Microsatellite Marker Analysis for Laboratory Mice Profiling

Abstract

Background: The key point in the production procedure of inbred animals is checking the genetic purity. Skin grafting and coat color test are used traditionally to prove genetic purity, but they have some disadvantages. Recent advances in DNA profiling have enabled scientists to check easily the genetic purity of laboratory animals. In the current study, a set of microsatellite markers was designed to check the purity of inbred laboratory mice. **Materials and Methods:** Twenty microsatellites located on 20 chromosomes were employed to create a distinctive genetic profile for parentage analysis. Each individual primer was designed based on distinguishable colors and separable sizes. **Results:** Twenty specific microsatellite markers were used in the polymerase chain reaction mixture to identify inbred BALB/cJ strains. Our results confirmed that the designed microsatellites are excellent genetic markers for testing inbred BALB/cJ strain in laboratories. **Conclusion:** Our study showed that genetic profiling using microsatellite markers allows us to detect the genetic differences of laboratory mouse species in quality control tests and validation steps.

Keywords: *BALB/cJ, genetic profile, inbred, laboratory mice, microsatellite*

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Introduction

Animals have popularly used in biomedical and behavioral research due to remarkable benefits for human health. For example, the use of animals in experimental medicine increased notably life expectancy in the United States.[1] Among laboratory animals, small rodents, particularly rats and mice, are frequently used in quality control (QC) tests.[2] The latter also plays an important role in scientific researches.[2] Moreover, with advances in gene-editing technologies, the number of available transgenic mouse models is growing fast, and the art of managing mouse colonies is more important than ever. Therefore, colony managers often request mouse husbandry experts to develop economical and efficient techniques to determine genetically well-defined animals.[3]

Two major classes of laboratory mice include inbred and outbred strains. The first defines as a genetically homologous strain which the heterogeneity in the genome is <1% among the colony. This strain is produced by mating within a family over at least 20 generations. Due to this procedure, many genetic variants become fixed, and a specific and reliable genetic background is created in the colony. The use of such inbred mouse strains could decrease experimental variations and increase reproducibility and repeatability of *in vivo* tests.[4] Although inbred mice are treated as genetically identical, errors in DNA replication and germline transmissions invariably create genetic variations within an inbred strain. As a result, each mouse harbors new variations in the genome (approximately 60 loci). Although this amount of variation may not significant when considering the size of the genome, these mutations will accumulate and may become fixed in the population, causing an inbred strain to drift genetically over time.[5] This means that any inbred strain maintained for >20 generations will become genetically distinct from its parental strain. In the last 30 years, many animal vendors began to prevent this drift by the refreshing breeding stock of inbred strains from the cryopreserved embryos at defined intervals to reset this genetic clock and maintain the strain with a consistent genetic background over time.[3] Therefore, genetic drift is no longer an issue for most commercial mice.

Nonetheless, the identification of drift during the breeding is a critical step for applying such an approach to prevent the

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Quick Response Code:

No.	Marker	Sequence	Initial size (bp)	Fluorescent color (Size)	Final size (bp)
1	D10Mit230	F: TGGCCTTGGTCTGCAAAAGT	146	FAM (39bp)	185
		R: TTAAGAAGGGTAACAGTTGT			
2	D15Mit14	F: TCCACACATATAAGCTCACAGATA	186		225
		R: AACCCATCAATGAACCATGTAGCC			
3	D3Mit55	F: AGTTAGCTTTGAAATGGCATC	264		303
		R: GATCTAAGCATAAACCATACAT			
4	D12Mit4	F: AAACCATCTCAAGACCAATCCAA	303		342
		R: TACAGTGATGCCCCATTTCAGA			
5	D8Mit304	F: GCATGCACGCACACATGTG	119	HEX (38bp)	157
		R: TTGAGGAATGAAGTCCAGGTA			
6	D12Mit56	F: GCAGAGGCCACTTACTGCTGTGTC	159		197
		R: CACTGTAGAACAAAAGATGAGTCC			
7	D17Mit70	F: AATTTTGACAACTAAGACTTA	177		215
		R: CCACCCACCAACTGTCTACA			
8	D8Mit200	F: GCAGTACCTTGTCTAAGAATTAGAAGC	209		247
		R: TGCTGCTGATGTTGATGTTG			
9	D15Mit16	F: AAATCCGTGGTACCTAAGAGGG	255		293
		R: TACACCTATCTGCTTTATTTTGCCC			
10	D11Mit167	F: GCCTGAATCCTGCCAAGAG	322		360
		R: TCCTTGGCAAAATCCTTGAG			
11	D16Mit110	F: CAGAGAATCCCCACCTTGAA	96	NED (39bp)	135
		R: GGTACCATGGATATCAAGTATGAGC			
12	D11Mit179	F: TTTGGCATTCACATGTAGGTC	135		174
		R: TTGCTTTATAATCTTTCTCTGTGTG			
13	D11Mit224	F: CCTCAAGCCCTGAGTTTGAT	214		253
		R: CTCCTTTTTAAGACAAAACATCAACTA			
14	D9Mit2	F: TTCTGCTGGATTCTGTTGA	289		328
		R: AAATGGAGACAGGTAAAAACA			
15	D13Mit78	F: ATCAAAGTGTAAAGTGCTTG	297		336
		R: GGTTGCCAGCTATGCCTGCCAG			
16	D12Mit263	F: TCCCCTGGAGCATATTTGAC	130	PET (39bp)	169
		R: TCAGATCTCAGCAGATAAATACTTGG			
17	D1Mit17	F: CAAGCTTGTACACAAAATGC	175		214
		R: TCCCCTGCTGGCCTCCTTGG			
18	D13Mit66	F: CCAACTTCAGCCATAAGACAG	214		253
		R: ACTATGGACAAGGGTTGAAGC			
19	D2Mit51	F: ATTTCAGGGCCTGGGGAGATGG	285		324
		R: CCCTTATTGTTTTGAGACGGGGTC			
20	D1Mit15	F: TCCATGAAGAAACCCATGC	320		359
		R: CCAAGAGAAGAAATATCAGC			

accumulation of genetic variations in an inbred strain. Traditional methods, such as skin grafting and coat color test, are used to prove genetic purity, but these methods have some disadvantages including low accuracy, high cost, being laborious, and requirement of technical expertise.[6] On the other hand, genetically approaches have been developed and popularly recommended for this purpose as an easy, simple, and accurate method for genetic detections in many fields of medicine and health sciences.[6] Among genetic-based approaches, there has been a great deal of attention toward microsatellites. Microsatellites are repetitive short sequences with single-nucleotide regions that are abundantly distributed in the genome of eukaryotes and are used popularly as markers in genetic studies. Microsatellite markers, also known as short tandem repeats (STRs), were discovered in the 1990s.[7] They have been very valuable for gene identification by means of linkage study and gene segregation analysis for prenatal diagnosis.[8] They were used for human identification as early as 2001.[9] Since then, STR markers have been used for various purposes including quantitative fluorescence polymerase chain reaction (PCR),[10] autozygosity studies, animal parentage testing as well as animal identification.[11] STR markers are now a powerful tool for testing if an animal (e.g. laboratory animal species) are inbred or not? Therefore, in the current study, we aimed to develop a novel
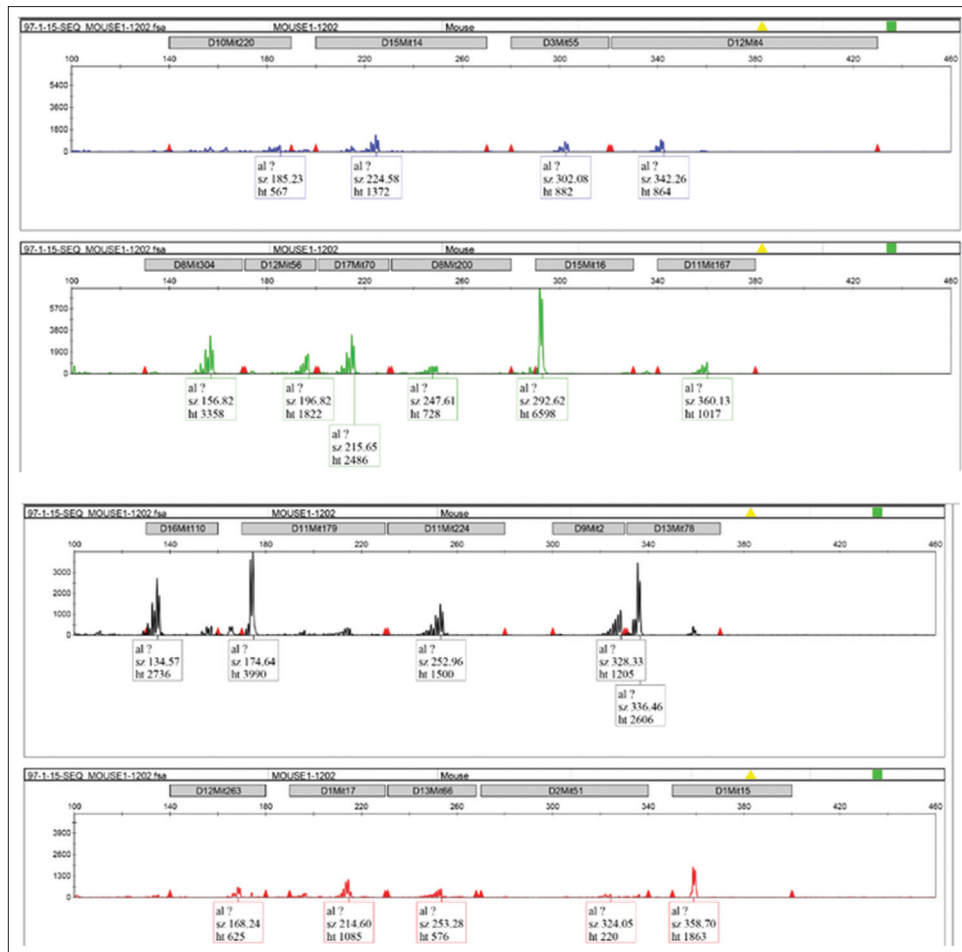
Figure 1: DNA microsatellite graphs regarding to mouse number 1

panel of STR markers selected from different chromosomes for testing whether different breeds of laboratory mice are inbred or not.

Materials and Methods

Microsatellite locus analysis and primer design

Using the NCBI database and Mouse Genome Informatics, locus information and sequence details of all microsatellites were obtained.[11] Primers were designed using Gene Runner software version 6.5.51 (Copyright © 1992-2018 Frank Buquicchio) according to the Whitehead Institute's MIT database [Table 1].

DNA samples

The number of mice was computed using the following formula to detect the herd contamination with a prevalence of 10%.

S = log (p)/log (U)

Fifty BALB/cJ mice (6–8 weeks) were used for the study, and samples were obtained by cutting the ends of the mice's tail after local anesthesia. DNA purification was performed from the samples as follow. 288 μl lysis buffer (584 mg

NaCl, 93 mg ethylenediaminetetraacetic acid disodium, 125 mg sodium dodecyl sulfate, 5 ml 1M tris buffer, up to 50 ml) and 6 μl proteinase K were added to 1.5 ml microtube containing 2 mm of the tail. The samples were then incubated at 55°C for overnight. After incubation, 1 ml of ethanol 100% was added to the mixture and mixed well. The samples were then centrifuged for 30 min at 16,000 g. The precipitate was washed with 1 ml 70% alcohol and re-centrifuged 16,000 g for 20 min. The supernatant was discarded, and the precipitate was dissolved in 300 μl ddH$_2$O by a 2 h incubation at 55°C. Finally, the samples were kept at −20°C for further analysis.

Multiplex polymerase chain reactions

At first, distilled water, 10x buffer, 100 mM MgCl$_2$, 40 mM dNTP, forward and reverse primers (100 pM), *Taq* DNA polymerase (1 unit), and DNA of each sample (200 ng/μl) were added into the 0.2 μl microtubes and mixed well. The microtubes were then inserted into a thermocycler device, and multiplex PCR was performed according to the following program (95°C for 5 min [1 cycle]; 95°C for 1 min, 60°C for 1.5 min, and 70°C for 2 min [30 cycles]; and 70°C for 15 min [1 cycle]). To ensure multiplex PCR

works correctly, the PCR products were electrophoresed on 1% agarose gel. Then, the samples were subjected to a genetic analyzer (ABI 3130XL Genetic Device) for further analysis.

Examination of polymerase chain reaction products using genetic analyzer

PCR products were evaluated to measure the amount of each marker using the ABI 3130XL Genetic Device. Briefly, 17 µl methanol was added into the samples and heated to 95°C for 5 min. Then, the samples were placed on ice for 3 min, and 1 µl of each product was applied to the device. The GeneMapper software was used to analyze the results in FASTA format.

Results

Genomic DNA from the samples was purified and subjected to the multiplex PCR. After confirmation by agarose gel electrophoresis, the samples were applied to the capillary electrophoresis, and the data were analyzed by a genetic analyzer. For example, DNA microsatellite graphs for mouse numbers 1 and 19 were shown in Figures 1 and 2, respectively. The size of each amplified

marker is presented in Table 2. The results were matched well with the data compared to Jackson database for inbred BALB/cJ species. The only marker that has a slight difference between the study and database was D16Mit110 (135 bp vs. 139 bp), which did not adequate for the difference in heredity.

Discussion

Today, biomedical research using laboratory animal is widespread and has great importance in diagnosis and drug development.[2] Therefore, scientists are mainly looking for animal models that have the lowest genetic variation to gain accurate and reliable results.[12] Using inbred species is one of the most important ways to achieve this goal.[13] Inbred populations are in fact colonies generated by at least 20 subsequent mating cycles from the first generation. In this system, the populations are genetically similar in their genomic natures. In fact, the strains obtained are ideal models for biological, pharmaceutical, and clinical assays. In another word, the genetic changes affecting the biomedical experiments in these species are very low. Different parentage verification and/or monitoring systems were developed and tested for inbred horses. However,
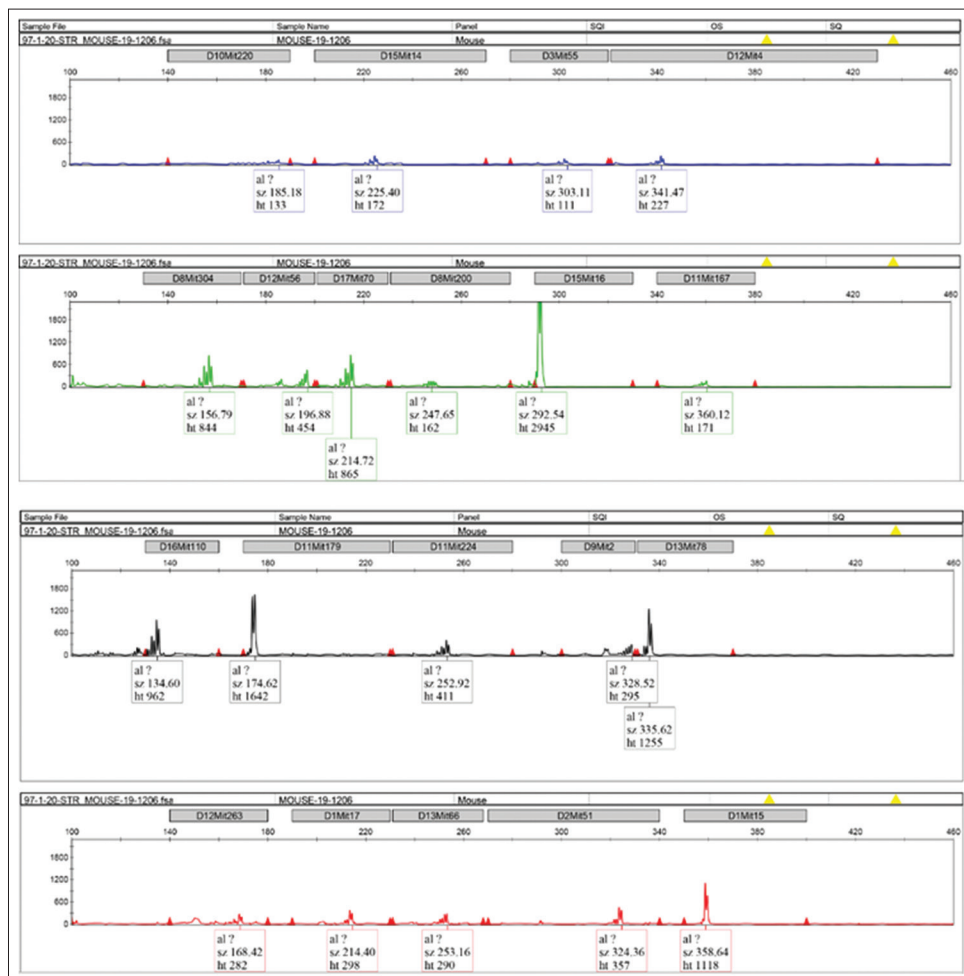

Figure 2: DNA microsatellite graphs regarding to mouse number 19

Table 2: The PCR product size (bp) of each marker obtained for the samples

Mice no.	D1Mit3	D15Mit5	D18Mit17	D1Mit46	D13Mit53	D11Mit2	D9Mit2	D13Mit78	D16Mit10	D11Mit179	D11Mit224	D9Mit110	D15Mit16	D17Mit167	D12Mit200	D8Mit56	D8Mit30	D12Mit4	D3Mit4	D15Mit55	D10Mit230
1	359	324	253	214	169	253	328	336	253	174	135	360	293	247	215	197	157	342	303	225	185
2	359	324	253	214	169	253	328	336	253	174	135	360	293	247	215	197	157	342	303	225	185
3	359	324	253	214	169	253	328	336	253	174	139	360	293	247	215	197	157	342	303	225	185
4	359	324	253	214	169	253	328	336	253	174	135	360	293	247	215	197	157	342	303	225	185
5	359	324	253	214	169	253	328	336	253	174	135	360	293	247	215	197	157	342	303	225	185
6	359	324	253	214	169	253	328	336	253	174	135	360	293	247	215	197	157	342	303	225	185
7	359	324	253	214	169	253	328	336	253	174	135	360	293	247	215	197	157	342	303	225	185
8	359	324	253	214	169	253	328	336	253	174	135	360	293	247	215	197	157	342	303	225	185
9	359	324	253	214	169	253	328	336	253	174	139	360	293	247	215	197	157	342	303	225	185
10	359	324	253	214	169	253	328	336	253	174	135	360	293	247	215	197	157	342	303	225	185
11	359	324	253	214	169	253	328	336	253	174	135	360	293	247	215	197	157	342	303	225	185
12	359	324	253	214	169	253	328	336	253	174	135	360	293	247	215	197	157	342	303	225	185
13	359	324	253	214	169	253	328	336	253	174	139	360	293	247	215	197	157	342	303	225	185
14	359	324	253	214	169	253	328	336	253	174	135	360	293	247	215	197	157	342	303	225	185
15	359	324	253	214	169	253	328	336	253	174	135	360	293	247	215	197	157	342	303	225	185
16	359	324	253	214	169	253	328	336	253	174	135	360	293	247	215	197	157	342	303	225	185
17	359	324	253	214	169	253	328	336	253	174	135	360	293	247	215	197	157	342	303	225	185
18	359	324	253	214	169	253	328	336	253	174	135	360	293	247	215	197	157	342	303	225	185
19	359	324	253	214	169	253	328	336	253	174	135	360	293	247	215	197	157	342	303	225	185
20	359	324	253	214	169	253	328	336	253	174	135	360	293	247	215	197	157	342	303	225	185
21	359	324	253	214	169	253	328	336	253	174	135	360	293	247	215	197	157	342	303	225	185
22	359	324	253	214	169	253	328	336	253	174	135	360	293	247	215	197	157	342	303	225	185
23	359	324	253	214	169	253	328	336	253	174	135	360	293	247	215	197	157	342	303	225	185
24	359	324	253	214	169	253	328	336	253	174	135	360	293	247	215	197	157	342	303	225	185
25	359	324	253	214	169	253	328	336	253	174	135	360	293	247	215	197	157	342	303	225	185
26	359	324	253	214	169	253	328	336	253	174	135	360	293	247	215	197	157	342	303	225	185
27	359	324	253	214	169	253	328	336	253	174	139	360	293	247	215	197	157	342	303	225	185
28	359	324	253	214	169	253	328	336	253	174	135	360	293	247	215	197	157	342	303	225	185
29	359	324	253	214	169	253	328	336	253	174	135	360	293	247	215	197	157	342	303	225	185
30	359	324	253	214	169	253	328	336	253	174	135	360	293	247	215	197	157	342	303	225	185
31	359	324	253	214	169	253	328	336	253	174	135	360	293	247	215	197	157	342	303	225	185
32	359	324	253	214	169	253	328	336	253	174	135	360	293	247	215	197	157	342	303	225	185
33	359	324	253	214	169	253	328	336	253	174	135	360	293	247	215	197	157	342	303	225	185
34	359	324	253	214	169	253	328	336	253	174	139	360	293	247	215	197	157	342	303	225	185
35	359	324	253	214	169	253	328	336	253	174	135	360	293	247	215	197	157	342	303	225	185
36	359	324	253	214	169	253	328	336	253	174	135	360	293	247	215	197	157	342	303	225	185
37	359	324	253	214	169	253	328	336	253	174	135	360	293	247	215	197	157	342	303	225	185
38	359	324	253	214	169	253	328	336	253	174	139	360	293	247	215	197	157	342	303	225	185
39	359	324	253	214	169	253	328	336	253	174	135	360	293	247	215	197	157	342	303	225	185
40	359	324	253	214	169	253	328	336	253	174	139	360	293	247	215	197	157	342	303	225	185

Table 2: Contd...

D1Mit		D3Mit	D4Mit	D5Mit	D6Mit	D13Mit78	D9Mit2	D11Mit224	D11Mit179	D16Mit110	D11Mit110	D11Mit167	D15Mit10	D8Mit200	D17Mit70	D2Mit56	D12Mit4	D3Mit55	D15Mit14	D10Mit230	Mice no.
359	253	324	214	253	169	336	328	253	174	135	360	293	247	215	197	157	342	303	225	185	41
359	253	324	214	253	169	336	328	253	174	135	360	293	247	215	197	157	342	303	225	185	42
359	253	324	214	253	169	336	328	253	174	135	360	293	247	215	197	157	342	303	225	185	43
359	253	324	214	253	169	336	328	253	174	135	360	293	247	215	197	157	342	303	225	185	44
359	253	324	214	253	169	336	328	253	174	135	360	293	247	215	197	157	342	303	225	185	45
359	253	324	214	253	169	336	328	253	174	135	360	293	247	215	197	157	342	303	225	185	46
359	253	324	214	253	169	336	328	253	174	135	360	293	247	215	197	157	342	303	225	185	47
359	253	324	214	253	169	336	328	253	174	135	360	293	247	215	197	157	342	303	225	185	48
359	253	324	214	253	169	336	328	253	174	139	360	293	247	215	197	157	342	303	225	185	49
359	253	324	214	253	169	336	328	253	174	139	360	293	247	215	197	157	342	303	225	185	50

among them, the usefulness of microsatellite markers was frequently proved by many investigations on different animal species. In a study, 17 STRs on different locations in the chromosomes were tested for parentage analysis of polish cold blood and Hucul horses. Although their parents were only excluded in a single locus, deviations were mainly observed in the loci. They concluded that DNA typing complications of some STR sequences, sex linkage of STRs as well as the null allelic occurrence were the source of wrong parentage exclusion.[14] In another study, 45 Caspian horses were genotyped for individual identification. Genotype evaluations were carried out by PCR using seven microsatellite markers. All studied microsatellite markers have high polymorphic information content value. They successfully used DNA typing for parentage testing and individual identification of Iranian horses.[15] In a similar investigation, 79 Jeju horses were genotyped using 20 microsatellites for parentage testing. The number of alleles and the polymorphic information contents ranged from 5 to 11 and 0.335–0.816, respectively. The total exclusion probability of the microsatellites was approximately one, and therefore, genotyping of Jeju horses using developed microsatellites was useful for tracking any genetic deviation in the species.[16] In a study by Takasu *et al.,* 31 microsatellite DNAs were employed for genotyping 125 endangered Kiso horses, which 83% of horses were breed. They found that inbreeding level was low and the horses have experienced a rapid loss of population. They concluded that parentage testing using designed microsatellites was highly reliable with a probability of exclusion.[17] Their work confirmed microsatellite markers are also useful for monitoring of wild genetic diversity and could be considered as a powerful tool in maintenance management of wild-type species. In another study by Moshkelani *et al*., 14 microsatellite markers were successfully used for identification and parentage testing of Iranian Arab horses. The average heterozygosity and the expected of heterozygosity were 0.656 and 0.697 in the population, respectively.[18] Luis *et al.* used six microsatellites for parentage testing in different Portuguese autochthonous horse breeds. Microsatellite loci were selected based on the polymorphism detected in other breeds. They also compared traditional techniques such as blood groups and protein polymorphisms with microsatellite DNAs in the same breeds. Surprisingly, the results showed that the microsatellite sets are comparable for paternity assignment of two breeds as shown by the high average exclusion probability values.[19]

Conclusion

In this study, BALB/cJ laboratory mice were evaluated in term of genetic purity for biotechnological applications using microsatellite markers. In our research work, 20 microsatellites located on 20 chromosomes were employed to create a distinctive genetic profile. Each

individual primer was designed based on distinguishable colors and separable sizes. Our data proposed that the designed STR markers could be considered for genetic testing of BALB/cJ mice in QC tests of pharmaceutical laboratories.

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Conflicts of interest

There are no conflicts of interest.

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