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Genetic monitoring of breeding colony through evaluation of genetic diversity within and between the inbred lines by using microsatellite markers

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Abstract
BALB/c strain is the most commonly used animal research model in biology/Bio-medical research. From BALB/c strain many of inbred, congenic and transgenic models were established and being used for the betterment of humans as well as domestic animals. At CSIR-IIIM, Jammu animal house facility conducted study for evaluation genetic diversity of inbred lines of BALB/c strain. Animals were maintained in sterile condition with routine health monitoring. Selection was conducted based on growth and reproduction performance of the mother's. Four selected lines and an unselected control line were maintained throughout the experiment. After, 20th generation of full sib mating results obtain three different lines. All the three lines of BALB/c strain shows standardized litter size of 8 pups at birth. All line mice were genotyped by using established 14 pair of microsatellite markers and genetic diversity analyzed. The Effective number of alleles was reported 1.021±0.016 in overall experimental population. Shannon's Index for within lines was 0 and among lines was 0.019±0.014. The Estimated Diversity (u) was 0 for within lines, 0.052 ±0.036 for control lines and 0.013±0.009 for among population. Polymorphic information content (PIC) observed in D11Mit260. Line1 was showing very little Genetic distance with Line2 (0.154) followed by control line (0.114) and Line3 (0.074). Control line and Line2 were observed very close genetic distance (0.010). It is concluded that BALB/c strain has no diversity within line and has very little diversity between lines after 20th generation of full-sib mating.

Keywords: mouse, litter size, genetic diversity, microsatellite marker

Introduction
Albino mice of a, b, c Stocks acquired by H.Bagg in 1913. In 1932, Snell added the /c. Now widely distributed and among the top 2-3 most widely used inbred strains. Used as a general-purpose strain in many different disciplines. Good breeding performance and long reproductive life-span. BALB/c strains derived from inbreeding (full sib or sister x brother mating) at CSIR-IIIM, Jammu. Four selected lines and an unselected control line were maintained throughout the experiment. After, 20th generation of full sib mating results obtain three different lines. All the three lines of BALB/c strain shows standardized litter size of 8 pups at birth. Which are genotypically identical and have high level of homozygosity. For quality control CSIR-IIIM, Jammu has established numerous production procedures and Genetic Monitoring Program that minimize the risk of genetic contamination.

Genetic Monitoring Program through *Molecular characterization* helps to determine the breeding behavior of species, individual reproductive success and the existence of gene flow, that is, the movement of alleles within and between populations of the same or related species and its consequences (Papa and Gepts, 2003). Previously Restriction fragment length polymorphism (RFLPs) has been the choice for many species to measure genetic diversity (Bonstein *et al*., 1980). But this technique is slow, cumbersome and it requires a large amount of DNA sample. So the use of SNPs (Single nucleotide polymorphism) has replaced the need of RFLP but they are less informative as less allele are present and use a large amount of DNA samples and data analysis.

So overcoming the hurdles of RFLP and SNP, the more efficient technique used is microsatellite which are the arrays of repeat sequences that display length variations, different alleles containing different number of repeat units. This markers is locus specific, highly polymorphic, co-dominant [1] amenable to analysis by PCR as PCR typing which is much quicker and more accurate as due the high heterozygosities and very high mutation rates of 10^3 and 10^4 thus, microsatellite markers are appropriate for the study of molecular taxonomy [2, 3],

evolution and population genetics [4] and also applied for DNA typing for individual identification and for assessing the degree of genetic relatedness between individuals. The data interpretation of microsatellite assays is also straight forward and based on visual identification of the polymorphisms. Thus, we implemented microsatellite marker based genetic monitoring program to evaluate the genetic diversity within and between the lines of BALB/c strains after 20th generation of full-sib mating.

Materials and Methods

Selection of inbred strain and tail tissue sampling

Four lines of BALB/C strain were selected for the present study that belonged to the CSIR-Indian Institute of Integrative Medicine, Jammu, (CSIR-IIIM). All the breeding mice utilized in the present study were maintained as breeder stock. A total of 80 mice were sampled as 10 individuals per lines with equal representation of each sex were chosen randomly as shown in Table 1.

Table 1: Sample (different lines of BALB/c strains) collection from CSIR-IIIM Animal House

Strains	Type	Selected traits	Filial Generation	Institute	No. of female	No. of male	Total sample
BALB/c line1	Inbred (full-sib mating)	Litter size	>20	CSIR-IIIM	10	10	20
BALB/c line2	Inbred (full-sib mating)	High weaning weight	>20	CSIR-IIIM	10	10	20
BALB/c line3	Inbred (full-sib mating)	Low weaning weight	>20	CSIR-IIIM	10	10	20
BALB/c control	Inbred (full-sib mating)	No selection	>20	CSIR-IIIM	10	10	20
					40	40	80

DNA Extraction and Selection of Microsatellite Markers

The tail samples were subjected to DNA extraction by using standard protocol of Sambrook and Russel (1989). In the present study, a set of 14 microsatellite markers were used. Some of these markers selected from mouse genome informatics (MGI) and were already used in different studies to determine the genetic diversity of mice as shown in Table 2. The primers were synthesized from Sigma.

Table 2: Primer sequences and other information of the microsatellite markers used.

Primer	Repeat Motif	Genetic location	Sequence (5'-3')	(%)GC Content	Fragment Range (bp)
D1Mit16	(CA)23	1	F:AGAGTTAGCTGCCTAGCTTGAGTG	50	162-197
			R:TGGAAAGATCTAGGGTTGTCAAAA	37.5	
D1Mit17	(GT)14	1	F: GTGTCTGCCTTTGCACCTTT	50	170-190
			R: CTGCTGTCTTTCCATCCACA	50	
D1Mit136	(GT)19	1	F:TAGCCCTACACACTGTAGAAATGC	45.8	86-108
			R: TGAACACAAAGTAGTAAATGCGTG	37.5	
D1Mit171	(CA)12	1	F: TGCAGATTCAGTCTGCCTTG	50	148-200
			R: AGCCATGGGAACACTCTCAC	55	
D1Mit356	(GT)30	1	F: GGGAGAACCTGTCAAGACCA	55	112-152
			R: TTTTGGAAATGAGTGTCTGGC	42.8	
D2Mit75	(CA)24	2	F: TCAGCATGTGGATGAATACACA	40.9	16-112
			R: AACTTTTTAAAAACTACGAGCGTG	33.3	
D3Mit200	(GT)23	3	F: CAACTTCAGTTTCTCATTTGAATTG	32	99-131
			R: GCAAATGGAAGAGGTTTCTCC	47.6	
D7Mit259	(CA)22	7	F: CCCCTCCTCCTGACCTCTT	63.1	116-152
			R: GTCTCCATGGGAACCACACT	55	
D11Mit227	(CA)34	11	F: CCAGCATTTGAACCCTGATT	45	116-188
			R: AAACCCATAGCCTGCATCTG	50	
D11Mit260	(CA)19	11	F: ACTTTGCCTTTATACTATATGGTGG	36	74-118
			R: CATTTGTTTAGTTCTCAGCACCA	39.1	
D13Mit130	(GT)24	13	F: TCTGCTGAAGGCCAGGAC	61.1	139-148
			R: TTGAAGTGTCATGTTGATTTTAATG	28	
D16Mit5	(GT)21	16	F: CGGGGATCATCCCTAAAAAC	50	132-161
			R: TCCCCAATTCCTCTTGTGTC	50	
D17Mit62	-	17	F: CCACATCTTCTAATCCTGCTCA	45.4	158-176
			R: CATATAGCCTGAGACATTCTGCC	47.8	
D17Mit124	(CA)15	17	F: TGTTGATGAGATCTTAAATCAGCC	37.5	149-163
			R:TTTAACTAGTTGTTATTGCATGTGTG	30.7	

PCR Amplification

After the extraction of DNA, the microsatellite loci were amplified though polymerase chain reaction (PCR) using primer set for selected microsatellite markers (Table 2). A total of 25µL reaction mixture was prepared for each reaction which included 12.5µl of GoTaq® Green Master Mix, 1µl of 2X upstream primer (10µM), 1µl of downstream primer (10µM), 2µl of DNA template (50ng/µl), 6.5µl of Nuclease-Free Water was used for the thermocycling of the PCR reactions. Initial denaturation at 95 °C for 5 minutes, and then for each of 35 cycles following procedure was used, denaturation at 94 °C for 0.45 seconds, annealing at primer specific temperature for 0.30 second, 72°C for 30 minute for extension process that followed by a final extension at 72 °C for 10 minutes. After DNA amplified product can be loaded directly onto agarose gels. Using promega's GoTaq™ DNA Polymerase with Green GoTaq™ Reaction Buffer eliminating the need to add loading dye to the PCR sample before electrophoresis. PCR product for each sample was loaded along with 50 bp standard ladder onto the 2% agarose gel and allowed to run on the gel at 80volts for 2 hours.

Statistical Analysis

Each PCR product was genotyped and gel documentation data was analyzed to work out the standard parameters of genetic diversity among lines of BALB/c strain under study. For statistical analysis of genetic diversity studies have done by GenAlEx 6.5 (2015) population genetics software (Smouse *et al*, 2015). This software can access easily through online which facilitated to work within Microsoft excel sheet. So, easily can analysis of numbers of alleles, Shannon information, heterozygosity and genetic distance (Nei) among different population [7, 8, 9].

Results

Our Genetic Monitoring Program is grounded in the identification of points in the production program where there is a higher risk of an undetected accidental mating of different lines. By focusing genetic diversity analysis methods on these critical high-risk points, while scrutinizing quality control throughout, a genetic monitoring program can prevent a single mistake from becoming catastrophic. At IIIM, Jammu used strict strategies at three levels for accurate genetic monitoring program:

Level 1. Visual Phenotypic characteristics of strains

Level 2. Careful record keeping, line segregation and strict pedigree record maintenance.

Level 3. Molecular characteristics and Genetic diversity analysis by using Microsatellite Markers

Results of all the three levels of Genetic Monitoring Program

1. Visual characteristics of BALB/c strain (coat color, litter size, weaning weight, growth curve)

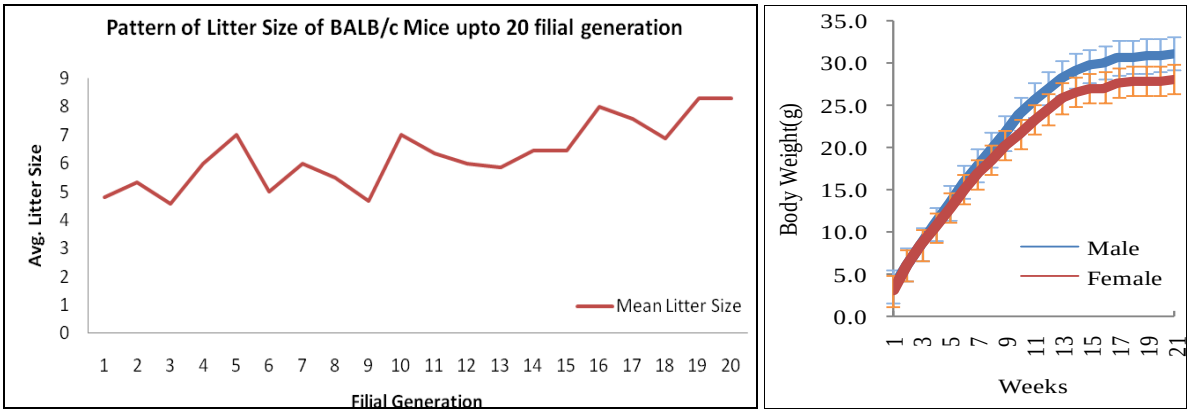


Fig 1: Mean litter size and mean growth curve of BALB/c strain at CSIR-IIIM, Jammu.

2. Production procedures that minimize co-localization and/or movement of lines of BALB/c Strain and that promote careful record keeping, line segregation and pedigree maintenance.

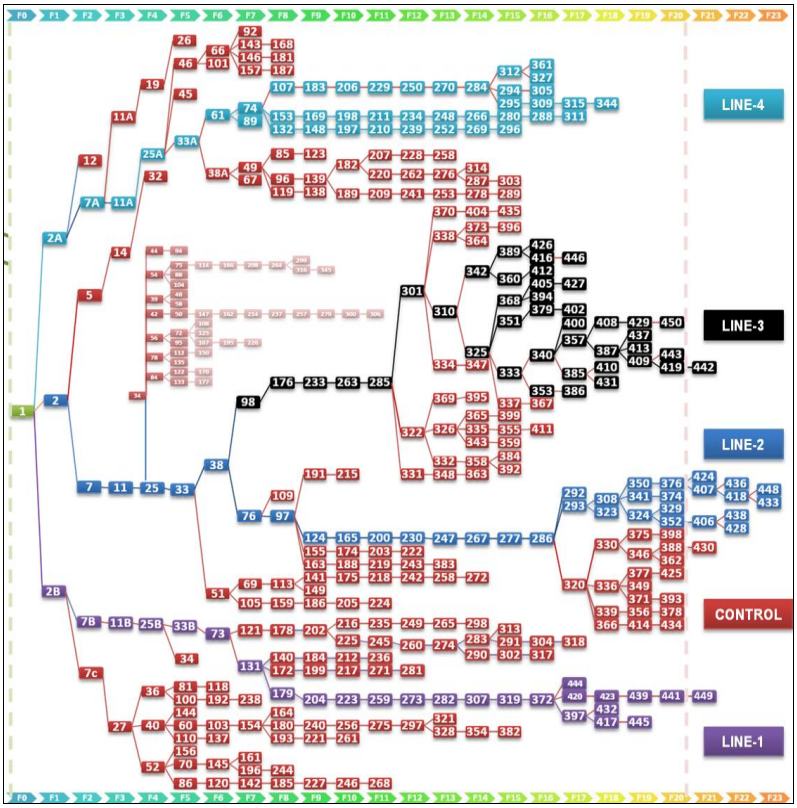
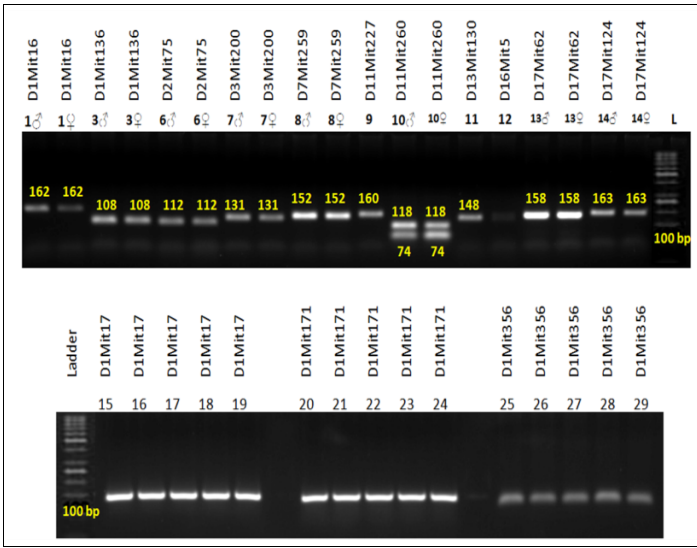


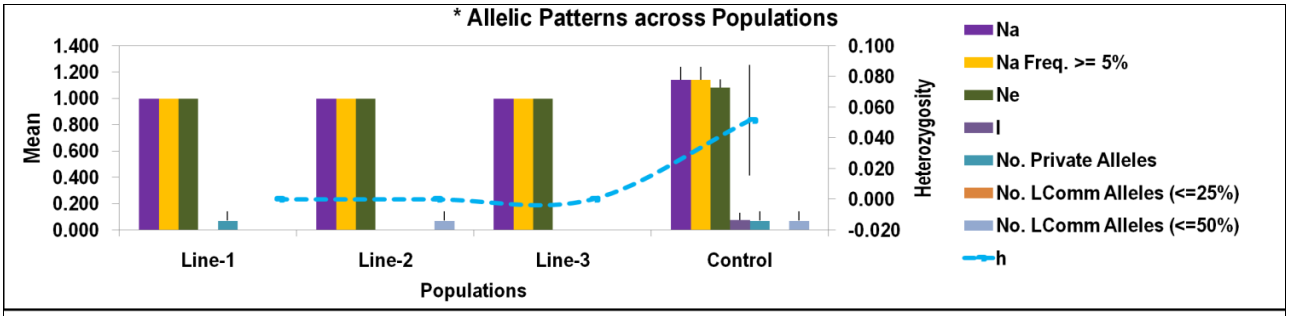
Fig 2: pedigree chart of BALB/c strain at CSIR-IIIM, Jammu.

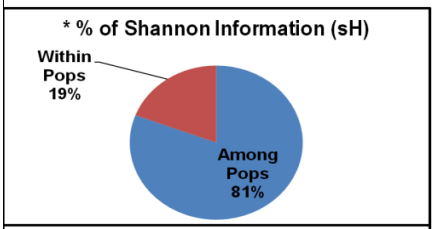
3. Genetic monitoring program outlining g 14 different microsatellite markers for 4 different lines of BALB/c mice strain revealed genetic diversity within and between lines of BALB/C mice.

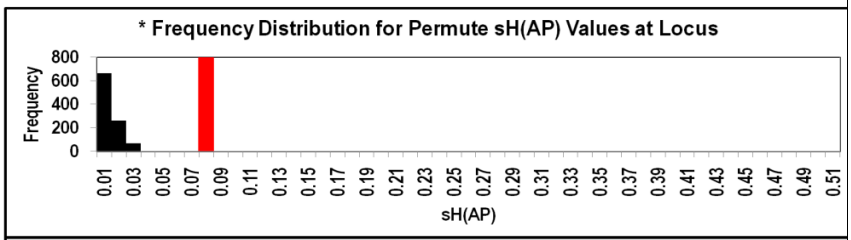


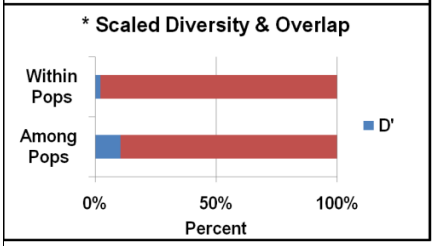
Locus	Allele	Line1	Line 2	Line 3	Control
D1Mit16	1	1.000	1.000	1.000	1.000
D11Mit227	1	1.000	1.000	1.000	1.000
D11Mit260	1	0.000	1.000	0.000	0.667
	2	1.000	0.000	1.000	0.333
D13Mit130	1	1.000	1.000	1.000	1.000
D16Mit5	1	0.000	1.000	1.000	1.000
	2	1.000	0.000	0.000	0.000
D17Mit124	1	1.000	1.000	1.000	1.000
D17Mit62	1	1.000	1.000	1.000	1.000
D1Mit136	1	1.000	1.000	1.000	1.000
D1Mit17	1	1.000	1.000	1.000	1.000
D2Mit75	1	1.000	1.000	1.000	1.000
D3Mit200	1	1.000	1.000	1.000	1.000
D7Mit259	1	1.000	1.000	1.000	1.000
D1Mit71	1	1.000	1.000	1.000	0.833
	2	0.000	0.000	0.000	0.167
D1Mit356	1	1.000	1.000	1.000	1.000

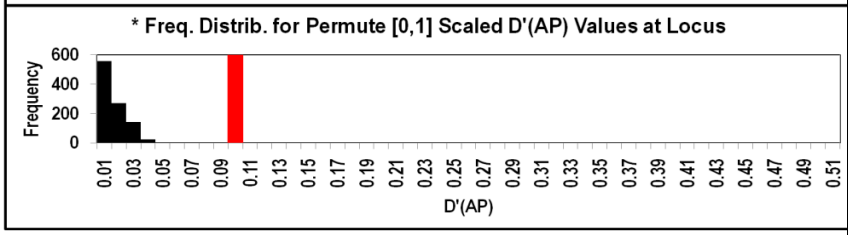
Fig 3: Allelic patterns of Male & female BALB/c strain in 2% agarose gel











* Mean Allelic Patterns Across Populations					
Population	Line1	Line 2	Line 3	Control	Mean Pop
No of allele (Na)	1.000 ±0.000	1.000 ±0.000	1.000 ±0.000	1.143 ± 0.097	1.036±0.025
Effective no. of allele (Ne)	1.000 ±0.000	1.000 ±0.000	1.000 ±0.000	1.085 ±0.061	1.021±0.016
Shannon's Information Index (I)	0.000 ±0.000	0.000 ±0.000	0.000 ±0.000	0.078 ±0.054	0.019±0.014
No. Private Alleles	0.071 ±0.071	0.000 ±0.000	0.000 ±0.000	0.071 ±0.071	0.004±0.004
No. LComm Alleles (<=25%)	0.000 ±0.000	0.000 ±0.000	0.000 ±0.000	0.000 ±0.000	0.000 ±0.000
No. LComm Alleles (<=50%)	0.000 ±0.000	0.071 ±0.071	0.000 ±0.000	0.071 ±0.071	0.004±0.004
Diversity (u)	0.000 ±0.000	0.000 ±0.000	0.000 ±0.000	0.052 ±0.036	0.013±0.009
Unbiased Diversity (uh)	0.000 ±0.000	0.000 ±0.000	0.000 ±0.000	0.062 ±0.043	0.015±0.011

* Genetic Distance (Nei) - Pairwise Population Matrix				
Line1	Line 2	Line 3	Control	
0.000				Line1
0.154	0.000			Line2
0.074	0.074	0.000		Line3
0.114	0.010	0.035	0.000	Control

Fig 4: Genetic diversity analysis of different lines of BALB/c strain by GenAlEx 6.5 population genetics software

Discussion

All line mice were genotyped by using established 14 pair of microsatellite markers and genetic diversity analyzed (Figure 3). The Effective number of alleles was reported 1.021±0.016 in overall experimental population. Shannon's Index for within lines was 0 and among lines was 0.019±0.014. The Estimated Diversity (u) was 0 for within lines, 0.052 ±0.036 for control lines and 0.013±0.009 for among population.

Polymorphic information content (PIC) observed in D11Mit260. Line1 was showing very little Genetic distance with Line2 (0.154) followed by control line (0.114) and Line3 (0.074). Control line and Line2 were observed very close genetic distance (0.010). It is concluded that BALB/c strain has no diversity within line and has very little diversity between lines after 20th generation of full-sib mating [10].

Conclusion

Our experimental results would be easy to obtain using GenAlEx 6.5 (2015) population genetics software which simplifies the genetic diversity analysis from the direct gel results of PCRs with Microsatellite Markers samples. Therefore, The PCR-based microsatellite analysis has considerable advantages over biochemical and immunological methods as a tool for the genetic monitoring of inbred mice lines. Also the basis of our data it can be concluded that inbred lines of BALB/c strain of mice have very low level of genetic diversity within control lines in term of numbers of alleles, Polymorphic information contents, heterozygosity and genetic distance (Nei). But there is no genetic diversity within the lines of Line-1, Line-2, Line-3 of BALB/c stain at CSIR-IIIM, Jammu. The present study indicated that with the limited number of samples and marker we obtained very little or negligible level of genetic diversity among the lines, under study, was observed. It is recommended to extend the study with higher number of samples with the higher number of markers for complete genetic diversity studies.

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