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PCR Protocol Part

SNP PolTaq DNA Polymerase 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 10X 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).
- SNP PolTaq DNA polymerase can also be used for realtime cycling when adding a suitable realtime PCR fluorescence dye.
- SNP PolTaq DNA polymerase can be used together with probe-based assays.
- 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 your convenience, the SNP Pol 2X Master Mix (M3061) already contains a premixed solution containing SNP Pol DNA Polymerase, 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.
   Keep the master mix on ice.

Table 1: Recommendations for PCR and qPCR / Reaction Setup (50µL PCR reaction)

Components	Volume	Final concentration
SNP PolTaq DNA Polymerase	0.5µL	2.5 units/50µL reaction
Nucleotides	1µL	of a 10mM ready-to-use dNTP mix (M3016).
10X amplification buffer	5µL	1X buffer
primer forward (10µM)*:	1µL	0.2 µM (0.05 – 1.0µM)
primer reverse (10µM):*	1µL	0.2 µM (0.05 – 1.0µM)
Probe**	x µL	0.2µM (0.05 – 0.3µM)
Template DNA / sample extract	y µL	<10ng plasmid DNA or <500ng genomic DNA
sterile, bidestilled water	z µL	up to 50µL total volume

Keep all components on ice.
Spin down and mix all solutions carefully before use.
* Primers should ideally have a GC content of 40-60%. For optimal results we recommend amplicon lengths in the range of 60 to 300bp.
** The necessary concentration of probe depends very much on the probe sequence and the kind of probe. Please test for optimum!

This product is compatible for the use with hydrolysis probe systems, e.g. TaqMan probes and qPCR cycler not requiring a passive reference dye!

Table 2: Final MgCl2 concentration in a **50µL reaction**

Additional MgCl2 conc. in reaction (mM)	0.5	1.0	1.5	2.0
Additional volume (µL) of 25mM MgCl2 per 50µL reaction.	1	2	3	4

Note: The Mg2+ concentration provided by the supplied SNP PolTaq PCR-buffer will produce satisfactory results in most cases. However, in some cases, e.g. amplicon size >500bp reactions may be improved by increasing the final Mg2+ concentration according to Table 2.

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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** 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. **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:	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.

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	