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For a complete description of primers, PCR programs and a discussion of the PCR conditions please consult: *Andrologia* **26**: 97-106 (1994) and *Biotechniques* **23**: 504-511 (1997). Click **here** to get the Biotechniques paper in PDF format.

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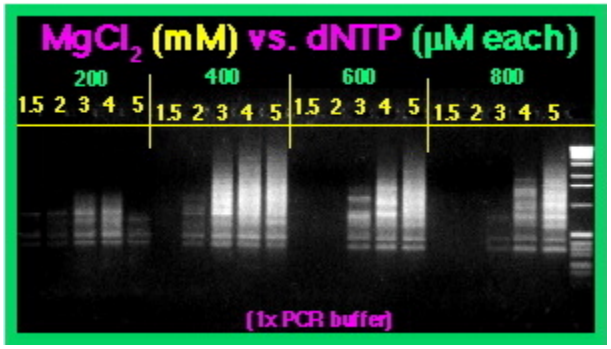
$MgCl_2$ concentration

Relationship between $MgCl_2$ and dNTP concentration (also on page 13)

dNTP concentrations of about 200µM each are usually recommended for the Taq polymerase, at 1.5mM $MgCl_2$ (Perkin Elmer Cetus). In a 25 µl reaction volume, theoretically these nucleotides should allow synthesis of about 6-6.5 µg of DNA. This amount should be sufficient for multiplex reactions in which 5 to 8 or more primer pairs are used at the same time. To work properly (besides the magnesium bound by the dNTP and the DNA), Taq polymerase requires free magnesium. This is probably the reason why small increases in the dNTP concentrations can rapidly inhibit the PCR reaction (Mg gets "trapped")whereas increases in magnesium concentration often have positive effects.

The relationship between the concentration of magnesium and that of the dNTPs was investigated by performing PCR with a degenerate primer in reactions that contained 200, 400, 600 and 800 µM each dNTP, combined with 1.5, 2, 3, 4 or 5 mM $MgCl_2$ (Fig. 34).

This test confirmed that any increase in dNTP concentration requires an increase in the concentration of magnesium ions in order for the reaction to work. At 200 µM each dNTP, reaction worked at all magmesium concentrations, but for this primer it worked better at 3 mM (which is about double the recommended magnesium concentration for the amount of dNTP). At 800 µM each dNTP, reaction worked only aboove 3 mM magnesium.



(also shown on page 13)

Fig. 34. PCR with a degenerate primer at different Mg and dNTP concentrations. Each of the Mg concentrations (1.5, 2, 3, 4, 5 mM) were combined with each of the following dNTP concentrations (each): 200µM, 400µM, 600µM and 800µM. Results indicate that increasing dNTP concentrations require increasing Mg concentrations for the PCR reactions to work.

Relationship between $MgCl_2$ and buffer (or salt) concentration

Two of the most important ingredients influenceing the results of a PCR reaction are the buffer (especially salt) and the magnesium concentrations. To study their relationship, a multiplex PCr was performed using mixture C (Fig. 36, below). Two sets of reactions were performed at two "extreme" concentrations of salt (KCl), 1x (50mM) and 3x (150 mM), and various magnesium concentrations (yellow values). Two other sets of reactions were performed at two "extreme" magnesium concentrations, 1.5 and 10.8 mM and various salt (KCl) concentrations (blue values). The dNTP concentration was kept constant, at 200 mM each deoxynucleotide. The following observation can be drawn:

- at 1x salt concentration and 200 mM each dNTP, reaction worked best at about 1.5 mM magnesium. At higher magnesium concentrations unspecific products appeared, but they gradually decreased in intensity towards 21.6 mM (probably because $MgCl_2$ is a salt, decreasing the stringency of the buffer - same way KCl does).
- at 3x salt concentration and 200 mM each dNTP, reaction worked best between 1.5 and 3.5 mM magnesium. As the stringency of the buffer was already lower than usual (due to the high KCl concnentration), further increase in $MgCl_2$ increased the "combined" stringency of teh reaction even more. Thus, fewer long unspecific products were obtained and the reaction was almost completely inhibited towards 21.6 mM magnesium.
- at 10.8 mM $MgCl_2$ and 200 mM each dNTP, reaction worked best around 2x salt (KCl) concetration (mostly specific products amplified). However, it is obvious that overall amount of PCR product is reduced compared to the reactions taking place at 1.5 mM magnesium. In this respect, high magnesium concentrations seem to inhibit the reaction more than high KCl (3x) concentrations. Therefore, it is likely that this magnesium inhibition is more than just a reduction in stringency of the reaction mixture.
- at 1.5 mM magnesium and 200 mM each dNTP, reaction worked best around 2x salt (KCl) concentration (all products amplified, few unspecific products visible). Overall product amount is higher than in the reactions taking place at 10.8 mM magnesium.

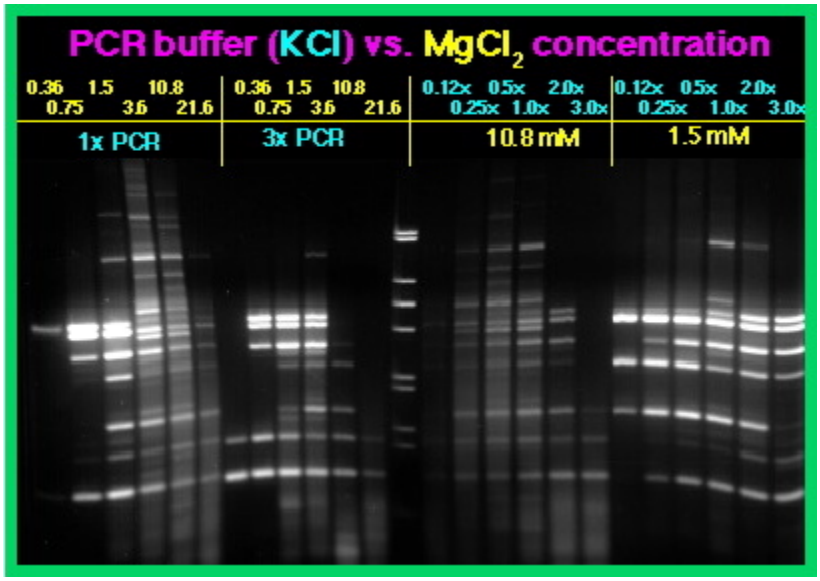


Fig. 36. Realtionship between magnesium and salt (KCl) concentration in PCR reactions. For a detailed description of the figure, please read text above.

Effects of variations in $MgCl_2$ concentration only

A recommended $MgCl_2$ concentration in a standard PCR reaction is 1.5mM, at dNTP concentrations of around 200µM each. To test the influence of $MgCl_2$, a multiplex PCR with mixture C was performed, keeping dNTP concentration at 200µM each and gradually increasing $MgCl_2$ from 1.8 to 10.8 mM (Fig. 37). The overall amplification became gradually more "specific" (unspecific bands disappeared) and the products acquired comparable intensities (at 10.8mM). However, higher concentrations of $MgCl_2$ appeared to inhibit the polymerase activity, decreasing the amount of all products. Taking into consideration the amount of PCR products, the best magnesium concentration should be between 1.8 and 3.6 mM. The large unspecific product (arrow) appeared due to the lower annealing temperature at which the reaction took place.

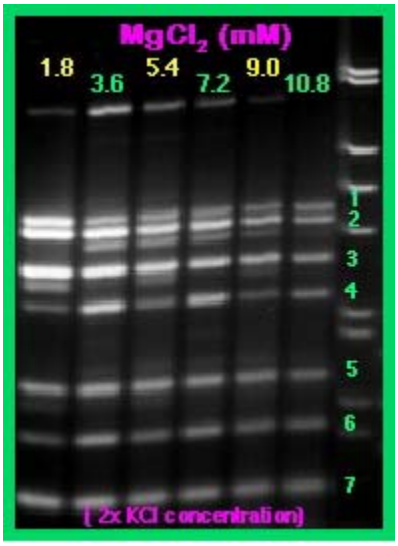


Fig. 37. Multiplex PCR amplification with mixture C at 2x KCl and increasing magnesium concentrations. Overall reaction becomes more specific at 10.8 mM magnesium, but the products are reduced in intensity. The most optimal magnesium concentration is somewhere between 1.8 and 3.6 mM where the PCR product amount is higher. The unspecific product (arrow) appears due to a lower than usual annealing temperature used for this reaction.