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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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## Nucleotides (dNTP)

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### dNTP "instability"

One important observation, coming from experiments with multiplex PCR, is that dNTP stocks are very sensitive to cycles of thawing/freezing. After 3-5 such cycles, multiplex PCR reactions usually did not work well. To avoid such problems, **small aliquots** (2-5  $\mu$ l) of dNTP (25 mM each), lasting for only a couple of reactions, can be made and kept frozen at  $-20^{\circ}$  C. However, during long-term freezing, small amounts of water evaporate on the walls of the vial changing the concentration of the dNTP solution. Before using, it is essential to **centrifuge** these vials at high speed in a microfuge.

This low stability of the dNTP is not so obvious when single loci are amplified.

Another important observation is that, anytime nucleotides are diluted in water, the solution should be buffered (for example with **10mM Tris pH 7.7-8.0**, final concentration). Otherwise, an acid pH will promote hydrolysis of dNTP into dNDP and dNMP and will render them useless for enzymatic DNA polymerizing reactions.

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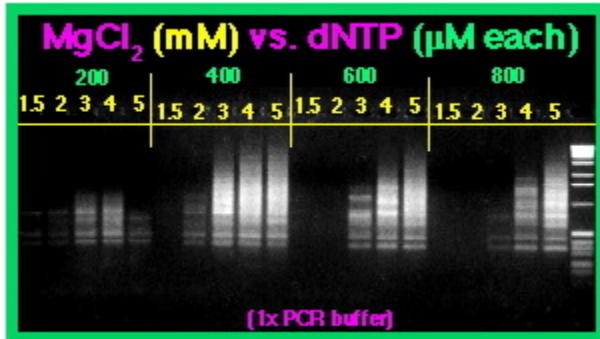
### Relationship between $MgCl_2$ and dNTP concentration (also on page [14](#))

dNTP concentrations of about 200 $\mu$ M each are usually recommended for the Taq polymerase, at 1.5mM  $MgCl_2$  (Perkin Elmer Cetus). In a 25  $\mu$ l reaction volume, theoretically these nucleotides should allow synthesis of about 6-6.5  $\mu$ g 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  $\mu\text{M}$  each dNTP, combined with 1.5, 2, 3, 4 or 5 mM  $\text{MgCl}_2$  (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  $\mu\text{M}$  each dNTP, reaction worked at all magnesium 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  $\mu\text{M}$  each dNTP, reaction worked only above 3 mM magnesium.

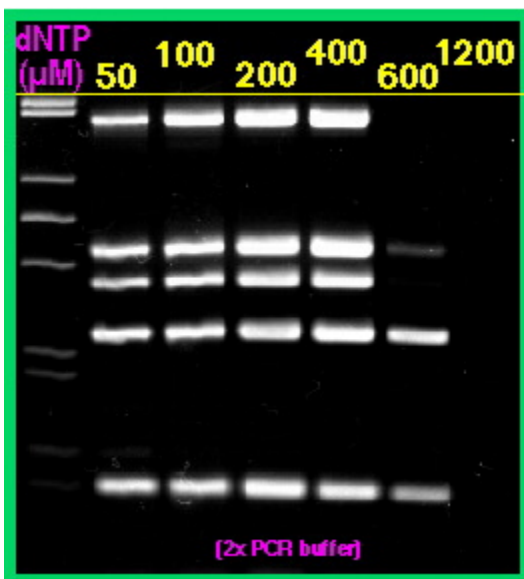


(also shown on page 14)

**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): 200 $\mu\text{M}$ , 400 $\mu\text{M}$ , 600 $\mu\text{M}$  and 800 $\mu\text{M}$ . Results indicate that increasing dNTP concentrations require increasing Mg concentrations for the PCR reactions to work.

### Common dNTP use in PCR and multiplex PCR

In another test aimed at examining the proper dNTP concentration, a multiplex PCR using primer mixture D was performed. The  $\text{MgCl}_2$  concentration was kept constant (3mM) while the dNTP concentration was increased stepwise from 50 to 100, 200, 400, 600 and 1200  $\mu\text{M}$  each deoxynucleotide (Fig. 35). The best results were achieved at 200 and 400  $\mu\text{M}$  dNTP; reaction was rapidly inhibited after these values. Lower than usual dNTP concentrations still allowed PCR amplification, but with somewhat less efficiency (lane "50").



**Fig. 35.** Multiplex PCR amplification of mixture D in 2x PCR buffer (3 mM Mg) using increasing concentrations of dNTP (50 $\mu\text{M}$ , 100 $\mu\text{M}$ , 200 $\mu\text{M}$ , 400 $\mu\text{M}$ , 600 $\mu\text{M}$  and 1200 $\mu\text{M}$  each). Most efficient amplification is seen at concentrations of 200-400 $\mu\text{M}$  each dNTP. Further increase in the dNTP concentration inhibits the reaction when  $\text{MgCl}_2$  is kept constant.