

Brief Report

Not peer-reviewed version

Allele Frequencies and the Probability of Identity for 18 STR Loci Generated via the Canine Genotypes™ Panel 2.1 Kit in Golden Retriever, Miniature Dachshund, and Shiba Inu Groups for Forensic Applications

Fubuki Kunita , Chihiro Udagawa , Takeshi Inagaki , Hideto Suzuki , Makoto Bonkobara , [Toshinori Omi](#) *

Posted Date: 1 December 2023

doi: 10.20944/preprints202312.0089.v1

Keywords: dog DNA identification; STR; golden retriever; miniature dachshunds; shiba inus



Preprints.org is a free multidiscipline platform providing preprint service that is dedicated to making early versions of research outputs permanently available and citable. Preprints posted at Preprints.org appear in Web of Science, Crossref, Google Scholar, Scilit, Europe PMC.

Copyright: This is an open access article distributed under the Creative Commons Attribution License which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Brief Report

Allele Frequencies and the Probability of Identity for 18 STR Loci Generated via the Canine Genotypes™ Panel 2.1 Kit in Golden Retriever, Miniature Dachshund, and Shiba Inu Groups for Forensic Applications

Fubuki Kunita ^{1,†}, Chihiro Udagawa ^{1,†}, Takeshi Inagaki ², Hideto Suzuki ², Makoto Bonkobara ^{3,4} and Toshinori Omi ^{1,4,*}

¹ Faculty of Veterinary Medicine, School of Veterinary Nursing and Technology, Department of Basic Science, Nippon Veterinary and Life Science University, Tokyo 180-8602, Japan; fubukilinkshyde@gmail.com (F.K.); cudagawa@nvlu.ac.jp (C.K.)

² Department of Legal Medicine, Jichi Medical University, Shimotsuke-shi 329-0498, Japan; 98010ti@jichi.ac.jp (T.I.); suzuki.hideto@jichi.ac.jp (H.S.)

³ Faculty of Veterinary Medicine, School of Veterinary Medicine, Department of Veterinary Clinical Pathology, Nippon Veterinary and Life Science University, Tokyo 180-8602, Japan; bonkobara@nvlu.ac.jp

⁴ Research Center for Animal Life Science, Nippon Veterinary and Life Science University, Tokyo 180-8602, Japan

* Correspondence: t.omi@nvlu.ac.jp; Tel.: +81-42-2314151

† Authors contributed equally to this work and were co-first authors.

Simple Summary: Dogs (*Canis lupus familiaris*) are popular companion animals in Japan. According to the Japan Pet Food Association, approximately 7 million dogs were bred and raised in Japan in 2022. For those dog owners, forensic dog remains (e.g., hair samples) could be used to identify lost or missing animals, such as those lost to major earthquakes and tsunamis. In addition, such remains could also be used in criminal investigations to link persons of interest to a crime scene. Using Thermo Scientific Canine Genotypes™ Panel 2.1 Kit (Canine Kit) targeted short tandem repeats (STRs), we show the forensic identity of 18 autosomal STR loci from non-related 50 Golden Retrievers, 50 Miniature Dachshunds and 50 Shiba Inus bred in Japan. Random Match probability based on 18 STR loci was subsequently estimated to be 3.257×10^{-16} in Golden Retrievers, 3.933×10^{-18} in Miniature Dachshunds, and 2.107×10^{-18} in the Shiba Inus breed. There are a few studies that have used this kit in Japan. The results suggest that the kit with 18 autosomal STR loci and one sex marker is effective in forensic applications.

Abstract: Similarly to Europe and the United States, the need for the forensic DNA identification of dogs is increasing in Japan. Because few studies use commercial genotyping kits, we examine the effectiveness of individual DNA identification using the Canine Genotypes Panel 2.1 Kit on limited samples of dogs bred in Japan. We used Genomic DNA extracted from blood in non-related 50 Golden Retrievers, 50 Miniature Dachshunds, and 50 Shiba Inus bred in Japan. We investigated 18 canine STR markers (PEZ02, PEZ17, FH2017, FH2309, PEZ05, FH2001, FH2328, FH2004, FH2361, PEZ21, FH2054, FH3377, FH2107, FH2088, vWF.X, FH2010, PEZ16, and FH3313) and one sex-related marker (ZFX.Y) according to the manufacturer's instructions. Allele frequency, He, Ho, *p*-value, PD, PE, PIC, and MP were calculated for each marker. Random Match probability based on 18 STR loci was subsequently estimated to be 3.257×10^{-16} in Golden Retrievers, 3.933×10^{-18} in Miniature Dachshunds, and 2.107×10^{-18} in the Shiba Inus breed. There are a few studies that have used this kit in Japan. The results suggest that the kit with 18 autosomal STR loci and one sex marker is effective in forensic applications.

Keywords: dog DNA identification; STR; golden retriever; miniature dachshunds; shiba inus

1. Introduction

According to the Japan Pet Food Association (JPFA), approximately 7 million dogs were bred and raised in Japan in 2022 [1]. According to the report, 9.7% of Japanese households had one or more purebred dogs. The human–dog (or human–companion animal) bond is strong in Japan. Dogs are very attached to human society. Forensically relevant cases that involve dogs include dog bite accidents and the identification of affected or illegally dumped animals. The estimate for dog bites is more than 40,000 cases in the last 10 years in Japan [2]. According to the JPFA report, approximately 6,500 dogs were either killed or separated from their owners due to the Great East Japan Earthquake that struck on March 11, 2011 [3]. The total number of dog abandonments reported in the newspapers exceeds 100 dogs [4].

The utility of mitochondrial DNA (mtDNA) [5–16] and/or short tandem repeat (STR) polymorphisms in forensic analysis has been extended in recent years [17–25]. In Japan, very little research on forensic genetics has focused on STR typing in dogs. Recently, the Canine genotype™ panel 2.1 Kit (Thermo Fisher Scientific, Waltham, MA, USA) can be used to determine 18 STR loci and sex, and it is commercially available and can be purchased in Japan. Then, we first show the identity data for 18 primarily autosomal STR loci tetranucleotide markers, and we use the kit on 150 purebred dogs: 50 non-related Golden Retrievers, 50 Miniature Dachshunds, and 50 Shiba Inus.

2. Materials and Methods

The material used comprised DNA stored in our laboratory, which was previously extracted from blood using the QIAamp DNA Blood Mini Kit (QIAGEN, Germantown, MD, USA). The blood samples were obtained from the Department of Veterinary Clinical Pathology at Nippon Veterinary Life Science University (NVLU) in Musashino, Tokyo; they were originally collected at the Veterinary Medical Teaching Hospital at NVLU with the written consent of each owner.

We selected two purebred breeds that are relatively popular in Japan and that have been analyzed before using the Canine Genotypes Panel 1.1 Kit (Thermo Fisher Scientific, Waltham, MA, USA). Then, we used dog DNA from non-related 50 Golden Retrievers, 50 Miniature Dachshunds, and 50 Shiba Inus. DNA concentrations were measured with Nano Drop (Thermo Fisher Scientific).

The amplification of 18 STR loci (PEZ02, PEZ17, FH2017, FH2309, PEZ05, FH2001, FH2328, FH2004, FH2361, PEZ21, FH2054, FH3377, FH2107, FH2088, vWF.X, FH2010, PEZ16, and FH3313) and one sex-determinant locus (ZFX and ZFY) was performed using the Canine Genotypes Panel 2.1 Kit (Thermo Fisher Scientific) in accordance with the manufacturer’s instructions (Table 1). Capillary electrophoresis was conducted using a 3730xl DNA Analyzer (Applied Biosystems, Waltham, MA, USA) in accordance with the manufacturer’s instructions.

Table 1. Allele frequencies of the 18 canine STR loci in Golden Retriever, Miniature Dachshund, and Shiba Inu groups in Japan.

PEZ02				PEZ17				FH2017			
Frequency				Frequency				Frequency			
Allele Size	G.R.	M.D.	Shiba	Allele Size	G.R.	M.D.	Shiba	Allele Size	G.R.	M.D.	Shiba
	(n = 43)	(n = 50)	(n = 50)		(n = 50)	(n = 50)	(n = 50)		(n = 50)	(n = 50)	(n = 50)
106	-	0.001	-	196	0.040	-	0.010	254	0.060	0.020	-
114	-	-	0.040	200	0.330	0.040	0.200	258	0.010	0.230	-
118	0.081	0.440	-	204	0.270	0.310	0.200	262	0.160	0.350	0.580
122	0.442	0.040	0.120	208	0.250	0.350	0.250	266	0.670	0.350	0.410
126	0.454	0.390	0.280	212	0.070	0.270	0.300	270	0.100	0.050	0.010
130	0.023	0.110	0.090	216	0.030	0.030	0.040				
134	-	-	0.010	220	0.010	-	-				
138	-	0.010	0.020								
FH2309				PEZ05				FH2001			
Frequency				Frequency				Frequency			
Allele Size	G.R.	M.D.	Shiba	Allele Size	G.R.	M.D.	Shiba	Allele Size	G.R.	M.D.	Shiba
	(n = 49)	(n = 50)	(n = 50)		(n = 49)	(n = 50)	(n = 46)		(n = 44)	(n = 50)	(n = 48)

343	0.020	-	-	101	0.367	0.540	0.228	120	0.114	0.200	0.156
351	-	0.170	-	105	0.133	0.080	0.500	124	0.011	0.200	-
355	-	-	0.020	109	0.490	0.280	0.228	128	0.171	0.140	0.052
363	0.020	0.050	0.001	113	0.010	0.100	-	132	-	0.300	0.021
367	-	0.050	0.440	117	-	-	0.044	136	0.080	0.020	0.042
371	0.061	-	0.001					140	0.375	0.100	0.240
375	0.020	-	0.080					144	0.171	0.020	0.073
379	0.245	-	-					148	0.080	0.020	0.104
383	0.092	0.360	0.080					152	-	-	0.313
387	0.306	-	0.010								
391	0.031	0.020	0.070								
395	0.010	0.240	0.070								
399	0.082	-	0.210								
403	-	0.020	-								
407	-	0.090	-								
411	0.112	-	-								
FH2328				FH2004				FH2361			
Frequency				Frequency				Frequency			
Allele Size	G.R.	M.D.	Shiba	Allele Size	G.R.	M.D.	Shiba	Allele Size	G.R.	M.D.	Shiba
	(n = 50)	(n = 50)	(n = 48)		(n = 50)	(n = 50)	(n = 50)		(n = 50)	(n = 50)	(n = 50)
173	0.010	0.110	-	232	0.030	0.010	0.210	331	-	0.080	-
177	0.010	0.340	0.021	236	0.230	0.460	0.220	335	0.090	-	-
181	-	0.020	0.146	240	0.080	0.050	0.520	339	0.010	0.140	0.130
185	0.180	0.170	0.177	244	0.510	0.010	0.030	343	0.040	0.190	0.260
189	0.080	0.310	0.052	248	0.010	-	0.010	347	0.340	0.210	0.140
193	0.040	0.030	0.385	292	-	-	0.010	351	0.270	0.040	0.090
197	0.506	0.020	0.083	300	0.010	0.440	-	355	0.120	0.160	0.190
201	0.120	-	-	312	-	0.020	-	359	0.050	0.080	0.090
205	-	-	0.115	324	0.130	0.010	-	363	0.020	0.090	0.050
209	-	-	0.021					367	0.040	0.010	-
								371	0.010	-	0.040
								379	0.010	-	-
								383	-	-	0.010
PEZ21				FH2054				FH3377			
Frequency				Frequency				Frequency			
Allele Size	G.R.	M.D.	Shiba	Allele Size	G.R.	M.D.	Shiba	Allele Size	G.R.	M.D.	Shiba
	(n = 50)	(n = 50)	(n = 48)		(n = 48)	(n = 50)	(n = 50)		(n = 48)	(n = 50)	(n = 50)
83	0.030	-		143	0.291	0.090	-	181	0.052	0.010	-
87	0.230	0.290	0.167	147	0.052	0.220	0.070	186	-	0.470	0.250
91	0.340	0.120	0.125	151	0.167	0.130	0.130	191	0.250	0.010	0.170
95	0.400	0.340	0.490	155	0.083	0.030	0.350	196	0.073	0.080	0.080
99	-	0.250	0.219	159	0.021	0.070	0.170	201	0.010	0.130	0.010
				163	0.229	0.110	0.100	206	-	0.010	0.040
				167	0.416	0.070	0.160	211	0.021	-	-
				171	0.010	0.270	0.020	216	-	-	0.020
				175	-	0.010	-	231	0.010	0.030	0.180
								236	0.010	0.210	0.010
								241	0.542	0.040	-
								246	0.031	-	0.020
								256	-	0.010	-
								271	-	-	0.120
								276	-	-	0.020
								286	-	-	0.070
								291	-	-	0.010
FH2107				FH2088				vWF.X			
Frequency				Frequency				Frequency			
Allele Size	G.R.	M.D.	Shiba	Allele Size	G.R.	M.D.	Shiba	Allele Size	G.R.	M.D.	Shiba

	(n = 50)	(n = 50)	(n = 50)		(n = 50)	(n = 50)	(n = 49)		(n = 49)	(n = 50)	(n = 50)
359	0.460	0.040	0.010	111	0.080	-	0.133	150	-	-	0.010
363	0.040	0.060	0.220	119	0.120	0.270	0.255	156	0.560	0.670	0.220
367	0.020	0.280	0.040	123	0.220	-	0.367	162	0.367	0.270	0.290
371	0.060	0.040	0.250	127	0.450	0.450	0.214	168	0.031	0.040	0.440
375	0.180	0.210	0.140	131	0.030	0.050	0.031	174	-	-	0.040
379	0.160	0.180	0.030	135	0.100	0.230	-	186	0.051	0.020	-
383	0.050	0.070	0.130								
387	0.020	0.100	0.080								
391	0.010	-	0.020								
395	-	0.020	-								
399	-	-	0.080								
FH2010				PEZ16				FH3313			
Frequency				Frequency				Frequency			
Allele Size	G.R.	M.D.	Shiba	Allele Size	G.R.	M.D.	Shiba	Allele Size	G.R.	M.D.	Shiba
	(n = 44)	(n = 50)	(n = 49)		(n = 46)	(n = 50)	(n = 48)		(n = 46)	(n = 50)	(n = 45)
224	0.011	-	0.204	287	0.022	0.020	0.031	349	-	-	0.156
228	0.125	0.160	0.225	291	0.685	0.280	0.573	353	-	-	0.011
232	0.705	0.490	0.102	295	0.044	0.220	0.333	357	-	-	0.011
236	0.148	0.350	0.459	299	0.207	0.150	0.063	361	-	-	0.256
240	0.011	-	0.010	303	-	0.020	-	365	-	-	0.122
				307	0.033	0.130	-	369	0.022	0.130	0.178
				311	-	0.040	-	373	0.152	0.070	0.056
				315	-	0.120	-	377	0.011	0.010	0.033
				319	0.011	0.020	-	381	0.054	0.050	0.022
								383	0.022	0.050	-
								387	0.022	0.010	0.011
								391	0.228	0.090	0.011
								395	0.130	0.130	0.033
								399	0.055	0.230	0.033
								403	0.011	0.070	0.011
								407	0.022	0.070	-
								411	-	0.020	-
								415	0.152	0.050	0.022
								419	0.044	-	-
								423	0.011	0.010	0.022
								427	-	-	0.011
								431	0.033	-	-
								435	0.033	-	-
								443	-	0.010	-

G.R. : Golden Retrievers, M.D. : Miniature Dachshunds, Shiba : Shiba Inus.

To carry out fragment analysis, Peak Scanner Fragment Analysis 2 (Thermo Fisher Scientific) was used. Moreover, statistical analysis was performed using Cervus (<http://www.fieldgenetics.com/pages/home.jsp>, accessed on 1 October 2023). Cervus was used to determine expected heterozygosity (He), observed heterozygosity (Ho), *p*-value (P) for the Hardy–Weinberg equilibrium exact test using a Markov chain, the power of the discriminant (PD), the power of exclusion (PE), polymorphic information content (PIC), and matching probability (MP). The accumulated power of discrimination (APD), which is a comprehensive PD, was calculated from the PD of 18 loci. Similarly, APE was also calculated using the PE of 18 loci.

3. Results and Discussion

The allele frequencies of the 18 canine STR loci in 50 Golden Retrievers, 50 Miniature Dachshunds, and 50 Shiba Inu groups are shown in Table 1. A total of 130 alleles were observed with respect to 18 STR loci in the purebred Golden Retriever group, with allele frequency distributions from 4 to 16; 126 alleles were observed in the purebred Miniature Dachshund group, with frequency

distributions from 3 to 15; 131 alleles were observed in the Shiba Inu group, with allele frequency distributions from 3 to 17. The average number of alleles per locus was 7.2 in the Golden Retriever group, 7.0 in the Miniature Dachshunds group, and 7.3 in the Shiba Inu group. In the purebred group, FH3313 exhibited the largest allele numbers, with a total of 16 in the Golden Retriever group, 15 in the Miniature Dachshund group, and 17 in the Shiba Inu group. The lowest number of alleles was observed in the following: PEZ02, PEZ05, PEZ21, and vWF.X in the Golden Retriever group; FH2010 in the Miniature Dachshund group; and FH2017 in the Shiba Inu group.

Table 2 also shows that the forensic application values of 18 STR loci in the Golden Retriever, Miniature Dachshund, and Shiba Inu groups were explored by calculating forensic parameters, including the power of the discriminant (PD), power of exclusion (PE), polymorphic information content (PIC), matching probability (MP), and accumulated power of discrimination (APD). In the Golden Retriever group, the He value ranged from 0.471 to 0.882 with an average of 0.677. The Ho value ranged from 0.340 to 0.780 with an average of 0.588. PIC values ranged from 0.428 to 0.860 with an average of 0.626. The PD, PE, and RMP values ranged from 0.677 to 0.972, 0.258 to 0.748, and 0.028 to 0.323, respectively. APD, which was calculated via the PD of 18 STR loci was $1-3.257 \times 10^{-16}$. In other words, Random Match probability (RMP) was 3.257×10^{-16} for 18 STR loci in Golden Retrievers groups. In the Miniature Dachshund group, the He values ranged from 0.481 to 0.891 with an average of 0.727. The Ho values ranged from 0.380 to 0.860 with an average of 0.652. PIC values ranged from 0.409 to 0.872 with an average of 0.675. The PD, PE, and MP values ranged from 0.658 to 0.976, 0.232 to 0.767, and 0.024 to 0.342, respectively. APD, which was calculated via the PD of 18 loci, was $1-3.933 \times 10^{-18}$. RMP was 3.933×10^{-18} for 18 STR loci in Miniature Dachshunds groups. In the Shiba Inu group, He values ranged from 0.500 to 0.865 with an average of 0.732. Ho values ranged from 0.354 to 0.820 with an average of 0.604. PIC values ranged from 0.382 to 0.840 with an average of 0.682. The PD, PE, and MP values ranged from 0.632 to 0.964, 0.196 to 0.717, and 0.036 to 0.368, respectively. APD, which was calculated via the PD of 18 loci, was $1-2.107 \times 10^{-18}$. RMP was 2.107×10^{-18} for 18 STR loci in the Shiba Inus groups.

Table 2. Forensic parameters of 18 STR loci in Golden Retrievers, Miniature Dachshunds, and Shiba Inus in Japan.

G.R.							
Loci	He	Ho	P	PD	PE	PIC	MP
PEZ02	0.599	0.558	0.923	0.747	0.304	0.506	0.253
PEZ17	0.756	0.740	0.663	0.894	0.519	0.706	0.106
FH2017	0.517	0.340	0.144	0.726	0.300	0.476	0.274
FH2309	0.821	0.612	0.077	0.942	0.642	0.790	0.058
PEZ05	0.614	0.653	0.472	0.768	0.323	0.592	0.232
FH2001	0.784	0.682	0.718	0.921	0.580	0.747	0.079
FH2328	0.638	0.580	0.235	0.828	0.411	0.595	0.172
FH2004	0.669	0.480	0.021	0.843	0.430	0.620	0.157
FH2361	0.791	0.780	0.395	0.924	0.593	0.754	0.076
PEZ21	0.677	0.580	0.360	0.825	0.391	0.604	0.175
FH2054	0.812	0.729	0.658	0.934	0.615	0.776	0.066
FH3377	0.641	0.583	0.074	0.823	0.406	0.591	0.177
FH2107	0.729	0.520	0.178	0.892	0.517	0.691	0.108
FH2088	0.725	0.740	0.526	0.884	0.500	0.681	0.116
vWF.X	0.564	0.510	0.917	0.719	0.278	0.473	0.281
FH2010	0.471	0.477	0.524	0.677	0.258	0.428	0.323
PEZ16	0.49	0.370	0.141	0.691	0.270	0.441	0.309
FH3313	0.882	0.652	0.278	0.972	0.748	0.860	0.028
Average	0.677	0.588		0.834	0.449	0.626	0.166
M.D.							
Loci	He	Ho	P	PD	PE	PIC	MP
PEZ02	0.647	0.620	0.935	0.802	0.370	0.572	0.198
PEZ17	0.713	0.700	0.293	0.857	0.444	0.649	0.143
FH2017	0.706	0.720	0.453	0.852	0.436	0.642	0.148

FH2309	0.778	0.780	0.553	0.915	0.567	0.738	0.085
PEZ05	0.62	0.480	0.071	0.793	0.359	0.556	0.207
FH2001	0.807	0.720	0.089	0.931	0.607	0.771	0.069
FH2328	0.753	0.680	0.146	0.894	0.519	0.704	0.106
FH2004	0.598	0.500	0.216	0.749	0.309	0.507	0.251
FH2361	0.864	0.860	0.683	0.962	0.710	0.838	0.038
PEZ21	0.731	0.720	0.711	0.872	0.468	0.671	0.128
FH2054	0.839	0.820	0.652	0.951	0.668	0.810	0.049
FH3377	0.716	0.760	0.139	0.881	0.497	0.675	0.119
FH2107	0.831	0.700	0.030	0.947	0.654	0.801	0.053
FH2088	0.676	0.540	0.088	0.830	0.401	0.609	0.170
vWF.X	0.481	0.380	0.144	0.658	0.232	0.409	0.342
FH2010	0.618	0.540	0.120	0.772	0.324	0.534	0.228
PEZ16	0.825	0.800	0.698	0.942	0.64	0.792	0.058
FH3313	0.891	0.420	0.000	0.976	0.767	0.872	0.024
Average	0.727	0.652		0.866	0.498	0.675	0.134
Shiba Inu							
Loci	He	Ho	P	PD	PE	PIC	MP
PEZ02	0.711	0.600	0.125	0.868	0.471	0.659	0.132
PEZ17	0.774	0.640	0.022	0.906	0.540	0.726	0.094
FH2017	0.500	0.380	0.090	0.632	0.196	0.382	0.368
FH2309	0.746	0.640	0.157	0.903	0.542	0.710	0.097
PEZ05	0.651	0.565	0.399	0.814	0.380	0.585	0.186
FH2001	0.808	0.354	0.000	0.933	0.614	0.773	0.067
FH2328	0.783	0.604	0.326	0.923	0.585	0.748	0.077
FH2004	0.642	0.600	0.122	0.812	0.381	0.581	0.188
FH2361	0.848	0.820	0.513	0.955	0.682	0.820	0.045
PEZ21	0.676	0.417	0.000	0.842	0.422	0.620	0.158
FH2054	0.799	0.780	0.693	0.929	0.601	0.764	0.071
FH3377	0.856	0.640	0.007	0.959	0.699	0.830	0.041
FH2107	0.845	0.800	0.280	0.954	0.678	0.817	0.046
FH2088	0.743	0.714	0.278	0.885	0.497	0.690	0.115
vWF.X	0.679	0.740	0.267	0.832	0.405	0.612	0.168
FH2010	0.694	0.551	0.203	0.852	0.439	0.637	0.148
PEZ16	0.562	0.354	0.019	0.725	0.285	0.479	0.275
FH3313	0.865	0.667	0.549	0.964	0.717	0.84	0.036
Average	0.732	0.604		0.872	0.507	0.682	0.128

We investigated the genetic polymorphisms of 18 STR loci using the Canine Genotypes Panel 2.1 Kit in three purebred Golden Retriever, Miniature Dachshund, and Shiba Inu groups. The results of the genotype showed that this kit would be informative for the identity testing of canines in Japan. We also previously applied the Canine Genotypes Panel 1.1 Kit on 40 Golden Retrievers and 40 Miniature Dachshunds, and APD was estimated to be $1-1.4337 \times 10^{-14}$ (G.R.), $1-1.61466 \times 10^{-15}$ (M.D.) [27]. Both kits performed equally well with respect to obtaining genotyping results for the identity testing of canines. Moreover, the interruption of the peak's size was easier to distinguish in Panel 2.1, which was based on tetranucleotides, than in Panel 1.1, which was based on dinucleotide structures, in our laboratory. Our findings correspond with a previous paper by Kanthaswamy et.al [24].

Fang et al. have reported that the 19 STR loci have forensic application values in Shiba Inu groups via the commercial PBG Canine Genotype STR system (Beijing Protect Baby Technology, BeijingChina) [26]. In this study, we also showed population data with high genetic polymorphisms relative to the Shiba Inu breed in Japan, which was determined using the Canine Genotypes Panel 2.1 Kit. The Shiba Inu is an ancient breed of Japanese origin. The STR analysis is also informative for pedigree management.

4. Conclusions

In this study, 18 STR loci exhibited high genetic polymorphism in Golden Retrievers, Miniature Dachshunds, and Shiba Inus using the Canine Genotypes Panel 2.1 Kit. This is the first time Japanese canine population data were reported using this kit. The results of the genotype showed that the Panel 2.1 Kit could be informative for the identity testing of canines in Japan.

Author Contributions: Conceptualization, T.I., H.S. and T.O.; methodology, F.K., M. B., C.U., and T.O.; validation, F.K., C.U., and T.O.; formal analysis F.K., and T.O.; resources, M.B.; writing—original draft preparation, F.K.; writing—review and editing, T.O. and C.U. F.K and C.U contributed equally to this work and were co-first authors. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are available from the corresponding author upon reasonable request.

Acknowledgments: The authors are grateful to the Dr. Kyoichi Tamura in Department of Veterinary Clinical Pathology, Nippon Veterinary and Life Science University for sample collection and Dr. Toshiaki Yamamoto in Department of Applied Science Nippon Veterinary and Life Science University for a part of data analysis.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Japan Pet Food Association. Available online: <https://petfood.or.jp/data/chart2022/index.html> (accessed on 1 April 2023).
2. Ministry of the Environment. Available online: https://www.env.go.jp/nature/dobutsu/aigo/2_data/statistics/files/r03/2_3_1.pdf (accessed on 1 April 2023).
3. Japan Pet Food Association. Available online: <https://petfood.or.jp/data/chart2011/01.html> (accessed on 1 April 2023).
4. Ministry of the Environment. Available online: <https://www.env.go.jp/gyakutai2022> (accessed on 1 April 2023).
5. Savolainen, P.; Rosén, B.; Holmberg, A.; Leitner, T.; Uhlén, M.; Lundeberg, J. Sequence analysis of domestic dog mitochondrial DNA for forensic use. *J. Forensic Sci.* **1997**, *42*, 593–600.
6. Schneider, P.M.; Seo, Y.; Rittner, C. Forensic mtDNA hair analysis excludes a dog from having caused a traffic accident. *Int. J. Legal Med.* **1999**, *11*, 315–316.
7. Savolainen, P.; Arvestad, L.; Lundeberg, J. A novel method for forensic DNA investigations: Repeat-type sequence analysis of tandemly repeated mtDNA in domestic dogs. *J. Forensic Sci.* **2000**, *45*, 990–999.
8. Wetton, J.H.; Higgs, J.E.; Spriggs, A.C.; Roney, C.A.; Tsang, C.S.; Foster, A.P. Mitochondrial profiling of dog hairs. *Forensic Sci. Int.* **2003**, *133*, 235–241.
9. Baute, D.T.; Satkoski, J.A.; Spear, T.F.; Smith, D.G.; Dayton, M.R.; Malladi, V.S.; Goyal, V.; Kou, A.; Kinaga, J.L.; Kanthaswamy, S. Analysis of forensic SNPs in the canine mtDNA HV1 mutational hotspot region. *J. Forensic Sci.* **2008**, *53*, 1325–1333.
10. Himmelberger, A.L.; Spear, T.F.; Satkoski, J.A.; George, D.A.; Garnica, W.T.; Malladi, V.S.; Smith, D.G.; Webb, K.M.; Allard, M.W.; Kanthaswamy, S. Forensic utility of the mitochondrial hypervariable region 1 of domestic dogs, in conjunction with breed and geographic information. *J. Forensic Sci.* **2008**, *53*, 81–89.
11. Webb, K.M.; Allard, M.W. Mitochondrial genome DNA analysis of the domestic dog: Identifying informative SNPs outside of the control region. *J. Forensic Sci.* **2009**, *54*, 275–288.
12. Webb, K.M.; Allard, M.W. Identification of forensically informative SNPs in the domestic dog mitochondrial control region. *J. Forensic Sci.* **2009**, *54*, 289–304.
13. van Asch, B.; Albarran, C.; Alonso, A.; Angulo, R.; Alves, C.; Betancor, E.; Catanesi, C.I.; Corach, D.; Crespillo, M.; Doutremepuich, C.; et al. Forensic analysis of dog (*Canis lupus familiaris*) mitochondrial DNA sequences: An interlaboratory study of the GEP-ISFG working group. *Forensic Sci. Int. Genet.* **2009**, *4*, 49–54.
14. Imes, D.L.; Wictum, E.J.; Allard, M.W.; Sacks, B.N. Identification of single nucleotide polymorphisms within the mtDNA genome of the domestic dog to discriminate individuals with common HVI haplotypes. *Forensic Sci. Int. Genet.* **2012**, *6*, 630–639.
15. Sugiyama, S.; Chong, Y.H.; Shito, M.; Kasuga, M.; Kawakami, T.; Udagawa, C.; Aoki, H.; Bonkobara, M.; Tsuchida, S.; Sakamoto, A.; et al. Analysis of mitochondrial DNA HVR1 haplotype of pure-bred domestic dogs in Japan. *Leg. Med.* **2013**, *15*, 303–309.

16. Thai, Q.K.; Chung, D.A.; Tran, H.-D. mtDNA HV1 database: A web-based tool for collecting and surveying Canis mtDNA HV1 haplotype in public database. *BMC Genet.* **2017**, *18*, 60.
17. Brauner, P.; Reshef, A.; Gorski, A. DNA profiling of trace evidence—Mitigating evidence in a dog biting case. *Forensic Sci.* **2001**, *46*, 1232–1234.
18. Pádár, Z.; Angyal, M.; Egyed, B.; Füredi, S.; Woller, J.; Zöldág, L.; Fekete, S. Canine microsatellite polymorphisms as the resolution of an illegal animal death case in a Hungarian zoological gardens. *Int. J. Legal Med.* **2001**, *115*, 79–81.
19. Kanthaswamy, S.; Tom, B.K.; Mattila, A.M.; Johnston, E.; Dayton, M.; Kinaga, J.; Erickson, B.J.; Halverson, J.; Fantin, D.; DeNise, S.; et al. Canine population data generated from a multiplex STR kit for use in forensic casework. *J. Forensic Sci.* **2009**, *54*, 829–840.
20. Tom, B.K.; Koskinen, M.T.; Dayton, M.; Mattila, A.-M.; Johnston, E.; Fantin, D.; Denise, S.; Spear, T.; Smith, D.G.; Satkoski, J.; et al. Development of a nomenclature system for a canine STR multiplex reagent kit. *Forensic Sci.* **2010**, *55*, 597–604.
21. Ogden, R.; Mellanby, R.J.; Clements, D.; Gow, A.G.; Powell, R.; McEwing, R. Genetic data from 15 STR loci for forensic individual identification and parentage analyses in UK domestic dogs (*Canis lupus familiaris*). *Forensic. Sci. Int. Genet.* **2012**, *6*, e63–e65.
22. Berger, B.; Berger, C.; Hecht, W.; Hellmann, A.; Rohleder, U.; Schleenbecker, U.; Parson, W. Validation of two canine STR multiplex-assays following the ISFG recommendations for non-human DNA analysis. *Forensic. Sci. Int. Genet.* **2014**, *8*, 90–100.
23. Tsuji, A.; Ishiko, A.; Kimura, H.; Nurimoto, M.; Kudo, K.; Ikeda, N. Unusual death of a baby: A dog attack and confirmation using human and canine STRs. *Int. J. Legal Med.* **2008**, *122*, 5962.
24. Kanthaswamy, S.; Oldt, R.F.; Montes, M.; Falak, A. Comparing two commercial domestic dog (*Canis familiaris*) STR genotyping kits for forensic identity calculations in a mixed-breed dog population sample. *Anim. Genet.* **2019**, *50*, 105–111.
25. Lee, M.; Hwang, I.K.; Jung, J.Y.; Kim, J.Y.; Chang, M.; Moon, S. Construction of an in-house allelic ladder for Canine Genotypes™ Panel 2.1 Kit. *J. Forensic. Sci.* **2021**, *6*, 2362–2368.
26. Fang, Y.; Yang, J.; Deng, Y.; Zhu, B. Forensic Application Evaluation of a Novel Canine STR System in Pembroke Welsh Corgi and Shiba Inu Groups. *Biomed. Res. Int.* **2021**, 5355109.
27. Kawakami, T. Study on DNA identification by Short Tandem Repeat analysis in dog. *Bull. Nippon Vet. Life Sci. Univ.* **2012**, *61*, 148.

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.