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PCR Protocol Part

SNP PolTaq DNA Polymerase 2x Master Mix PCR Protocol

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.
- Spin down and mix all solutions carefully before use.
- SNP PolTaq DNA Polymerase 2X PCR Master Mix buffer is optimized for short amplicon length (about 60 – 200bp), but also longer amplicons are possible. The addition of additional MgCl2 (0.5 – 1.5mM) might be needed in case of longer amplicons (>500bp).
- The SNP PolTaq DNA Polymerase 2X PCR Master Mix can also be used for realtime cycling when adding a suitable realtime PCR fluorescence dye or probe).
- Use disposable tips containing hydrophobic filters to minimize cross-contamination.

Procedure

1. **Thaw SNP PolTaq DNA Polymerase 2X PCR Mastermix,** (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. A negative control (without template DNA) should always be included in every experiment. 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 (amplicon >500bp), reactions may be improved by increasing the final Mg2+ concentration according to Table 2.

Table 1: PCR reaction components (**25µL PCR reaction**)

Components	Components	Final concentration
SNP PolTaq 2X PCR Master Mix	12.5 µL	1X
Primer forward (10µM)*:	0.5µL	0.2 µM (0.05 – 1.0µM)
Primer reverse (10µM):	0.5µL	0.2 µM (0.05 – 1.0µM)
Template DNA	x µL	<10ng plasmid DNA or <500ng genomic DNA
Nuclease-free water	x µL	up to 50µL total volume
25mM MgCl2**	x µL	only if necessary

* Primers should ideally have a GC content of 40-60%
** should only be necessary for amplicon lengths of >500bp

Table 2: Final MgCl2 concentration in a **25µL reaction**

Additional MgCl2 conc. in reaction (mM)	0.5	1.0	1.5	2.0
Additional volume of 25mM MgCl2 per 50µL reaction (µL)	0.5	1	1.5	2

Note: The optimal Mg2+ concentration should be determined empirically but in most cases the supplied 10X buffer 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.

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Table 3: PCR conditions (**Thermal cycler**)

Step	time	temperature	comments
Initial denaturation:	2 min.	95°C	
3-step cycling			
Denaturation:	15 sec.	95°C	
Annealing:	10 sec.	54 - 72°C	approximately 3°C to 5°C* below lower Tm of primers.
Extension:	30 sec./250bp	72°C	approx. 0.5kbp per minute extension rate
Number of Cycles	25 - 40		
Hold		<10°C	

Note: After amplification, samples can be stored at +2°C to +8°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	

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