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## Standard DNA amplification assay

### Suggested Protocol using RedTaq DNA Polymerase

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

Pipette the following into a PCR tube, mix and make up to a final volume of 50  $\mu$ L. We recommend dispensing all reagents on ice, adding the enzyme last. It is important to vortex all buffers and MgCl<sub>2</sub> solutions before use to remove any gradients that may result from repeated freeze/thaw steps. If you do have already your own PCR-Protocol established, please use your existing pipetting scheme and Thermocycler protocol.

Table 1: PCR reaction components

Components	Quantities
Template DNA	1 ng - 10 ng plasmid DNA or 5 ng - 500 ng genomic DNA
Nucleotides	1 $\mu$ L (10 mM) each of dATP, dCTP, dGTP, dTTP
10X amplification buffer	5 $\mu$ L
25 mM MgCl <sub>2</sub>	1.5 $\mu$ L (if no complete buffer is used)
primer 1:	4-7 $\mu$ L of 3 $\mu$ M solution (10 - 20 pmol absolute)
primer 2:	4-7 $\mu$ L of 3 $\mu$ M solution (10 - 20 pmol absolute)
sterile, bidestilled water	up to 50 $\mu$ L
Taq-Polymerase	1.0 - 4.0 $\mu$ L (1 - 4 units)

Table 2: MgCl<sub>2</sub> concentration in a 50  $\mu$ L reaction (complete buffer)

Final MgCl <sub>2</sub> conc. in reaction (mM)	1.5	2.0	2.5	3.0	3.5	4.0	4.5
Additional volume of 25 mM MgCl <sub>2</sub> per reaction ( $\mu$ L)	0	1	2	3	4	5	6

Table 3: MgCl<sub>2</sub> concentration in a 50  $\mu$ L reaction (incomplete buffer)

Final MgCl <sub>2</sub> conc. in reaction (mM)	1.5	2.0	2.5	3.0	3.5	4.0	4.5
Additional volume of 25 mM MgCl <sub>2</sub> per reaction ( $\mu$ L)	3	4	5	6	7	8	9

### Notes:

- Drops should be collected by centrifugation and 50  $\mu$ L of mineral oil should be layered upon the reaction mixture.
- Program the thermal cycler according to the manufacturer's instructions. Each programme should start with an initial heat incubation step at 94°C for 5 minutes!
- Recommended elongation time is 1 minute per 1kb of target!
- For maximum yield and specificity, temperatures (annealing) and cycling times should be optimised for each new template target or primer pair.