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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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Taq polymerase

Different concentrations of a Taq polymerase were tested using primer mixture C (Fig. 32). The most efficient enzyme concentration seemed to be around 0.4μl or 2 Units/25μl reaction volume. Too much enzyme, possibly because of the high glycerol concentration in the stock solution, resulted in an unbalanced amplification of various loci and a slight increase in the background. too little enzyme resulted in the lack of some of the amplification products.

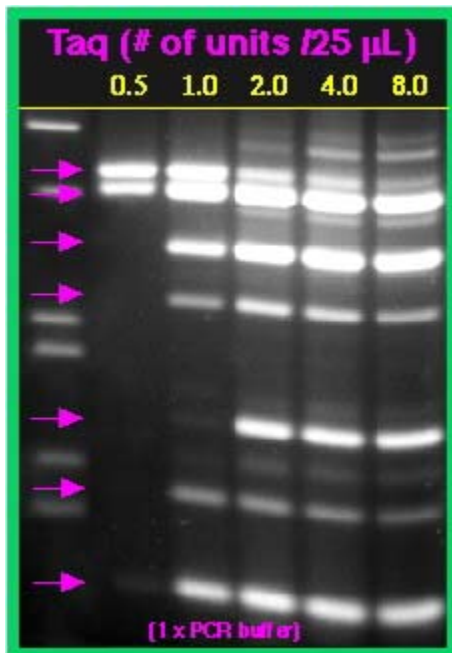
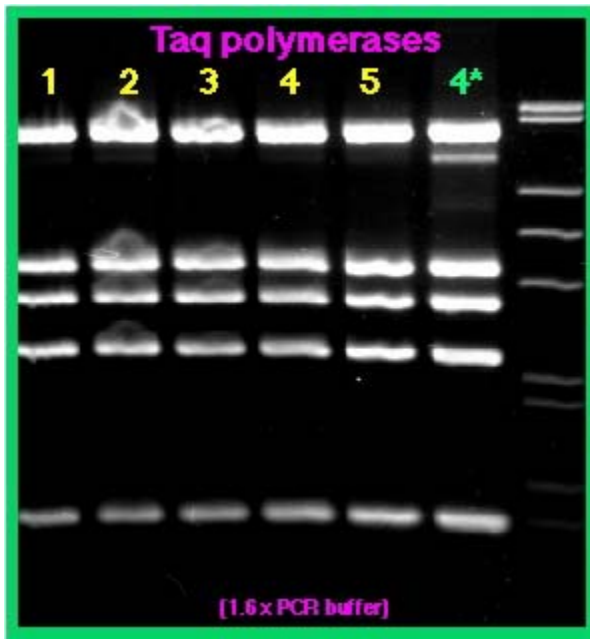


Fig. 32. Amplification products of mixture C, using 0.5 Units/25μl, 1 Unit/25μl, 2Units/25μl, 4 Units/25μl and 8 Units/25μl reaction volume are shown. Arrows indicate the expected positions of the amplification products. The most appropriate enzyme concentration was between 1-2 Units/25μl.

Five native Taq polymerases, from five different sources, were used to amplify multiplex mixture D in 1.6x PCR buffer using 2Units enzyme/25μl reaction (Fig. 33). In the same buffering conditions, all these enzyme performed similarly.

Fig. 33. Multiplex PCR of mixture D in 1.6x PCR buffer using Taq polymerases from five sources. Lanes 1 to 5 indicate that all enzymes work similarly



at the same concentration. Lane 4* (green) shows the products obtained when the enzyme from lane 4 was used in the buffer provided by the vendor. An unspecific product appeared, indicating that buffer composition influences the results.