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PAPER

Isolation and characterisation of a dinucleotide microsatellite set for a parentage and biodiversity study in domestic guinea pig (*Cavia porcellus*)

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Abstract

The domestic guinea pig is a valuable genetic resource because it is part of local folklore and food tradition in many South American countries. The economic importance of the guinea pig is due to its high feed efficiency and the quality of animal protein produced. For these reasons, our study is aimed to design a complete dinucleotide microsatellite marker set following international recommendation to assess the genetic diversity and genealogy management of guinea pigs. We selected a total of 20 microsatellites, looking for laboratory efficiency and good statistical parameters. The set was tested in 100 unrelated individuals of guinea pigs from Ecuador, Peru, Colombia, Bolivia and Spain. Our results show a high degree of polymorphisms with a total of 216 alleles and a mean number of 10.80±3.49 for markers with a combined exclusion probability of 0.99.

Introduction

The guinea pig (*Cavia porcellus*), also called *cavy*, is originally from the Andean regions of southern Colombia, Ecuador, Peru, and Bolivia, where the species was domesticated between 7000 and 5000 BC (Morales, 1995). Today, a stable population of 35 million animals is reared in this area (DAD-IS, 2014). The guinea pig has several uses and is a valuable economic resource for indigenous populations in the South American marginal areas where they originate. The guinea pig is a unique source of food due to their ability to convert poor vegetable resources to protein. Additionally, the guinea pig has a strong presence in local folklore and in popular medicine and is an important resource in the cultural patrimony of local nations, especially the Quechuas and Aymaras. The guinea pig has been introduced to other countries since the Spanish colonisation of the American continent, and today, they are used as exotic pets or for scientific experimentation (Guerrini, 2003). Owing to it great capacity of growing and the poor feeding needs, many efforts have also been made to promote guinea pig husbandry in developing countries. The guinea pig was introduced in several West African countries. Even if no official statistics are available (Manjeli *et al.,* 1998), there are some stable reared populations in Cameron, Democratic Republic of Congo and Tanzania (Maass *et al.,* 2005, 2010; Matthiesen *et al.,* 2011). To date, no complete genetic study has been carried out on the domestic guinea pig although great advances have been reached with the completion of the genomic sequence (http://www.ensembl.org/Cavia_porcellus/Info/Index; Broad Institute, 2015). Only a few studies have been conducted looking at microsatellites in guinea pigs, and they have centred on wild subspecies of the *Cavia* genus such as *Cavia aperea* and *Cavia magna* (Kanitz *et al.,* 2009) or have been limited to a small marker panel (Burgos-Paz *et al.,* 2011). The large number of guinea pig animals and breeds reared in South America necessitated the development of molecular tools to perform genetic characterizations and population structure studies as well as a parentage testing strategy for modern breeding approaches. To respond to this demand, the aims of our study were to design a polymorphic set of dinucleotide microsatellites useful both for analysing the genetic diversity of the domestic *Cavia* and as for parentage control, following the Food and Agriculture Organization (FAO) and International Society for Animal Genetics (ISAG) recommendations on this type of research in domestic animals.

Materials and methods

Samples used and DNA extraction

Hair samples from a total of 100 unrelated animals belonging to several domestic guinea pig populations were used in our study. Some samples were collected from several breeding lines from Ecuador (40) divided in 10 sample for type/line (Andina, Peru, Inti and commercial local type) and others from Colombia (15), Bolivia (13) and Perú (15); also, some samples were collected in Spain from commercial lines (20) reared as pets. DNA was obtained by incubating 3 hair roots in the presence of 100 µL of 5% Chelex® (Biorad, Göttingen, Germany) resin suspension at 95°C for 10 minutes and 99°C for 3 min.

In silico identification of microsatellites and primer design

The cavPor3 (high-coverage 6.79X assembly) genome release of the guinea pig (*Cavia porcellus*) was used to search for microsatellite sequences (http://www.ensembl.org/Cavia_porcellus/Info/Index) using the NCBI finder tool (Appendix Table 1). Sequence repeat motifs of ≥18 bp including poly AG, AC, AT, TC, CA, and GT were searched. A total of 25 sequences were selected. The primer pairs used for polymerase chain reaction (PCR)

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Key words: *Cavia porcellus*; STRs; Biodiversity; Power of exclusion.

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amplification were designed using Primer3 software version 0.4.0 (Rozen and Skaletsky, 2000). Our parameter sets included an optimum primer size of 20±5 bp, an optimum melting temperature of ~60±5°C and a GC content between 20 and 80%. The software was allowed to design primer pairs with expected PCR product sizes of 80 to 350 bp.

Microsatellite locus selection

Our primer pairs were synthesised by Stabvida, Costa de Caparica (Portugal) without further modifications. PCR was performed separately for each locus in a reaction volume of 25 µL containing ~10-30 ng of genomic DNA, 0.2 µM each primer pair, 1X NH_4SO_4 PCR buffer, 2.5 mM $MgCl_2$, 200 µM each dNTP, and 1U Taq polymerase (AIDLAB, Beijing, China). The annealing temperature was 56°C for 35 cycles. PCR products were visualised on a 3% agarose gel, stained with ethidium bromide, in TBE buffer at 150 V/cm, using a 100-bp ladder as a reference (Thermo Fisher Scientific Inc., Waltham, MA, USA).

Based on the amplification efficiency and the absence of a nonspecific PCR product, the samples were sequenced using the BigDye cycle sequencing kit 2.0 (Life Technologies, Carlsbad, CA, USA), and the sequences were deposited in GenBank (Table 1) after sequencing a control sample from the original clone (Appendix Table 1). Additionally, four microsatellite loci (Kanitz *et al.,* 2009) were included in our study with some modifications and discarding tetranucleotide repeat motifs loci (Table 1).

Microsatellite typing

A final set of 20 polymorphic microsatellites was selected from the microsatellites we tested. The forward primer for each locus was 5' end labelled with fluorescent dye (Figure 1). PCR was performed separately for each locus in a final reaction volume of 25 µL containing ~10-30 ng of genomic DNA, 0.2 µM each primer pair, 1X NH_4SO_4/KCl PCR buffer, 3 mM $MgCl_2$, 200 µM each dNTP, and 1U Taq polymerase (AIDLAB, Beijing, China). Multiplex reactions were performed following the size range and dye availability using ABI dye set D (Figure 1). The optimal annealing temperature was established by a gradient amplification of 8 samples (annealing temperature from 50 to 62°C) on a Biometra Tgradient Thermal cycler (Biorad).

The sizes of the microsatellite alleles were visualised using an ABI PRISM 3130 Genetic Analyzer (Life Technologies), using a POP7 polymer and the internal size standard GeneScan500-Rox (Life Technologies). Genotypes were read with the ABI PRISM GeneScan 3.1.2 software (Applied Biosystems, Carlsbad, CA, USA) and interpreted with the ABI PRISM Genotyper 3.7 NT software (Applied Biosystems).

Statistical analysis

The mean number of alleles, observed and unbiased expected estimates of gene diversity, and their standard deviations, together with the polymorphic information content (PIC) were obtained using MICROSATELLITE TOOLKIT software (Park, 2001). We estimated non-exclusion probabilities considering the first (NE-1P), second (NE-2P) or parent pairs (NE-PP) and individual (NE-I) and sib identity (NE-SI) as well as the Hardy Weinberg Equilibrium (HWE), using Cervus software version 3.0.3 (Kalinowski *et al.,* 2007). The combined posterior probability (PEC) was calculated with the algorithm of Jamieson (1994). Deviations from HWE and F_{is} based on locus by locus AMOVA calculations were assessed using ARLEQUIN 3.5.1.3 (Excoffier and Lischer, 2010).

Results

Fluorescent polymerase chain reaction design and microsatellite genotyping

Based on amplification efficiency, success rate, and the absence of non-specific amplification of our primer pairs, a total of 16 microsatellites were selected for the panel design. We named these microsatellites CUY1,CUY2, CUY3, CUY4, CUY5, CUY6, CUY7, CUY8, CUY9, CUY10, CUY12, CUY16, CUY17, CUY18, CUY20, and CUY22. Additionally, 4 din-

Table 1. Summary of the general characteristics of the twenty selected microsatellite loci.

Locus	GB	RP	MX	Tm	SR	Forward primer (5'-3')	Reverse primer (5'-3')	Reference
CUY1	KP115879	GT	2	55	271-285	ctttcaggcaataggcatcc	gcagcttggactacagagca	This work
CUY2	KP115880	CA	2	55	250-262	caagatgccatcaactttcgt	tgttgctgagatgctgcttt	This work
CUY3	KP115881	GT	1	55	212-252	gcaagtcaaattcatccctga	gagtcctgccaagcaaaatc	This work
CUY4	KP115882	GT	2	55	210-230	tcatctcgcttcagcatttg	aatggtcaggggctaggatt	This work
CUY5	KP115883	CA	2	55	141-163	ggccaaagcaggaatgtcta	tagggcaagcattgatgatg	This work
CUY6	KP115884	CA	4	55	158-168	tggcttgctttctctttggt	ctgtgctcagcattgcattt	This work
CUY7	KP115885	CA	2	55	183-197	gatgcagtgcagaggagtca	tgtgtggttttgtgtgtgagg	This work
CUY8	KP115886	TC	1	55	181-217	tgattgcacctgagaagtgg	ccaagtgttcttggtgcttg	This work
CUY9	KP115887	GT	2	55	116-130	gctggaatgcaagacaagc	tgagttttcagctgtgatgagt	This work
CUY10	KP115888	GT	1	55	106-128	ttccaagcatttcagaaaaca	tgacttcccaaccaaggaaa	This work
CUY12	KP115889	AG	4	55	232-250	ggaatggtggcaaactccta	tctcctcctcctcctccttc	This work
CUY16	KP115890	AT	3	60	223-247	tttgagtcaagccgtgaaca	gcctgttttgaaactgttttactg	This work
CUY17	KP115891	TC	4	55	152-170	tgatggacaatatactgggaacc	tagcatgcatgaagccctaa	This work
CUY18	KP115892	CA	2	55	176-214	tgtcacttctcactccacca	tcccaaacctcttgtttgct	This work
CUY20	KP115893	AT	4	55	218-258	tcttggaaatggcctacatttt	tggtctctaggggtatccatt	This work
CUY22	KP115894	TC	4	55	206-232	cgaacatgccaagcagatta	acaccagttccttgccacat	This work
Cavy2	AJ496560	AC	2	55	124-154	ggccattatgccccccaac	agctgctccttgtgctgtag	Kanitz *et al.* (2009)
Cavy3	AJ496561	CT	1	55	195-225	acagcgatcacaatctgcac	gcagtggtaacccagaatgg	Kanitz *et al.* (2009)
Cavy11	AC192015	CT	1	55	140-180	ccgtgcttttcctgtctttg	tggaccccaatctgacatag	Kanitz *et al.* (2009)
Cavy12	AC182323	AG	1	55	143-187	agaatgcctttgggactgg	agatcttgcctctgcacttg	Kanitz *et al.* (2009)

GB, GenBank accession number; RP, microsatellite repeat motive; MX, polymerase chain reaction multiplex reaction where the locus amplified; Tm, annealing temperature of polymerase chain reaction; SR, size range in base pairs.

ucleotide markers were selected from the Kanitz *et al*. (2009) based on sequence length and marker polymorphisms (Table 1) with no modification except for Cavy11 and Cavy 12, where the primer sequence was re-designed to improve the melting temperature parameter. A 4 colour system (ABI D Dye set) and a ~20 bp minimum predicted distance between loci was used to design the electrophoresis pattern. The unusually large distance between loci was designed because of a lack of references about this species, specifically information about expected allelic range. The panel of PCR amplification resulted in four PCR multiplexes divided into three electrophoresis sets (Figure 1). The gradient amplification resulted in an optimal hybridisation temperature, based on the broadness of the band, of 55±0.5°C for all of the multiplexes, with the exception of the CUY 16 maker (60±0.5°C).

Marker polymorphism and quality

The allelic range (a region of the electropherogram where a locus specific allele can be found) we obtained was generally high. The mean difference between two alleles in the same individual ranged from 1.5 in CUY7 to 10.91 Cavy2.

A total of 216 alleles were found with a mean value of 10.80±3.49. All microsatellites were highly polymorphic with a minimum of 6 alleles (CUY6) and a maximum of 19 (Cavy12). The allelic richness ranged from a minimum of 4.002 for CUY9 and a maximum of 9.969 for Cavy12. We found observed and expected heterozygosity to have an average mean value of 0.590±0.115 and 0.778±0.080, respectively, which is considered high (Table 2). To evaluate the polymorphisms of each marker, the PIC value was calculated and found to range from 0.503 for CUY9 and 0.902 for Cavy12. Deviations from HWE were found in 9 of the 20 loci (Appendix Table 2); Cavy12 and CUY7 were found in disequilibrium in 6 populations, CUY2, CUY10 and CUY17 ($P<0.05$). The sample from Bolivia showed the highest number markers in disequilibrium (8) while the Spanish population showed the lowest ones (2). F_{is} values with a total mean value of 0.173.

Panel set power statistics

In Table 3, the non-exclusion probability values are shown. The first two values (NE-1P and NE-2P) give the non-exclusion probability when the parents were considered one by one (the first parent and then the second parent of the opposite sex, respectively). In both cases, the higher value was for CUY9 (0.84 and 0.68), and the lower value was for Cavy12 (0.31 and 0.18). When parent pairs were considered, the results were comparable for identity and sibling identity non-exclusion probability, with a maximum value obtained for CUY9 (0.50, 0.25, and 0.54, respectively) and a lower probability for Cavy12 (0.05, 0.02 and 0.30, respectively).

Following the Jamieson (1994) algorithm the combined posterior probability (PEC) was calculated (Table 3). The results show a high value for all types (0.99) but a smaller value for the sibling identity exclusion combined probability (0.84).

Discussion

The aim of our study was to construct a polymorphic marker panel of microsatellites that would be useful for both genetic diversity studies and kinship and parentage analysis in *Cavia porcellus* populations. Microsatellites are very powerful genetic markers that can be used for identifying the genetic structure, pedigree analysis and genetic variation of closely related species. Until the present work, only a few studies had been carried out on wild guinea pigs using either a reduced microsatellite loci panel (Asher *et al.,* 2008; Kanitz *et al.,* 2009; Kouakou *et al.,* 2015) or AFLP loci (Burgos-Paz *et al.,* 2011). Some biodiversity studies have been carried out in Africa using the Kanitz *et al.* (2009) marker panel, such in Côte d'Ivoire (Kouakou *et al.,* 2015) although these authors did not find clear genetic differences among the three analysed populations. The most complete study on the genus *Cavia* was performed on mitochondrial DNA (Dunnum and Salazar-Bravo, 2010). Domestic

Table 2. Descriptive statistics of the twenty designed microsatellite marker loci.

Locus	NA	AR	Ho	He	PIC	HW	Fis
Cavy02	9	5.093	0.670	0.723	0.675	ns	-0.038
Cavy03	13	6.469	0.573	0.816	0.788	**	0.228
Cavy11	17	8.193	0.777	0.872	0.854	ns	0.068
Cavy12	18	9.969	0.500	0.913	0.902	nd	0.427
CUY01	8	5.196	0.588	0.756	0.718	*	0.076
CUY02	7	5.131	0.447	0.728	0.682	**	0.283
CUY03	11	5.997	0.650	0.790	0.756	ns	0.034
CUY04	9	5.832	0.500	0.708	0.680	**	0.186
CUY05	12	6.863	0.728	0.835	0.810	ns	0.097
CUY06	6	4.266	0.461	0.723	0.668	**	0.270
CUY07	7	4.551	0.373	0.690	0.639	**	0.457
CUY08	17	8.166	0.621	0.860	0.841	**	0.180
CUY09	7	4.002	0.398	0.547	0.503	ns	0.242
CUY10	11	6.299	0.573	0.790	0.760	ns	0.232
CUY12	9	5.278	0.703	0.754	0.716	ns	0.039
CUY16	11	7.229	0.767	0.829	0.808	ns	0.025
CUY17	10	6.857	0.713	0.840	0.816	ns	0.097
CUY18	10	6.287	0.578	0.800	0.770	**	0.212
CUY20	14	6.800	0.578	0.737	0.713	ns	0.178
CUY22	10	7.068	0.590	0.847	0.825	*	0.193
Mean±SD		6.270±1.430	0.590±0.115	0.778±0.080	0.750±0.100	**	0.173

NA, total number of alleles; AR, allelic richness; Ho, observed heterozygosity; He, expected heterozygosity; PIC, polymorphic information content; HW, deviation from Hardy-Weinberg equilibrium; Fis, fixation index within populations; SD, standard deviation. *P<0.05; **P<0.01; ns, not significant.

guinea pigs were included in these studies as an out-group. Our main objective was to compare the genetic diversity of the domestic guinea pig to the overall rearing area of the species. For this reason, we designed a panel of microsatellite markers to examine recent evolutionary events to infer the population structure and the genetic differentiation among different commercial lines and locally recognised guinea pig breeds. In addition, the importance of the guinea pig for the rural economy of several Latin American countries increases the need for molecular tools to further initiatives for their genealogical management and breeding design (Mommens *et al.,* 1998; Tozaki *et al.,* 2001; Bonnet *et al.,* 2002). Despite the diffusion into local communities and the low technological level needed for guinea pig farming, there exists intense commercial activity for these animals. Dinucleotide microsatellites are being used as genetic markers for the identification of population structure, genome mapping, and pedigree analysis and to resolve taxonomic ambiguities in many other animals in addition to the guinea pig (Xu and Liu, 2011; Martinez *et al.,* 2012; Gama *et al.,* 2013; Abdul-Muneer, 2014).

We successfully isolated, by scaffold genome sequencing, 25 microsatellite sequences, of which 16 were selected for the final panel based on their technical quality. All markers proposed here can be easily amplified in multiplex PCR reactions using crude sample lysates. Generally, all of the loci had a very high number of alleles (10.8±3.40), which was higher than the values found by Kanitz *et al.* (2009) and Kouakou *et al.* (2015), as well as a high mean allelic range (25 bp). Even if only 11 loci out of 20 were in HWE in overall sample, the F index values were very high (0.173). These findings, despite the high number of alleles, can be explained by the small sample number used in this preliminary study, possibly leading to the maximization of heterozygous excess values (Wahlund, 1928), as highlighted also by the HW disequilibrium calculated by separated populations that showed a significant value for the sixth population only in the markers Cavy12 and CUY7. These results can be due by the particular mating system based on using inbreeding animals added to the great interchange of males and females in the country markets. The total combined exclusion probability highlighted that the 20 loci are enough to obtain a good efficiency for parentage testing and traceability purposes in this species.

Conclusions

We have identified a set of 16 microsatellite loci for domestic *Cavia porcellus* genetic diversity research, and we have also established their standardised genotype analysis parameters. These markers could potentially resolve parentage and individual assignment cases. The high degree of genetic diversity and poly-

Table 3. Summary statistics for the non-exclusion probability values.

Locus	NE-1P	NE-2P	NE-PP	NE-I	NE-SI
Cavy02	0.683	0.510	0.321	0.123	0.421
Cavy03	0.538	0.363	0.181	0.059	0.359
Cavy11	0.417	0.262	0.102	0.031	0.324
Cavy12	0.309	0.183	0.053	0.015	0.299
CUY01	0.640	0.460	0.269	0.096	0.398
CUY02	0.677	0.502	0.313	0.119	0.417
CUY03	0.586	0.409	0.222	0.076	0.376
CUY04	0.678	0.489	0.279	0.112	0.426
CUY05	0.502	0.332	0.156	0.049	0.347
CUY06	0.702	0.532	0.356	0.130	0.423
CUY07	0.723	0.552	0.365	0.146	0.443
CUY08	0.440	0.281	0.113	0.035	0.331
CUY09	0.836	0.676	0.499	0.249	0.540
CUY10	0.578	0.399	0.208	0.072	0.375
CUY12	0.643	0.462	0.271	0.097	0.399
CUY16	0.502	0.330	0.147	0.048	0.350
CUY17	0.493	0.323	0.149	0.047	0.344
CUY18	0.565	0.389	0.202	0.068	0.369
CUY20	0.632	0.446	0.234	0.092	0.406
CUY22	0.478	0.311	0.139	0.043	0.339
PEC	0.99	0.99	0.99	0.84	0.99

NE-1P, non-exclusion of one candidate parent; NE-2P, candidate parent given the genotype of a known parent of the opposite sex; NE-PP, candidate parent pair; NE-I, identity of two unrelated individuals; NE-SI, identity of two siblings; PEC, combined exclusion probability calculated using the Jamieson formula (Jamieson, 1994).

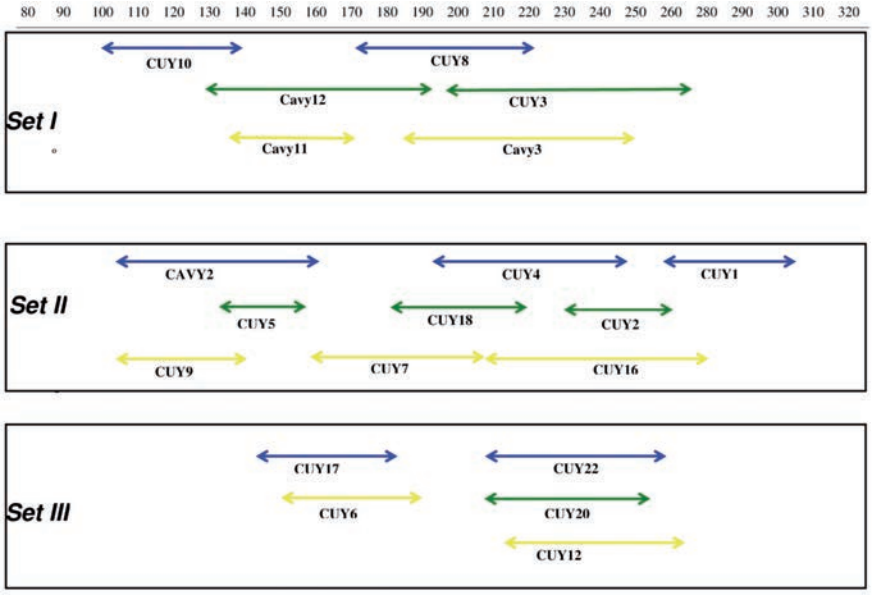

Figure 1. Electrophoresis set up of the twenty Cavia *porcellus* microsatellites based on allelic range and fluorescent dye for ABI D set. Blue=6FAM; Green=HEX; Yellow=ATTO550 (ROX as internal ladder).

morphisms indicate the potential of this microsatellite panel to be employed in future extended studies on the biodiversity of the cavy population. Therefore, genotype analyses with these standardised microsatellite panels will enhance cavy genetic selection by providing individual identification to increase the precision of measured phenotypes and for the construction of pedigrees to support the measurement of genetic estimates of phenotypic variation across generations.

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APPENDIX

Appendix Table 1. Total primers pair designed in available scaffold sequence of *Cavia porcellus*.

Origin sequence description	Accession number	Repeat motive°	Amplicon length	Forward oligo	Reverse oligo
Cavia porcellus clone CH234-497P15, complete sequence	AC171739.3	GT(18)	273	ctttcaggcaataggcatcc	gcagcttggactacagagca
Cavia porcellus clone CH234-9K24, complete sequence	AC173430.3	CA (22)	258	caagatgccatcaactttcgt	tgttgctgagatgctgcttt
Cavia porcellus, clone *Cavia porcellus*-24932957J7, complete sequence	AC165221.3	GT (18)	228	gcaagtcaaattcatccctga	gagtcctgccaagcaaaatc
Cavia porcellus clone CH234-14J14, complete sequence	AC175208.3	GT(22)	223	tcatctcgcttcagcatttg	aatggtcaggggctaggatt
Cavia porcellus clone CH234-497P15, complete sequence	AC171739.3	CA(20)	156	ggccaaagcaggaatgtcta	tagggcaagcattgatgatg
Cavia porcellus clone CH234-9K24, complete sequence	AC173430.3	CA(18)	162	tggcttgctttctctttggt	ctgtgctcagcattgcattt
Cavia porcellus clone B64 microsatellite sequence	GU045442.1	CA(18)	187	gatgcagtgcagaggagtca	tgtgtggttttgtgtgtgagg
Cavia porcellus clone CH234-402D11, complete sequence	AC175781.3	TC(21)	190	tgattgcacctgagaagtgg	ccaagtgttcttggtgcttg
Cavia porcellus clone CH234-334G9, complete sequence	AC181988.3	GT(18)	120	gctggaatgcaagacaagc	tgagttttcagctgtgatgagt
Cavia porcellus clone CH234-9K24, complete sequence	AC173430.3	GT(21)	117	ttccaagcatttcagaaaaca	tgacttcccaaccaaggaaa
Cavia porcellus clone C15 microsatellite sequence	GU045440.1	TG(20)	156	aaaatgtgtccatggggatg	gcatgtgtttatcgcgtctg
Cavia porcellus clone CH234-34N9, complete sequence	AC174609.3	AG(28)	242	ggaatggtggcaaactccta	tctcctcctcctcctccttc
Cavia porcellus clone CH234-34N9, complete sequence	AC174609.3	AG(24)	273	tgccaaatgagaatggatga	ggggttaatggcaatgtgtc
Cavia porcellus clone CH234-386E16, complete sequence	AC216606.3	CA(22)	250	agcaagaggcacacaagtca	ggggttaatggcaatgtgtc
Cavia porcellus clone CH234-14J14, complete sequence	AC175208.3	AG(25)	153	aaagctttggactgcgaaga	ttccttccttccttccttcc
Cavia porcellus clone CH234-14J14, complete sequence	AC175208.3	AT(25)	248	tttgagtcaagccgtgaaca	gcctgttttgaaactgttttactg
Cavia porcellus clone CH234-33F4, complete sequence	AC174824.3	TC(19)	154	tgatggacaatatactgggaacc	tagcatgcatgaagccctaa
Cavia porcellus clone CH234-33F4, complete sequence	AC174824.3	CA(21)	210	tgtcacttctcactccacca	tcccaaacctcttgtttgct
Cavia porcellus clone CH234-261L8, complete sequence	AC181987.3	TC(22)	196	tcccaaaggctgagcatatc	tggtcaaatttgtcttcatgtg
Cavia porcellus clone CH234-261L8, complete sequence	AC181987.3	AT(22)	231	tcttggaaatggcctacatttt	tggtctctaggggtatccatt
Cavia porcellus clone CH234-176E17, complete sequence	AC171368.3	TC(27)	262	atctttcctgccccttcttc	tggtgccacacacctgtaat
Cavia porcellus clone CH234-487I3, complete sequence	AC171142.3	TC(21)	248	cgaacatgccaagcagatta	acaccagttccttgccacat
Cavia porcellus clone CH234-487I3, complete sequence	AC171142.3	CA(21)	195	gcaaatgtgccatcttgtgt	aagttggttttgggggattt
Cavia porcellus clone CH234-176E17, complete sequence	AC171368.3	CA(27)	222	tgctgcagcctctttgaata	ccacagtggtaaatgatcgag
Cavia porcellus clone CH234-497P15, complete sequence	AC171739.3	CA(23)	108	aaatcgcctacagcaacattc	tttatggcaccagagagagc

°Repeat sequence length in National Center for Biotechnology Information sequence.

Appendix Table 2. Hardy Weinberg disequilibrium P value significance for each population.

	Andinean line	Inti line	Peru line	Commercial line	Peru	Bolivia	Colombia	Spain
CAVY02								
CAVY03							*	
CAVY11						**		
CAVY12	**		**	**	**	**	**	
CUY01								
CUY02				*		**	*	**
CUY03						**		
CUY04				*				
CUY05								
CUY06				**		*		
CUY07	*	*	*		**	**		
CUY08				*			**	**
CUY09		*						
CUY10				**		**	**	
CUY12								
CUY16								
CUY17	*					*	**	
CUY18								
CUY20								
CUY22								

*P<0.05; **P<0.01.