

Evaluation of a 49 InDel Marker HID panel in two specific populations of South America and one population of Northern Africa

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Abstract The majority of STR loci are not ideal for the analysis of forensic samples with degraded and/or low template DNA. One alternative to overcome these limitations is the use of bi-allelic markers, which have low mutation rates and shorter amplicons. Human identification (HID) InDel marker panels have been described in several countries, including Brazil. The commercial kit available is, however, mostly suitable for Europeans, with lower discrimination

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power for other population groups. Recently, a combination of 49 InDel markers used in four different ethnic groups in the USA has been shown to be more informative than another panel from Portugal, already tested in a Rio de Janeiro sample. However, these 49 InDels have yet to be applied to other admixed or isolated populations. We assessed the efficiency of this panel in two urban admixed populations (Rio de Janeiro, Brazil; Tripoli, Libya) and one isolated Native Brazilian community. All markers are in Hardy-Weinberg equilibrium (HWE) after the Bonferroni correction, and no Linkage disequilibrium was detected. Assuming loci independence and no substructure effect, cumulative RMP was 2.7×10^{-18} , 1.5×10^{-20} , and 4.5×10^{-20} for Native Brazilian, Rio de Janeiro, and Tripoli populations, respectively. The overall F_{st} value was 0.05512. Rio de Janeiro and Tripoli showed similar admixture levels, however for Native Brazilians one parental cluster represented over 60 % of the total parental population. We conclude that this panel is suitable for HID on these urban populations, but is less efficient for the isolated group.

Keywords Forensic genetics · Tripoli · Libya · Rio de Janeiro · Brazil · Amerindian

Introduction

In human forensic genetics, short tandem repeat (STR) loci have been used to characterize biological evidence and as the core markers to establish a variety of databases. Despite their appealing features, amplicon size of most STR loci is between 100 and 450 bp, which is not ideal for the analysis of degraded and/or low template DNA samples [1]. In addition, STR loci have relatively high mutation rates that can complicate kinship analyses. An alternative to overcome these limitations of STRs is the use of bi-allelic markers, such as Single

Nucleotide Polymorphisms (SNPs) and insertion/deletion polymorphisms (InDels). These markers can be detected in shorter amplicons and have low mutation rates. Indeed, Kayser and de Knijff [2] point out that SNPs and InDels are alternative markers that perform substantially better than STRs for the analysis of degraded DNA samples. SNPs were the first bi-allelic markers developed [3]. However, this system requires the use of complicated methods, which often are laborious and do not allow a quantitative analysis of the data [4, 5]. In contrast, InDels can be analyzed using a protocol that is more technically convenient than those used for SNPs, because the capillary electrophoresis platform can be used for typing [6].

Various human identification (HID) panels using InDels have been reported [6–11]. The panel of InDels available in the Qiagen Investigator DIPplex® kit is most suitable for the identification of European individuals [7, 8, 12, 13] and has lower discrimination power for Chinese [14, 15], Taiwanese [13], Korean [16, 17], Somali [8], and Bangladeshi populations [18]. Recently, LaRue et al. [19] used a combination of 49 InDel markers to investigate four major ethnic groups in the USA. After correcting for the number of markers used, they concluded that their panel has a discrimination power three orders of magnitude higher than another panel with 38 InDels from Portugal [10], which has already been tested in a Rio de Janeiro sample group [11]. However, these 49 InDel markers with high discrimination power for the major populations of North America have yet to be assessed for other admixed or isolated populations.

In this study, the efficiency of the 49 InDel markers was investigated in two urban admixed population samples—Rio de Janeiro, Brazil, and Tripoli, Libya—and one isolated Native Brazilian community. This panel is suitable for HID on these urban populations, but is less efficient for the isolated group.

Material and methods

Samples

Buccal swabs and dried blood spots on FTA paper from unrelated individuals, 62 Native Brazilians from São Gabriel da Cachoeira, Amazon Basin, Brazil; 93 residents from Rio de Janeiro, Brazil; and 77 from Tripoli, Libya, were previously characterized by microsatellite markers [20–22]. The samples were collected and anonymized in accordance with the methods approved by the Ethics in Research Committee of Clementino Fraga Filho Hospital/UFRJ (CEP N° 536/10).

The region of the Northwest Amazon, which covers the basin of the Upper Rio Negro, between Brazil and Colombia, has been inhabited for the past 2000 years by ethnic groups who speak languages belonging to three linguistic families:

Arawak, Tukano, and Maku (<http://www.socioambiental.org>. Accessed 09/28/2014). The São Gabriel da Cachoeira area has a population of 37,896 individuals, 77 % of self-declared Native Americans (Brazilian National Population Survey, 2010). Samples were collected at the Militar Hospital and the ethnic groups were 79 % of Native Brazilians (Baré, Tukano, Pira-Tapuya, Baniwa-Kuripako, Tariana, Desana, Kubeo, Wanana, and Tuyuka) and 21 % did not declare or did not know their ethnicity.

DNA extraction

DNA was isolated from buccal swabs and dried blood spots using their respective manual protocols [23] or EZ-1 Instrument with DNA Investigator Kit (Qiagen, Hilden, Germany) according to the manufacturer's recommendations. The quantity of DNA was determined by qPCR using the Quantifiler Quantification Kit and 7500 Real-Time PCR System (Life Technologies). Samples were normalized to 400 pg/uL and stored at -20°C until amplification.

Amplification and analysis of the 49 InDel Markers

Samples containing 500 pg of DNA were analyzed. Each of four preliminary multiplexed primer sets using a Geneamp 9, 700 (Life Technologies) were amplified with an initial step at 95°C for 11 min followed by 28 cycles of 20 s at 94°C for denaturation and 3 min at 59°C for annealing/extension. A final extension step of 60°C for 60 min was employed to promote terminal adenylation. Each sample was prepared immediately prior to electrophoretic analysis and run on a 3500×1 Genetic Analyzer (Life Technologies) with an injection time of 10 s and an injection voltage of 3 kV. Electrophoretic data were analyzed using GeneMapper IDX (Life Technologies).

Statistical analysis

Allele frequencies were determined by the gene counting method. Population genetic parameters were analyzed using Genetic Data Analysis software [24]. Departures from Hardy–Weinberg Equilibrium (HWE) and Linkage Disequilibrium were tested using Fisher's exact test. Bonferroni correction for multiple comparisons and population substructure parameter (F_{st}) was estimated by the methods described in Weir & Cockerham [25].

Admixture analysis was performed using STRUCTURE v2.3 software [26] (Pritchard 2000) with no prior information on the origin of ancestral populations, employing the Admixture Model with correlated allelic frequencies and considering $K=3$. Results are presented for the replicate run with highest $\text{Ln P(D)}_{K=3} = -74.935$ (100,000 burns plus 100,000 MCMC repetitions).

Table 1 Description, location, and distribution of 49 InDels markers in three populations

Marker number	rs number	Native Brazilians, Brazil (<i>n</i> =62)				Rio de Janeiro, Brazil (<i>n</i> =93)				Tripoli, Libya (<i>n</i> =77)				
		Frequency of Deletion	H ₀	HWE (<i>p</i> value)	RMP	Frequency of Deletion	H ₀	HWE (<i>p</i> value)	RMP	Frequency of Deletion	H ₀	HWE (<i>p</i> value)	RMP	Fst
1	4187	0.3852	0.4754	1.0000	0.3892	0.5000	0.5435	0.5316	0.3750	0.4804	0.5098	1.0000	0.3754	0.0077
2	16,402	0.4262	0.3934	0.1235	0.3806	0.6141	0.4674	1.0000	0.3890	0.2192	0.3562	0.7368	0.4912	0.1586
3	16,458	0.5917	0.4500	0.5998	0.3838	0.4946	0.5109	1.0000	0.3750	0.5577	0.5385	0.5903	0.3784	0.0036
4	140,809	0.5574	0.4918	1.0000	0.3784	0.5924	0.4239	0.2806	0.3840	0.2945	0.3151	0.0489	0.4279	0.0986
5	1,160,886	0.3250	0.4500	1.0000	0.4113	0.3043	0.4565	0.6213	0.4221	0.4071	0.4714	0.8091	0.3841	0.0067
6	1,610,871	0.1750	0.2167	0.0678	0.5476	0.3352	0.4505	1.0000	0.4066	0.4933	0.6133	0.0669	0.3750	0.0919
7	2,067,140	0.4561	0.4211	0.2773	0.3769	0.3681	0.3846	0.1122	0.3942	0.4231	0.5000	1.0000	0.3811	0.0015
8	2,067,191	0.4083	0.4500	0.5964	0.3838	0.5163	0.5978	0.0932	0.3753	0.5704	0.4930	1.0000	0.3801	0.0170
9	2,307,507	0.3214	0.4643	0.7633	0.4130	0.5163	0.4674	0.5433	0.3753	0.3516	0.3906	0.2742	0.3999	0.0427
10	2,307,526	0.7000	0.4333	1.0000	0.4246	0.4022	0.5000	0.8322	0.3851	0.3630	0.4247	0.4468	0.3959	0.1097
11	2,307,579	0.3667	0.4333	0.5868	0.3947	0.3152	0.5217	0.0579	0.4161	0.4054	0.5135	0.6392	0.3844	0.0032
12	2,307,603	0.1083	0.2167	1.0000	0.6696	0.5435	0.5000	1.0000	0.3769	0.5486	0.4861	1.0000	0.3774	0.2108
13	2,307,656	0.5094	0.5660	0.4111	0.3751	0.4780	0.5165	0.8371	0.3755	0.5224	0.5075	1.0000	0.3755	-0.0049
14	2,307,696	0.5083	0.4167	0.2069	0.3751	0.5761	0.4565	0.5272	0.3810	0.3750	0.4722	1.0000	0.3921	0.0377
15	2,307,700	0.5351	0.5439	0.6023	0.3762	0.3913	0.4348	0.3938	0.3877	0.4342	0.5263	0.6359	0.3794	0.0134
16	2,307,710	0.2083	0.3500	1.0000	0.5035	0.3913	0.5652	0.1248	0.3877	0.3267	0.3867	0.2946	0.4105	0.0305
17	2,307,839	0.0833	0.1333	0.3270	0.7295	0.3696	0.4348	0.5077	0.3938	0.1233	0.1918	0.2837	0.6377	0.1417
18	2,307,850	0.1833	0.3333	0.6767	0.5356	0.1957	0.2826	0.3214	0.5191	0.2254	0.3099	0.3261	0.4846	-0.0042
19	2,308,112	0.3509	0.4912	0.7678	0.4002	0.3098	0.4891	0.2254	0.4190	0.5417	0.4444	0.3542	0.3768	0.0593
20	2,308,189	0.6140	0.4561	0.7855	0.3890	0.6467	0.3587	0.0385	0.3993	0.4167	0.4722	0.8140	0.3822	0.0577
21	2,308,196	0.5948	0.3276	0.0153	0.3845	0.3804	0.5000	0.6555	0.3905	0.7115	0.4231	1.0000	0.4318	0.1118
22	2,308,232	0.2105	0.3158	0.6931	0.5009	0.3098	0.3587	0.1451	0.4190	0.3359	0.5156	0.2734	0.4063	0.0113
23	2,308,276	0.4917	0.4167	0.1997	0.3751	0.4565	0.5217	0.6722	0.3769	0.4851	0.5224	0.8102	0.3752	-0.0055
24	2,308,292	0.4583	0.5500	0.6010	0.3768	0.2880	0.3804	0.4604	0.4320	0.1833	0.3333	0.6677	0.5356	0.0703
25	3,038,530	0.5877	0.5789	0.1839	0.3830	0.4130	0.4130	0.1895	0.3829	0.4437	0.6056	0.0903	0.3782	0.0246
26	3,042,783	0.6316	0.4912	0.7820	0.3941	0.5879	0.5385	0.3865	0.3831	0.7571	0.3143	0.2015	0.4674	0.0290
27	3,045,264	0.2193	0.3333	1.0000	0.4910	0.3641	0.3587	0.0421	0.3955	0.3116	0.3913	0.5801	0.4181	0.0172
28	3,047,269	0.4250	0.4500	0.6008	0.3808	0.4620	0.3587	0.0106	0.3765	0.5608	0.4730	0.8107	0.3788	0.0118
29	3,838,581	0.9000	0.2000	1.0000	0.6886	0.4457	0.5217	0.6725	0.3780	0.4375	0.4375	0.4554	0.3790	0.2351
30	3,841,948	0.1083	0.1833	0.5228	0.6696	0.3750	0.4457	0.6542	0.3921	0.4470	0.4697	0.8040	0.3779	0.1173
31	4,646,006	0.6250	0.4167	0.4147	0.3921	0.4185	0.5761	0.1309	0.3819	0.4769	0.4308	0.3155	0.3755	0.0369
32	10,623,496	0.3158	0.4561	0.7663	0.4158	0.3000	0.4222	1.0000	0.4246	0.4028	0.5556	0.2306	0.3850	0.0080
33	10,688,868	0.4500	0.4667	0.7935	0.3775	0.3132	0.4725	0.4745	0.4172	0.3151	0.4384	1.0000	0.4162	0.0174
34	13,447,508	0.3667	0.4000	0.2799	0.3947	0.3043	0.4348	1.0000	0.4221	0.2985	0.3284	0.0810	0.4255	-0.0011
35	17,859,968	0.5250	0.5167	1.0000	0.3756	0.3859	0.4457	0.6582	0.3890	0.4318	0.4697	0.8028	0.3798	0.0131
36	28,923,216	0.6583	0.3167	0.0228	0.4038	0.5489	0.4239	0.2108	0.3774	0.5833	0.4091	0.2109	0.3822	0.0057
37	33,951,431	0.8500	0.2000	0.1099	0.5875	0.6793	0.5109	0.1567	0.4134	0.5149	0.4627	0.6199	0.3752	0.1027
38	34,051,577	0.4000	0.5600	0.3682	0.3856	0.6703	0.3736	0.1502	0.4091	0.5571	0.3714	0.0516	0.3783	0.0622
39	34,495,360	0.3167	0.4333	1.0000	0.4154	0.5707	0.4891	1.0000	0.3801	0.7308	0.3846	1.0000	0.4453	0.1406
40	34,510,056	0.7917	0.3167	0.7031	0.5035	0.4837	0.4891	0.8420	0.3753	0.4318	0.6212	0.0449	0.3798	0.1246
41	34,511,541	0.7373	0.3220	0.1842	0.4503	0.3750	0.5109	0.5029	0.3921	0.4467	0.3867	0.0603	0.3779	0.1221
42	34,528,025	0.3158	0.3509	0.2212	0.4158	0.3846	0.4176	0.2715	0.3894	0.3099	0.3662	0.2666	0.4190	0.0016
43	34,535,242	0.7456	0.3684	0.7455	0.4572	0.6033	0.5543	0.1948	0.3863	0.7222	0.3611	0.3903	0.4390	0.0220
44	34,795,726	0.7895	0.3158	0.7021	0.5009	0.5380	0.5326	0.5425	0.3765	0.5643	0.4429	0.4778	0.3792	0.0640
45	34,811,743	0.9167	0.1333	0.3433	0.7295	0.6304	0.4565	0.8217	0.3938	0.7292	0.3750	0.7645	0.4441	0.0954
46	35,605,984	0.6852	0.3704	0.3438	0.4163	0.4286	0.5055	0.8295	0.3803	0.5423	0.4930	1.0000	0.3768	0.0560
47	35,716,687	0.7250	0.4167	1.0000	0.4410	0.5489	0.5761	0.1510	0.3774	0.5224	0.4776	0.8017	0.3755	0.0374
48	36,062,169	0.5167	0.5000	1.0000	0.3753	0.6413	0.5000	0.5012	0.3974	0.5000	0.5758	0.3235	0.3750	0.0201
49	60,901,515	0.5877	0.5789	0.1821	0.3830	0.6154	0.5714	0.0772	0.3894	0.6181	0.3750	0.0851	0.3901	-0.0060

Results and discussion

Population data and forensic parameters

A panel of 49 InDel loci was typed in three population samples: Native Brazilians ($n=62$) from the Amazon Basin in Brazil and two admixed samples from Rio de Janeiro ($n=93$) and Tripoli, Libya ($n=77$). A sample electropherogram of one of these multiplexes is shown in Supplementary Fig. 1. The results indicated that all markers are in Hardy–Weinberg equilibrium (HWE) except for one marker in Native Brazilians (marker 36, rs28923216); three markers for the Rio de Janeiro population (markers 20, rs2308189; 27, rs3045264; and 28, rs3047269), and one marker for the Libyans (marker 40, rs34510056) (Table 1). This number of departures is no more than would be expected to occur by chance. In addition, there were no significant departures from HWE after the Bonferroni correction ($\alpha' \approx 0.05/49, p < 0.001$). To measure population differentiation due to substructure, the three groups were analyzed for Wright's F_{st} estimates. Even though these three populations are not expected to mix due to their distance apart and characteristics, when combined for analysis purposes only, the combined F_{st} value of the populations is relatively low, $F_{st}=0.05512$. This value was higher than that reported for the North American sample populations [19]. Overall, the results reveal that the markers constitute a suitable system for HID to be used with the two urban groups. However, the panel was less efficient for the isolated community.

The power of an InDel panel is related to the number of markers with a Random Match Probability (RMP) near or below 0.4 (considering the ideal value of $p=q=0.5$, RMP is 0.375). For all markers, the RMP varied from 0.3 to 0.73 in the populations tested (Table 1). Assuming loci independence and no substructure effect, cumulative RMPs were 2.7×10^{-18} , 1.5×10^{-20} , and 4.5×10^{-20} for Native Brazilian, Rio de Janeiro, and Tripoli populations, respectively. The number of InDel loci above and below, an RMP of 0.4, was varied by population (Fig. 1). There were 23 markers above this threshold in Native Brazilians, and only 12 and 16 markers in the Rio de Janeiro and Tripoli samples, respectively.

Linkage disequilibrium (LD) was determined using Fisher's exact test, with 10,000 shuffling [24]. For the 49 InDel markers, there were 1176 possible pairwise comparisons per population sample. A total of four pairs had a detectable LD at the 0.05 level, two from Rio de Janeiro and two from Native Brazilians (Supplementary Table S1). This proportion of detectable LD was lower than expected by chance. In addition, there were no significant departures from independence after the Bonferroni correction. The lack of detectable LD supports that the product rule can be applied. Cumulative RMP values were 1.5×10^{-20} and 4.5×10^{-20} for the Rio de Janeiro and Tripoli populations, respectively. These

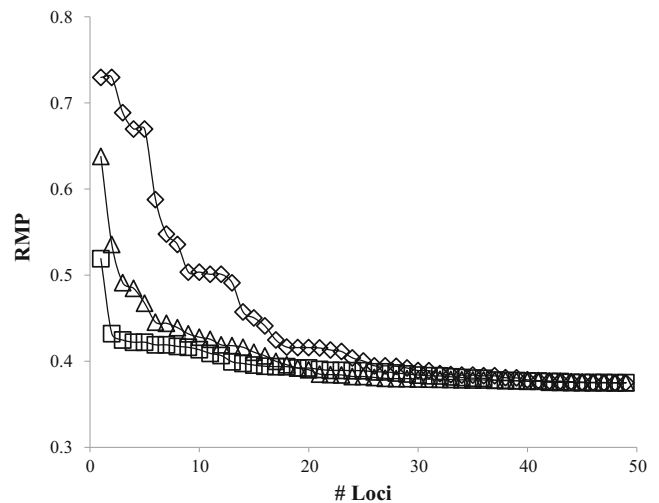


Fig. 1 Random Match Probability (RMP) plot against the Number of Loci (#Loci), in three population samples: Native Brazilians (white diamond); Rio de Janeiro (white square); and Tripoli (white triangle)

values are one order of magnitude higher than those reported for the North American ethnic groups, and two orders of magnitude higher than the value for isolated Native Brazilians. This finding suggests that higher degrees of isolation leading to genetic bias and lower diversity may reduce the efficiency of the 49 InDel marker panel. Similar reductions in efficiency of other InDel panels have been reported elsewhere [8, 14, 15].

Population comparison

Population substructure was analyzed using the Structure software. The highest likelihood was achieved for $K=3$ (three parental populations, data not shown). The percentages of admixture within each of the populations are presented in Fig. 2. The Rio de Janeiro and Tripoli sample populations

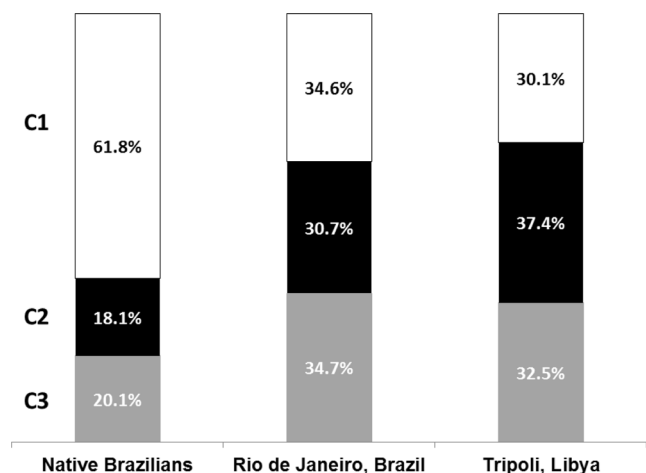


Fig. 2 Schematic representation of the average substructure population. Using Structure v2.3, assuming $K=3$ and admixture model, three clusters (C1, C2, and C3) are represented for each population. The percentage of admixture is denoted inside the corresponding bars

showed similar proportions of admixture levels for each of their respective parental clusters (30 %). However, for Native Brazilians, one of the parental clusters (C1) represented over 60 % of the total parental population. This substructure cannot be explained by differences in sample size, because the other population samples (Rio de Janeiro, $n=93$ and Libya, $n=77$) had equivalent sizes and showed no bias towards any cluster.

Substructure analysis reveals that admixed urban samples had equal contributions from three parental clusters. Native Brazilians had a major contributing cluster (over 60 %), indicating that some of these loci could behave as ancestry Amerindian markers. Previous results using the only commercially available InDel panel in two Asian groups, namely Tibetans and Koreans, also revealed loci behavior that closely resembles that of ancestry markers in these populations [16, 27].

In conclusion, the 49 InDel marker panels could be used for HID and genetic studies in general, but caution should be exercised in the case of isolated, potentially substructured populations in which the RMP will not be as informative as in admixed populations. For the use of the 49 InDels within isolated populations, the use of additional suitable loci should be investigated to improve efficiency.

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