------|--------------|-------------|-----------------------------------------------------------------------------------------------------------------------------------------------------|
| Initial denaturation: | 3 min. | 94°C | |
| 3-step cycling | | | |
| Denaturation: | 0.5 - 1 min. | 94°C | Approximately 5°C* below lower T _m 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 | 10 min. | 72°C | |

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

7. **For simplified hot start**, proceed as described in step 8. Otherwise, place the PCR tube in the thermal cycler and start program.
8. **Simplified hot start:** Start PCR program. Once the thermal cycler has reached 94°C, place PCR tubes in the thermal cycler. In many cases, this simplified hot start improves specificity of PCR. For highly specific and convenient hot start PCR please use the Genaxxon bioscience HotStart (M3006), SuperHot Taq-Polymerase (M3307) or the HotStart Mastermix (M3007).

Table 4: Recommendations for Standard PCR-Primers

| | |
|-----------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Length: | 18-30 nucleotides |
| GC-Content: | 40-60% |
| T_m: | Design primer pairs with similar T _m values. Optimal annealing temperature may be above OR below the estimated T _m . As a starting point, use an annealing temperature of 3°C to 5°C below T _m of the primer with the lower T _m -Value. |
| Sequence: | Avoid complementarity 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 | |