WHAT'S HIDING BETWEEN THE PRIMERS? USING MASSIVELY PARALLEL SEQUENCING TO CAPTURE STR REPEAT REGION AND FLANKING REGION SEQUENCE VARIATION

Nicole Novroski[1], Jennifer Churchill[1], Jonathan King[1], and Bruce Budowle[1, 2]

[1]Institute of Applied Genetics, Department of Molecular and Medical Genetics, University of North Texas Health Science Center, 3500 Camp Bowie Blvd., Fort Worth, Texas 76107, USA
[2]Center of Excellence in Genomic Medicine (CEGMR), King Abdulaziz University, Jeddah, Saudi Arabia

Correspondence should be sent to Nicole Novroski, Nicole.novroski@live.unthsc.edu

Introduction

The current standard methodology in forensic DNA typing relies on amplification of short tandem repeat (STR) markers by the polymerase chain reaction (PCR) and allele sizes (i.e., length-based) determined for each locus using capillary electrophoresis (CE). Massively parallel sequencing (MPS), also known as next generation sequencing (NGS), allows high throughput sequencing of STR amplicons, which can identify nominal length-based (LB) genetic variation but equally as well inter-allelic sequence (sequence-based; SB) variation. The increased effective number of alleles per marker for some STR loci improves discrimination power, which may be invaluable in some cases of kinship analysis and for mixture de-convolution. Furthermore, allelic variation captured using MPS may be useful towards understanding of STR mutations and their rates and may contribute to evolutionary studies using STR markers.

One issue with current massively parallel sequencing (MPS) is the need to capture flanking region sequence variation to exploit the full power of forensically relevant STR loci. The application of such data will increase our knowledge and understanding of each locus, gain additional genetic information about population specific genetic parameters, increase the power of discrimination of a locus, and potentially aid in mixture de-convolution efforts. The underlying genetic variation needs to be described through studies on various population groups.

Allele sequence variation that resides within repeat and flanking regions will be presented on 27 autosomal, 7 X chromosome and 24 Y chromosome STRs identified in 777 unrelated individuals from four population groups. These markers were sequenced using the ForenSeq™ DNA Signature Prep Kit (Illumina) and MiSeq (Illumina) benchtop sequencer using the Forensic Genomics (FGx)™ software system. Data were analyzed with the supporting ForenSeq™ Universal Analysis Software (UAS) and supplemented with STRait Razor and in-house excel workbooks.

Methods

Samples, Extraction and Quantification

Whole blood samples were obtained from 777 unrelated individuals from four major population groups (US Caucasian, N=210; Hispanic, N=198; African American, N=200; and East Asian, i.e., Chinese, N=169). DNA was extracted using the Qiagen® QIAamp™ DNA Mini Kit and quantitated using the Qubit® 2.0 Fluorimeter.

ForenSeq™ Library Preparation and MiSeq Sequencing

The ForenSeq™ DNA Signature Prep Kit was used to barcode and create libraries for DNA sample batches of 32-34 samples per library, including controls. Sequencing was performed on the MiSeq Desktop Sequencer using the MiSeq FGx Forensic Genomics System (Illumina).

Sequence Variation Identification

Characterization of STR data was performed using a modified version of STRait Razor 2.0 and in-house Excel-based workbooks. A minimum coverage threshold of 5X was used for STR allele-calling. A stutter threshold of 20% was used for initial screening. Alleles were reported using nomenclature recommended by ISFG (Parson *et al*). Sequence variants were described as either pre-existing or novel, based on whether or not they have been characterized in the literature, and further defined by the location of the sequence variant, as either a repeat region (RR) variant or a flanking region (FR) variant.

Concordance Testing

Concordance testing was performed by CE generated genotypes using the GlobalFiler® PCR Amplification Kit and AmpFLSTR® Yfiler® PCR Amplification Kit on a subset of the population samples (n=170 for Globalfiler; n=59 for Yfiler). Discordance was defined as any instance in which an allele detected by one approach was not observed above the operationally defined coverage threshold by the comparison approach and/or the sequence was not the same.

Results

Novel sequence variants were identified in STR repeat regions and flanking regions. Four general categories of variation were observed with the SB information for the 777 population samples.

First, the effective increase in allele number due to sequence variation compared with alleles characterized by repeat number (or length) alone was due predominately to internal sequence variation present within the repeat regions of some of the STR loci. Consistent with the literature, the loci D2S1338, D12S391, and D21S11 exhibit the largest contribution to increased diversity via sequence variation in the RR. Among the four populations studied, there were 50 additional alleles detected in the D2S1338 locus; 60 additional alleles found at the D12S391 locus; and 63 additional alleles observed in the D21S11 locus. Other loci, such as D8S1179 and D6S1043, also had a number of sequence variant alleles, and most variants observed were different from the currently published repeat motifs.

Second, some loci demonstrated an effective increase in allele number only when FR sequence information was included. This category of variation includes STR loci in which the repeat regions of the alleles did not display sequence differences, but did show substantial variability in the flanking regions surrounding the repeat regions of interest. For example, the loci D7S820, D13S317, and D22S1045 did not have many sequence variant alleles observed within their RRs; however within the FRs, all three loci exhibited at least a 40% increase in the total number of alleles.

Third, several loci showed either RR variation or FR sequence variation in the SB alleles. The loci D18S51, DXS10135, and DYS385a-b are three examples in which there were alleles that contained both RR sequence and FR sequence variation.

Fourth, some loci such as DYS643, Y-GATA-H4 and TPOX did not display any effective increase in diversity using MPS beyond that which was observed by CE.

Of note, ~400 alleles from 46 loci were observed in this study which have not yet been reported in the scientific literature, illustrating the additional degree of genetic variation that exists within

some of the most commonly used STR markers. By capturing flanking sequence information, correct repeat annotation for loci, such as vWA and D13S317, is possible. This method also allowed for resolution of indels present within the primer regions of traditional PCR-CE kits, allowing for better characterization of repeat regions of alleles and increased power of discrimination due to these flanking region variants. Allele frequencies, heterozygosities, and other forensic parameters by population were calculated.

There were examples of MPS data that rely solely on RR variation that were discordant with operationally defined CE-based data. In total, there were only 2 instances out of 7980 comparisons (170 samples x 20 loci x 2 alleles, assuming 2 alleles for a homozygote plus 59 samples x 18 Y-STR loci x 1 allele, assuming hemizygous loci and 59 samples X 1 Y-STR locus X 2 alleles (DYS385)) where a "potential mismatch" in allele designation occurred. The first was an allele with repeat [TATC]11 at the D7S820 locus where an upstream single nucleotide deletion caused the allele designation by CE to be a 10.3. If MPS analysis only targets the repeat region, this allele would be called an 11. The other example was observed at the D22S1045 locus that was designated as a 15.1 allele by CE while the true RR number is a 15.

Conclusions

The population data described in this study demonstrate that there is variation and substantial novel variation within repeat regions and/or flanking regions of a number of STR markers, whereas a few loci present little to no additional discrimination power using MPS. While the current forensically relevant STR loci were not selected based on total genetic variation, moving forward it may be worthwhile to consider inclusion of STR loci that offer additional discrimination power in the form of RR and/or FR sequence variation.

Current PCR-CE genotyping methods are able to address the major portion of the current needs of the forensic community. However higher throughput, increased resolution, and better mixture de-convolution of complex biological samples are still needed. While the data described herein are sufficient to use for calculating the strength of STR results from casework evidence, the data are still only a small representation of the variation that likely exists for these loci. Sequence data currently does not exist in DNA databases, therefore sequence-based DNA profiles will likely for the time being be used for 1:1 comparisons of suspect or victim to evidence.

This study is the first of its kind to thoroughly evaluate flanking and repeat region sequence variation for 58 STR loci in five populations, which will prove invaluable to the forensic community for future investigations, including kinship analyses and mixture de-convolution assays. Continued efforts to establish population-based databases of these markers are essential for a greater understanding of STR diversity.

Related Publication

Novroski, N.M., King, J.L., Churchill, J.D., Seah, L.H., Budowle, B. Characterization of genetic sequence variation of 58 STR loci in four major population groups, Forensic Sci. Int. Genet. 25 (2016) 214-226.

Acknowledgements

This work was supported in part by award no. 2015-DN-BX-K067, awarded by the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this publication are those of the authors and do not necessarily reflect those of the U.S. Department of Justice.

References

1. K.B. Gettings, R.A. Aponte, P.A. Vallone, J.M. Butler. STR allele sequence variation: Current knowledge and future issues. Forensic Sci. Int. Genet. 18 (2015) 118-130.
2. C. Børsting, N. Morling. Next generation Sequencing and its applications in forensic genetics, Forensic Sci. Int.Genet. 18 (2015) 78-89.

3. S. Dalsgaard, E. Rockenbauer, C. Gelardi, C. Børsting, S.L. Fordyce, N. Morling. Characterization of mutations and sequence variations in complex STR loci by second generation sequencing. Forensic Sci. Int.Genet. Supplement Series 4 (2013) e218-e219.

4. C. Van Neste, F. Van Nieuwerburgh, D. Van Hoofstat, D. Deforce. Forensic STR analysis using massively parallel sequencing. Forensic Sci. Int. Genet 6 (2012) 810-818.

5. D.W. Craig, J.V. Pearson, S. Szelinger, A. Sekar, M. Redman, J.J. Corneveaux, T.L. Pawlowski, T. Laub, G. Nunn, D.A. Stephan, N. Homer, M.J. Huentelman. Identification of genetic variants using barcoded multiplex sequencing. Nature Methods (2008) 887-893.

6. S.L. Friis, A.Buchard, E. Rockenbauer, C. Børsting, N. Morling. Introduction of the Python script STRinNGS for analysis of STR regions in FASTQ or BAM files and expansion of the Danish STR sequence database to 11 STRs, Forensic Sci. Int. Genet. 21 (2016) 68-75.

7. K.B. Gettings, K.M. Kiesler, S.A. Faith, E. Montano, C.H. Baker, B.A. Young, R.A. Guerrieri, P.M. Vallone. Sequence variation of 22 autosomal STR loci detected by next generation sequencing, Forensic Sci. Int. Genet. 21 (2016) 15-21.

8. W. Parson, D. Ballard, B. Budowle, J.M. Butler, K.B. Gettings, P. Gill, L. Gusmão, D.R. Hares, J.A. Irwin, J.L. King, K.P. de Knijff, N. Morling, M. Prinz, P.M. Schneider, C. Van Neste, S. Willuweit, C. Phillips. Massively Parallel Sequencing of forensic STRs: Considerations of the DNA Commission of the International Society for Forensic Genetics (ISFG) on minimal nomenclature requirements, Forensic Sci. Int. Genet. 22 (2016) 54-63.

9. M. Klintschar, Z. Kozma, N. Al Hammadi, M. Abdull Fatah, C. Nöhammer. A study on the short tandem repeat systems HumCD4, HumTH01 and HumFIBRA in population samples from Yemen and Egypt, Int. J. Legal Med. 111 (1998) 107-109.

10. F.R. Wendt, J.D. Churchill, N.M.M. Novroski, J.L. King, J. Ng, R.F. Oldt, K.L. McCulloh, J.A. Weise, D.G. Smith, S. Kanthaswamy, B. Budowle. Genetic analysis of the Yavapai Native Americans from West-Central Arizona using the Illumina MiSeq FGx™ forensic genomics system, Forensic Sci. Int. Genet. 24 (2016) 18-23.

11. K.J. van der Gaag, R.H. de Leeuw, J. Hoogenboom, J. Patel, D.R. Storts, J.F.J. Laros, P. de Knijff. Massively Parallel Sequencing of Short Tandem Repeats – Population data and mixture analysis results for the PowerSeq™ system, Forensic Sci. Int. Genet. 2016 in press.

12. M.C. Kline, C.R. Hill, A.E. Decker, J.M. Butler. STR Sequence analysis for characterizing normal, variant, and null alleles, Forensic Sci. Int. Genet. 5 (2011) 329-332.

13. C. Allor, D.D. Einum, M. Scarpetta. Identification and Characterization of Variant Alleles at CODIS STR Loci, J. Forensic Sci. 50 (2005) 1128-1133.

14. A. Moller, E. Meyer, B. Brinkmann. Different types of structural variation in STRs: HumFES/FPS, HumVWA, and HumD21S11, Int. J. Legal Med. 106 (1994) 319-323.

15. B. Brinkmann, A. Sajantila, H.W. Goedde, H. Matsumoto, K. Nishi, P. Wiegand. Population genetic comparisons among eight populations using allele frequency and sequence data from three microsatellite loci, Eur. J. Hum. Genet. 4 (1996) 175-182.

16. M.D. Barber, B.J. McKeown, B.H. Parkin. Structural variation in the alleles of a short tandem repeat system at the human alpha fibrinogen locus, Int. J. Legal Med. 108 (1996) 180-185.

17. B. Brinkmann, E. Meyer, A. Junge. Complex mutational events at the HumD21S11 locus, Hum. Genet. 98 (1996) 60-64.

18. A.M. Lins, K.A. Micka, C.J. Sprecher, J.A. Taylor, J.W. Bacher, D. Rabbach, R.A. Bever, S. Creacy, J.W. Schumm. Development and population study of an eight-locus short tandem repeat (STR) multiplex system, J. Forensic Sci. 43 (1998) 1168-1180.

19. R.A.L. Griffiths, M.D. Barber, P.E. Johnson, S.M. Gillbard, M.D. Haywood, C.D. Smith, J. Arnold, T. Burke, A. Urquhart, P. Gill. New reference allelic ladders to improve allelic designation in a multiplex STR system, Int. J. Legal Med. 111 (1998) 267-272.

20. A. Kido, M. Hara, Y. Yamamoto, H. Kameyama, R. Susukida, K. Saito, A. Takada, M. Oya. Nine short tandem repeat loci analysis in aged semen stains using the AmpFLSTR Profiler Kit and description of a new vWA variant allele, Leg. Med (Tokyo) 5 (2003) 93-96.

21. C. Cruz, T. Ribeiro, C. Vieira-Silva, I. Lucas, R. Espinheira, H. Geada. vWA STR locus structure and variability, International Congress Series 1261 (2004) 248-250.

22. E.M. Dauber, W. Bär, M. Klintschar, F. Neuhuber, W. Parson, E. Mueller-van der Spruit, W.R. Mayr. New sequence data of allelic variants at the STR loci ACTBP2 (SE33), D21S11, FGA, vWA, CSF1PO, D2S1338, D16S539, D18S51 and D19S433 in Caucasoids, International Congress Series 1261 (2004) 191-193.

23. P. Grubwieser, R. Muhlmann, H. Niederstatter, M. Pavlic, W. Parson. Unusual variant alleles in commonly used short tandem repeat loci, Int. J. Legal Med. 119 (2005) 164-166.

24. M. Heinrich, H. Felske-Zech, B. Brinkmann, C. Hohoff. Characterisation of variant alleles in the STR systems D2S1338, D3S1358 and D19S433, Int. J. Legal Med. 119 (2005) 310-313.

25. S. Hering, R. Nixdorf, J. Edelmann, C. Thiede, J. Dreβler. Further sequence data of allelic variants at the STR locus ACTBP2 (SE33): Detection of a very short off ladder allele, International Congress Series 1288 (2006) 810-812.

26. E.M. Dauber, G. Dorner, S. Wenda, E.M. Schwartz-Jungl, B. Glock, W. Bär, W.R. Mayr. Unusual FGA and D19S433 off-ladder alleles and other allelic variants at the STR loci D8S1132, vWA, D18S51 and ACTBP2 (SE33), Forensic Sci. Int. Genet. Suppl. Series 1 (2008) 109-111.

27. E.M. Dauber, E.M. Schwartz-Jungl, S. Wenda, G. Dorner, B. Glock, W.R. Mayr. Further allelic variation at the STR-loci ACTBP2 (SE33), D3S1358, D8S1132, D18S51 and D21S11, Forensic Sci. Int. Genet. Suppl. Series 2 (2009) 41-42.

28. C. Phillips, L. Fernandez-Formoso, M. Garcia-Magarinos, L. Porras, T. Tvedebrink, J. Amigo, M. Fondevila, A. Gomez-Tato, J. Alvarez-Dios, A. Freire-Aradas, A. Gomez-Carballa, A. Mosquera-Miguel, A. Carracedo, M.V. Lareu. Analysis of global variability in 15 established and 5 new European Standard Set (ESS) STRs using the CEPH human genome diversity panel, Forensic Sci. Int. Genet. 5 (2011) 155-169.

29. C. Phillips, S. Kind, L. Fernandez-Formoso, M. Gelabert-Besada, A. Carracedo, M.V. Lareu Global population variability in Promega PowerPlex CS7, D6S1043, and Penta B STRs. Int. J. Legal Med. 127 (2013) 901-906.

30. M.V. Lareu, S. Barral, A. Salas, C. Pestoni, A. Carracedo. Sequence variation of a hypervariable short tandem repeat at the D1S1656 locus, Int. J. Legal. Med. 111 (1998) 244-247.

31. A. Morales-Valverde, S. Silva-De La Fuente, G. Nun˜ez-Rivas, M. Espinoza-Esquivel. Characterisation of 12 new alleles in the STR system D18S51. Forensic Sci. Int. Genet, SS2 (2009) 43-44.

32. M.V. Lareu, C. Pestoni, F. Barros, A. Salas, A. Carracedo. Sequence variation of a hypervariable short tandem repeat at the D12S391 locus. Gene 182 (1996) 151-153.

33. J.A. Bright, K.E. Stevenson, M.D. Coble, C.R. Hill, J.M. Curran, J.S. Buckleton. Characterising the STR locus D6S1043 and examination of its effect on stutter rates, Forensic Sci. Int. Genet. 8 (2014) 20-23.

34. C. Gelardi, E. Rockenbauer, S. Dalsgaard, C. Borsting, N. Morling. Second generation sequencing of three STRs D3S1358, D12S391 and D21S11 in Danes and a new nomenclature for sequenced STR alleles, Forensic Sci. Int. Genet. 12 (2014) 38-41.

35. L. Wang, X.C. Zhao, J. Ye, J.J. Liu, T. Chen, X. Bai, J. Zhang, Y. Ou, L. Hu, B.W. Jiang, F. Wang. Construction of a library of cloned short tandem repeat (STR) alleles as universal templates for allelic ladder preparation, Forensic Sci. Int. Genet. 12 (2014) 136-143.

36. J.D. Churchill, S.E. Schmedes, J.L. King, B. Budowle. Evaluation of the Illumina® Beta Version ForenSeq™ DNA Signature Prep Kit for use in genetic profiling, Forensic Sci. Int. Genet. 20 (2016) 20-29.

37. W. Wang, T. Kishida, M. Fukuda, Y. Tamaki. The Y-27H39 polymorphism in a Japanese population, Int. J. Legal Med. 109 (1996) 157-158.

38. Reference tables to: Evaluation of Y-chromosomal STRs: a multicenter study (Kayser et al.) and Chromosome Y microsatellites: population genetic and evolutionary aspects (de Knijff et al.), Int. J. Legal Med. 110 (1997) 141-149.

39. T. Kumoro, H. Tsutsumi, R. Mukoyama, M. Nakamura. Repeat Structure of DYS389 Locus, Nippon Hoigaku Zasshi (The Japanese Journal of Legal Medicine) 52(4) (1998): 227-232. [Article in Japanese]

40. Q. Ayub, A. Mohyuddin, R. Qamar, K. Mazhar, T. Zerjal, S.Q. Mehdi, C. Tyler-Smith. Identification and characterization of novel human Y-chromosomal microsatellites from sequence database information, Nucleic Acids Research 28(2) (2000): e8.

41. J.M. Butler, R. Schoske, P.M. Vallone, M.C. Kline, A.J. Redd, M.F. Hammer. A novel multiplex for simultaneous amplification of 20 Y chromosome STR markers, Forensic Sci. Int. 129 (2002) 10-24.

42. A.J. Redd, A.B. Agellon, V.A. Kearney, V.A. Contreras, T. Karafet, H. Park, P. de Knijff, J.M. Butler, M.F. Hammer. Forensic value of 14 novel STRs on the human Y chromosome, Forensic Sci. Int. 3460 (2002) 1-15.

43. R. Schoske, P.M. Vallone, M.C. Kline, J.W. Redman, J.M. Butler. High-throughput Y-STR typing of U.S. populations with 27 regions of the Y chromosome using two multiplex PCR assays, Forensic Sci. Int. 139 (2004) 107-121.

44. J.M. Butler, A.E. Decker, P.M. Vallone, M.C. Kline. Allele frequencies for 27 Y-STR loci with U.S. Caucasian, African American, and Hispanic samples, Forensic Sci. Int. 156 (2006) 250-260.

45. T. Komuro, H. Tsutsumi, R. Mukoyama, M. Nakamura. Repeat Structure of DYS389 Locus, Nippon Hoigaku Zasshi (The Japanese Journal of Legal Medicine) 52 (1998) 227-232.

46. M.E. D'Amato, L. Ehrenreich, K. Cloete, M. Bejeddou, S. Davison. Characterization of the highly discriminatory loci DYS449, DYS481, DYS518, DYS612, DYS626, DYS644 and DYS710, Forensic Sci. Int. Genet. 4 (2010) 104-110.

47. E. Bosch, A.C. Lee, F. Calafell, E. Arroyo, P. Henneman, P. de Knijff, M.A. Jobling. High resolution Y chromosome typing: 19 STRs amplified in three multiplex reactions. Forensic Sci. Int. 125 (2002) 42-51.

48. P.S. White, O. L. Tatum, L.L. Deaven, J. L. Longmire. New, Male-Specific Microsatellite Markers from the Human Y Chromosome, Genomics 57 (1999) 433-437.

49. P.M. Schneider, S. Meuser, W. Waiyawuth, Y. Seo, C. Rittner. Tandem repeat structure of the duplicated Y-chromosomal STR locus DYS385 and frequency studies in the German and three Asian populations. Forensic Sci.Int. 97 (1998) 61-70.

50. D.H. Warshauer, J.D. Churchill, N. Novroski, J.L. King, B. Budowle. Novel Y-chromsome Short Tandem Repeat Variants Detected Through the use of Massively Parallel Sequencing, Genomics Proteomics Bioinformatics 13 (2015) 250-257.

51. J. Edelmann, S. Hering, M. Michael, R. Lessig, D. Deichsel, G. Meier-Sundhausen, L. Roewer, I. Plate, R. Szibor. 16 X-chromosome STR loci frequency data from a German population, Forensic Sci. Int. 124 (2001) 215-218.

52. J. Edelmann, D. Deichsel, S. Hering, I. Plate, R. Szibor. Sequence variation and allele nomenclature for the X-linked STRs DXS9895, DXS8378, DXS7132, DXS6800, DXS7133, GATA172D05, DXS7423 and DXS8377, Forensic Sci. Int. 129 (2002) 99-103.

53. M.T. Zarrabeitia, T. Amigo, C. Sañudo, M.M. de Pancorbo, J.A. Riancho. Sequence structure and population data of two X-linked markers: DXS7423 and DXS8377, Int. J. Legal Med. 116 (2002) 368-371.

54. S. Hering, C. Augustin, J. Edelmann, M. Heidel, J. Dressler, H. Rodig, E. Kuhlisch, R. Szibor. DXS10079, DXS10074 and DXS10075 are STRs located within a 280-kb region of Xq12 and provide stable haplotypes useful for complex kinship cases, Int. J. Legal Med. 120 (2006) 337-345.

55. I. Gomes, R. Pereira, W.R. Mayr, A. Amorim, A. Carracedo, L. Gusmão. Evaluation of DXS9902, DXS7132, DXS6809, DXS7133, and DXS7423 in humans and chimpanzees: sequence variation, repeat structure, and nomenclature, Int. J. Legal Med. 123 (2009) 403-412.

56. J.E. Sim, H.Y. Lee, W.I. Yang, K-J. Shin. Population genetic study of four closely-linked X-STR trios in Koreans, Mol Biol Rep 37 (2010): 333-337.

57. T.M. Diegoli, M.D. Coble. Development and characterization of two mini-X chromosomal short tandem repeat multiplexes, Forensic Sci. Int. Genet. 5 (2011) 415-421.

58. I. Gomes, A. Brehm, L. Gusmão, P.M. Schneider. New sequence variants detected at DXS10148, DXS10074 and DXS10134 loci, Forensic Sci. Int. Genet. 20 (2016) 112-116.

59. J.V. Planz, K.A. Sannes-Lowery, D.D. Duncan, S. Manalili, B. Budowle, R. Chakraborty, S.A. Hofstadler, T.A. Hall. Automated analysis of sequence polymorphism in STR alleles by PCR and direct electrospray ionization mass spectrometry, Forensic Sci. Int. Genet. 6 (2012) 594-606.

60. M.D. Barber, B.H. Parkin. Sequence analysis and allelic designation of the two short tandem repeat loci D18S51 and D8S117, Int. J. Legal Med. 109 (1996) 62-65.

61. P. Gill, C.P. Kimpton, A. Urquhart, N.J. Oldroyd, E.S. Millican, S.K. Watson, T.J. Downes. Automated short tandem repeat (STR) analysis in forensic casework--a strategy for the future, Electrophoresis 16 (1995) 1543-1552.

62. A. Amorim, L. Gusmao, M.J. Prata, Population and formal genetics of the STRs TPO, TH01 and VWFA31/A in North Portugal. Adv. in For. Haemogenetics 6 (1996) 486-488

63. E. Momhinweg, C. Luckenbach, R. Fimmers, H. Ritter. D3S1358: Sequence analysis and gene frequency in a German population, Forensic Sci. Int. 95 (1998) 173-178.

64. R. Szibor, S. Lautsch, I. Plate, K. Bender, D. Krause. Population genetic data of the STR HumD3S1358 in two regions of Germany, Int. J. Legal. Med. 111 (1998) 160-161.

65. H-G. Zhou, K. Sato, Y. Nishimaki, L. Fang, H. Hasekura. The HumD21S11 system of short tandem repeat DNA polymorphisms in Japanese and Chinese, Forensic Sci. Int. 86 (1997) 109-118.

66. S.J. Walsh, S.L. Robinson, G.R. Turbett, M.P. Davies, A.N. Wilton. Characterisation of variant alleles at the HumD21S11locus implies unique Australasian genotypes and re-classification of nomenclature guidelines, Forensic Sci. Int. 135 (2003) 35-41.

67. K.K. Kidd, A.J. Pakstis, W.C. Speed, R. Legacé, J. Chang, S. Wootton, E. Haigh, J.R. Kidd. Current sequencing technology makes microhaplotypes a powerful new type of genetic marker for forensics, Forensic Sci. Int. Genet. 12 (2014) 215-224.

68. D. Becker, H.Rodig, C. Augustin, J. Edelmann, F. Götz, S. Hering, R. Szibor, W. Brabetz. Population genetic evaluation of eight X-chromosomal short tandem repeat loci using Mentype Argus X-8 PCR amplification kit. Forensic Sci. Int. Genet. 2 (2008) 69-74.

69. Qiagen, QIAamp® DNA Mini and Blood Mini Handbook, June 2012.

70. Thermo Fisher Scientific, Qubit® 2.0 Fluorimeter User Manual, October 2010.

71. Illumina® ForenSeq™ DNA Signature Prep Reference Guide, August 2014.

72. Illumina® ForenSeq™ Universal Analysis Software Guide, June 2015.

73. D.H.Warshauer, J.L. King, B. Budowle. STRait Razor v2.0: The improved STR Allele Identification Tool – Razor, Forensic Sci. Int. Genet. 14 (2015) 182-186.

74. Genetic Data Analysis software, 1996, http://en.bio-soft.net/dna/gda.html

75. X. Zeng, J.L. King, M. Stoljarova, D.H. Warshauer, B.L LaRue, A. Sajantila, et al., High sensitivity multiplex short tandem repeat loci analyses with massively parallel sequencing, Forensic Sci. Int. Genet. 16 (2015) 38-47.

76. Integrative Genomics Viewer (IGV) – Broad Institute (2013) https://www.broadinstitute.org/igv/

77. National Institute of Justice STRBase, www.cstl.nist.gov/strbase/

78. F.R. Wendt, X. Zeng, J.D. Churchill, J.L. King, B. Budowle. Analysis of Short Tandem Repeat and Single Nucleotide Polymorphism Loci from Single-Source Samples Using a Custom HaloPlex Target Enrichment System Panel, Am J Forensic Med Pathol. 37 (2016) 99-107.

79. R. Szibor, S. X-chromosomal markers: Past, present and future, Forensic Sci. Int. Genet. 1 (2007) 93-99.

80. J. Cullen, K. Nordtvedt. World Haplogroup & Haplo-I Subclade Predictor, 2008, http://members.bex.net/jtcullen515/haplotest.htm

81. M. Bodner, I. Bastisch, J.M. Butler, R. Fimmers, P. Gill, L. Gusmão, N. Morling, C. Phillips, M. Prinz, P.M. Schneider, W. Parson. Recommendations of the DNA Commission of the International Society for Forensic Genetics (ISFG) on quality control of autosomal Short Tandem Repeat allele frequency databasing (STRidER), Forensic Sci. Int. Genet. 24 (2016) 97-102.