



ORIGINAL ARTICLE

Forensic parameters of the Powerplex® 21 system (Promega Corp.) in mestizo populations from the West of Mexico[☆]



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Abstract

Introduction: Microsatellites or short tandem repeats (STR) are the markers of choice in human identification. For this purpose, they must be analyzed in populations. This task has been scarcely performed for new generation human identification systems, which is critical in Mexican mestizo populations, because a significant genetic structure has been demonstrated due to admixture differences.

Objective: To validate the system in mestizo populations from the west region of Mexico.

Methods: A total of 374 unrelated Mexican-mestizos from the west region were analyzed with the Powerplex® 21 system. Genetic data were analyzed using different softwares and including additional related populations for comparison purposes.

Results and conclusions: We estimated allele frequencies, and genotype distribution of all markers was in agreement with Hardy-Weinberg expectations; the linkage disequilibrium test discarded association between all pair of loci. The following forensic parameters were estimated: power of discrimination (PD), power of exclusion (PE), heterozygosity (Het), polymorphism information content (PIC), and typical paternity index (PI); its combined power of discrimination and exclusion was ~100% and 99.99999473%, respectively. This constitutes an

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important increment with respect to typical 15 STR systems for the same population/region. This report validates the Powerplex® 21 system in western Mexican-mestizos for confident interpretation of DNA profiles in forensic cases and paternity testing. Sistema Powerplex® 21; México; Identificación humana; Poblaciones.

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PALABRAS CLAVE

Short Tandem
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Identificación
humana;
Poblaciones

Parámetros forenses del sistema Powerplex® 21 (Promega Corp.) en población mestiza del occidente de México

Resumen

Introducción: Los microsatélites o *short tandem repeats* son los marcadores de elección en identificación humana, para lo cual se deben analizar en las poblaciones. Esta tarea se ha realizado escasamente con los sistemas genéticos de nueva generación, lo cual es crítico en las poblaciones mestizas de México, ya que se ha demostrado una subestructura genética significativa por diferencias en sus componentes de mezcla.

Objetivo: Hacer la validación poblacional del sistema de análisis genético Powerplex® 21 en mestizos de la región occidente de México.

Material y métodos: Se analizaron 374 individuos no emparentados residentes de la región occidente de México con el sistema Powerplex® 21. Los datos genéticos se analizaron con diversos programas estadísticos y se compararon con poblaciones de referencia.

Resultados y conclusiones: Se determinaron las frecuencias alélicas y la distribución de genotipos de todos los marcadores estuvieron en equilibrio Hardy-Weinberg; la prueba de desequilibrio de ligamiento descartó asociaciones entre pares de loci. Se estimaron los siguientes parámetros de interés forense para el sistema PowerPlex® 21: poder de discriminación (PD), poder de exclusión (PE), heterocigosidad (Het), contenido de información polimórfica (PIC) e índice de paternidad (IP) típico, cuyo poder de discriminación y exclusión combinado fue -100% y 99,99999473%, respectivamente. Esto constituye un incremento importante respecto a lo ofrecido por sistemas tradicionales con 15 short tandem repeats para la misma población/región. La información reportada valida el uso del sistema PowerPlex® 21 en mestizos del occidente de México para interpretar de forma confiable perfiles de ADN en pruebas de paternidad y casos forenses.

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Introduction

Microsatellites, or short tandem repeats (STRs) are elective human identification markers, the most important of which are the 13 STRs of the CODIS (Combined DNA Index System) that the FBI has identified for creating databases for administering justice.¹ Also of note are the 15 autosomal STRs included in the AmpF/STR Identifier kit² of *Applied Biosystems* (now *Life Technologies*) and the Powerplex 16 System³ of *Promega, Corp.*, the main validated genetic systems that have become the normal tool for resolving forensic tests and paternity tests around the world.

In addition to technical validation,^{2,3} population studies also need to be conducted in order to implement STRs, which include estimates of various statistical parameters with a forensic interest.⁴ Within populations, allele frequencies and minimum allele frequencies need to be estimated, and the population in which the genetic analysis system is going to be used needs to be in Hardy-Weinberg equilibrium (HWE), since this will make it possible to use the squared

binomial formula to estimate the frequency of genotypes based on allele frequencies. In addition to this, it is also necessary to discard associations between pairs of loci by verifying the linkage disequilibrium (LD), which it makes it possible to use the product rule to estimate the frequency of genetic profiles, inter alias.⁴ In addition to this, it is also helpful to estimate the statistical parameters that are of forensic interest that indicate the a priori usefulness expected for each locus and for the genetic system as a whole, such as heterozygosity (Het), power of exclusion (PE), power of discrimination (PD), polymorphic information content (PIC) and typical paternity index (TPI). Meanwhile, with regard to inter-population verifications, validation of STRs usually includes making comparisons with nearby populations to define their genetic relationships, structure and even an understanding of their origins. In short, the forensic parameters and the population validation make it possible to reliably use these systems to estimate the frequency of a genetic profile in a criminal case or to calculate the probability of paternity when an alleged father matches a child

in a paternity test. This is why population studies have been conducted and databases have been generated throughout the world using STRs.¹

In spite of the elevated power of exclusion and discrimination of these kits, there are situations where the 15 STRs are insufficient for resolving certain cases, such as paternity tests where the mother has not participated,⁵ and others. Therefore, more powerful genetic analysis systems have been developed, such as the Powerplex® 21, the Powerplex® Fusion (Promega) and the Globalfiler™ (Life Technologies).⁶ The problem with implementing these new kits is the scant amount of databases, which is particularly acute in Mexico due to the significant differences that have been shown between geographical regions.⁷ In order to understand the origin of these differences, it is important to understand that the majority of the Mexican population (~90%) is mestizo due to the biological interbreeding that started with the discovery of America around 500 years ago, which occurred between Europeans, mainly Spaniards, Native American or indigenous populations, and to a lesser degree, Africans who had been initially brought as slaves following the conquest of Mexico.⁷ The National Institute of Anthropology and History defines a mestizo as someone who was born in Mexico with a surname derived from a Spanish surname, and whose family has Mexican ancestors who can be tracked back to three generations. Indeed, the STRs used in human identification have shown an increase in the European component in populations in the northwest region, whereas the indigenous

component increases towards central and south-eastern Mexico.⁷

In view of this, in this study we will report on the forensic parameters of the Powerplex® 21 system commercial kit (Promega, Corp),⁸ which includes the 13 STRs of the CODIS (D3S1358, TH01, D21S11, D18S51, D7S820, D5S818, D13S317, D16S539, CSF1PO, VWA, D8S1179, TPOX, FGA), five other tetranucleotide markers (D1S1656, D6S1043, D2S1338, D12S391, D19S433), and two pentanucleotide markers (Penta E and Penta D), plus amelogenin as a gender marker. The results were compared with reference populations available in the literature.

Methodology

Population sample

374 bloodstains were analyzed from unrelated individuals residing in various states in the western region of Mexico. The following numbers of individuals were included from the following states: Aguascalientes (n=71), Colima (n=16), Jalisco (n=207), Michoacán (n=65) and Nayarit (n=17) (Fig. 1). All of them declared themselves to be Mexican mestizos as they did not belong to an indigenous group nor were they foreigners. Before being included in the study, the participants signed a letter of informed consent in accordance with the Helsinki Declaration. These populations have



Figure 1 Geographic location and size of the sample (n) of mestizo population in western Mexico analysed in this study, and of the only population studied previously.

been classified as being in the western region due to previous studies in Mexican mestizos,⁷ in which a significant differentiation was observed between geographical regions but not between populations in the same region.

Laboratory tests

The DNA that was used came from dry bloodstains on FTA® paper that was washed with FTA Purification Reagent solution (Whatman Inc.). A punch was taken from the FTA paper that was directly used as a DNA template for amplification with the Powerplex® 21 System (Promega, Corp). The PCR products were subjected to capillary electrophoresis in an ABI Prism® 3130 Genetic Analyzer sequencer (Applied Biosystems), and the genotypes were defined using GeneMapper® ID Software v3.2 (Applied Biosystems). The protocols were based on the manufacturer's recommendations.⁸

Statistical analysis

The allele frequencies were determined and the genotype frequency matches were verified with the HWE for each STR, and the DL between loci pairs were verified using exact Fisher assays with 5000 permutations using the programme *Genetic Data Analysis* (GDA v 1.3).⁹ The statistical parameters of forensic interest (PD, PE, IPT, Het and PIC) were determined using a PowerStats spreadsheet.¹⁰ For the inter-population study, results from the previous study on mestizos in Mexico City were added,¹¹ together with those of different North American racial groups (U.S.A.).¹² GDA was also used to estimate the co-ancestry coefficient between population pairs as a measure of genetic distance. These data were represented graphically on a Neighbour Joining tree using the programme Treeview.¹³

Results and discussion

The allele frequencies of the 20 autosomal STRs in the Powerplex® 21 System were estimated (Table 1

). Even though they are the most basic parameter, allele frequencies are nevertheless the most useful data that forensic geneticists use for bio-statistical interpretation of each of their paternity tests and forensic cases where there are matches. In these tests, minimum allele frequencies are crucial for conservatively interpreting cases of null alleles or rare alleles: i.e., giving the accused the benefit of the doubt.⁴

The genotype distribution of the 20 STRs was in HWE ($p \geq 0.0205$). The DL assay likewise had insignificant results in most of the 182 loci pair combinations (data not shown). It bears noting that the five combinations with the lowest probability results for the DL assay included D21S11 ($0.00031 \leq p \leq 0.00281$). Yet when the Bonferroni correction was applied, and due to the findings being isolated ($5/182 = 2.74\%$), there was no evidence showing DL between loci. On one hand, the results validate the use of the squared binomial formula for estimating the genotype frequency based on the allele frequencies (HWE) or vice versa. On the other hand, they also support the use of the product rule for estimating the frequency of a genetic profile or for calculating paternity indices with the 20 STRs of the Powerplex®

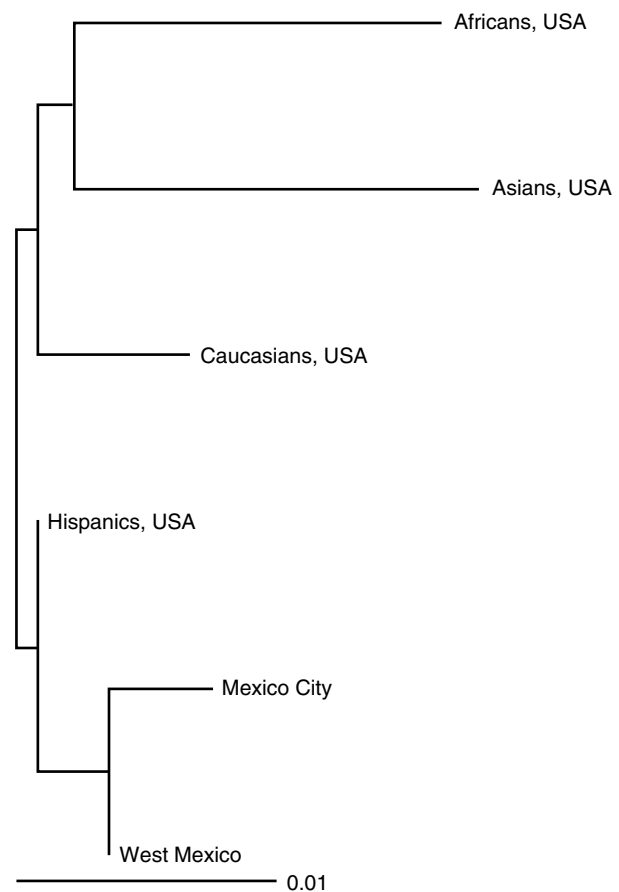


Figure 2 Neighbour joining tree representing the genetic distances between two Mexican mestizo populations (western Mexico and Mexico City) and racial groups of North America (U.S.A., USA in the figure).

21 system. In addition to this, the adjustment to HWE and the linkage equilibrium validate the grouping that was done in this project with populations from the western region, as described in the preliminary comprehensive study of Mexican mestizo populations.⁷

The various statistical parameters of forensic interest were estimated for each of the 20 loci (Table 2). For the system of 20 STRs, the combined PD was practically 100%, while the combined PE was 99.99999473%. These percentages represent a significant advance compared to what was reported with 15 STRs in three Mexican populations (including western Mexico), where the lower limit of the combined PD was noted to be lower >99.9999% as was that of the combined PE >99.9987%.¹⁴

The genetic distances were estimated (not shown) that are shown in a Neighbour Joining tree (Fig. 2). In general, a result can be observed that is congruent with the historical/evolutionary background history, since Mexican populations are most closely related with each other, followed by Spaniards, Caucasians (Europeans), Asians and Africans respectively. There were no anthropological discussions of these findings since this was not the purpose of the study, and due to the limits on the number of reference populations for conducting a more in-depth analysis, such as miscegenation or structure.

Table 1 Allele frequencies of the Powerplex® 21 system (Promega) in the mestizo population of western Mexico.

Alleles	D3S1358	D1S1656	D6S1043	D13S317	Penta E	D16S539	D18S51	D2S1338	CSF1PO	Penta D
5					0.029					
6										
7					0.082				0.001	0.001
8				0.080	0.033	0.009			0.008	0.012
9				0.183	0.007	0.110			0.013	0.144
9.3										
10		0.004	0.008	0.084	0.028	0.171	0.007		0.271	0.223
10.3										
11		0.033	0.180	0.223	0.080	0.258	0.012		0.291	0.144
11.2										
12	0.001	0.096	0.168	0.249	0.190	0.298	0.122		0.345	0.177
12.2										
13	0.019	0.079	0.116	0.118	0.087	0.144	0.114		0.063	0.204
13.2										
14	0.071	0.096	0.182	0.062	0.084	0.009	0.171	0.001	0.004	0.060
14.2										
14.3		0.003								
15	0.412	0.140	0.024	0.001	0.098		0.178		0.003	0.013
15.2										
15.3		0.029								
16	0.258	0.213	0.007		0.088		0.096	0.029		
16.2										
16.3		0.041								
16.4					0.003					
17	0.135	0.041	0.040		0.067		0.128	0.174		
17.2										
17.3		0.154								
17.4					0.003					
18	0.098	0.009	0.084		0.032		0.071	0.076		
18.3		0.041								
19	0.005	0.001	0.056		0.021		0.055	0.217		
19.2										
19.3		0.015	0.004							
20	0.001		0.031		0.025		0.015	0.152		
20.2										
20.3		0.001	0.012				0.011	0.020		
21			0.005		0.017					
21.2										
21.3			0.053							
22			0.013		0.016		0.013	0.067		0.020
22.2										
22.3			0.012							
23			0.003		0.008		0.005	0.138		
23.2										
24					0.001		0.003	0.071		
24.3										
25								0.047		
26		0.001						0.008		
27										
28										
29										
30										
30.2										
31										
31.2										
32										
32.2										
33										
33.2										
35										
35.2										
FAM	0.0076	0.0084	0.0086	0.0083	0.0087	0.0080	0.0085	0.0081	0.0077	0.0082

Table 1 (Continued)

Alleles	TH01	VWA	D21S11	D7S820	D5S818	TPOX	D8S1179	D12S391	D19S433	FGA
5	0.001									
6	0.255					0.004				
7	0.312			0.019	0.060					
8	0.078			0.106	0.007	0.537	0.005			
9	0.131			0.076	0.040	0.064	0.005		0.003	
9.3	0.209									
10	0.015			0.267	0.046	0.059	0.107		0.001	
10.3				0.001						
11				0.277	0.445	0.233	0.052		0.003	
11.2									0.004	
12				0.187	0.284	0.102	0.099	0.001	0.074	
12.2									0.012	
13		0.004		0.057	0.109	0.001	0.344		0.202	
13.2									0.076	
14		0.072		0.007	0.007		0.243		0.263	
14.2									0.055	
14.3										
15		0.079		0.001	0.001		0.120	0.021	0.148	0.001
15.2									0.086	
15.3										
16		0.326			0.001		0.024	0.024	0.037	
16.2									0.027	
16.3										
16.4										
17		0.282						0.075	0.005	
17.2									0.003	
17.3								0.021		
17.4										
18		0.167						0.194	0.001	0.013
18.3								0.015		
19		0.063						0.225		0.058
19.2										0.003
19.3								0.004		
20		0.007						0.175		0.074
20.2										0.001
20.3										
21								0.096		0.126
21.2				0.001						
21.3										0.001
22								0.068		0.146
22.2										0.004
22.3										0.003
23								0.041		0.130
23.2										0.005
24								0.021		0.166
24.3										0.003
25								0.016		0.153
26								0.001		0.074
27			0.020							0.029
28			0.108							0.007
29			0.187							0.003
30			0.242							
30.2			0.016							
31			0.084							
31.2			0.120							
32			0.020							
32.2			0.142							
33			0.001							
33.2			0.055							
35			0.001							
35.2			0.003							
FAM	0.0078	0.0077	0.0084	0.0080	0.0074	0.0070	0.0079	0.0085	0.0083	0.0088

FAM: minimum allele frequency.

Table 2 Parameters of forensic interest for the Powerplex® 21 system (Promega) in the mestizo population of western Mexico.

STR	D3S1358	D1S1656	D6S1043	D13S317	PentaE	D16S539	D18S51	D2S1338	CSF1PO	PentaD
PD	0.885	0.971	0.970	0.942	0.983	0.917	0.970	0.964	0.861	0.949
PE	0.480	0.700	0.732	0.674	0.759	0.588	0.727	0.623	0.512	0.626
Het	0.732	0.852	0.869	0.839	0.882	0.794	0.866	0.812	0.751	0.814
PIC	0.691	0.867	0.863	0.800	0.902	0.748	0.864	0.846	0.665	0.808
TPI	1.87	3.40	3.81	3.11	4.25	2.42	3.74	2.67	2.01	2.69
HWE	0.7713	0.5895	0.5376	0.2081	0.1849	0.4778	0.4914	0.1467	0.8032	0.7695
STR	TH01	VWA	D21S11	D7S820	D5S818	TPOX	D8S1179	D12S391	D19S433	FGA
PD	0.909	0.915	0.957	0.927	0.864	0.821	0.923	0.961	0.956	0.970
PE	0.535	0.502	0.706	0.588	0.411	0.283	0.538	0.721	0.679	0.780
Het	0.764	0.746	0.855	0.794	0.689	0.593	0.766	0.863	0.842	0.892
PIC	0.734	0.736	0.831	0.767	0.659	0.595	0.754	0.842	0.826	0.867
TPI	2.12	1.96	3.46	2.42	1.60	1.23	2.14	3.66	3.16	4.66
HWE	0.0852	0.5255	0.4215	0.5385	0.0205	0.0220	0.2469	0.4530	0.2815	0.0993

HWE: hardy Weinberg equilibrium, exact probability of the assay; TPI: typical paternity index; Het: heterozygosity; PD: power of discrimination; PE: power of exclusion; PIC: polymorphic information content.

In conclusion, this is the first study to conduct a population validation of the Powerplex® 21 system in mestizos from western Mexico to verify its reliability for implementation for human identification.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

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References

- Butler JM. Genetics and genomics of core short tandem repeat loci used in human identity testing. *J Forensic Sci.* 2006;51:253–65.
- Collins PJ, Hennessy LK, Leibelt CS, Roby RK, Reeder DJ, Foxall PA. Developmental validation of a single-tube, amplification of the 13 CODIS STR loci, D2S1338, D19S433, and amelogenin: the AMPF®STR Identifiler PCR amplification kit. *J Forensic Sci.* 2004;49:1–13.
- Krenke BE, Tereba A, Anderson SJ, Buel E, Culhane S, Finis CJ, et al. Validation of 16-locus fluorescent multiplex system. *J Forensic Sci.* 2002;47:1–13.
- García Fernández O. Parámetros en identificación forense y criminalística biológica. In: Martínez Jarreta MB, editor. *La prueba del ADN en medicina forense: la genética al servicio de la ley en el análisis de indicios criminales y en la investigación biológica de la paternidad.* Barcelona: Masson; 1999. p. 286–95.
- von Wurmb-Schwark N, Mályusz V, Simeoni E, Lignitz E, Poetsch M. Possible pitfalls in motherless paternity analysis with related putative fathers. *Forensic Sci Int.* 2006;159:92–7.
- Butler JM, Hill CR, Coble MD. Variability of new STR loci and kits in U.S. population groups. Profiles in DNA. Available from: <http://www.promega.com/resources/articles/profiles-in-dna/2012/variability-of-new-str-loci-and-kits-in-us-population-groups> [accessed 29.03.15].
- Rubi-Castellanos R, Martínez-Cortés G, Muñoz-Valle JF, González-Martín A, Cerda-Flores RM, Anaya-Palafox M, et al. Pre-Hispanic Mesoamerican demography approximates the present-day ancestry of Mestizos throughout the territory of Mexico. *Am J Phys Anthropol.* 2009;139:284–94.
- Powerplex® 21 System Technical Manual (TMD034) Revised 7/14. Available from: <https://worldwide.promega.com/resources/protocols/technical-manuals/101/powerplex-21-system-protocol> [accessed 29.03.15].
- Lewis PO, Zaykin D. Genetic Data Analysis (GDA): computer program for the analysis of allelic data version 1.1. Available from: <http://www.eeb.uconn.edu/people/plewis/software.php> [accessed 29.03.15].
- Tereba A. PowerStats. A computer program for the analysis of population statistics, 1999. Available from: <http://www.promega.com/geneticidtools> [accessed 29.03.15].
- Ramírez-Flores E, Saiz M, Villegas-Carmona D, Alvarez-Cubero MJ, Alvarez JC, Vega-Navarrete L. Genetic variation of 24 STR loci in a Mexican Mestizo population from Mexico D.F. *Forensic Sci Int Genet.* 2014;10:e4–6.
- Hill CR, Duewer DL, Kline MC, Coble MD, Butler JM. US population data for 29 autosomal STR loci. *Forensic Sci Int Genet.* 2013;7:e82–3.
- Page RD. Treeview 1.6.6. 2001. Available from: <http://taxonomy.zoology.gla.ac.uk/rod/rod.html> [accessed 29.03.15].
- Rubi-Castellanos R, Anaya-Palafox M, Mena-Rojas E, Bautista-España D, Muñoz-Valle JF, Rangel-Villalobos H. Genetic data of 15 autosomal STRs (Identifiler kit) of three Mexican Mestizo population samples from the States of Jalisco (West), Puebla (Center), and Yucatan (Southeast). *Forensic Sci Int Genet.* 2009;3:71–6.