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AmpliTaq Gold™

Improves Short Tandem Repeat Amplifications of Highly Degraded DNA

By S. Hummel,[1] J. Burger,[1,2] J. Rameckers,[1] C. Lassen,[1] W. Schmerer,[1] B. Herrmann[1,3]

[1] Institut für Anthropologie, Georg-August-University", Bürgerstr. 50, 37073 Göttingen, Germany. [2] Institut für Anthropologie Johannes Gutenberg-University", Saarstr. 21, 55099 Mainz, Germany. [3] Wissenschaftskolleg, Institute for Advanced Studies, Wallotstr. 19, 14193 Berlin, Germany.

Artifact Products in Short Tandem Repeat Amplifications

Many DNA samples encountered in forensic and biological investigations exhibit severe degradation and contamination caused by both biological and non-biological factors as well as by DNA of exogenous origin.[1,2,3] Thus, for molecular analysis, the application of standard DNA extraction procedures and amplification protocols can be limited. Although these samples usually contain fragmented target DNA in the picogram range up to a few nanograms, most of them are still suitable for genetic analysis; even for single copy DNA target sequences if specially adapted protocols are applied.

For example, molecular sex determination through the amplification of a short sequence of the amelogenin gene consisting of only 106/112 basepairs[4] can easily be achieved.[5] Even polymorphic short tandem repeat (STR) microsatellite DNA with product lengths ranging from less than 100 basepairs up to 300 basepairs can be amplified from numerous samples.[2,6,7,8]

However, due to the tandem repeat structure of the core units consisting of 2–6 bases, artifact products appearing as so-called "shadow bands" are often co-amplified very efficiently.[9,10,11,12] These non-intended products are assumed to be created by DNA polymerase slippage[13] or may be due to random hybridization of fragmented DNA.[7] This phenomenon is well-known even from microsatellite amplifications of contemporary DNA. In amplifications of ancient DNA (aDNA) these artifact products can occur in similar quantities as those found in true alleles. If the artifact product occurs during the early cycles, they may even dominate the total yield of amplification products. In either case, the genetic typing of ancient biological specimens by short tandem repeat (STR) amplifications and fragment length determination is complicated or even impossible.

Improvement of Product Yield and Amplification Specificity

Using the AmpliTaq Gold™ enzyme, the main obstacles in genetic typing of ancient and degraded samples (*i.e.*, low product yield and low amplification specificity) can be overcome. As already reported for amplifications of contemporary DNA,[14] the amplifications of the STR loci HUMCD4, HUMVWA31A, and HUMFES/FPS[15,16,17] from aDNA revealed remarkably higher product yields by using the AmpliTaq Gold enzyme (Figure 1 a–c). In addition, it is the improvement of amplification specificity that now enables reproducible genetic typing from aDNA. STR amplifications from severely degraded DNA often exhibit strong artifact bands when using standard *Taq* DNA polymerase, while aliquots of the same DNA extraction samples reveal specific amplifications of the indigenous alleles using the AmpliTaq Gold enzyme. The former artifact bands are reduced to the level of background; by this means, alleles can be identified easily.

Automated DNA Extraction

The bone and teeth samples used in this investigation were derived from archaeological skeletal material ranging between 200 and 3000 years of age. In order to exclude false positive results due to modern contamination, all samples underwent the standard procedures for aDNA work[1,6] (*i.e.*, handling of sample material with disposable gloves and face masks, complete removal of sample surface material, and the separation of DNA extraction, amplification process, and electrophoretic analysis to three

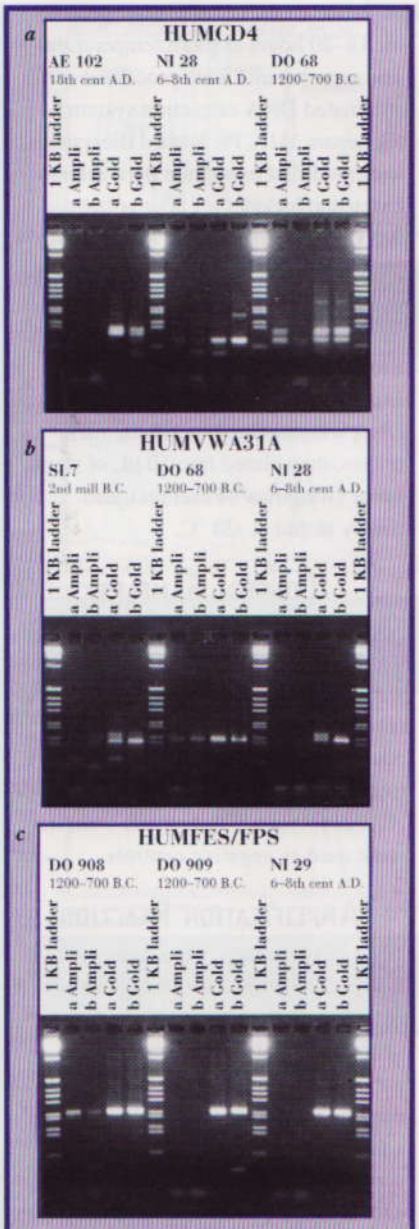

Figure 1 (a–c): Each agarose gel shows three aDNA samples amplified with AmpliTaq® DNA Polymerase and the AmpliTaq Gold™ enzyme, respectively. Regardless of the Short Tandem Repeat system employed, the product yields using the AmpliTaq Gold enzyme are remarkably higher than using AmpliTaq DNA Polymerase. The fact that aliquots of the same mix (Figure 2) can reveal different product intensities (e.g. HUMCD4, Do 68, Ampli a, b) is due to the stochastic nature of target distribution working with a very small number of targets.

Size ranges of amplification products:

HUMCD4	88–123 basepairs
HUMVWA31A	135–167 basepairs
HUMFES/FPS	213–237 basepairs

different laboratory rooms). Roots of the teeth and small pieces of compact bone (femur) were ground to powder in a mixer mill, and 0.3 g aliquots were subjected to a decalcifying extraction with 1.5 mL of 0.5 M EDTA (pH 8.3) for 18–20 hours at room temperature. The supernatants were processed in an automated DNA extraction system (Genepure 341A, PE Applied Biosystems) with modified extraction parameters. Extraction includes a 1 hour proteinase K digestion, separate phenol and chloroform extraction steps, and the adsorptive binding of DNA to a silica powder suspension (Glassmilk™, Geneclean® II, Bio 101) in presence of isopropanol and sodium acetate. The Glassmilk-DNA was collected on filtrate membranes, then eluted into 50 µL of sterile water (Ampuwa®, Fesenius), and finally stored at -20 °C.

Control Samples

For positive control DNA, extracts derived from saliva of the investigators were used. Thus, not only was a correct reaction process proven, but also, contamination of the ancient samples through handling could be excluded by STR genotypes. No-template samples were used as negative controls.

Amplification Reaction

The experiments were designed as duplicate reactions for each sample/enzyme combination with all component concentrations and reaction parameters being invariant, except the enzyme and the specific mode of addition. For this reason, the reaction mixes were set up as master mixes for each STR primer system. After division of master mixes, the aDNAs were added in order to achieve a concentration of target DNA, which was as homogeneous as possible. A further subdivision was made before the enzymes were added. The preparation of the duplicate amplification samples is demonstrated in Figure 2 with final component concentrations as follows: 50 mM KCl, 10 mM Tris-HCl (GeneAmp® 10X PCR Buffer II), 2.0 mM $MgCl_2$ (both PE Applied Biosystems),

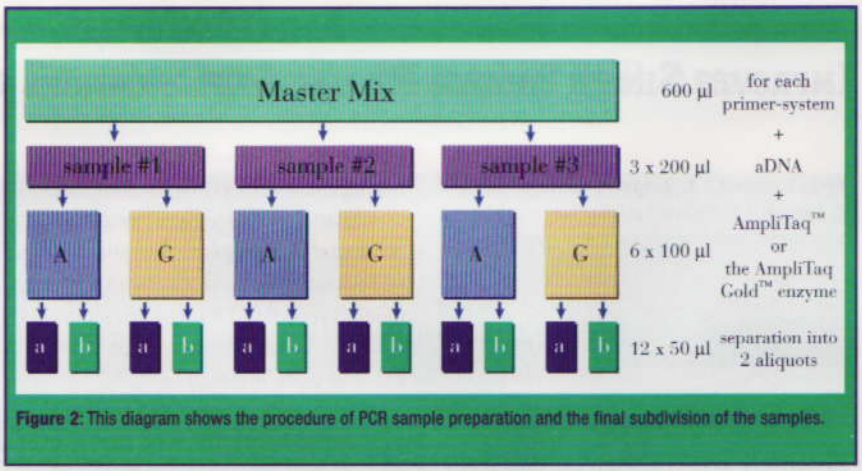

Figure 2: This diagram shows the procedure of PCR sample preparation and the final subdivision of the samples.

175 µM each dNTPs (Boehringer Mannheim), primers: 0.12 µM HUMVWA31A, or 0.12 µM HUMCD4, or 0.14 µM HUMFES/FPS, and 1–10 ng of aDNA. In degraded ancient samples, the latter value neither allows conclusions to be drawn as to the amount of intact target DNA, nor the possibility of *Taq* DNA polymerase inhibitors within the sample. Two units of the AmpliTaq Gold enzyme per sample were pipetted at room temperature, before the samples were overlayed with mineral oil. The cycling reactions were started with an initial 94 °C delay for 10 minutes. In contrast, 2 units of the regular AmpliTaq® DNA Polymerase were added in a routine 94 °C Hot Start, after an overlay with mineral oil.

Cycling parameters in a DNA Thermal Cycler (PE Applied Biosystems) for HUMVWA31A and HUMFES/FPS: 60 cycles consisting of 94 °C 1 min., 50 °C 1 min., 72 °C 1 min. For HUMCD4: 60 cycles consisting of 94 °C 1 min., 53 °C 1 min., 72 °C 1 min.

Electrophoretic Analysis

To establish the yield of amplification products, 10 µL of each sample were run on a 3% agarose gel (Agarose, Ultra Pure, Life Technologies). The gels were pre-stained with ethidium bromide and the DNA visualized by 254 nm UV transillumination (Figure 1 a–c). By these preliminary electrophoretic results, the amounts of PCR product to be analyzed on denaturing 6% PAA gels were gathered. The typing of 0.5 µL to 4.0 µL of each sample was performed by using 12 cm well-to-read gels in the automated fluorescence fragment length detection system (GeneScan® 672) on the ABI Prism™ 373A stretch DNA Sequencer (both PE Applied Biosystems). Each sample was determined using the internal lane standard GeneScan 350-ROX (data not shown). ■

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