



Species identification in routine casework samples using the SPInDel kit

Filipe Pereira^{a,*}, Cíntia Alves^b, Cátia Couto^a, Lourdes López Díaz^c, David Parra^c, Sandra Furfuro^d, Mercedes Aler^e, Luis Burillo Borrego^f, Tereza Olekšáková^g, Filipa Balsa^h, Lisa Sampaio^h, Maria João Anjos Porto^h, Heloisa Afonso Costaⁱ, Cristina Arévalo Voss^j, Mariela Caputo^k, Daniel Corach^k, Óscar García^l, Susana Pedrosa Moro^m, Rui Pereiraⁿ, António Amorimⁿ

^a IDENTIFICA, Science and Technology Park of the University of Porto - UPTEC, Porto, Portugal

^b University of Porto, Faculty of Sciences, Porto, Portugal

^c Servicio de Criminalística de la Guardia Civil, Departamento de Medio Ambiente, Madrid, Spain

^d Universidad Nacional de Cuyo Facultad de Ciencias Médicas, Laboratorio de Análisis de ADN, Mendoza, Argentina

^e Servicio de Laboratorio, Instituto de Medicina Legal y Ciencias Forenses, Sección de genética y Criminalística, Valencia, Spain

^f Instituto de Medicina Legal y Ciencias Forenses de Valencia, Sección de Biología, Servicio de Laboratorio, Valencia, Spain

^g Institute of Criminalistics, Prague, Czech Republic

^h Delegação do Centro- Instituto Nacional de Medicina Legal e Ciências Forenses- I.P., Serviço de Genética e Biologia Forenses, Coimbra, Portugal

ⁱ Delegação do Sul do Instituto Nacional de Medicina Legal e Ciências Forenses, Serviço de Genética e Biologia Forenses, Lisboa, Portugal

^j Comisaría general de Policía Científica- Cuerpo Nacional de Policía Spain, Laboratorio de ADN, Madrid, Spain

^k Universidad de Buenos Aires- Facultad de Farmacia y Bioquímica, Departamento de Microbiología- Inmunología- Biotecnología y Genética - Cátedra de Genética Forense y Servicio de Huellas Digitales Genéticas SHDG - CONICET., Buenos Aires, Argentina

^l Basque Country Police-Ertzaintza, Forensic Science Unit- Forensic Genetics Section, Erandio-Bizkaia, Spain

^m NASERTIC, Unidad de Laboratorio de Navarra de Servicios y Tecnologías, Villava, Spain

ⁿ Universidade do Porto, Instituto de Investigação e Inovação em Saúde- I3S, Porto, Portugal

ARTICLE INFO

Keywords:

Non-human DNA
Forensics
mtDNA

ABSTRACT

The identification of species in casework samples is of fundamental importance for forensic investigations. Laboratories are increasingly compelled to provide accurate and fast identifications in trace materials left on crime scenes, wildlife poaching, illegal trade of protected species, fraudulent food products cases, etc. However, the field of nonhuman forensic genetics is still working on the standardization of typing methods and practices. Here we describe the successful implementation of the Species Identification by Insertions/Deletions (SPInDel) method in routine casework analyses in 11 laboratories worldwide. The SPInDel was developed to detect human DNA, at the same time that identifies common animal species. The fragment size analysis of six mtDNA regions allows identification in suboptimal DNA samples, including mixtures, with no need for sequencing. The samples were collected from 2013 to 2018 and included hair, blood, meat, saliva, faeces, bones, etc. The SPInDel kit successfully identified > 95% of the samples, being dog, human and pig the most frequently detected species. The six SPInDel loci were successfully amplified in mixtures and degraded samples (river water, sand, stains in clothes, etc.). Interestingly, several species that were not originally targeted by SPInDel primers were also identified (e.g., red fox, brown bear, fallow deer and red deer). In conclusion, the SPInDel kit was successfully used in crime scene investigations (often involving human DNA detection) and in cases of poaching, environmental contamination and food fraud. It is now becoming a useful tool for the routine analysis of nonhuman DNA samples within the high quality standards of forensic genetics.

1. Introduction

The Species Identification by Insertions/Deletions (SPInDel) method is a multiplex PCR for species identification purposes that uses conventional genotyping methodology similar to that employed with Short

Tandem Repeats (STRs). Species are identified by numeric profiles of fragment lengths determined using capillary electrophoresis [1]. The method has been validated in several laboratories worldwide [2,3].

* Corresponding author.

E-mail address: fpereirapt@gmail.com (F. Pereira).

<https://doi.org/10.1016/j.fsigss.2019.09.070>

Received 15 September 2019; Accepted 24 September 2019

Available online 27 September 2019

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2. Methods

The SPInDel was used according to the recommended protocol described before [2,3]. Data regarding the use of the methods was collected in a questionnaire sent to all participating laboratories.

3. Results

The SPInDel was used in 11 laboratories from Spain (n = 20), Argentina (n = 5), Portugal (n = 3) and Czech Republic (n = 1). The method was used to identify the species present in 29 samples. The cases were analysed in 2013 (n = 3), 2015 (n = 4), 2016 (n = 6), 2017 (n = 9) and 2018 (n = 7). The samples were collected in crime scenes (n = 12), environmental sets (n = 9), food products (n = 3) or other situations (n = 5). DNA collected from various types of biological samples or materials were successfully analysed: blood (n = 4), hairs (n = 4), clothes (n = 4), muscle (n = 4), swabs (n = 4), vomit (n = 1), scats (n = 1), bone (n = 1), sand (n = 1), water (n = 1), among others (n = 5).

The SPInDel was able to identify the species in 28 out of the 29 samples. Mixtures were detected in eight samples: human and dog (n = 2); human and horse (n = 1); cat and dog (n = 1); human and sheep (n = 1), human, cat and dog (n = 1), human, pig and goat (n = 1), cattle, pig and sheep (n = 1). A total of 12 species were detected in the samples: dog (n = 8), human (n = 7), pig (n = 6), cattle (n = 4), goat (n = 3), cat (n = 2), sheep (n = 2), brown bear (n = 2), red deer (n = 1), fallow deer (n = 1), horse (n = 1) and red fox (n = 1).

The laboratories obtained complete profiles (i.e., amplification of six regions) in 25 out of the 29 samples. One sample was identified with five SPInDel markers and two samples with four markers.

4. Discussion

The SPInDel kit proved to be a useful laboratory procedure, often as a preliminary test prior to other analyses. For example, the kit proved that hairs found in a fire pit were from horse, not a missing person in the area. In another case, a heart was found in a box and the SPInDel

proved it was from a pig. The method was also used in cases of poaching. For example, the SPInDel proved that the blood in the trousers of a suspect of poaching was from a protected wild goat. In another case, the SPInDel proved that a hunting trap had fox DNA.

5. Conclusion

The SPInDel provided results in 97% of the casework samples. Successful amplifications were achieved in low quality DNA samples. Moreover, mixtures were easily identified, including cases of mixtures of three species. Interestingly, species not tested before using SPInDel were successfully identified, such as the brown bear, red deer or fallow deer. Overall, the method proved to be a useful laboratory tool for the detection of human DNA in forensic samples. Moreover, it allowed the identification of the species in a single reaction, helping forensic laboratories to answer their requests in a fast and rigorous way.

Declaration of Competing Interest

Nothing to declare.

Acknowledgments

We thank all the participating laboratories for providing the data necessary for this study.

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