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Assessment of Genetic Purity of Inbred Strains of Mice using Microsatellite Markers

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Abstract

Monitoring of genetic make-up of inbred strains of mice is one of the most essential and basic requirements to assure the continued purity and homogeneity of the strains. It further helps in maintaining phenotypic characteristics of mice strain, which in turn, ensures the reproducibility of research outcomes between the group of mice and over time. In the present study, the phenotypic and genotypic characteristics of various inbred strains of mice maintained in our Animal Facility were assessed to ascertain their purity. The mice exhibited strain-specific phenotypic characters and skin coat colours in accordance with the published profile of the specific strains. The average litter size of C57BL/6 and BALB/c mice, the most commonly used strains of mice, was calculated over different generations and found to be 5 to 6.6 and 4 to 6, respectively. For checking the genetic purity of mice, more than 1700 tissue samples (ear punched tissue) were collected from various strains of mice. Genomic DNA was isolated from the tissue samples and subjected to PCR-based microsatellite analysis. Amplified PCR products of genomic DNA of tested mice showed identical amplicon base-pair length as per the published profile in the MGI database, indicating the purity and uniformity of the mice.

Keywords: Genetic Monitoring, Inbred Mice, Microsatellite Markers, C57BL/6, BALB/c

1. Introduction

Toxicology is the study of the adverse consequences of chemicals and biologicals on living organisms. Regulatory toxicology includes the collection, processing, and evaluation of epidemiological as well as experimental toxicology data. This in turn authorizes the researchers to take preventive decisions directed towards the safety of health against the deleterious effects of chemical substances. In 1970, the United States Food and Drug Administration (USFDA) investigated many toxicology laboratories and established that there were deficiencies in experimental planning and substandard managerial practices that have resulted in unreliable outcomes of animal toxicity studies. After the establishment of the Organization for Economic Cooperation and Development (OECD), the Good Laboratory Practice (GLP) regulations came into existence in 1978. The expert group of OECD developed and laid down the principles of GLP and made the way for researchers to produce internationally acceptability data of safety testing by adhering to these principles from different countries[1]. To achieve reproducible, repeatable, and reliable test results in regulatory studies, it is essential to have sound, healthy, valid, unbiased test systems, i.e., research laboratory animals.

Genetic, microbial, and parasitic monitoring are the three primary types of test systems used for animals in research. Genetic monitoring, specifically checks the genetic purity of mice strains or different alleles in the mutant stock

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and ensures genetic integrity. The genetic monitoring program has been practical in achieving successful individual reproductive and breeding behavior, along with determining the movement of alleles and gene flow[2].

In mouse colony management, genetic monitoring has been established as a testing system to assure the genetic purity of laboratory animals and to detect and avoid contamination by checking various gene loci or another polymorphic locus in the genome[3]. Historically, hemagglutination, biochemical markers, skin grafting, Restriction Fragment Length Polymorphism (RFLP) were used for genetic monitoring along with Single Sequence Length Polymorphism (SSLP) and Single Nucleotide Polymorphism (SNP)[4]. Biomedical research is incomplete without the use of laboratory mice due to significant advantages for human health. Laboratory animals are used in Quality Control (QC) tests and plays an essential role in scientific research[5]. Mice have anatomical, genetic, and physiological similarities to humans, which is ideal for biomedical research animal models. Merits of mice over other rodents include their small size, easy maintenance, short reproductive cycle, and ample genetic resources and literature. Reduced genetic variation and a specific as well as reliable genetic background in the colony of mice help in getting increased reproducibility and repeatability of animal studies[6]. Inbred mice are alike genetically at all of their loci. Genetic homogeneity allows them to react uniformly to experimental treatments. Owing to the usage of inbred mice, researchers throughout the world can analyze and compare their experimental studies with each other[7]. In addition, genetic homogeneity will reduce the number of mice per experimental group, complying with the 3Rs principle of animal research given by Russel and Burch[8].

Microsatellites are short repeat sequences with single-nucleotide regions present numerously in the genome of eukaryotes, and hence, has been attaining a great deal of attention. The microsatellite markers were discovered in the 1990s as powerful tools for testing inbred animals and analysis for prenatal diagnosis with identification of genes through linkage studies and gene segregation[9]. The presence of microsatellites in the genome can be assessed using automated analysis, which makes them an indispensable tool for genotyping[10]. Polymerase chain reaction (PCR), a modern molecular biology technique is used to amplify a single copy of a DNA (Deoxyribonucleic acid) fragment into millions of copies[11]. The mouse's genetic integrity and homologous genome are essential for the generation of uniform, reproducible, and repeatable data, which is to be internationally accepted. This study describes our efforts to maintain mice of a uniform genotype necessary for good science using PCR-based microsatellite markers. "Use of quality animals will facilitate our scientific community to carry out excellent science for the benefit of human and animal health", signifying the importance of genetic monitoring of mice used for research.

2. Material and Methods

2.1 Animals

IMTECH Centre for Animal Resources & Experimentation (iCARE) CSIR-IMTECH, Chandigarh, is a barrier-maintained animal facility approved by the Committee for the Purpose of Supervision of Experiments on Animals (CPCSEA), houses several inbred strains of mice viz. BALB/c, C57BL/6, C3H/He, and DBA/2 in individually ventilated cage (IVCs) system in different rooms. All mice are maintained under standard environmental conditions, the temperature of 24-25^{0}C with a relative humidity of 30–70% and 12 hrs light/dark cycle and are provided feed and water *ad libitum*. All protocols performed in this study were approved by the Institutional Animal Ethics Committee (IAEC) of IMTECH, Chandigarh (approval number IAEC/QC/1, dated November 16, 2006). The principles and guidelines of CPCSEA, Ministry of Fisheries, Animal Husbandry and Dairying, India, were followed to minimalize pain and distress in animals using analgesics and/or anaesthetics.

2.2 Sample Collection

The tissue samples were collected during the ear punching procedure used for the identification of mice. This pierced ear tissue is enough to accomplish a genomic analysis of mice using PCR-based genotyping.

2.3 DNA Isolation

DNA from tissue samples was extracted either with proteinase K (Promega, USA) digestion method or using DNeasy Blood and Tissue Kit (Qiagen, Germany) as per the manufacturer's instructions.

2.4 Polymerase Chain Reaction

The 25µl of the reaction mixture was prepared using master mix (Buffer, MgCl2, dNTP, Taq DNA polymerase, New England Biolabs, USA; MP Biomedicals, USA) and primers. DNA (~100 ng/µl) was added to each reaction, and the volume was made up with nuclease-free water. Then the reaction tubes were kept in a thermal cycler. The protocol was set; initial denaturation at 94^{0}C for 10 minutes, denaturation at 94^{0}C for 40 seconds, annealing at 55^{0}C for 40 sec., extension at 72^{0}C for 1 min, repeated for 35 cycles,

and final extension at 72^{0}C for 5 min. The microsatellite primers were selected from the Mouse Genome Informatics (MGI) database and synthesized commercially. The primer sequence details and amplicon size are given in Table 1.

Table 1. Sequence details and amplicon size of microsatellite primers

Primer	Sequence 5' – 3'	Amplification (bp)	MGI Accession ID
D1Mit21	CGCTGGACAATCTTATAATTGCA TCGAATCCCAACAACCACAT	C3H/He: 230bp DBA/2:250bp	MGI:706769
D6Mit39	CTTGACTTCCCCAGGTATGTG TTGACTGCCTTTTTTTTTTGG	C3H/He:118bp DBA/2:118bp	MGI:706334
D10Mit35a	GTGCTGCTTCACTGTTTGGA CAAGCAAGGTAAATTGGAAAGG	C57BL/6: 228bp BALB/c:240bp C3H/He: 228bp DBA/2:228bp	MGI:705597
D12Mit5	CACATAGACCAGACAGGCATGCGT CAAGGTCACGTTGCTAGCTAGGAA	C57BL/6:176bp BALB/c:160bp C3H/He:160bp DBA/2:160bp	MGI:702642
J:34136 Whitehead Institute at MIT, *et al.*, Genetic and Physical Maps of the Mouse, Database Release. Database Release. 1999			

2.5 Agar Gel Electrophoresis

Agar gel (2%) was prepared by mixing agarose powder in TAE (Tris-acetate-EDTA) buffer, and ethidium bromide was added to the gel to facilitate visualization of DNA after electrophoresis. When adequate migration has occurred, the gel is placed on an ultraviolet Tran's illuminator to observe the bands of amplified products. The gel documentation was done, and images were taken.

3. Results

The genetic monitoring protocol was approved by IAEC of the institute for quality control; since then, more than 1700 tissue samples were collected for genotyping in the last fifteen years. The phenotypic characters and skin coat colours were monitored and found that C57BL/6 (Black), BALB/c (Albino), C3H/He (Agouti), and DBA/2 (Brown) were the same as their parent generation's published literature. Inbred C57BL/6 and BALB/c mice are the primarily utilized strains in our institute for research. Hence, we maintained the pedigree record of their foundation colonies, including average litter size as well as the average number of males and females obtained at the time of weaning of mice, which is presented in Figures 1, 2, and 3. The pedigree record and its maintenance are vital to avoid genetic contamination. The average litter size in C57BL/6 and BALB/c mice ranges from 5.00 - 6.67 and 4.00 - 6.40, respectively. The C3H/He and DBA/2 mice were used only for experimental work, but we still checked their genetic quality.

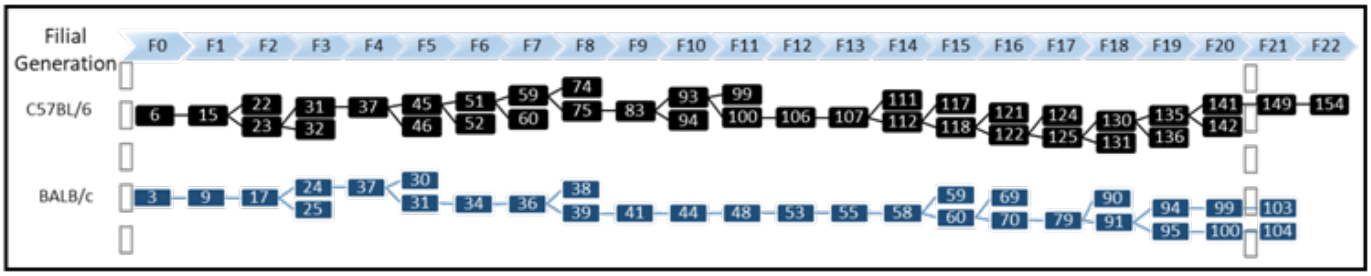


Figure 1. Pedigree chart of C57BL/6 and BALB/c mice strain at iCARE, CSIR-IMTECH, Chandigarh.

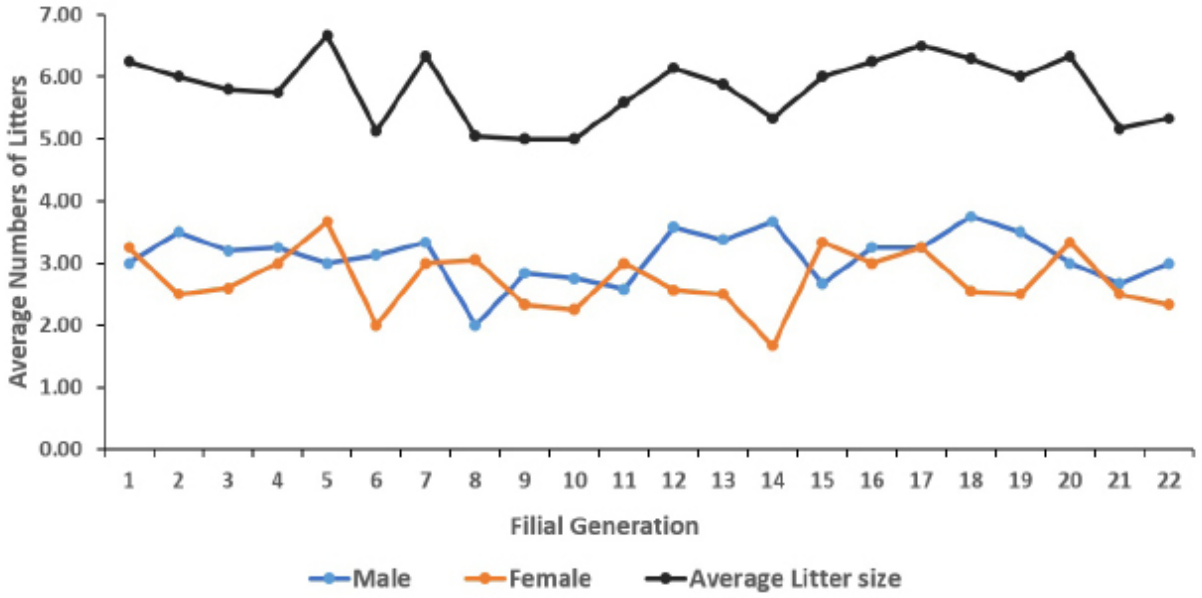


Figure 2. Pattern of average litter size, average number males and females of C57BL/6 mice up to 22 generations.

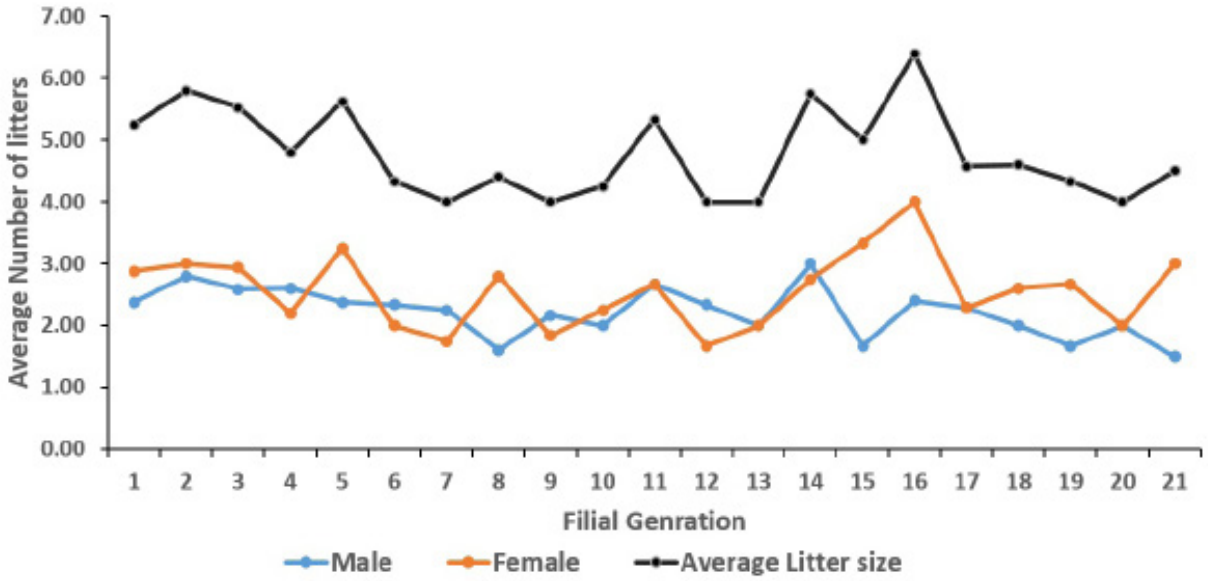


Figure 3. Pattern of average litter size, average number of males, and females of BALB/c mice up to 21 generations.

The genetic monitoring of mice is an integral component of the quality control program designed for mice maintained and bred in our iCARE facility. The isolated genomic DNA from the tissue samples was subjected to PCR. After confirmation by agarose gel electrophoresis, the amplicon base-pair lengths published in the MGI database were compared and presented in Figure 4. The base-pair length in our results compared with the standard base-pair length mentioned in the MGI database for inbred C57BL/6, BALB/c, C3H/He, and DBA/2 mice strains were found identical. Differentiation of these four strains maintained at iCARE was done based on the four selected microsatellite markers as they are having the same amplified base-pair length in more than two markers.

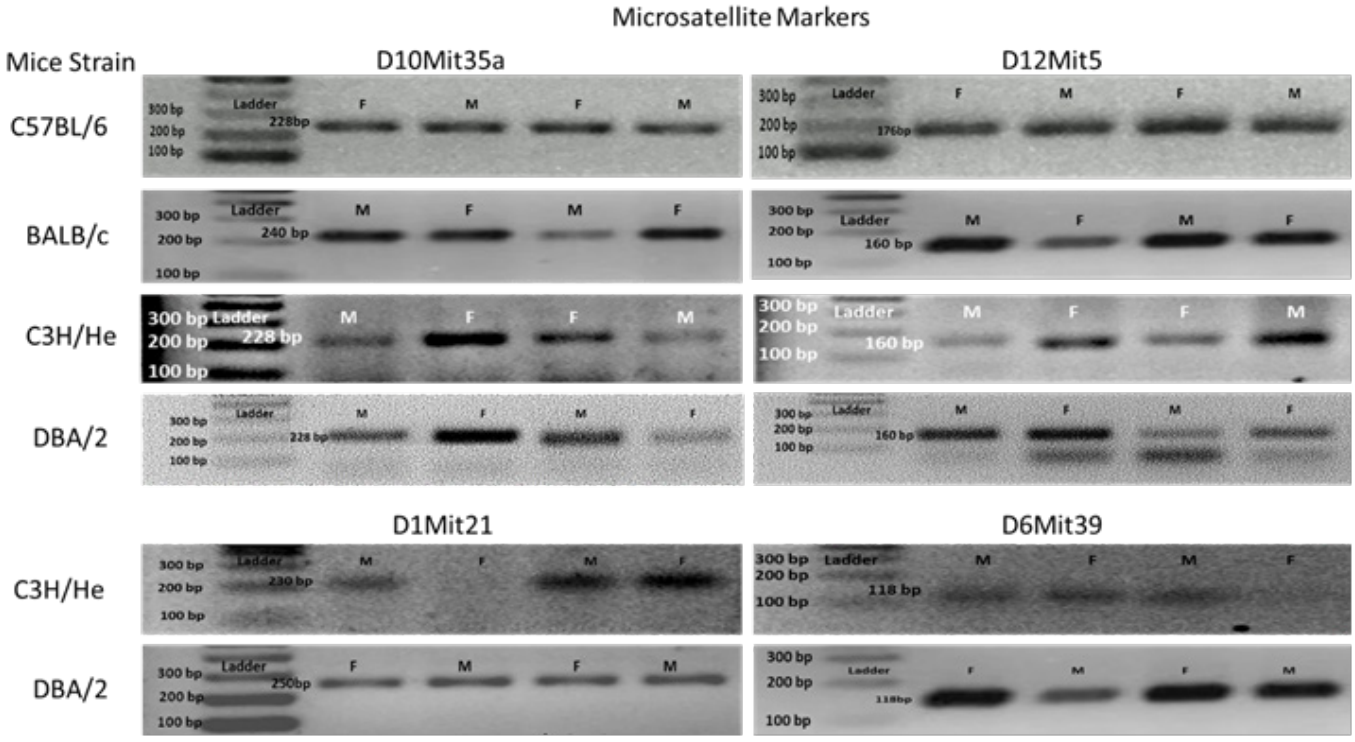


Figure 2. Representative gel images from the mice were genotyped using different microsatellite markers.

4. Discussion

Currently, laboratory animals are extensively used in immunological, toxicological, pharmaceutical, and biomedical research and have immense importance in drug development and disease diagnosis. Therefore, researchers are looking for animal models with low genetic variation to get precise, uniform, and consistent results[12]. The solution to this problem is to use inbred mice to achieve health benefits for human beings. Inbred animals are generated by sibling mating for at least 20 generations and have a homozygous genome. These strains are ideal for biological, pharmaceutical, and clinical assays as their homogeneity of genetic materials will not affect the experimental protocols[13]. Different monitoring systems were established and tried for inbred mice; however, the effective use of microsatellite markers for monitoring was demonstrated by many investigations on various animal species[9]. Microsatellite markers in the mice genome can be used for mapping different genes and reviewing segregation patterns. PCR-based methods can identify and genotype different strains, thus permitting for exact genotypes to be assigned[14]. Cohan *et al.* (2019)[9], used 20 microsatellite markers designed to check genetic purity by PCR. The genetic profiling of the BALB/cJ mice using microsatellite markers allowed the researchers to detect the genetic purity of the strain in quality control tests. In another study, the authors used a phenotypic character, pedigree record, line segregation, and genetic analysis by microsatellite markers as tools to prove that there is no diversity among the different lines of BALB/c mice maintained[15]. The genetic monitoring using microsatellite marker of inbred strains of mice has several advantages compared to traditional markers such as histocompatibility and isoenzyme variations. The genetic relationship between inbred, outbred, and transgenic strains was evaluated with different 14 microsatellite markers. Inbred strains have significantly low genetic variability and pups of inbred mice are suitable for expanding purebred lines to study genetic variation, cloning, gene mapping, and mutation[16]. Gurumurthy and co-workers validated 400 sets of SSLP markers from the MGI database on 10 distinct mouse inbred strains. They described the cost-effectiveness of these markers using PCR and the agarose gel electrophoresis technique. This assay and analysis of amplified products can be accomplished in any basic molecular biology laboratory[17]. In the present study, using 4 microsatellite markers, we have analyzed the genetic purity of the pedigree maintained mouse lines in our facility. The continued genotyping of these mice has supported a breeding program designed to supply quality, genetically characterized mice for the biomedical, pharmaceutical, and toxicological research programs of our institute as well as various other CPCSEA registered institutes.

5. Conclusion

PCR-based microsatellite analysis results are compared to check the genetic purity of different inbred mouse strains bred and maintained at iCARE, CSIR-IMTECH. This study inferences that by utilizing the number of samples and microsatellite markers, the genetic diversity within and between the strains can be prevented and assist in maintaining pure inbred lines of mice.

6. Acknowledgement

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7. Conflict of Interest

The authors declare that they have no conflict of interest.

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