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Efficiency comparison of seven different *Taq* polymerases used in hemogenetics

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Abstract. Currently, STR typing is the most efficient and the fastest way for identification of biological material samples from human. However, efficacy of DNA analysis largely depends on DNA degradation which is related to sample aging and storage conditions. Objective of the study was to compare the enzymatic efficiency of polymerases used for analysis of degraded, low concentration DNA templates. Seven different polymerase brands have been used. Significant differences in polymerase efficiency in relation to DNA template degradation were observed.

Keywords: Forensic science; Polymerase chain reaction optimization; Short tandem repeat; Archival samples

1. Introduction

Analysis of tandemly repeated DNA sequences, called STR or microsatellites, was a revolution in a forensic biology. It allowed a very precise identification of the individuals, based on a small sample of extracted DNA. In addition, degraded DNA, due to the small sizes of microsatellite sequences, could be used for STR analysis. This technique has a great importance in criminal cases, and enables to match a suspected person, to a trace found on crime scene. Another important aspect of STR analysis is an identification of unidentified people and unknown corpses.

Reference material acquired from the suspects, gives a large amounts of non-degraded DNA, but the situation is different when we deal with the samples collected at the crime scenes or from the unidentified bodies, where many different factors (physical, chemical or

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biological) can damage DNA. In many cases even some of the best polymerases are not able to amplify the longer fragments of heavily degraded material.

STR plays a key role in polymerase. Currently, many commercial polymerases designated to the different reactions are available. These enzymes are able to amplify numerous STR loci in a single polymerase chain reaction. At the same time, every amplified DNA fragment is marked with a specific dye, which enables the analysis in a sequencer, and gives a unique DNA profile, based on different number of tandem repeats in every analyzed locus. Results are visualized as the pattern of peaks and strength of their signal is directly proportional to the number of allele copies amplified in each locus. The low level of amplification can affect lost of heterozygosity, which makes it impossible to determine complete genetic profile.

The main objective was to find out if it is possible to acquire full genetic profile form amount of template DNA lower than 0.50 ng, which is the minimum suggested by polymerases' manufacturers.

The first criterion was the ability of polymerases to amplify all of the possible alleles. The second was the level of the amplification measured with the height of analyzed peaks.

2. Material and methods

Blood samples were collected in 1955 from 30 non-related individuals at the Department of Forensic Medicine, Medical University of Bialystok. Dried samples were packed in paper envelopes and stored at room temperature with constant humidity.

For DNA extraction, 0.02 g of dried blood was used for each sample. DNA extraction was performed with QIAamp DNA Mini Kit (Qiagen), according to manufacturers' protocol.

Total amount of isolated DNA was measured fluorometrically using PicoGreen dsDNA Quantitation Reagent (Invitrogen, Molecular Probes) and Fluoroscan Ascent FL (Labsystems). The quality of DNA was assessed by 2% ethidium bromide agarose gel electrophoresis.

Seven different *Taq* polymerases were tested: Ampli*Taq* Gold DNA Polymerase (Applied Biosystems), DNA Polymerase–gel form (Biotools), *Taq* Polymerase Recombinant (Invitrogen), *Taq* Platinum Polymerase (Invitrogen), JumpStart *Taq* Polymerase (Sigma), *Taq* DNA Polymerase (New England BioLabs), and *Taq* DNA Polymerase (Promega). The PCR amplification was performed on GenAmp PCR System 9700 thermocycler (Applied Biosystems), with AmpF*l*STR SGM Plus Amplification kit (Applied Biosystems). The total amount of DNA used for the reaction was 1.25, 0.50 and 0.25 ng in a total volume of 25 μl reaction mix with 2.5 U of each polymerase. Control, non-degraded DNA from AmpF*l*STR SGM Plus was used for comparison against the samples. Conditions of PCR reaction [1,2,3] were: 28 cycles (denaturation 94 °C, 1 min; annealing 59 °C, 1 min; elongation 72 °C, 1 min), final extension 60 °C, 45 min. Each HotStart polymerase had the step of the initial denaturation according to the manufacturers' protocol.

Primer sequences were marked with fluorescent dyes that enable detection of the product in the sequencer. Length of amplified products varied between 90 and 360 bp.

Electrophoresis was performed on ABI PRISM® 3100 Sequencer (Applied Biosystems), with the use of 36 cm capillary and Performance Optimised Polymer™ POP-4

Table 1
Comparison of the polymerases

Polymerase	*C*	S	L	S.D.	%FP
Ampli*Taq* Gold® (Applied Biosystems)	312.6	69.16	5.8	31,76	73,64
Taq (New England BioLabs)	52.8	0.58	21.6	1,16	1,82
Gel Form (Biotools)	216.9	61.53	8	24,42	63,64
JumpStart™ *Taq* (Sigma)	259	74.41	6.6	37,67	70
Taq (Promega)	184.7	32.66	11.2	11,42	49,09
Platinum® *Taq* (Invitrogen)	348.2	31.92	12.2	15,98	44,55
Taq (Invitrogen)	182.4	65.75	4	11,03	81,82

C = mean peak height ratio for the control (RFU), *S* = mean peak height ratio for the sample (RFU), *L* = mean number of lost alleles, S.D. = standard deviation for the *S* value, %FP = percentage of full profile.

(Applied Biosystems). Collected data was analysed in a default mode (peak amplitude thresholds 50 pts) with Genescan 3.7 and Genotyper 3.7 software (Applied Biosystems).

3. Results and discussion

The results of amplifications when total amount of template DNA were 0.50 and 1.25 ng showed no statistically important differences in the polymerases efficiency.Polymerase efficiency, with total amount of sample and control DNA equal to 0.25 ng, is shown in Table 1. First differences between polymerases are observed in case of control DNA. The largest efficiency in control samples does not correspond with the small number of lost alleles. Best profiles from samples were given by *Taq* DNA (Invitrogen), Ampli*Taq* Gold, JumpStart *Taq* and Gel Form polymerases. *Taq* DNA polymerase (New England BioLabs) lost practically all of the alleles.

Lost of alleles was observed in longest amplified fragments of SGM Plus loci, D2S1338, D18S51, FGA, which is shown in other publications. Bender et al. proves that artificially degraded DNA has an exclusion size for DNA molecules larger than 220 bp and seems to include only a small portion of molecules up to 300 bp [4].

Holt et al. found that a reaction with less DNA (e.g., < 1 ng template) may amplify well and produce interpretable signal [5]. Schneider et al. determined that successful amplification (of 0.25–2.5 ng of degraded DNA in standard PCR conditions) does not necessarily mean that the typing results were correct for all loci [6]. Moretti et al. shows that some samples containing little DNA can be typed successfully [7].

Our work shows that even small amount of DNA template (0.25 ng), from very old blood sample, can give up to 70–80%, depending on the used polymerase.

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