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Oznaczanie loci zestawu PowerPlex Y w wybranych tkankach przechowywanych w zróżnicowanych warunkach środowiska zewnętrznego

Typeability of PowerPlex Y (Promega) profiles in selected tissue samples incubated in various environments

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Celem pracy było określenie możliwości oznaczania układów zestawu PowerPlex Y (Promega) w próbkach narządów jamy brzusznej pobranych ze zwłok i przechowywanych w różnych warunkach środowiska zewnętrznego. Próbki narządów zostały pobrane do badań w czasie badania sekcyjnego ze zwłok o znanym czasie zgonu, nie dłuższym niż 14 godzin. Tkanki pobierane były od osób młodych w wieku 20-30 lat, zmarłych śmiercią gwałtowną i przechowywane w temperaturze 4°C i 21°C w pojemnikach zamkniętych o objętości 40 ml wypełnionych wodą słoną, słodką, ziemią ogrodową i piaskiem oraz w pojemnikach zamkniętych i otwartych nie zawierających żadnego podłoża, w temperaturze 4°C i 21°C. Profile porównawcze oznaczano w próbkach krwi. DNA izolowano metodą organiczną w odstępach 7 dniowych. Amplifikacja i genotypowanie z użyciem multipleksowego systemu PowerPlex Y-STR Kit and ABI 310 zgodnie z instrukcją producenta. W przedstawionych badaniach najwcześniej obserwowano zanik alleli w materiale przechowywanym w pojemnikach zamkniętych w temperaturze 21°C wypełnionych piaskiem i ziemią.

In cases of decomposed bodies, Y chromosomal STR markers may be useful in identification of a male relative. The authors assessed typeability of PowerPlex Y (Promega) loci in post mortem tissue material stored in various environments. Kidney, spleen and pancreas specimens were collected during autopsies of five persons aged 20-30 years, whose time of death was determined within the limit of 14 hours. Tissue material was incubated at 21°C and 4°C in various environmental conditions. DNA was extracted by the organic method from tissue samples collected in 7-day intervals and subsequently typed using the PowerPlexY-STR kit and ABI 310. A fast decrease in the typeability rate was seen in specimens incubated in peat soil and in sand. Kidney tissue samples were typeable in all PowerPlexY-STR loci within 63 days of incubation at 4°C. Faster DNA degradation was recorded in spleen and pancreas specimens. In samples with negative genotyping results, no DNA was found by fluorometric quantitation. Decomposed soft tissues are a potential material for DNA typing.

Key words: decomposed tissues, environmental conditions, PowerPlex Y.
Słowa kluczowe: tkanki rozłożone, warunki zewnętrzne, PowerPlex Y.

INTRODUCTION

Multiplex PCR-based STR kits with the fluorescence detection technology have been validated to produce rapid and robust amplification of several DNA loci from biological samples and thus have become one of the most reliable means of personal identification. Y-chromosome-specific short tandem repeat (Y-STR) analysis became a widely accepted tool for human identification. STR loci from Y chromosome represent information for patrilineage

tracking without recombination [1, 2]. This system is useful for paternity testing, human remains identification in mass disasters and felon identification [3, 4, 5, 6]. The aim of this study was the assessment of typeability of STR loci included in PowerPlex Y System in kidney, spleen and pancreas specimens depending on various environmental conditions.

MATERIAL AND METHODS

Kidney, spleen and pancreas specimens were collected during autopsies of five persons aged 20-30 years with the post mortem interval (PMI) limited to 14 hours, according to recommended anatomical body sections (abdomen). Early signs of body decomposition were prevented by storage in a morgue refrigerator. Tissue specimens 2x2x2cm in size were incubated at 4°C and 21°C in closed 40 ml containers and at 21°C in closed 40 ml containers filled with sand, garden peat soil, pond water or salt water, and at 21°C in open 40 ml containers. Five samples of each tissue were collected in 7-day intervals. DNA was extracted from 5 mg of tissue by the modified organic procedure [7]. Reference DNA profiles were typed in fresh blood samples collected from respective corpses on autopsy. Twelve polymorphic Y-STR systems: DYS19, DYS385a/b, DYS389I/II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438, DYS439 included in PowerPlex Y-system were amplified following the manufacturer's instructions (Promega) with the exception that all reaction reagents were reduced proportionally, so that the volume of the reaction mix was 10μl. Electrophoresis and genotyping were performed in an ABI310 Genetic Analyzer (Applera, USA) using the Genescan v3.11 and Genotyper v2.5 software. A signal of 150 RFU was adopted as a threshold value.

RESULTS

The authors evaluated the typeability of DYS19, DYS385a/b, DYS389I/II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438, DYS439 included in

Table I. Typeability of PowerPlex Y loci in kidney specimens.
Tabela I. Oznaczanie loci PowerPlex Y w nerce.

Conditions Warunki	DYS391	DYS3891	DSY439	DYS389I/II	DYS438	DYS437	DYS19	DYS392	DYS393	DYS390	DYS385a/b
4°C, closed container pojemnik zamknięty	98/126	91/112	77/105	70/91	112/133	63/91	77/105	63/91	105/126	98/127	70/98
21°C, closed container pojemnik zamknięty	77/105	70/91	56/84	49/70	91/112	42/70	56/84	42/70	84/105	77/105	49/77
21°C, open container pojemnik otwarty	84/112	77/98	63/91	56/77	98/119	49/77	63/91	49/77	91/112	84/112	56/84
21°C, salt water in closed container woda słona, pojemnik zamknięty	49/91	42/84	35/77	28/49	63/112	49/84	35/77	21/56	56/105	49/84	28/63
21°C, pond water in closed container woda słodka, pojemnik zamknięty	70/105	56/91	49/91	35/70	84/133	63/98	49/91	28/56	77/105	63/98	42/84
21°C, sand in closed piasek, pojemnik zamnknięty	14/28	14/28	7/28	7/28	21/28	14/28	7/28	7/28	21/28	14/28	14/28
21°C, peat soil in closed container ziemia, pojemnik zamknięty.	7/28	7/21	7/21	7/21	7/28	7/28	7/21	7/21	7/28	7/28	7/21

Table II. Typeability of PowerPlex Y loci in spleen specimens.
Tabela II. Oznaczanie loci PowerPlex Y w śledzionie

Conditions Warunki	DYS391	DYS3891	DSY439	DYS389I/II	DYS438	DYS437	DYS19	DYS392	DYS393	DYS390	DYS385a/b
4°C, closed container pojemnik zamknięty	56/98	49/91	42/77	28/63	70/133	49/98	42/77	28/56	63/105	49/98	35/63
21°C, closed container pojemnik zamknięty	35/77	28/70	21/56	121/49	49/112	28/77	21/56	14/21	42/84	28/77	17/42
21°C, open container pojemnik otwarty	42/84	35/77	28/63	28/56	56/119	35/84	28/63	21/28	49/91	35/81	21/49
21°C, salt water in closed container woda słona, pojemnik zamknięty	42/84	35/77	21/56	14/49	77/126	35/77	21/56	14/42	49/91	35/77	21/56
21°C, pond water in closed container woda słodka, pojemnik zamknięty	56/91	49/84	4277	28/49	63/112	49/91	42/77	28/49	56/105	49/91	28/49
21°C, sand in closed container piasek, pojemnik zamknięty	21/28	21/28	14/28	14/28	14/28	21/28	14/28	14/28	21/28	14/28	14/28
21°C, peat soil in closed container ziemia, pojemnik zamknięty	21/28	21/28	21/28	14/21	21/28	21/28	14/28	14/21	21/28	14/28	14/28

Table III. Typeability of PowerPlex Y loci in pancreas specimens
Tabela III . Oznaczanie loci PowerPlex Y w trzustce

Conditions Warunki	DYS391	DYS3891	DSY439	DYS389I/II	DYS438	DYS437	DYS19	DYS392	DYS393	DYS390	DYS385a/b
4°C, closed container pojemnik zamknięty	70/112	56/105	49/91	49/84	84/133	63/105	49/91	49/77	70/112	63/105	49/91
21°C, closed container pojemnik zamknięty	49/91	35/84	28/70	28/63	63/112	42/84	28/70	28/56	49/91	42/84	28/70
21°C, open container pojemnik otwarty	56/98	42/91	35/77	35/70	70/119	49/91	35/77	35/63	56/98	49/91	35/77
21°C, salt water in closed container woda słona, pojemnik zamknięty	35/77	28/63	21/56	14/42	49/98	35/70	21/56	14/42	35/91	35/70	21/42
21°C, pond water in closed container woda słodka, pojemnik zamknięty	56/91	49/84	42/70	28/63	63/112	49/91	42/77	28/49	56/105	49/91	28/63
21°C, sand in closed container piasek pojemnik zamknięty	14/28	14/28	7/28	7/28	21/28	14/28	7/28	7/28	21/28	14/28	14/28
21°C, peat soil in closed container ziemia pojemnik zamknięty	14/28	14/28	7/21	14/21	14/28	14/28	7/21	14/21	14/28	14/28	14/21

PowerPlex-Y systems in kidney, spleen and pancreas specimens incubated at 21°C and 4°C in various environmental conditions in the interval of 7 to 133 days. PowerPlex Y-STR typeability limits for the tissues under study are presented in Tables I, II, III. The values before the slash denote time limits in days, when full PowerPlex Y-STR profiles were typeable in all the samples. The values after the slash denote time limits in days, after which no PowerPlex Y-STR profiles were seen for the set of 5x5 samples as a whole. In the time spans between the two values, partial profiles were observed.

DISCUSSION

DNA was extracted using the organic method, commonly employed in genetic identification of mass disaster victims [8, 9]. PowerPlex-Y systems kit was validated as highly specific and sensitive for human DNA and suitable for typing of degraded samples [10]. Fast DNA degradation was observed in the studied material stored in peat soil or sand, which may have resulted from humus acid content, microbial action or acid pH [11, 12, 13]. On the other hand, an increased air access and higher temperature during our experiments favoured desiccation and preservation of the material [14], resulting in a prolonged typeability of full Power Plex profiles in specimens stored at 21°C in open containers when compared to these kept in closed containers. In our opinion, organs extracted from a corpse and placed in a water environment within a short time after death are devoid of body bacteria, show diluted enzyme activity and are prevented from air access, which decelerates decomposition process in relation to that in an intact body. Decomposed soft tissues are a potential material for DNA typing. Different typeability rates of PowerPlex loci are due to environmental conditions, rather than to organ type.

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