

Report of the European DNA profiling group (EDNAP)-an investigation of the hypervariable STR loci ACTBP2, APOAI1 and D11S554 and the compound loci D12S391 and D1S1656

Peter Gill^{a,*}, E. d'Aloja^b, B. Dupuy^c, B. Eriksen^d, M. Jangblad^e,
V. Johnsson^f, A.D. Kloosterman^g, A. Kratzer^h, M.V. Lareuⁱ, B. Mevag^c,
N. Morling^d, C. Phillips^j, H. Pfitzinger^k, S. Rand^l, M. Sabatier^m,
R. Scheithauerⁿ, H. Schmitter^o, P. Schneider^p, I. Skitsa^q, M.C. Vide^r

^a*Service Development, Forensic Science Service, Priory Home, Gooch Street North, Birmingham B5 6QQ, UK*

^b*Catholic University, Rome, Italy*

^c*Rettsmedisinsk Institut, Ricshospitalet 0027, Oslo, Norway*

^d*Department of Forensic Genetics, Institute of Forensic Medicine, University of Copenhagen, Copenhagen, Denmark*

^e*SKL, National Laboratory of Forensic Science, City, Sweden*

^f*National Bureau of Investigation, City, Finland*

^g*Netherlands Forensic Science Institute, Volmerlaan 17, Rijswijk, The Netherlands*

^h*Institute of Legal Medicine, University of Zurich, Zurich, Switzerland*

ⁱ*Institute of Legal Medicine, Faculty of Medicine, University of Santiago de Compostela, Santiago de Compostela, Spain*

^j*Department of Haematology, St. Bartholomews and the Royal London School of Medicine and Dentistry, Queen Mary and Westfield College, University of London, London, UK*

^k*Codgene, University of Strasbourg, Strasbourg, France*

^l*Institute of Legal Medicine, University of Munster, Munster, Germany*

^m*Laboratoire de Police Scientifique, Toulouse, France*

ⁿ*Institute of Forensic Medicine, University of Innsbruck, Innsbruck, Austria*

^o*Bundeskriminalamt, Wiesbaden, Germany*

^p*Institute of Legal Medicine, University of Mainz, Mainz, Germany*

^q*DNA laboratory, Hellenic Police, 14 Sevastoupoleos St., Athens, Greece*

^r*Instituto de Medicina Legal, Coimbra, Portugal*

Received 16 February 1998; received in revised form 20 August 1998; accepted 11 September 1998

*Corresponding author. Tel.: +44-121-6076800; fax: +44-121-6222051; e-mail: DNAPGill@compuserve.com

Abstract

This paper describes the results of three collaborative exercises which continues the EDNAP theme to explore whether uniformity of DNA profiling results could be achieved between European laboratories using STRs. In an earlier exercise, complex hypervariable AAAG-repeat STR loci were investigated, but reproducibility was found to be poor because of the variation of techniques used by participating laboratories. In the exercise reported here, an internal allelic ladder composed of ACTBP2 and D11S554 fragments was distributed. This ladder was used to size ACTBP2 analysed by a “singleplex” PCR amplification and D11S554 combined with APOA11 in a separate “duplex” reaction. Laboratories were asked to test 7 blood stains, one of which was a known control, and to report the results to the co-ordinating laboratory. The exercise demonstrated that ACTBP2 showed good reproducibility between laboratories, whereas further testing would be needed to validate APOA11 and D11S554 for interlaboratory comparisons. In separate exercises, the simple loci D12S391 and D1S1656 were tested; both of these showed excellent reproducibility between laboratories. © 1998 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Uniformity of DNA profiling results; STR loci; Result reproducibility

1. Introduction

Previously, the European DNA Profiling group (EDNAP) has carried out a number of collaborative experiments to determine which STR systems will produce results that are reproducible between laboratories. The first exercise [1] compared the complex AAAG-repeat ACTBP2 locus [2] with a simple STR repeat (HUMTH01). It was found that inter-laboratory reproducibility was poor for the former where sizing was carried out against an internal lambda marker which has a structure very different to that of tandem repeat DNA. As a result, it was apparent that ACTBP2 allele migration relative to the internal size standard was dependent upon the electrophoretic system, hence results between laboratories were divergent, although there was no reason to suppose that within laboratory designations were problematical.

To circumvent the problem of comparing AAAG-repeat alleles against a dissimilar size standard, Dupuy and Olaisen [3] developed a multiplexed internal sizing marker based on sequenced alleles derived from the loci ACTBP2 and D11S554 [4]. The markers span the range of alleles derived from the latter loci and APOA11. The ladder markers and primers to amplify ACTBP2, D11S554 and APOA11 were supplied to EDNAP members by B. Dupuy and B. Olaisen for testing and the results of this exercise are reported here. Additionally, primers and ladder markers for the loci D12S391 [5] and D1S1656 [6] were supplied by M.V. Lareu and A. Carracedo and circulated as a separate exercise.

2. Materials and methods

2.1. *ACTPB2, APOA11 and D11S554 exercise*

In common with previous exercises, specific recommendations of materials and methods were not issued. However all laboratories (fourteen in total) used either ABD 373 or 377 instrumentation with denaturing acrylamide gels. A ROX labelled allelic ladder marker was distributed [3] along with primers to amplify the locus ACTBP2 and the loci APOA11 and D11S554 in a separate duplex reaction. Specifically, the ROX-labelled ladder was constructed comprised of 25 alleles from ACTBP2 ranging between 233–333 bp and five alleles from D11S554 ranging between 176–225 bp. The format of the exercise followed that previously described [1]. Participants were asked to analyse seven blood-stains for the three loci using the reference internal allelic ladder standard.

2.2. *D12S391 and D1S1656 exercise*

In a separate exercise, D12S391 and D1S1656 primers were distributed to participants to test against the same blood stains. In this exercise, two laboratories used the Pharmacia ALF automated sequencer, one used a manual system (silver staining) and the remainder used ABD 373 or 377 automated sequencers.

2.3. *Sizing alleles*

All participants in the exercise used the Local Southern method of sizing.

3. Results

3.1. *AAAG-repeat loci*

In order to be able to precisely identify alleles, the measurement error (range) must be within ± 0.5 bp. An experimental estimate of 99% of the range (assuming a normal distribution) is given by $\pm 3 \times \text{SD}$. This was used as the benchmark to compare against. The best results were obtained by the ACTBP2 locus (Table 1); the SD varied between 0.1–0.2 bp, indicating a 99% range of up to ± 0.6 bp. The SD of ApoA11 0.2–0.7, indicating a much wider range of up to ± 2.1 bp. The greatest variation was with D11S554 where the SD varied between 0.5–0.8 bp (range up to ± 2.4 bp). Closer inspection of the data revealed that laboratories which obtained very close results with ACTBP2 and APOA11 were divergent by 1 bp with D11S554. Two laboratories collaborated to discover the cause of the anomaly. One possibility investigated was concurrent generation of ' n ' bands in the ladder and ' $n+1$ ' bands in the alleles in one of the laboratories (which would shift the sizing by one bp). However, it was confirmed that in both laboratories sample and ladder products were the correct ' $n+1$ ' bands. Afterwards, the primers were investigated in greater detail. It transpired that during the

Table 1
Compilation of results from AAAG-repeats

Sample no.	allele 1 Median size	allele 2 Median size	allele 1 Minimum	allele 2 Minimum	allele 1 Maximum	allele 2 Maximum	allele 1 Range	allele 2 Range	allele 1 SD	allele 2 SD
ACTBP2										
1	265.0	295.0	264.8	294.5	265.3	295.1	0.5	0.6	0.1	0.2
2	299.0	303.0	298.7	302.7	299.1	303.1	0.4	0.4	0.1	0.1
3	275.0	299.0	274.6	298.7	275.2	299.3	0.6	0.6	0.2	0.1
4	265.0	303.0	264.7	302.7	265.3	303.1	0.6	0.4	0.2	0.1
5	273.0	315.0	272.8	314.7	273.4	315.2	0.6	0.5	0.2	0.1
6	295.0	307.0	294.5	306.8	295.1	307.1	0.6	0.3	0.2	0.1
7	267.0	317.0	266.8	316.6	267.3	317.3	0.5	0.7	0.1	0.2
APOA11										
1	270.5	290.4	270.3	290.2	271.1	291.0	0.8	0.8	0.2	0.3
2	286.5	290.4	285.3	290.2	288.0	292.2	2.7	2.0	0.7	0.5
3	258.5	293.5	257.9	293.3	260.0	294.1	2.1	0.8	0.5	0.3
4	276.4	286.4	276.1	285.7	278.0	288.0	1.9	2.3	0.5	0.5
5	270.5	285.6	269.9	285.3	271.2	286.0	1.4	0.7	0.4	0.2
6	290.4	290.4	289.9	289.9	292.0	292.0	2.1	2.1	0.5	0.5
7	285.5	298.4	285.2	298.1	286.0	299.0	0.8	0.9	0.3	0.3
D11S554										
1	204.0	237.3	202.9	236.5	204.3	237.8	1.5	1.3	0.5	0.5
2	208.2	219.8	206.8	218.8	209.0	221.0	2.2	2.3	0.8	0.7
3	200.1	229.0	198.8	228.1	201.5	229.6	2.7	1.5	0.8	0.5
4	200.0	232.3	198.7	231.2	200.3	232.8	1.6	1.7	0.6	0.6
5	208.1	233.3	206.8	232.2	209.0	234.0	2.2	1.8	0.7	0.7
6	216.0	220.1	214.8	218.7	217.0	221.0	2.2	2.3	0.7	0.7
7	212.2	241.5	210.8	240.0	213.0	241.9	2.3	1.8	0.8	0.6

Results are compiled from returns from 13 different laboratories. For each sample the median size in base pairs, the minimum observed, maximum observed and range observed are compared to the calculated standard deviation (all figures in the table are in base pairs).

course of the exercise two different sets had been separately produced—one of the primers had an extra base erroneously attached (by the manufacturer) and this was the reason for the anomaly and the wide range observed.

Although ranges appeared to be wider than that necessary for definitive identification for APOA11, it was possible for some laboratories (Table 2) to obtain results which were very close (within 0.1 bp) to each other.

Dupuy and Olaisen [3] demonstrated that within their laboratory ACTBP2 gave results which were very close to the actual sequenced size in bp (within 0.06 bp); alleles were reproducibly sized since the SD varied between 0.04–0.11. Although the measured SD was greater for the EDNAP between laboratory exercise ($SD_{\max}=0.2$) this level of variation was just outside the range of ± 0.5 bases hence total between laboratory reproducibility could not be guaranteed given the experimental constraints of this exercise. However, for APOA11 and D11S554 the results suggested that there was still inconsistent variation of migration relative to the predominantly ACTBP2 allelic ladder.

Table 2

A comparison of results from two different laboratories, illustrating reproducibility of ACTBP2 and APOA11

Sample no.	Laboratory code				Differences (bp)	Differences (bp)
	3	3	16	16		
ACTBP2						
1	265	295	265.11	295	0.11	0
2	299	303	299.11	303.11	0.11	0.11
3	275.04	299.09	275.17	299.11	0.13	0.02
4	265	303.09	265	303.11	0	0.02
5	272.91	315.03	273.11	315.1	0.2	0.07
6	295	307	295.11	307.11	0.11	0.11
7	267.04	317	267.11	317.11	0.07	0.11
APOA11						
1	270.41	290.39	270.45	290.45	0.04	0.06
2	286.23	290.3	286.33	290.34	0.1	0.04
3	258.36	293.29	258.33	293.33	0.03	0.04
4	276.39	286.39	276.33	286.24	0.06	0.15
5	270.36	285.52	270.45	285.37	0.09	0.15
6	290.4	290.4	290.35	290.35	0.05	0.05
7	285.44	298.39	285.44	298.33	0	0.06
D11S554						
1	204.22	237.6	202.86	236.45	1.36	1.15
2	208.17	220.17	206.77	218.75	1.4	1.42
3	200.16	229.44	198.82	228.14	1.34	1.3
4	200.21	232.55	198.82	231.24	1.39	1.31
5	208.17	233.53	206.78	232.4	1.39	1.13
6	216.17	220.13	214.78	218.74	1.39	1.39
7	212.17	241.52	210.78	240.45	1.39	1.07

The reported primer anomaly has shifted the size of D11S554 alleles by approximately 1.3 bp greater in laboratory 3 compared to laboratory 16. For each allele, the figures given in the table are their measurements in base pairs.

Table 3
D12S391 results from participating laboratories

Sample no.	D12S391	
	allele 1	allele 2
1	17	17
2	21	21
3	16	20
4	16	19
5	17	18.3
6	18	25
7	18	24

The table shows allelic designations for samples 1–7. Three laboratories initially scored the 18.3 allele in sample 5 as 19.

Dupuy and Olaisen [3] demonstrated that sizing of these two loci did not give an absolute size-measurement in base pairs but had nevertheless achieved a reproducible within laboratory test. The EDNAP exercise suggested that between laboratory variation was greater than anticipated. Although similar equipment (ABD 373 and 377 instruments) was used it was possible that variations in protocols e.g. run temperature, voltage and acrylamide concentration may cause significant differences in migration rates. Differences appeared to be greatest with APOA11 and D11S554 alleles since these were compared against a ladder constructed predominantly of ACTBP2 alleles.

3.2. D12S391

A total of seven laboratories participated in this separate exercise (Table 3). Coincidentally, sample 5 contained a rare 18.3 allele which three laboratories scored as 19. On further analysis, using the rule that alleles can only be scored if within 0.5 bp of

Table 4
D151656 results from participating laboratories

Sample no.	D151656		Laboratory code	
	definitive results		9	10
	allele 1	allele 2	allele 2	allele 2
1	12	18.3	18.3	18.3
2	14	16.3	16.3	16.3
3	11	15.3	15.3	15.3
4	12	14	14	14
5	13	20.3	20.3	20.3 or 21
6	11	16.3	16.2 or 16.3	16.3
7	11	12	12	12

The Table shows allelic designations for samples 1–7. Two laboratories scored high molecular weight alleles (samples 5 and 6) inconclusively.

a putative allele ladder marker [7], the correct designation was obtained. This exercise emphasised the importance of using the criterion described.

3.3. *D1S1656*

A total of twelve laboratories participated. One laboratory reported sample 5 as genotype 13-(20.3 or 21). A different laboratory reported sample 6 as 11-(16.2 or 16.3). Otherwise there was complete agreement (Table 4). The two discrepancies reflected uncertainty of reporting results alleles varying by 1 bp in the laboratories concerned.

4. Discussion

Bär et al., [8] recommended the use of allelic ladders constructed from the relevant loci in combination with denaturing gels in order to minimise electrophoretic mobility shifts. It is well established that complementary strands of DNA migrate at different rates (even within denaturing gels), hence it is important to ensure that homologous strands of ladders and primers are dye-labelled [9,10]. In addition, there is great sequence diversity to be found within ACTBP2 [11–13]. Dupuy and Olaisen [3] suggested that D11S554 alleles containing AAGG repeats migrated differently to those with AAAG repeats when compared to an ACTBP2 allelic ladder. Lareu et al [14] demonstrated mobility differences of ACTBP2 alleles between different denaturing systems when compared to a pBR322/MspI digest.

We can generalise that anomalous mobility differences of alleles within a locus are negligible provided (a) that the allelic ladders are constructed from the commonest variants (b) denaturing systems are used (c) alleles in a sample are compared to an allelic ladder derived from alleles at the same locus. In addition, alleles not represented within the allelic ladder will migrate in predictable fashion provided that the sequences are similar to those found in adjacent ladder alleles. However, it is possible that some rare variants may have sequence differences sufficient to cause anomalous mobility shifts when compared against allelic ladders of different sequences [15]. Within a given laboratory, provided that the mobilities of any given allele are reproducible this is inconsequential, although it follows that electrophoretic systems may not always give an absolute indication of the size of an allele.

The conclusion of the first part of the exercise indicated that best reproducibility of results between laboratories was obtained when loci were compared directly to complementary allelic ladders derived from the same locus. Since the allelic ladder used in this exercise comprised alleles which were predominantly ACTBP2, this was the reason why results at this locus were much better than those obtained with APOA1 and D11S554.

Finally, the loci D12S351 and D1S656 were demonstrated to be suitable candidates for standardisation since they have been well characterised; in addition sequenced allelic ladders exist for both [5,6]. The exercise demonstrated the need to use windows which were ± 0.5 bp centred on the appropriate allelic ladder marker.

References

- [1] P. Gill, C. Kimpton, E. d'Aloja, J. Andersen, W. Bär, B. Brinkmann, S. Holgersen, V. Johnsson, A.D. Kloosterman, M.V. Lareu, L. Nelleman, H. Pfitzinger, C.P. Phillips, H. Schmitter, P.M. Schneider, M. Stenersen, Report of the European DNA profiling group (EDNAP)-towards standardisation of short tandem repeat (STR) loci, *Forensic Sci. Int.* 65 (1994) 51–59.
- [2] M.H. Polymeropoulos, D.S. Rath, C.R. Merrill, Tetranucleotide repeat polymorphism at the human β -actin related pseudogene H- β -Ac-psi-2 (ACTBP2), *Nucleic Acids Res.* 20 (1992) 1432.
- [3] B.M. Dupuy, B. Olaisen, A dedicated internal standard in fragment length analysis of hyperpolymorphic short tandem repeats, *Forensic Sci. Int.* 86 (1997) 207–227.
- [4] T. Phromchotikul, D. Brown, M. Litt, Microsatellite polymorphisms at the D11S554 and D11S569 loci, *Hum. Mol. Genet.* 3 (1992) 21.
- [5] M.V. Lareu, C. Pestoni, M. Schurenkamp, S. Rand, B. Brinkmann, A. Carracedo, A highly variable STR at the D12S391 locus, *Int J Legal Med* 109 (1996) 134–138.
- [6] M.V. Lareu, S. Barral, A. Salas, M.S. Rodriguez-Calvo, C. Pestoni, A. Canacedo, Further exploring of new STRs of interest for forensic genetic analysis, in: B. Olaisen, B. Brinkmann, P. Lincoln (Eds.), *Advances in Forensic Haemogenetics* 7, Elsevier, 1997, in press.
- [7] P. Gill, A. Urquhart, E. Millican, N. Oldroyd, S. Watson, R. Sparkes, C.P. Kimpton, A new method of STR interpretation using inferential logic-development of a criminal intelligence database, *Int. J. Leg. Med.* 109 (1996) 14–22.
- [8] W. Bär, B. Brinkmann, A. Carracedo, P. Gill, P. Lincoln, B. Olaisen, DNA recommendations. Further report of the DNA commission of the ISFH regarding the use of short tandem repeat systems, *Int. J. Leg. Med.* 110 (1997) 175–176.
- [9] H. Saitoh, U. Ueda, K. Kurosaki, M. Kiuchi, The different mobility of complementary strands depends on the proportion AC/GT, *Forensic Sci. Int.* 94 (1998) 155–156.
- [10] P. Gill, E. d'Aloja, J. Andersen, B. Dupuy, M. Jangblad, V. Johnsson, A.D. Kloosterman, A. Kratzer, M.V. Lareu, M. Meldegaard, C. Phillips, H. Pfitzinger, S. Rand, M. Sabatier, R. Scheithauer, H. Schmitter, P.M. Schneider, M.C. Vide, Report of the European DNA profiling group (EDNAP): an investigation of the complex STR loci D21S1 1 and HUMFIBRA (FGA), *Forensic Sci. Int.* 86 (1997) 25–33.
- [11] A. Moller, B. Brinkmann, Locus ACTBP2 (SE33): sequencing data reveal considerable polymorphism, *Int. J. Leg. Med.* 106 (1994) 262–267.
- [12] A. Müller, M. Schurenkamp, B. Brinkmann, Evaluation of an ACTBP2 ladder composed of 26 sequenced alleles, *Int. J. Leg. Med.* 108 (1995) 75–78.
- [13] B. Rolf, M. Schurenkamp, A. Junge, B. Brinkmann, Sequence polymorphism at the tetranucleotide repeat of the human beta-actin related pseudogene H-beta-Ac-psi-2 locus, *Int J. Leg. Med* 110 (1997) 69–72.
- [14] M.V. Lareu, C.P. Phillips, C. Pestoni, F. Berros, J. Munoz, A. Carracedo, Anomalous electrophoretic behaviour of HUMACTBP2 (SE33), in: W. Bär, A. Fiori, U. Rossi (Eds.), *Advances in Forensic Haemogenetics*, 1993, pp. 121–123.
- [15] P. Gill, B. Brinkmann, E. d'Aloja, J. Andersen, W. Bär, A. Carracedo, B. Dupuy, B. Eriksen, M. Jangblad, V. Johnsson, A.D. Kloosterman, P. Lincoln, N. Morling, S. Rand, M. Sabatier, R. Scheithauer, P. Schneider, M.C. Vide, Considerations of STR nomenclature by the European DNA Profiling group (EDNAP), *Forensic Sci. Int.* 87 (1997) 185–192.