

## FOR THE RECORD

*Jheelam Banerjee,<sup>1</sup> M.Sc.; R. Trivedi,<sup>1</sup> Ph.D.; and V. K. Kashyap,<sup>1</sup> Ph.D.*

# Polymorphism at 15 Short Tandem Repeat AmpF $\ell$ STR<sup>®</sup> Identifier<sup>TM</sup> Loci in Three Aboriginal Populations of India: An Assessment in Human Identification

---

**POPULATION:** Munda, Santal and Oraon are chosen in this study, as they constitute the primitive Austroloid population groups that inhabit Chotanagpur Plateau of Eastern India. Munda have lent their names to the "Mundari" branch of Austro-Asiatic language family. Santal, the third largest tribe in India also belongs to the Austro-Asiatic language family. The Oraon tribe speaks "Kurukh" classified under the Dravidian language family. These populations were hunter-gatherers but have maintained a high degree of endogamy (1)

**KEYWORDS:** forensic science, AmpF/STR<sup>®</sup> Identifier<sup>TM</sup>, short tandem repeat (STR), Munda, Santal, Oraon, Chotanagpur Plateau, population genetics, DNA typing, D5S818, FGA, D8S1179, D21S11, D7S820, CSF1PO, D3S1358, THO1, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51

STR polymorphisms have proven to be extremely useful in population genetics studies and human identification. The fifteen analysed STR loci were tetra-nucleotide repeats: D5S818, FGA, D8S1179, D21S11, D7S820, CSF1PO, D3S1358, THO1, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51.

Blood samples were collected randomly by venipuncture from 185 unrelated consenting individuals of 3 tribal population of Chotanagpur Plateau of eastern India, in accordance with the ethical guidelines of the institution involved. DNA was extracted using the standard phenol-chloroform method (2). Quantitation of extracted DNA was carried out using the Quantiblot-Kit (PE Biosystem). PCR amplification was performed using the AmpF/STR<sup>®</sup> Identifier<sup>TM</sup> PCR Amplification Kit (3). The amplified products were detected using denaturing gel cast on ABI Prism<sup>TM</sup> 377 DNA Sequencer. The reference ladder used were obtained along with AmpF/STR<sup>®</sup> Identifier<sup>TM</sup> PCR Amplification Kit. Allele designation and data analysis were carried out using the Genescan<sup>TM</sup> and Genotyper<sup>TM</sup> softwares.

Statistical analysis included calculations for allele frequencies, observed heterozygosity, probability of homozygosity, ex-

act test, likelihood ratio, power of exclusion and discrimination using DNATYPE Program (4). The average variance of the alleles at each locus was calculated using Microsat Software (<http://lotka.stanford.edu/microsat.html>). Results are given in Tables 1–4.

All the loci were observed to be highly polymorphic. Minimum of 120 chromosomes have been analyzed for each loci in the three tribal groups. Allele 29 at D21S11, allele 12 at CSF1PO, allele 9 at THO1, allele 8 at D13S317 and allele 11 at TPOX were found to be most frequent in the tribes. Of the 15 microsatellite loci, FGA, D8S1179, D7S820, D2S1338 and D19S433 are highly polymorphic (variance  $\geq 4$ ), while the other loci demonstrate a narrow range of variation. The tests show that most of these populations are following the Hardy-Weinberg Equilibrium and have not undergone any major population stochastic processes. The combined Power of Discrimination for the three populations Munda, Santal and Oraon are 0.972, 0.978 and 0.977 respectively. Our work demonstrates that 15 microsatellite/STR markers chosen for this study are highly suitable for individual identification and genetic characterization of highly endogamous aboriginal populations. The data generated in this study will augment the existing STR information already available for Indian populations (5–8).

The complete dataset is available on website <http://humstr.ndacdb.org> and also through electronic mail from communicating author: dtubprd@giascl01.vsnl.net.in, vkk2k@hotmail.com

<sup>1</sup> National DNA Analysis Centre, Central Forensic Science Laboratory, 30, Gorachand Road, Kolkata-700014, West Bengal, India.

TABLE 1—*Allele frequencies of fifteen STR loci in Munda (N = 128) Tribe of Chotanagpur Plateau, India.*

Allele	D5S818	FGA	D8S1179	D21S11	D7S820	CSF1PO	D3S1358	TH01	D13S317	D16S539	D2S1338	D19S433	vWA	TPOX	D18S51
6								0.123							
7								0.169	0.008						
8	0.031				0.198			0.100	0.461	0.094			0.269		
9	0.063				0.079	0.016		0.462	0.039	0.164		0.008	0.185		
9.3					0.016			0.131							
10	0.063		0.131		0.190	0.262		0.015	0.063	0.102			0.162	0.008	
10.2					0.008										
11	0.414		0.023		0.302	0.270			0.281	0.352		0.023	0.362	0.008	
12	0.305		0.069		0.175	0.373			0.109	0.172		0.062	0.023	0.049	
12.2														0.008	
13	0.125		0.108		0.032	0.048			0.039	0.086		0.323		0.148	
13.2												0.015		0.008	
14			0.208		0.008	0.024	0.055			0.031		0.231	0.200		0.320
14.2												0.031			
15			0.300				0.258					0.115	0.031	0.197	
15.2												0.108	0.008	0.008	
16	0.025	0.138					0.367					0.069	0.192		0.131
16.2							0.008					0.008			
17		0.023					0.250				0.032		0.215		0.033
17.2												0.008		0.008	
18	0.050						0.063				0.169		0.185		0.049
19	0.050										0.258		0.169		
19.2	0.008														
20	0.133										0.097			0.008	
21	0.058										0.056			0.016	
21.2	0.033														
22	0.208									0.056					
22.2	0.008														
23	0.200									0.177					
24	0.133									0.073					
25	0.050									0.056					
26	0.008									0.016					
26.2	0.008														
27	0.017									0.008					
28			0.055												
28.2			0.008												
29			0.297												
29.2			0.008												
30			0.188												
30.2			0.008												
31			0.039												
31.2			0.172												
32			0.039												
32.2			0.086												
33			0.031												
33.2			0.070												
H	0.641	0.850	0.862	0.828	0.730	0.619	0.766	0.739	0.594	0.719	0.790	0.769	0.769	0.862	0.754
HP	0.212	0.687	0.317	0.992	0.200	0.087	0.513	0.686	0.098	0.146	0.209	0.441	0.367	0.022	0.234
ET	0.224	0.002	0.107	0.763	0.225	0.287	0.313	0.038	0.009	0.020	0.102	0.088	0.765	0.102	0.553
LR	0.182	0.000	0.042	0.706	0.272	0.222	0.188	0.019	0.003	0.037	0.134	0.153	0.815	0.084	0.635
PD	0.711	0.868	0.813	0.827	0.795	0.716	0.729	0.715	0.690	0.792	0.848	0.807	0.813	0.736	0.813
PE	0.343	0.695	0.719	0.652	0.476	0.314	0.538	0.491	0.284	0.458	0.581	0.543	0.543	0.719	0.517

N: Number of chromosome; H: Observed heterozygosity; HP: Probability of homozygosity; ET: Exact test; LR: Likelihood ratio; PD: Power of discrimination; PE: Power of exclusion.

TABLE 2—Allele frequencies of fifteen STR loci in Santal ( $N = 122$ ) tribe of Chotanagpur Plateau, India.

Allele	D5S818	FGA	D8S1179	D21S11	D7S820	CSF1PO	D3S1358	TH01	D13S317	D16S539	D2S1338	D19S433	vWA	TPOX	D18S51
6								0.205							
7						0.008		0.189							
8	0.016					0.303		0.115	0.303	0.098				0.336	
8.2						0.033									
9	0.033					0.025	0.025		0.385	0.049	0.123		0.016	0.213	
9.3								0.082							
10	0.107		0.082			0.156	0.131		0.025	0.082	0.066			0.049	
10.2							0.008								
11	0.352		0.049			0.377	0.328			0.287	0.336		0.041	0.377	0.017
12	0.336		0.098			0.098	0.451			0.189	0.246		0.107	0.025	0.042
13	0.148		0.098				0.049			0.049	0.082		0.328		0.167
13.2													0.025		
14			0.221				0.008	0.049		0.041	0.049		0.189	0.221	0.275
14.2													0.041		
15	0.008		0.311					0.336					0.18	0.082	0.225
15.2								0.041					0.049	0.008	
16			0.115					0.262					0.025	0.148	0.158
17			0.016					0.262					0.057	0.189	0.067
18	0.025		0.008					0.049					0.238	0.246	0.033
19	0.066												0.18	0.098	
20	0.098												0.066	0.008	0.017
21	0.033												0.049		
21.2	0.016														
22	0.197												0.066		
23	0.287												0.189		
24	0.123												0.074		
24.2	0.025														
25	0.041												0.057		
25.2	0.025														
26	0.041												0.025		
26.2	0.016														
27			0.016												
28			0.082												
29			0.246												
29.2			0.008												
30	0.008		0.139												
30.2			0.025												
31			0.057												
31.2			0.148												
32			0.033												
32.2			0.148												
33.2			0.09												
34.2			0.008												
H	0.754	0.82	0.803	0.885	0.607	0.623	0.754	0.623	0.738	0.836	0.902	0.82	0.771	0.705	0.75
HP	0.651	0.616	0.861	0.52	0.03	0.446	0.842	0.018	0.462	0.362	0.273	0.785	0.351	0.886	0.21
ET	0.139	0.101	0.373	0.121	0.597	0.352	0.363	0.115	0.498	0.755	0.538	0.007	0.376	0.828	0.172
LR	0.065	0.089	0.556	0.075	0.644	0.364	0.493	0.244	0.273	0.516	0.486	0.012	0.333	0.922	0.162
PD	0.728	0.843	0.813	0.856	0.73	0.669	0.743	0.753	0.777	0.788	0.852	0.806	0.817	0.697	0.813
PE	0.517	0.637	0.605	0.765	0.299	0.319	0.517	0.319	0.489	0.667	0.8	0.637	0.546	0.436	0.51

N: Number of chromosome; H: Observed heterozygosity; HP: Probability of homozygosity; ET: Exact test; LR: Likelihood ratio; PD: Power of discrimination; PE: Power of exclusion.

TABLE 3—*Allele frequencies of fifteen STR loci in Oraon (N = 120) tribe of Chotanagpur Plateau, India.*

Allele	D5S818	FGA	D8S1179	D21S11	D7S820	CSF1PO	D3S1358	TH01	D13S317	D16S539	D2S1338	D19S433	vWA	TPOX	D18S51	
4													0.025			
5													0.008			
6													0.125			
7						0.017							0.2			
8	0.017		0.008			0.35	0.008			0.125	0.325	0.108			0.008	
9	0.067					0.042				0.433	0.1	0.192			0.3	
9.2						0.008									0.108	
9.3													0.083			
10	0.133		0.15			0.242	0.142				0.075	0.083		0.059	0.1	
11	0.275		0.042			0.233	0.358				0.208	0.225		0.017	0.442	0.008
11.2														0.017		
12	0.292		0.092			0.008	0.358	0.025			0.233	0.233		0.068	0.042	0.008
12.2														0.008		
13	0.2		0.108			0.008	0.092	0.033			0.058	0.117		0.314	0.017	0.149
14	0.008		0.308			0.017	0.042	0.1				0.042		0.224	0.192	0.368
14.2														0.042		0.008
15	0.008		0.175					0.308						0.093	0.108	0.167
15.2														0.059	0.025	
16			0.1					0.283						0.059	0.183	0.114
16.2														0.034		
17		0.017						0.225					0.058		0.158	0.114
18	0.017							0.025					0.133		0.2	0.018
18.2	0.008															
19	0.1												0.192		0.092	0.008
19.2	0.008															
20	0.108												0.083		0.025	
21	0.042												0.05			0.008
21.2	0.008															
22	0.225												0.033			0.008
22.2	0.017															
23	0.233												0.233			
24	0.117												0.133			
25	0.075												0.042			
26	0.008												0.017			
27	0.017												0.025			
27.2			0.008													
28			0.067													
29			0.283													
29.2			0.008													
30	0.008		0.217													
30.2			0.005													
31			0.042													
31.2	0.008		0.133													
32			0.008													
32.2			0.108													
33.2			0.067													
34.2			0.008													
H	0.833	0.9	0.85	0.833	0.733	0.8	0.817	0.633	0.85	0.817	0.783	0.746	0.733	0.75	0.79	
HP	0.292	0.286	0.54	0.943	0.693	0.136	0.317	0.08	0.177	0.879	0.105	0.126	0.02	0.326	0.966	
ET	0.004	0.848	0.005	0.825	0.102	0.088	0.021	0.312	0.778	0.076	0.592	0.568	0.13	0.038	0.333	
LR	0.002	0.805	0.003	0.844	0.083	0.198	0.018	0.154	0.853	0.11	0.633	0.482	0.231	0.038	0.351	
PD	0.777	0.851	0.82	0.832	0.762	0.713	0.762	0.734	0.778	0.824	0.857	0.824	0.843	0.691	0.787	
PE	0.662	0.795	0.695	0.662	0.481	0.599	0.631	0.332	0.695	0.631	0.568	0.503	0.481	0.51	0.581	

N: Number of chromosome; H: Observed heterozygosity; HP: Probability of homozygosity; ET: Exact test; LR: Likelihood ratio; PD: Power of discrimination; PE: Power of exclusion.

TABLE 4—Number of alleles and their variance among three primitive Austroloid tribal populations of India.

Populations	D5S818	FGA	D8S1179	D21S11	D7S820	CSF1PO	D3S1358	TH01	D13S317	D16S539	D2S1338	D19S433	vWA	TPOX	D18S51
Munda	6 (1.328)	11 (5.004)	8 (3.725)	6 (2.167)	7 (2.341)	6 (1.110)	5 (1.000)	5 (1.277)	7 (3.008)	7 (2.355)	11 (6.442)	8 (1.768)	6 (2.951)	5 (1.591)	11 (3.512)
Santal	6 (1.193)	9 (3.872)	8 (3.237)	7 (2.371)	5 (2.137)	5 (0.801)	5 (1.037)	5 (1.630)	7 (3.389)	7 (2.529)	10 (6.898)	7 (1.714)	6 (2.864)	5 (1.882)	9 (2.654)
Oraon	6 (1.529)	9 (3.630)	8 (4.928)	6 (2.732)	6 (13.739)	5 (1.668)	6 (1.291)	6 (5.287)	5 (1.190)	6 (2.749)	11 (8.427)	8 (14.024)	7 (3.054)	5 (1.960)	6 (1.791)
Average	6 (1.35)	9.66 (4.17)	8 (3.96)	6.33 (2.42)	6 (6.07)	5.33 (1.19)	5.33 (1.11)	5.33 (2.73)	6.33 (2.53)	6.66 (2.54)	10.66 (7.26)	7.66 (5.83)	6.33 (2.96)	5 (1.81)	8.66 (2.65)

### Acknowledgments

The authors are thankful to Ministry of Home Affairs, Government of India, for financial support to carry out the research work. Special thanks to Mrs. Nidhi Khare, District Commissioner of East Singhbhum for her cooperation, NGO's for helping in Sample collection and the people of Chotanagpur plateau.

### References

1. Singh KS. India's communities. National series. People of India. Oxford, U.K.: Oxford University Press, 1998.
2. Sambrook J, Fritsch EF, Maniatis T. Molecular cloning. A laboratory manual. 2nd ed. Cold Spring Harbour, NY: Cold Spring Harbor Laboratory Press, 1989.
3. Applied Biosystems. AmpF/STR® Identifiler™ PCR amplification kit user's manual, instruction for use of products.
4. DNATYPE, (Windows 95/NT ver), Chakraborty R, Stivers D, Zhong Y, CHG. Houston, Texas: University of Texas.
5. Sahoo S, Kashyap VK. Allele Frequency of data for Powerplex 16 loci in four major population of Orissa, India. *J Forensic Sci* 2002;47:912–5. [PubMed]
6. Ashma R, Kashyap VK. *Genetic polymorphism at 15 STR loci among three important subpopulation of Bihar, India*. *J Forensic Sci* 2002;130:58–62.
7. Gaikwad S, Kashyap VK. *Genetic diversity in four tribal groups of western India: a survey of polymorphism in 15 STR loci and their application in human identification*. *Forensic Sci Int* 2003;134:225–31. [PubMed]
8. Sarkar N, Kashyap VK. Allelic variation at 15 microsatellite loci in an important austroloid and two Indocaucasoid groups of Chattisgarh, India. *J Forensic Sci* 2004;49:184–8. [PubMed]

Additional information and reprint requests:

V. K. Kashyap, Ph.D.  
Director  
National Institute of Biologicals  
Ministry of Health and Family Welfare  
A-32, Sector-62  
Institutional Area  
Noida-201307  
India