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Different thermostable DNA polymerases may amplify different RAPD products

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The use of Random Amplified Polymorphic DNA (RAPD) markers has become a recent and powerful tool for fingerprinting anonymous genomes (1–4). From the very beginning the reproducibility of RAPD fingerprints has been a major concern to many investigators, and while many laboratories have succeeded to optimize the reaction conditions with respect to their particular DNA samples, chemicals and equipment used, the comparability of diagnostic RAPD markers produced from different laboratories remains unknown. The latter will prove of central importance especially for providing a central data base service on diagnostic RAPD markers for genome identification (e.g. RAPD data base from the *European Collection of Animal Cell Cultures at PHLS London, UK).*

By comparing the amplification patterns of a total of 13 commercially available thermostable DNA polymerases (Table 1) we found that all polymerases which derive from *Thermus aquaticus* produced similar (but not identical) amplification patterns, whereas all polymerases from other sources (except Tfl from *Thermus flavus*) amplified very few or no discrete products (data not shown). We subjected total genomic DNA of one clone of the cladoceran *Daphnia galeata* (5) to RAPD analysis using 3 different primers (Kit B from Operon Technologies; Alameda, CA) and 13 different polymerases. Each primer/polymerase combination was tested using the same template DNA dissolved in (i) TE buffer, (ii) ddH20, and (iii) low melting point agarose ('in gel' DNA; FMC NuSieve GTG, Biozym), which represent the main alternatives of template DNA solutions used in routine studies. All reactions were reproduced at least three times in a Perkin Elmer Cetus 9600 thermal cycler using 12.5 µl reaction volume and no oil overlay. 0.3 U polymerase were used per reaction; reaction buffers (adjusted to 2 mM $MgCl_2$, 0.1 mM each dNTP, 3 pmoles primer) were according to manufacturers recommendations for each enzyme. Samples were predenatured at 85°C for 2 min and subjected to 40 step cycles: 92°C for 20 s, 38°C for 15 s, ramp 3 s/°C to 72°C for 60 s. Amplification products were separated and visualized on 1.4% agarose gels (NuSieve 3:1) in TBE buffer containing 0.1 µg/ml ethidium bromide.

For each given primer/template/polymerase combination, clearly reproducible fingerprint patterns were achieved also under slightly different DNA template concentrations (Figure 1). Between different combinations, especially with respect to the type of polymerase used, both qualitative and quantitative differences in the amplification patterns were found. Seven DNA polymerases, which derive from *Thermus aquaticus* and one from *Thermus flavus* amplified clear and reproducible banding patterns. As can be seen from Figure 1, there was little variation within the amplification patterns for any given enzyme even under slightly different reaction conditions. However, between enzymes some obvious differences were found, which relate to both, the number and relative amounts of amplified products. In addition, enzymes differed significantly with respect to sensitivity to slight changes in the $MgCl_2$ concentration as introduced by template DNA which has been resuspended in TE buffer instead of water, whereas 'in gel' DNA template had little or no effect on the amplification patterns. In essence, there were generally 2–4 major RAPD markers, which got amplified by all *Thermus aquaticus* derived polymerases, but there were also always 1–4 unambiguous markers, which got amplified only by one or a few enzymes (cf. Figure 1).

Although the exact reasons for the differences in the amplification patterns of the polymerases are unknown, it may be argued that both the activity and specificity of different polymerases depend on slightly different temperature and reaction preferences, which affect the outcome of possible competition reactions between the products amplified in the first and most critical cycles. Thus, it may be possible to generate similar or even identical amplification patterns by using polymerases from different sources by varying the reaction conditions by means of try and error experiments. However, this would be of little or no use for most application studies.

Our results unambiguously demonstrate that the outcome of a RAPD fingerprint pattern may depend on the type of polymerase used. Thus, diagnostic RAPD markers identified in different laboratories may not necessarily be interchangeable and suited for a RAPD marker data base, unless the same reaction conditions (including template DNA in ddH_2O, TE buffer or 'in gel') and in particular the same type of polymerase has been used.

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Table 1. Thermostable DNA polymerases used in the study

commercial name	source	supplier
Taq DNA polymerase	*Thermus aquaticus*	Amersham (Amh)
Tfl DNA polymerase	*Thermus flavus*	Biozym (Bioz)
Taq DNA polymerase	*Thermus aquaticus*	Boehringer Mannheim (BM)
Taq DNA polymerase	*Thermus aquaticus*	Gibco BRL (Gib)
AmpliTaq® , AS	*Thermus aquaticus*	Perkin Elmer Cetus (PCA)
Native Taq DNA polymerase	*Thermus aquaticus*	Perkin Elmer Cetus (PCN)
Taq DNA polymerase I	*Thermus aquaticus*	Promega I (Pro I)
Taq DNA polymerase II	*Thermus aquaticus*	Promega II (Pro II)
Tth DNA polymerase	*Thermus thermophilus*	Biozym
Replitherm® DNA polymerase	unknown	Biozym
Tth DNA polymerase	*Thermus thermophilus*	Boehringer Mannheim
$Vent_R$® DNA polymerase	*Thermococcus litoralis*	New England Biolabs
$DeepVent_R$™ DNA polymerase	*Pyrococcus spec.*	New England Biolabs

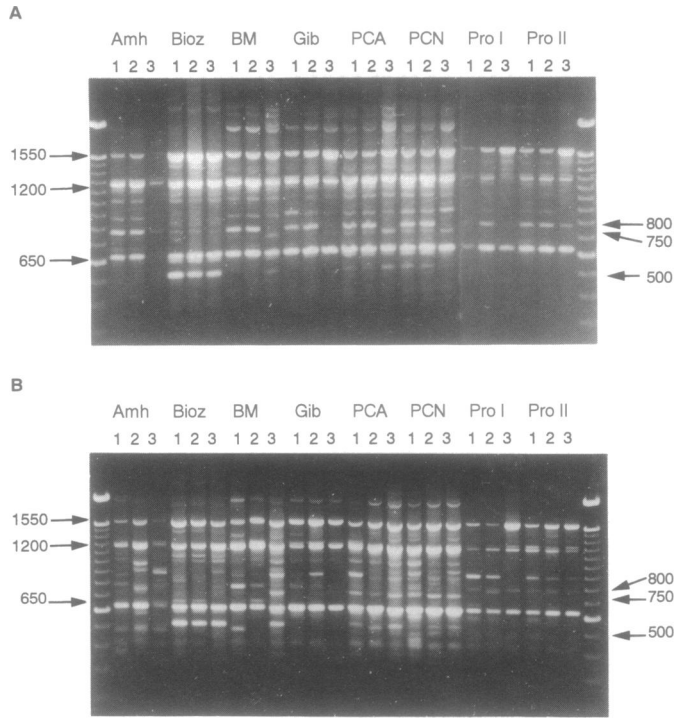

Figure 1. RAPD fingerprint patterns of eight thermostable DNA polymerases (Amh = Amersham, Bioz = Biozym, BM = Boehringer Mannheim, Gib = Gibco, PCA = Perkin Elmer Cetus Amplitaq, PCN = Perkin Elmer Cetus Native Taq, Pro I, II = Promega; see table 1) at different reaction conditions. The same primer/template DNA combination (primer OPB-04, *Daphnia galeata* clone 144) was used and each gel shows three amplification patterns for each enzyme. (A): (1) 1 ng and (2) 3 ng template DNA in ddH_2O and (3) 1.5 ng template DNA in TE; (B) same as in (A) except that the template DNA has been collected in low melting point agarose and diluted to working solutions (1 ng/μl) with ddH_2O or TE buffer, respectively. Control reactions without template DNA did not produce amplification products with any enzyme (not shown). Three major RAPD fragments at 1550 bp, 1200 bp, and 650 bp were amplified by all enzymes under almost all reaction conditions. Although each single amplification pattern for a given polymerase was reproducible under the named conditions, some fragments got amplified only by certain polymerases. A 500 bp fragment always got amplified by Bioz independent of the reaction conditions, but never by four other enzymes (Amh, Gib, Pro I, Pro II). On the other side, Bioz never revealed a 800 bp fragment which got amplified by all other enzymes (at least under certain conditions). Furthermore, a 750 bp fragment always got amplified by Amh and PCN, but no other enzyme except BM (lanes 3).