

Identification of a Deceased Crew Member From a Capsized Vessel

The Usefulness of Y-STR Markers to Exclude Nonpaternal Lineages

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The MS Rocknes capsized in Vattlestraumen on January 19, 2004. Twelve crew members were rescued shortly after the accident, while 18 crew members, almost all of Asian origin, were either deceased (5) or missing (13). The lowest point of the capsized vessel was 25 meters under sea level (Figure 1). Three weeks later, divers managed to bring up 11 deceased crew members from inside the boat. One more deceased was found inside the ship when it was turned. One crew member is still missing.

Most of the deceased crew members were identified using traditional methods based on odontological criteria or information from the medical examination. However, one of the deceased, designated 542B, could not be identified using traditional methods, and we decided to apply DNA analysis to try to establish the identity of this person.

Reference samples were received from 2 unrelated males (A.B. and M.M.), each of them missing a brother. Samples from the deceased and the 2 unrelated males (A.B. and M.M.), both putative brothers of 542B, were initially typed using the STR markers in the SGM[®]Plus multiplex kit. The likelihood ratio that the reference person was a brother of deceased 542B was calculated using the software package FAMILIAS (www.math.chalmers.se/~mostad/familias/). Genotypes of the two reference persons are shown in Table 1. Based on these likelihood ratios, it was 14 times more likely that A.B. was 542B's brother rather than an unrelated individual and 10 times more likely that M.M. was a brother rather than an unrelated individual. Based on the results of



Figure 1: The capsized MS Rocknes

these autosomal markers, both of the reference persons could be brothers of the deceased, and the small likelihood ratios were not sufficient to establish the identity of the missing person.

To obtain additional genetic information, we successfully typed samples from each of the 3 males using the 12 Y-STR markers of the PowerPlex[®] Y multiplex kit. The Y haplotypes of the deceased and the 2 putative brothers are given in Table 2. There was a match between 542B and A.B. (identical Y-haplotypes), while M.M. could be excluded as brother of 542B (different alleles in 7 of the 12 markers).

The Y haplotype of the deceased was not previously observed in our population database of 1760 Norwegian males typed in the "minimal haplotype markers". The Y haplotype in the Norwegian database with the closest similarity to the one observed for 542B differed by a total of 4 one-step repeat mutations at 3 different loci. This observation was consistent with the information that the missing persons were Asian (Filipino). Using the Y-STR haplotype

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Sample ID	D3S1356	vWA	D16S539	D2S1338	Amelo	D8S1179	D21S11	D18S51	D19S433	TH01	FGA
542B (deceased)	18, 17	16, 19	10, 10	24, 20	XY	16, 13	30, 30.2	12, 20	15.2, 14	6, 7	23, 26
A.B.	16, 17	14, 19	9, 10	17, 20	XY	11, 13	30, 30.2	14, 15	13, 14	6, 8	22, 25
M.M.	16, 17	17, 17	10, 10	25, 20	XY	13, 13	29, 29	14, 16	15.2, 14	6, 9	23, 21

Table 1: Results of typing the autosomal STR markers in the SGM[®]Plus multiplex kit

Sample ID	DYS391	DYS389I	DYS439	DYS389II	DYS438	DYS437	DYS19	DYS392	DYS393	DYS390	DYS385a/b
542B (deceased)	10	12	12	28	10	14	16	12	15	23	12,14
A.B.	10	12	12	28	10	14	16	12	15	23	12,14
M.M.	10	12	13	28	10	14	15	13	12	24	13,15

Table 2: Results of typing the Y-STR markers in the PowerPlex[®] Y System

reference database (YHRD, www.yhrd.org), we searched for a match in the Filipino population database (211 males) and the pooled South East Asian population database (532 males). No match was found in these populations or in a world-wide database search (20.000 males in 175 populations). However, using the "neighbour function", 3 haplotypes with a

similarity of +/-1 repeat in 1 marker were found in the Asian population. Again, the results were in accordance with an Asian origin of the deceased. The low frequency of this haplotype strongly supported the idea that deceased 542B and reference person A.B. shared paternal lines, as expected if they were brothers.

In conclusion, Y-specific STR analysis contributed important genetic evidence in the present case and demonstrated the usefulness of Y-STR markers in identification cases. Deceased 542B was identified as the brother of A.B. based on the results from the DNA analysis, and in particular, the results from the Y-STR markers.

Strategy to Improve Reliability in the Laboratory

Addition of FGA Primers to Multiplexes PowerPlex Y and FFFL, and to Monoplex D5S2360

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Abstract

In this article, we demonstrate the successful addition of FGA primers to kits available for multi- and monoplex amplification of STR markers used in DNA typing.

Introduction

Sample mix-up is still a problem in laboratories. Forensic DNA laboratories routinely employ commercially available kits for multiplex amplification of autosomal short tandem repeat (STR) markers, e.g., PowerPlex® 16 System (Promega Corp.) and/or SGM Plus® (Applied Biosystems). In these two kits, 8 markers overlap, meaning that the same 8 loci are typed and a sample mix-up would easily be detected. In special cases however, it is necessary to analyse more markers by means of additional kits having no locus in

common with the two kits mentioned above. This is a situation where a sample mix-up could go undetected, due to the lack of overlapping markers. In this article, we demonstrate the successful addition of FGA primers to the PowerPlex Y System, FFFL Multiplex and the D5S2360 monoplex kit.

Materials and Methods

Reaction Conditions

FGA primers were manufactured according to Urquhart *et al.* (1) and labeled as follows: FGA Primer 1 [PET (or 6-FAM, when used in combination with D5S2360)]:

5`-GCCCATAGGTTTTGAACTCA-3`

FGA Primer 2: 5`-TGATTTGTCTGTAATTGC-CAGC-3`.

The (FGA) primer concentration was 0.2µM, and AmpliTaq Gold® DNA polymerase was used.

Amplification conditions for the D5S2360 plus FGA were as follows:

FGA (6-FAM) primer concentration: 0.1µM

D5S2360 (HEX) primer concentration: 0.5µM.

The PowerPlex Y and FFFL cycling conditions

were set as described in the manufacturer's protocols (Promega) or the literature (2).

Matrix (Colour deconvolution)

To analyse PowerPlex Y plus FGA and FFFL plus FGA (PET labeled) on an ABI PRISM® 310 Genetic Analyzer, the following matrix standards were used with dye set G5: FL, JOE, and TMR from the Promega PowerPlex Matrix Standards, 310/377, along with PET and LIZ® from Applied Biosystems Matrix Standard Set DS-33.

We recommend following the chapter "Creation of the Gene Scan Matrix File" in the *ABI PRISM 310 Genetic Analyzer user's guide*. PCR products can now be detected by using dye set G5.

Applied Biosystems LIZ is used as internal lane standard. After the first application, definition is required.

For analysing D5S2360 plus FGA (either HEX or 6-FAM labeled), a matrix was created using dye set A (Applied Biosystems) 6-FAM, HEX and NED (Matrix Standard Sets) along with the Promega dye CXR400 (PowerPlex® Matrix Standards), which served as internal lane standard.

Adjustments in the Genotyper® Software:

Data interpretation using the Genotyper software required appropriate adjustments: FGA alleles must be added, and macros have to be expanded.

Internal lane standard must be replaced by LIZ; a 5-dye sample sheet must be selected.

Conclusions

In the wake of the foreseeable lack of RFLP-VNTR technique for parentage testing, laboratories have to become aware of the

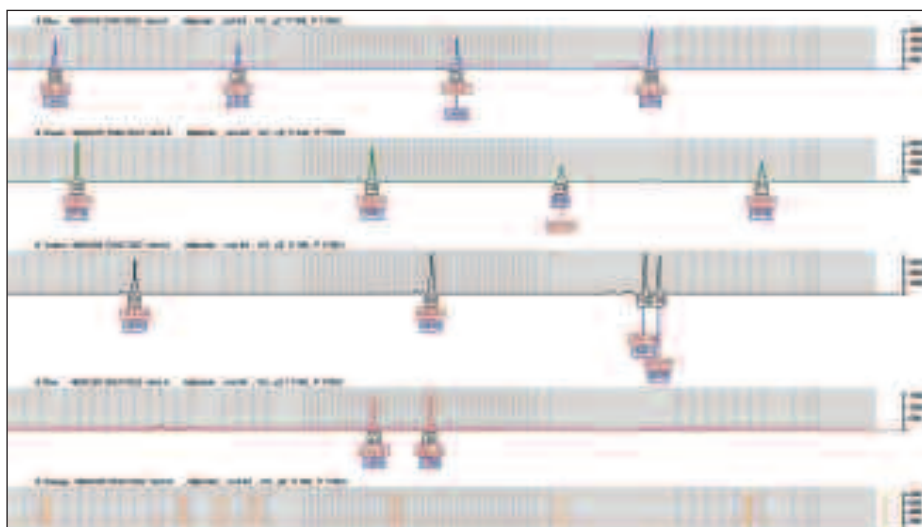


Figure 1: A DNA profile obtained with the PowerPlex Y System (12 loci) plus FGA primers and analysed using the ABI PRISM 310 Genetic Analyzer.

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need for additional, highly discriminating STR systems. This is especially true in complicated kinship cases.

Additional kits for multiplex amplification of either autosomal or Y-chromosome STR markers should include overlapping markers in order to enforce redundancy.

Addition of FGA primers to kits lacking overlapping markers can be useful to establish reliability by means of redundancy.

We chose the FGA locus for this purpose

because it is highly polymorphic and can be found in most commercial kits for autosomal STR markers.

Technical Comments

1. FGA ladders can be obtained by amplifying the Promega FGA allelic ladder by using the above mentioned FGA primers.

2. When Y-chromosome STR markers are analysed in a mixed DNA source, the amount of added FGA primers can be

reduced in order to avoid 'bleedthrough' artefacts (extra peaks).

References

1. Urquhart, A. *et al.* (1995) Highly discriminating heptaplex short tandem repeat PCR system for forensic identification. *BioTechniques* **18**, 116–121.
2. Henke, L. *et al.* (2001) Sequence analysis and population data on the new short tandem repeat locus D5S2360. *Forensic Sci. Int.* **116**, 55–58.



Participants attending the forensic workshop at the C.S.S. Mendel Institute in Rome.

DNA IQ™ and Differex™ Workshop in Rome

In February 2005, Promega Italy organised a one-day forensic workshop in Rome on the topic "DNA extraction and purification for casework samples". The workshop was held at the C.S.S. Mendel Institute. Fourteen enthusiastic participants from nine different laboratories attended the workshop where

the DNA IQ™ System and the new Differex™ Systems were presented to the community. The workshop included a practical session in the laboratory where samples provided by customers were processed for DNA purification. Samples included various bones, teeth, tissues, swabs, semen stains

and hair. Attendees were asked to preprocess some of the samples. Overnight incubation was performed for bone samples with the Bone Incubation Buffer plus Proteinase K, while tissue samples were incubated in proteinase K and Incubation Buffer. Extracted DNA was amplified by attendees and results made available for evaluation. The workshop included a round-table session where attendees discussed their experience with the DNA IQ System by presenting a number of actual cases.

The meeting was a good opportunity for attendees to learn more about new Promega products and to observe their at-the-bench application. The workshop was also an opportunity for members of different labs to get together and share their experiences.

If you would like to host a similar workshop in your area, please contact your Promega branch office or distributor to organise an event specifically designed for your laboratory.

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Upcoming Meetings of Interest:

Spring Conference Homicide Investigation

April 22–24, 2005
Leeds, UK
conferences@forensic-science-society.org.uk

MAFS Meeting

June 15–18, 2005
Monastir, Tunisia

16th International Symposium on Human Identification

September 26–29, 2005
Grapevine, Texas
www.promega.com/geneticsymp16

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How to install bin and panels files in GeneMapper® ID, versions 3.1 and 3.2

To facilitate analysis of data generated with the PowerPlex® Systems, we have created bin and panel files to allow automatic assignment of genotypes using the ABI GeneMapper ID® software, versions 3.1 and 3.2. After samples have been amplified using the PowerPlex System and detected using the ABI PRISM® 310 or 3100 Genetic Analyzer, the sample files can be imported into the GeneMapper ID software and analyzed. To correctly process the sample files, it is necessary to import and install the bin and panel files. Those files are available by request from the Promega regional technical support contacts and are now available for download from the Promega web site. (www.promega.com/geneticidtools/panels_bins/) Files specific for GeneMapper ID software, versions 3.1 and 3.2, are available.

To install these files, follow these steps:

1. Copy the bin and panel files to the computer you are using.
2. Open the GeneMapper ID software program.
3. Select "Tools" then "Panel Manager".
4. Highlight the "Panel Manager" icon in the upper left tile (Navigation Panel).
5. Select "File" and then "Import Panels".
6. Navigate to the files that you copied in Step 1. Select Promega_Panels_ID3.1.0 or Promega_Panels_ID3.2.0, depending on the GeneMapper ID software version installed. Click "Import".
7. In the Navigation Panel, highlight the PowerPlex Panels folder that you just imported in Step 6.
8. Select "File", then "Import Bin Set".
9. Navigate again to the files that you copied in Step 1. Select Promega_Bins_ID3.1.0 or Promega_Bins_ID3.2.0, depending on the GeneMapper ID software version installed. Click "Import".
10. At the bottom of the "Panel Manager" window, select "Apply" then "OK". The "Panel Manager" window will automatically close.

New Improved PowerPlex® Matrix Standards, 3100 – Custom

Spectral calibration is a crucial step in analyzing results generated with PowerPlex Systems on different detection platforms. We have responded to customer input to improve the quality of spectral calibrations on the ABI PRISM 3100 and 3100-Avant Genetic Analyzers. Promega has assembled a team of expert scientists to respond to hardware and software revisions on these instruments. As a result of our efforts, we have developed a set of matrix standards that improves spectral resolution on the ABI PRISM 3100 and 3100-Avant Genetic Analyzers.

The improved matrix standards, PowerPlex Matrix Standards, 3100 – Custom (Cat.# X3121), is now available through your local branch or distributor. The PowerPlex Matrix Standards, 3100 (Cat. # DG3650), has not been discontinued.

The PowerPlex Matrix Standards, 3100 – Custom, contains two tubes of JOE-labeled fragments, designated JOE A Matrix, Custom, and JOE B Matrix, Custom. Use JOE A to generate a matrix for the PowerPlex Y and PowerPlex ES Systems; use JOE B for the PowerPlex 16 System. The PowerPlex Matrix Standards, 3100 – Custom, protocol (GE163) has instructions for use of these new matrix standards.

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*In the U.S., effective March 29, 2005, U.S. Pat. Nos. 4,683,195, 4,965,188 and 4,683,202 will expire. In Europe, effective March 28, 2006, European Pat. Nos. 2,011,184 and 2,003,362 will expire.

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