International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2013): 6.14 | Impact Factor (2014): 5.611

SSR Molecular Marker and Analysis Report of Variety of Chicken for their Varying Weight

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Abstract: ***Microsatellites are easy to genotype and densely distributed throughout eukaryotic genomes, making them the preferred genetic marker for high resolution genetic mapping. The use of DNA marker technology in poultry as a strains identification has progressed rapidly during the last decade. In our case, Simple Sequence Repeats (SSR) analysis for 56 individuals male and female belonging to fast white broiler and Rainbow rooster multicolored dual purpose birds (fast white female(A), fast white male(B), Rainbow rooster female (C)and Rainbow rooster male(D) using four primers. A total of 480 bands in different loci is generated, range of molecular weight start from 97-2708.***

Keywords: Microsatellites, Germ Plasm, Miniprep, Genetic mapping, Dendrogram

1. Introduction

Commercial broiler production and layer farming is highly capital oriented and competitive. Both are serious business ventures and are beyond the reach of the small farmer. Grain and poultry feed become very expensive and native birds with low growth potential, broodiness and poor laying (only 60 eggs in an year) are no more viable. Dual purpose birds with all attributes of native, faster Growth, less Fat, Tasty Meat and more Eggs suit the small farmer . These birds also thrive on low inputs and meet the gap of nutritional security of densely population countries, they also add to the additional income for the house wife.

Rainbow rooster multicolored dual purpose breed cross, suitable for Back Yard rearing and "Organic Chicken" Production, Breed by Indbro Research & Breeding farms Pvt. Ltd. at Hyderabad (India) and distributed by vet Frontiers in East and central Africa with distribution centers in Eldoret, Kenya and kampala, Uganda . The Breeder stock are well vaccinated and maintained under strick Bio-security to produce a chick with good livability and excellent performance. The Rainbow Rooster chick is a product of fast growing broilers, high laying brown birds and disease resistant native Germ Plasm. fast growth potential in the first 6 weeks help in less brooding efforts, less fat, tasty and higher meat yield compared to Native Birds.

2. Materials and Methods

DNA Isolation

mdigDNA Miniprep kit were using to Obtaining highly pure gDNA from blood of 56 individuals male and female belonging to fast white broiler and Rainbow rooster multicolored dual purpose birds (fast white female(A), fast white male(B), Rainbow rooster female (C)and Rainbow rooster male(D) .

SSR PCR

Simple Sequence Repeats (SSR) was done by using four primers of ADL0306, MberA4-10,ADL0278 and SULT1B1 (Table 1) The mixtureof the PCR reaction had a final volume of 25 µl andcontained 2 ul of genomic DNA, 10x GC buffer -3 ul, dNTP - 1 ul, MgCl2 - 1 ul, SSR primer - 1 ul,Taq poly- 0.5 ul,ddH2O -16.5 ul , The PCR was performed in „Bio-Rad Thermo cycler" using the following cycling parameters: Cycle 1 denaturation (94°C) 5 minutes, annealing (49°C,53°C,55°C,57°C) 30 second and extension (72°C) 1 minutes followed by 36 cycle denaturation (95°C) 1 minute, and final extension (72°C) 10 minutes. The PCR products were tested with electrophoresis on 1.5% agarose gels in 0.5x TBE buffer at 90v for 1 hour. (Promega, USA) stained by ethidium bromide. An external DNA ladder was used to verify the band size and their relative position. The separated gel was pictured by using a Gel Documentation System.

Statistical Analysis

To analyze SSR results, a software Total lab was used. On the basis of molecular weight, generated dendrogram, so that similarity and dissimilarity among various chicken samples can be calculated easily.

3. Results

Table 1: Sequence of primers designed from SSR

	PRIMER	*Forward Sequence*	*Reverse Sequence*	*Primer Length*	*Annealing Temperature*
A	ADL0306	TCAGTTTGACTTTCCTTCAT	GTTACTGTATCTTGGCTCAT	20	49
B	MberA4-10	GTCCTTGCCAGAGGCTTC	TGGGTCAGACGGGCTTTG	18	53
C	ADL0278	CCAGCAGTCTACCTTCCTAT	TGTCATCCAAGAACAGTGTG	20	55
D	SULT1B1	CACTGTCTGGGTTGGGAATG	GGCTCCGTAACGCAGTCTGT	20	57

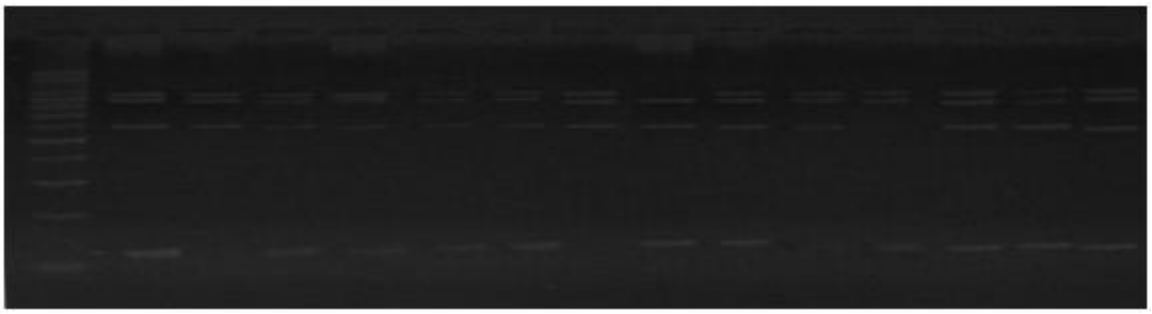

Plate 1: SSR gel profile generated for 14 accessions (AC) using primer (ADL0306)

Plate 2: SSR gel profile generated for 14 accessions(BB) using primer (MberA4-10)

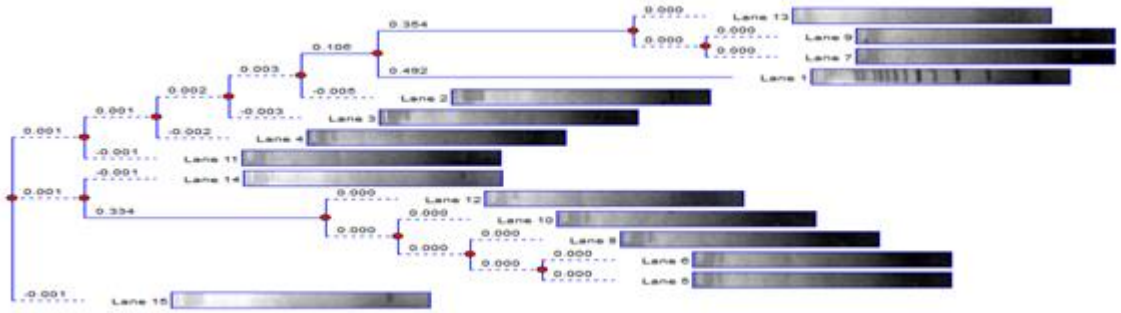

Figure 1: Dendrogram showing diversity of 14(CA) genotypes generated by SSR markers using primer (ADL0278)

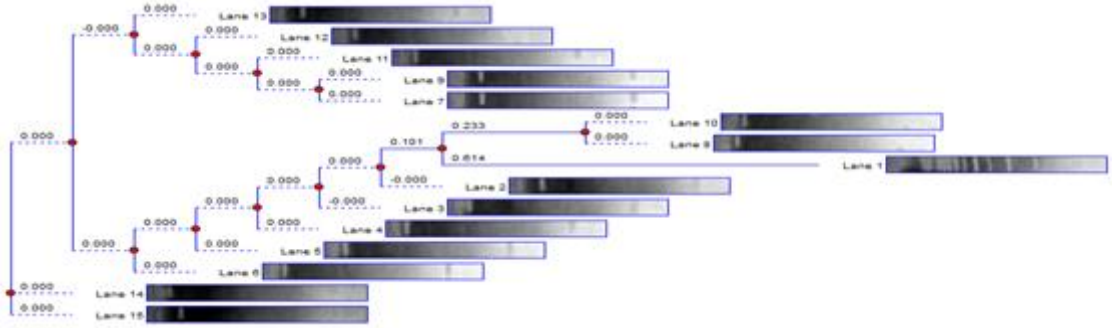

Figure 2: Dendrogram showing diversity of 14(DD) genotypes generated by SSR markers using primer (SULT1B1)

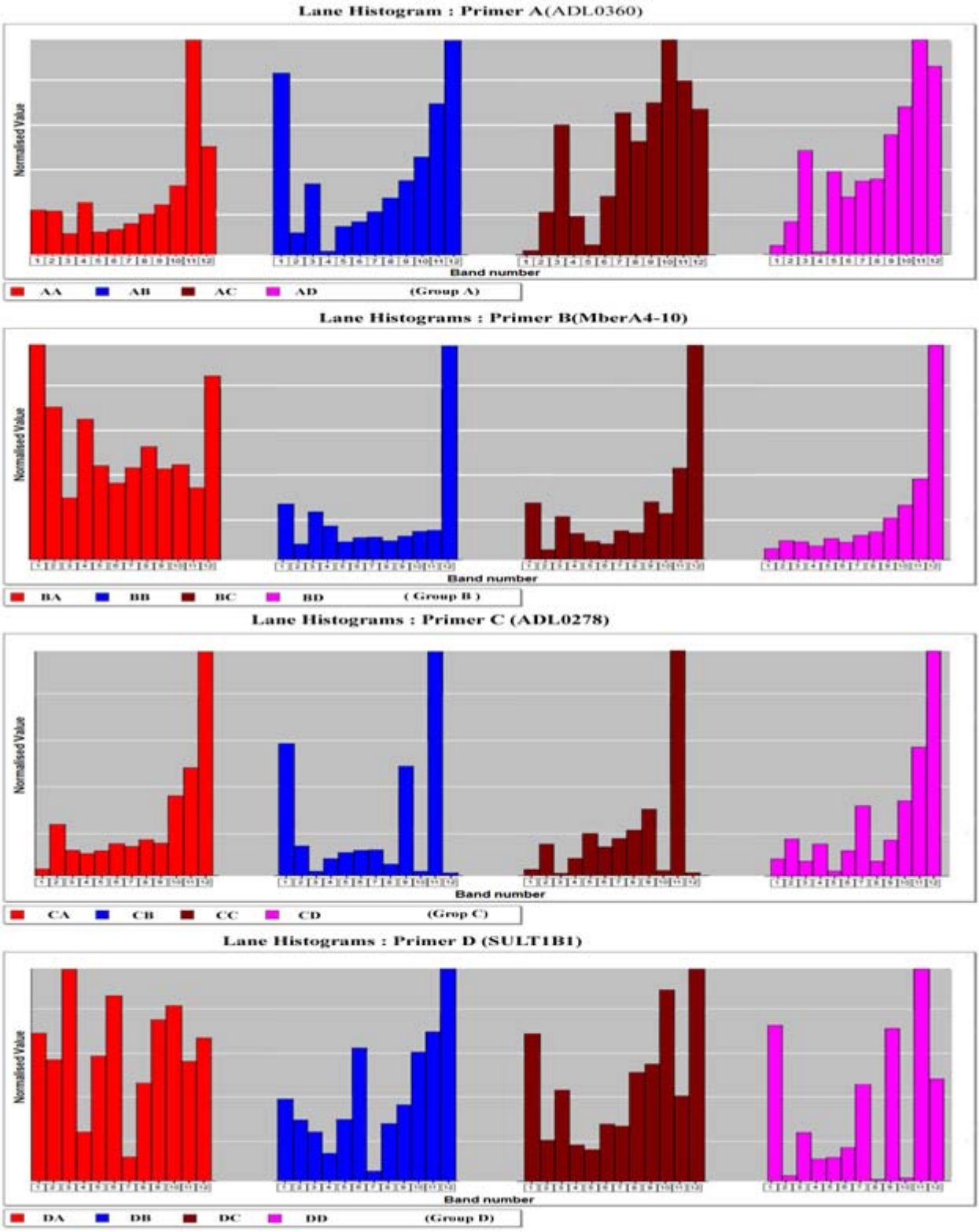

Figure 3: Histogram of (ADL0306) , (MberA4-10) , (ADL0278) and (SULT1B1) SSR Primer to show the similarity between four family chicken based on them band pattern

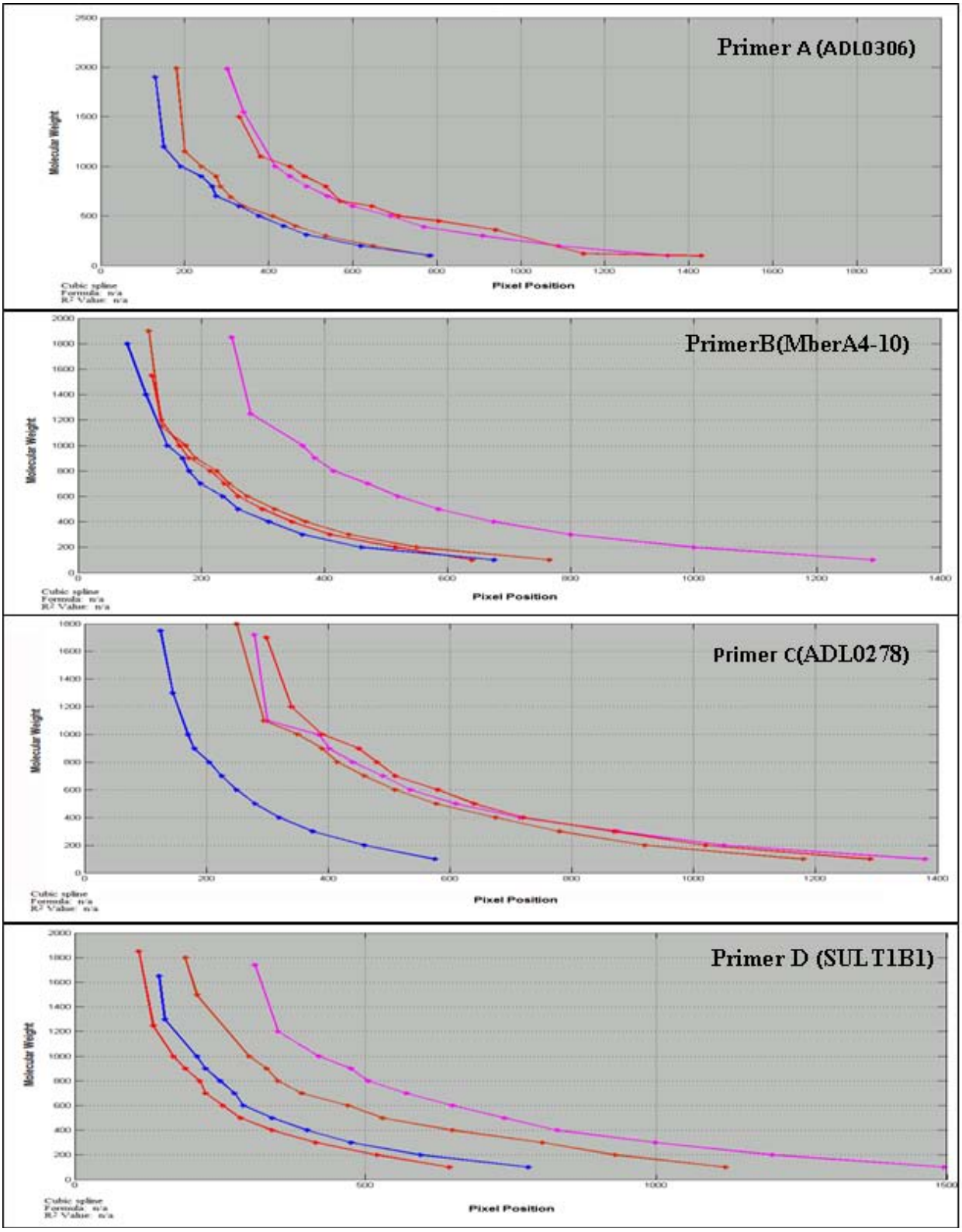

Figure 4: A cubic spline composed of 48 polynomial segments, this shape is used as a range of molecular weight of banding appear in (ADL0306) , (MberA4-10) , (ADL0278) and (SULT1B1) SSRprimer with 56 chicken samples

Table 2: details of four SSR Primers with four chicken generations

Sample																	
Lane No / Primers	Lane 1	Lane 2	Lane 3	Lane 4	Lane 5	Lane 6	Lane 7	Lane 8	Lane 9	Lane 10	Lane 11	Lane 12	Lane 13	Lane 14	Lane 15	Total band	Range of M.W
		Group A															
Primer A (ADL0306)	Ladder(M)	AA1	AA2	AA3	AA4	AA5	AA6	AA7	AA8	AA9	AA10	AA11	AA12	AA13	AA14	46	130-1022
		AB1	AB2	AB3	AB4	AB5	AB6	AB7	AB8	AB9	AB10	AB11	AB12	AB13	AB14	45	138-1173
		AC1	AC2	AC3	AC4	AC5	AC6	AC7	AC8	AC9	AC10	AC11	AC12	AC13	AC14	51	110-1046
		AD1	AD2	AD3	AD4	AD5	AD6	AD7	AD8	AD9	AD10	AD11	AD12	AD13	AD14	49	130-1046
		Group B															
Primer B (MberA4-10)		BA1	BA2	BA3	BA4	BA5	BA6	BA7	BA8	BA9	BA10	BA11	BA12	BA13	BA14	25	371-1000
		BB1	BB2	BB3	BB4	BB5	BB6	BB7	BB8	BB9	BB10	BB11	BB12	BB13	BB14	26	414-1461
		BC1	BC2	BC3	BC4	BC5	BC6	BC7	BC8	BC9	BC10	BC11	BC12	BC13	BC14	22	505-1051
		BD1	BD2	BD3	BD4	BD5	BD6	BD7	BD8	BD9	BD10	BD11	BD12	BD13	BD14	22	500-1032
		Group C															
Primer C (ADL0278)		CA1	CA2	CA3	CA4	CA5	CA6	CA7	CA8	CA9	CA10	CA11	CA12	CA13	CA14	20	104-2067
		CB1	CB2	CB3	CB4	CB5	CB6	CB7	CB8	CB9	CB10	CB11	CB12	CB13	CB14	26	109-2100
		CC1	CC2	CC3	CC4	CC5	CC6	CC7	CC8	CC9	CC10	CC11	CC12	CC13	CC14	26	111-2331
		CD1	CD2	CD3	CD4	CD5	CD6	CD7	CD8	CD9	CD10	CD11	CD12	CD13	CD14	22	504-992
		Group D															
Primer D (SULT1B1)		DA1	DA2	DA3	DA4	DA5	DA6	DA7	DA8	DA9	DA10	DA11	DA12	DA13	DA14	28	452-800
		DB1	DB2	DB3	DB4	DB5	DB6	DB7	DB8	DB9	DB10	DB11	DB12	DB13	DB14	23	411-817
		DC1	DC2	DC3	DC4	DC5	DC6	DC7	DC8	DC9	DC10	DC11	DC12	DC13	DC14	23	607-1035
		DD1	DD2	DD3	DD4	DD5	DD6	DD7	DD8	DD9	DD10	DD11	DD12	DD13	DD14	26	97-2708

4. Conclusion

Dendrogram generated by Totallab software clearly showing the dissimilarity among chicken sample which cause different weight genotypic. The tables generated on the basis of those differences are an attempt to make sure why they developed with different body size.

5. Future Scope

Poultry forms are one of the major sources of providing chicken meat in high populated country like India. Growing big size chicken will be one of the great steps in order to increase food productivity. With these information provided in this research paper will help how to increase and select different chicken species.

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