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# Ultimate shortening of the PCR product in the STR system TH01—a new perspective in testing of degraded forensic samples

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**Abstract**

The microsatellite TH01 system was flanked with newly designed primers which were as short as possible. One of the primers was 6FAM-labeled. Analysis of PCR products was performed using capillary electrophoresis with the ABI PRISM 310 genetic analyser. The homemade allelic ladder contained alleles 6, 7, 8, 9, 9.3 and 10 ranging from 69 to 85 bp. Using the newly designed primers and the AmpFl STR SGM Plus kit, 111 samples were examined. The obtained results were exactly the same in both tests. The possibility of amplification and analysis as short polymorphic fragments increases the chance of positive DNA typing from degraded forensic samples.


*Keywords:* TH01 STR; Degraded DNA

## 1. Introduction

Thyrosine hydroxylase intron 1 (TH01) is a short tandem repeat (STR) marker located in 11p15.5 containing a $(AATG)_{5-9}$ $(ATG)_{9.3}$ $(AATG)_{10-11}$ repeated sequence. The range of the allelic ladder in commercial testing kits is from 172 to 188 bp. The TH01 region was flanked with a newly designed primers which were as short as possible and products ranging from 69 to 85 bp were obtained.

## 2. Materials and methods

DNA from 111 dried blood stains was isolated with the Chelex 100 method [3]. The amplification was performed with the newly designed primers (accession D00269,

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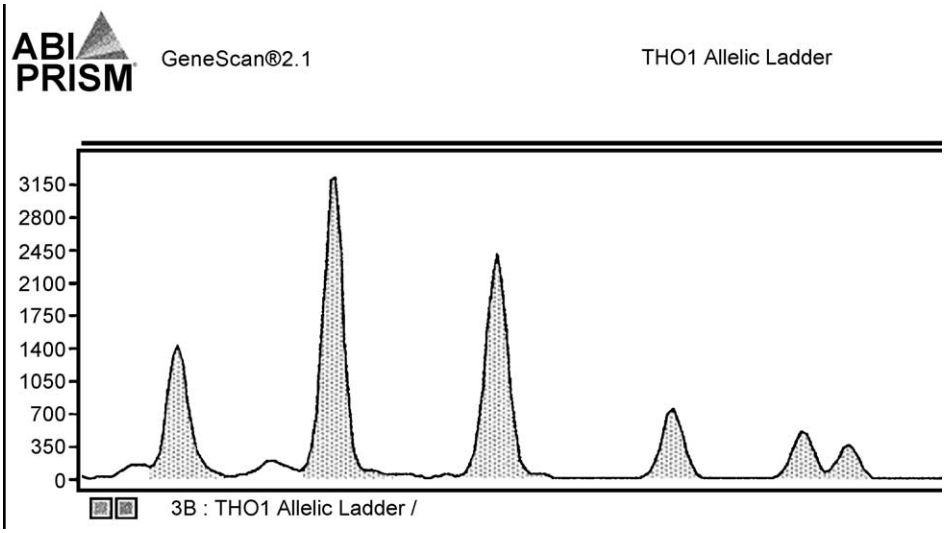


Fig. 1. GeneScan® 2.1 display of electropherogram and alleles size of TH01 allelic ladder.

position 1146) [1] and the AmpFlSTR SGM Plus PCR Amplification kit (Applera). The sequence of the new primers is shown below:

TH01LOC F 5′– TGG CCT GTT CCT CCC TTA TTT CCC – 3′

TH01LOC R 5′– CAC AGG GAA CAC AGA CTC CAT GGT – 3′

The amplification was conducted in 6.25-μl reaction volume consisting of 1 × PCR buffer (Applera) containing of 15 mM $MgCl_2$, 200 μM each dNTP, 0.25 U of AmpTaq DNA polymerase (Applera) and 0.5 μM of each primer . The primer TH01LOC F was 6FAM-labeled. PCR conditions were as follows: 94 °C 2 min [(94 °C 1 min, 54 °C 1 min, 70 °C 1.5 min) × 10, (90 °C 1 min, 54 °C 1 min, 70 °C 1.5 min) × 20] on a PTC 100 MJ Research thermocycler. Analysis of PCR products was performed using the capillary electrophoresis on an ABI PRISM 310 Genetic Analyzer and with ROX 350 as an internal marker of migration.

## 3. Results

The homemade allelic ladder contained alleles 6, 7, 8, 9, 9.3 and 10 (Fig. 1).

Table 1
The size of alleles according to the tabular data of the GeneScan® 2.1

| Allele | 6 | 7 | 8 | 9 | 9.3 | 10 |
|---|---|---|---|---|---|---|
| Size | 69.31 | 72.95 | 76.80 | 80.97 | 83.99 | 85.08 |

Table 2
Polymorphism of the STR TH01 in the Polish population

| STR TH01 phenotype | Number observed | Number expected | $\chi^2$ | Gene frequencies |
|---|---|---|---|---|
| 6/6 | 5 | 7.5 | 0.8 | $TH01^6 = 0.26$ |
| 6/7 | 13 | 9.2 | 1.6 | |
| 6/8 | 8 | 6.9 | 0.2 | |
| 6/9 | 9 | 11.5 | 0.4 | $TH01^7 = 0.16$ |
| 6/9.3 | 19 | 14.4 | 1.5 | |
| 6/10 | 1 | 0.6 | 0.3 | |
| 7/7 | 1 | 2.8 | 1.2 | $TH01^8 = 0.12$ |
| 7/8 | 4 | 4.3 | 0 | |
| 7/9 | 7 | 7.1 | 0 | |
| 7/9.3 | 9 | 8.9 | 0 | $TH01^9 = 0.20$ |
| 8/8 | 1 | 1.6 | 0.2 | |
| 8/9 | 5 | 5.3 | 0 | |
| 8/9.3 | 7 | 6.7 | 0 | $TH01^{9.3} = 0.25$ |
| 9/9 | 9 | 4.4 | 4.8 | |
| 9/9.3 | 6 | 11.1 | 2.3 | $TH01^{10} = 0.01$ |
| 9.3/9.3 | 7 | 6.9 | 0 | |
| Total | 111 | – | 13.3 | 1.00 |
| | | $df = 30$ | $p > 0.99$ | |

The obtained results were exactly the same for the AmpFlSTR SGM Plus test [2] and the new TH01 flanked fragments. Tables 1 and 2 show the TH01 allele frequencies in the Polish population sample and results of the $\chi^2$ test ($p$>0.99).

## 4. Discussion

The possibility of amplification and analysis of short polymorphic fragments increases the chance of positive DNA typing from authentic degraded forensic samples. The short TH01 fragments can be applied to forensic samples including blood, saliva, semen, hair, tissue, bone and even sexual assault swabs and stains.

## References

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