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# Case study: paternity testing—when 21 loci are not enough

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**Abstract.** A seemingly routine paternity case which involved the testing of a mother, child and man led to inconclusive results as an exclusion involving only a single repeat was found at one of the 14 loci tested. This led to the testing of further loci. Only the single exclusion was found after profiling a total of 21 loci, with the addition of a further single locus providing a second exclusion. Even with both mutation events incorporated into the calculation a paternity index (PI) of 4957 was calculated, still a significant figure. However, when a second likelihood ratio was calculated assessing the likelihood of the results if the biological father of the child was the tested man or the tested man's brother, then the results were not significant, only 0.15. This analysis led to the profiling of the tested man's brother who matched at all 19 loci that were profiled and was concluded to be the biological father. 

*Keywords:* Paternity testing; STR; Mutation

## 1. Introduction

Laboratories that are involved in undertaking paternity testing often work with limited information about individual cases; this at times can potentially lead to incorrect conclusions. The situation has been alleviated to some degree in recent years with the introduction of kits that allow the easy typing of multiple loci, the more advanced multiplex kits such as the Identifiler™ (ABI) and the Powerplex® 16 (Promega) simultaneously profiling 15 and 16 STR loci. However, in some cases, it is desirable to increase the number of loci that are profiled in order to resolve complex scenarios. Here, we present a case where 22 loci were typed before a case could be resolved.

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## 2. Material and methods

### 2.1. STR profiling

DNA was extracted from buccal swabs using QiAMP® DNA extraction Kit (Qiagen). The samples were profiled using the Powerplex® 1.1 and 2.1, Powerplex® 16 Powerplex® FFFL kits (Promega) and the ABI Identifiler™. The additional locus, SE33, was tested using custom made primers. Products were analysed using the Hitachi FMBIO® and the ABI Prism™ 310 Genetic Analyzer. Because of the highly variable nature of SE33 the PCR products from all the individuals involved in the case were sequenced using the ABI BigDye™ Terminator sequencing kit.

### 2.2. Statistical analysis

PIs were calculated according to standard formulas. The mutation frequencies were incorporated into the PIs as a likelihood ratio, using the formulae suggested by Charles Brenner [1]. A mutation frequency of 0.0025 was used for a single step D21 locus and 0.0015 for a multiple step SE33 locus [2].

Table 1
The DNA profiles of the mother, child and first tested man

| | Loci | Mother | | Child | | Tested Man 1 | | PI | PI* |
|---|---|---|---|---|---|---|---|---|---|
| 1 | D3S1358 | 16 | 16 | 16 | 16 | 14 | 16 | 2.222 | 1.384 |
| 2 | vWA | 14 | 17 | 16 | 17 | 14 | 16 | 2.531 | 1.459 |
| 3 | D16S539 | 11 | 12 | 11 | 12 | 11 | 11 | 1.68 | 0.772 |
| 4 | D2S1338 | 19 | 25 | 17 | 19 | 17 | 21 | 2.816 | 1.483 |
| 5 | D8S1179 | 10 | 13 | 10 | 13 | 12 | 13 | 0.719 | 0.589 |
| 6[a] | D21S11 | 30 | 30 | 28 | 30 | 29 | 29 | 0.0038 | 0.015 |
| 7 | D18S51 | 12 | 16 | 12 | 12 | 12 | 18 | 3.508 | 1.563 |
| 8 | D19S433 | 15.2 | 15.2 | 14 | 15.2 | 14 | 14 | 3.053 | 1.658 |
| 9 | THO1 | 9 | 9.3 | 8 | 9 | 6 | 8 | 5.405 | 1.688 |
| 10 | FGA | 23 | 23 | 20 | 23 | 20 | 24 | 3.077 | 1.509 |
| 11 | D7S820 | 11 | 11 | 8 | 11 | 8 | 8 | 5.714 | 1.742 |
| 12 | D13S317 | 10 | 12 | 10 | 11 | 11 | 13 | 1.6 | 1.299 |
| 13 | D5S818 | 11 | 12 | 12 | 13 | 12 | 13 | 3.03 | 1.505 |
| 14 | CSF1PO | 10 | 12 | 12 | 12 | 12 | 12 | 3.361 | 1.543 |
| 15 | TPOX | 11 | 11 | 8 | 11 | 8 | 8 | 2.326 | 1.399 |
| 16 | PENTA D | 9 | 9 | 9 | 14 | 13 | 14 | 9.26 | 1.761 |
| 17 | PENTA E | 15 | 16 | 12 | 16 | 7 | 12 | 2.146 | 1.365 |
| 18 | LPL | 10 | 10 | 10 | 12 | 12 | 12 | 3.716 | 1.582 |
| 19 | F13B | 8 | 10 | 8 | 9 | 9 | 10 | 2.146 | 1.365 |
| 20 | FES | 11 | 11 | 11 | 12 | 11 | 12 | 2.023 | 1.319 |
| 21 | F13A01 | 6 | 6 | 6 | 6 | 6 | 7 | 1.532 | 1.211 |
| 22[a] | SE33 | 23.2 | 28.2 | 23.2 | 27.2 | 15 | 24.2 | 0.0036 | 0.018 |
| Total PIs | | | | | | | | 4957 | 0.15 |

Two paternity indices are shown; the PI is the standard paternity index and is the likelihood ratio of the probability of the results if the tested man is the father compared to the probability of the results if an unrelated man is the father. The second PI* is the likelihood of the probability of the tested man being the father compared to the likelihood of the tested man's brother being the father.

[a] Tested loci that led to exclusions.

## 3. Results

The profiles of the mother, child and first tested man are shown in Table 1. All loci were successfully typed. Three of the loci, D2S1338, D19S433, PENTA D, initially profiled for the mother, child and first tested man were not repeated for the second tested man (results not shown). The alleles of the SE33 loci were sequenced to verify the interpretation from the allelic ladder, which can be difficult due to the complex nature of the loci; in all cases, the results of the sequencing confirmed the original findings.

With both mutation events incorporated into the calculation, a paternity index (PI) of 4957 was calculated; this is still relatively high due to the large number of matching loci. Without additional information and testing, it would be very difficult to make any firm conclusions about the results.

In this case, a dialog with the individuals involved in the test revealed that the tested man's brother could possibly be the biological father. Testing this hypothesis was possible and when the probability of the tested man being the father was compared to the probability that the tested mans brother was the father a much lower likelihood ratio was calculated, the results are presented in Table 1. A sample from the tested man's brother was obtained and profiled using 19 of the loci. No exclusions were found at any of the 19 loci, making it much more likely that he was the biological father, a PI of 140 million was calculated.

## 4. Discussion

This case illustrates one of the problems that face paternity testing laboratories when dealing with civil rather than criminal cases, namely that the information provided is often incomplete.

The case illustrates the value for a paternity testing laboratory to carry as many loci as possible in order to help to resolve complex cases. It also illustrates the benefit in trying to ascertain all the information that is relevant to the case before making any conclusions; however, because of the sensitive nature of many cases, such information will not always be forthcoming.

## References

[1] Brenner C. Mutations in paternity, 1998. http://dna-view.com.
[2] English Speaking Working Group Paternity Testing Workshop, 2003-personal communication.