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Research article

Results of the GEP-ISFG collaborative study on an X-STR Decaplex

L. Gusmão [1,a,*], C. Alves [1,a], P. Sánchez-Diz [1,b], M.T. Zarrabeitia [1,c], M.A. Abovich [1,d], I. Aragón [1,e], B. Arce [1,j], G. Arrieta [1,z], E. Arroyo [1,f], I. Atmetlla [1,A], C. Baeza [1,f], M.C. Bobillo [1,g], L. Cainé [1,h], R. Campos [1,i], L. Caraballo [1,k], E. Carvalho [1,l], M. Carvalho [1,m], R.M.B. Cicarelli [1,n], D. Comas [1,D], D. Corach [1,g], M. Espinoza [1,z], M.R. Espinheira [1,o], F. Rendo [1,p], O. García [1,q], I. Gomes [1,a,b], A. González [1,r], A. Hernández [1,s], M. Hidalgo [1,g], P. Lozano [1,e], M. Malaghini [1,t], D. Manzanares [1,r], B. Martínez [1,k], J.A. Martins [1,n], K. Maxzud [1,u], I. Miguel [1,p], N. Modesti [1,u], M. Montesino [1,v], R. Ortiz [1,w], J.J. Pestano [1,i], M.F. Pinheiro [1,h], L. Prieto [1,v], E. Raimondi [1,x], J.A. Riancho [1,c], M.B. Rodríguez [1,d], I. Salgado [1,y], N. Salgueiro [1,y], J.J. Sánchez [1,s], S. Silva [1,A], U. Toscanini [1,x], C. Vidales [1,B], C.V. Silva [1,o], M.C. Villalobos [1,w], C. Vullo [1,C], I. Yurrebaso [1,q], A.I. Zubillaga [1,B], A. Carracedo [1,b], A. Amorim [1,a]

[a] *IPATIMUP (Institute of Pathology and Molecular Immunology of University of Porto), Portugal*
[b] *Instituto de Medicina Legal, Universidade de Santiago de Compostela, A Coruña, Spain*
[c] *Universidad de Cantabria, Unidad de Medicina Legal and Departamento de Medicina, Spain*
[d] *Banco Nacional de Datos Genéticos, Buenos Aires, Argentina*
[e] *Biologia Molecular Ltda (BIOMOL Ltda), Bogota, Colombia*
[f] *Departamento de Toxicología y Legislación Sanitaria, Facultad de Medicina, Universidad Complutense de Madrid, Spain*
[g] *Servicio de Huellas Digitales Genéticos (SHDG) and Cátedra de Genética y Bioquímica Molecular, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Argentina*
[h] *Serviço de Genética e Biologia Forense, Delegação do Porto do Instituto Nacional de Medicina Legal, Portugal*
[i] *Laboratorio de Genética Forense, Instituto Anatómico Forense de Las Palma, ULPGC, Islas Canarias, Spain*
[j] *Departamento de Biología, Servicio de Criminalística, Dirección General de la Guardia Civil, Madrid, Spain*
[k] *Laboratorio de Genetica Molecular, Instituto de Investigaciones Inmunologicas de la Universidad de Cartagena, Colombia*
[l] *Laboratório de Diagnósticos por DNA, Instituto de Biologia, Universidade do Estado do Rio de Janeiro, Brazil*
[m] *Serviço de Genética e Biologia Forense, Delegação de Coimbra, Instituto Nacional de Medicina Legal, Portugal*
[n] *Laboratório de Investigação de Paternidade, Faculdade de Ciências Farmacêuticas, UNESP - Universidade Estadual Paulista, Araraquara, Brazil*
[o] *Serviço de Genética e Biologia Forense, Delegação de Lisboa. Instituto Nacional de Medicina Legal, Portugal*
[p] *Departamento de Genética, Antropología Física y Fisiología Animal, Unidad de Secuenciación y Genotipado, Servicio Genómica, Bizkaia, Univ. País Vasco (UPV/EHU), Spain*
[q] *Sección de Genética Forense, Area de Laboratorio Ertzaintza, Bizkaia, Spain*
[r] *ADF TecnoGen S.L., Madrid, Spain*
[s] *Instituto Nacional de Toxicología y Ciencias Forenses, Delegación de Canarias, Spain*
[t] *Lab. Frischmann Aisengart SA, Setor de Biologia Molecular, Curitiba, Brazil*
[u] *Laboratório de Biología Molecular do CEPROCOR - Agencia Córdoba Ciencia, Complejo Hospitalario Santa María de Punilla, Córdoba, Argentina*
[v] *Comisaría General de Policía Científica, Laboratorio de Biología, Madrid, Spain*
[w] *Unidad de Diagnóstico Molecular, Departamento de Bioquímica, Facultad de Medicina, Universidad Autónoma de Nuevo León, Monterrey, Mexico*
[x] *PRICAI-Fundación Favaloro, Buenos Aires, Argentina*
[y] *GDPN-Genética Médica e Diagnóstico Pré-Natal Prof. Doutor Sérgio Castedo, S.A. Porto, Portugal*

* Corresponding author at: IPATIMUP (Institute of Pathology and Molecular Immunology of University of Porto), Population Genetics, Rua Dr. Roberto Frias s/n, 4200-465 Porto, Portugal. Tel.: +351 225570700; fax: +351 225570799.
E-mail address: lgusmao@ipatimup.pt (L. Gusmão).
URL: http://www.ipatimup.pt
[1] On behalf of the GEP-ISFG (The Spanish-Portuguese Working Group of the International Society for Forensic Genetics).

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[z] Departamento de Ciencias Forenses, Sección de Bioquímica, Unidad de Genética Forense, Poder Judicial, Costa Rica
[A] Laboratorio de Análisis Clínicos y Moleculares, LACYM S.A. and CIBCM, San José, Costa Rica
[B] Unidad de Genética Molecular, Policlínica Gipuzkoa, San Sebastián, Spain
[C] Laboratorio de Inmunogenética y Diagnóstico Molecular (LIDMO), Córdoba, Argentina
[D] Unitat de Biologia Evolutiva, Universitat Pompeu Fabra, Barcelona, Spain

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Abstract

A collaborative work was carried out by the Spanish and Portuguese ISFG Working Group with a PCR multiplex for X chromosome STRs. Markers were selected among those described as polymorphic in humans and that have been used by some laboratories in forensics. Primers and various technical methods were investigated with the aim of optimizing a multiplex for the 10 selected X-STRs. Primer mix stock solutions were sent to the laboratories that were asked to analyse two female bloodstains, taking as reference the genetic profiles from 9947A, 9948 and NA3657 samples. In this work, we report the results obtained by 30 GEP-ISFG laboratories, using this Decaplex, as well as alternative technical conditions that also produced good results.

Keywords: X-STRs; GEP-ISFG; Multiplex PCR

1. Introduction

Many forensic laboratories have recently introduced the analysis of X-STR markers in their routine, due to the advantages in the use of these markers in cases of complex kinship analysis [1,2]. Nevertheless, PCR multiplex strategies, population data, mutation rates and other relevant genetic information concerning X-STRs are still scarce. In the last GEP-ISFG quality control (www.gep-isfg.org), in 2006, an increasing number of participating labs (11 out of 116) reported results on the analyses of X-STRs. However, from the 19 different markers reported, only 6 of them were typed by, at least, 5 labs (the minimum established by the GEP for a marker to be evaluated). Moreover, nomenclature discrepancies were detected in two loci: HPRTB and DXS8377.

In order to overcome these problems, the GEP decided to organize a collaborative exercise, consisting on the evaluation of an X-STR Decaplex including: DXS8378, DXS9898, DXS7133, GATA31E08, GATA172D05, DXS7423, DXS6809, DXS7132, DXS9902 and DXS6789 (Fig. 1).

2. Material and methods

Markers were selected taking into account the gene diversity values reported in different populations; and the potential for multiplexing. Preference was given to simple rather than complex STRs, following the ISFG recommendations concerning locus selection for forensic applications [3]. In order to reduce the potential PCR-generated slippage artifacts, trinucleotide repeats were avoided. To evaluate the optimized multiplex, primer mix stock solutions were sent to the participating laboratories and were asked to analyse two female bloodstains, using the protocol available at www.gep-isfg.org/ISFG/Castellano/Grupos_de_trabajo/Cromosomas_sexuales/crx.php.

3. Results and discussion

Of the 30 participating laboratories 23 followed the proposed amplification protocol. The remaining seven used alternative methods to perform the multiplex reaction with the same primer mix. Therefore, instead of the QIAGEN Multiplex PCR kit (Qiagen), some participants chose to setup the PCR

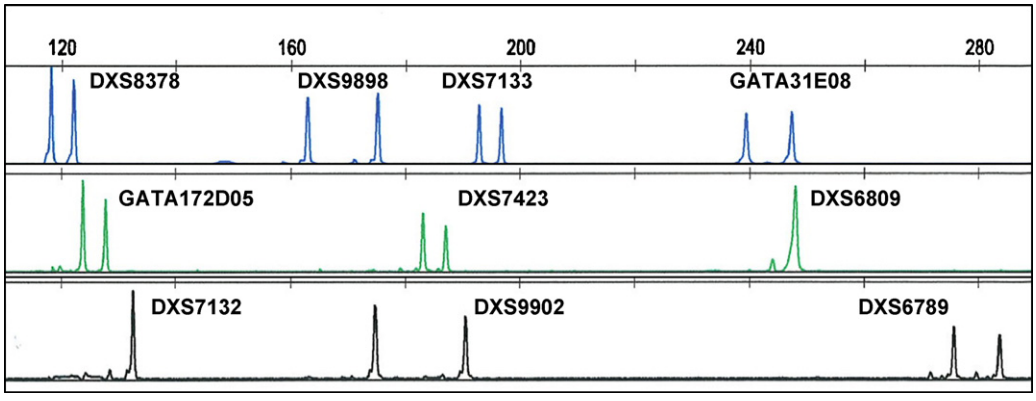

Fig. 1. Electropherogram of a female profile for the X-STR Decaplex.

multiplex using buffers and Taq DNA polymerases already available in their labs. Namely, three used Taq Gold Polymerase (Applied Biosystems); two used the Platinum Taq DNA Polymerase (Invitrogen); one used the Phusion High-Fidelity DNA Polymerase (BioLabs); and finally one used the Taq polymerase from Highway Molecular Biology (Argentina).

In spite of the different amplification methods employed, 27 out of the 30 participating laboratories reported identical results for the 2 control samples for all 10 markers.

Three labs presented different profiles for both control samples (in several markers), but in all cases the recommended protocol and/or nomenclature were not followed.

It is worth mentioning that two additional laboratories correctly typed the control samples in three PCR multiplex reactions, to allow detection using conventional electrophoresis and silver-staining techniques.

This newly constructed Decaplex has proved to be technically very robust since most laboratories successfully type the distributed samples.

Since population and mutation data are crucial for forensic evaluation of this new multiplex, and both are lacking for this set of markers, the next step of this collaborative work will be data compilation in Iberian and South American populations. In order to assure the quality of results, only the laboratories that correctly typed the distributed control samples in the first part of this work (27 out of the 30 participating laboratories) will collaborate in data collection.

Conflict of interest

None.

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