DOI: 10.21088/ijfmp.0974.3383.19326.1

Indian Journal of Forensic Medicine and Pathology
Volume 19, Number 3, July-September 2026

ORIGINAL ARTICLE

Evaluation of Forensic Genetic Efficiency Parameters of 23 Autosomal STR Markers (PowerPlex® Fusion 6C system) in a Population Sample of Hadoti Region

Bhawna Poonia[1], Sangita Gupta[2]

HOW TO CITE THIS ARTICLE:

Bhawna Poonia, Sangita Gupta. Evaluation of Forensic Genetic Efficiency Parameters of 23 Autosomal STR Markers (PowerPlex® Fusion 6C system) in a Population Sample of Hadoti Region. Indian J Forensic Med Pathol. 2026; 19(3): 279-291.

ABSTRACT

Rajasthan's southeastern part is known as "the land of Hada (Clan) Rajputs" i.e Hadoti. In this study, genetic structure of Hadoti population, who exhibited diverse ethanographic past, have been explored using 23 autosomal STR loci [PowerPlex® Fusion 6C (PPF) system] by the way of determining allele distribution and forensic efficiency parameters. Autosomal STR data was collected from the blood samples of 423 (194 females and 229 males) individuals of Rajasthani roots related to the Hadoti region. The STRs were amplified by using PPF 6C system (Promega Corporation) and the amplified samples were examined using Gene Mapper ID-X 1.6 software (Applied Biosystems) on a 3500xl Genetic Analyzer. Following Bonferroni correction, all the studied STR loci fulfilled the terms of the Hardy-Weinberg equilibrium. With the exception of Amelogenin and DYS391, DYS570, DYS576, this study found that among the 23 autosomal STR loci, SE 33 is the most informative (power of discrimination (PD) = 0.992), whereas TPOX locus (PD = 0.866)is the least. The obtained parameters of forensic interest demonstrated the compatibility of all the studied 23 STRs across Hadoti population of Rajasthan for forensic DNA identification and paternity testing.

KEYWORDS

• 23 STRs • PowerPlex® Fusion (PPF) 6C system • Genetic polymorphism • Population variations • Hadoti population

AUTHOR'S AFFILIATION:

[1] Ph.D. Scholar, Department of Science and Humanities (FSH), Poornima University, Jaipur, Rajasthan, India.

[2] Professor, Department of Science and Humanities (FSH), Poornima University, Jaipur, Rajasthan, India.

CORRESPONDING AUTHOR:

Bhawna Poonia, Ph.D. Scholar, Department of Science and Humanities (FSH), Poornima University, Jaipur, Rajasthan, India.

E-mail: pooniabhawna7@gmail.com

➢ **Received:** 11-04-2026 ➢ **Accepted:** 20-06-2026

INTRODUCTION

The north-west region of India was home to Rajasthan, once known as Rajputana or the domain of the Rajputs. Pakistan internationally landlocked the state to the south, while Gujarat shares the south-west border, Madhya Pradesh in south-east, Uttar Pradesh shares north-eastern border while Haryana and Punjab covers a very short border from the north. Rajasthan, which is nearly the size of Germany (342,239 sq km), has been the largest state in India.[1] The core middle location of Aravalli Hill ranges geographically split Rajasthan into eastern and north-western regions. Dhoondhar, Mewat, Hadoti and Mewar zones are situated in eastern flank while Marwar zone is on the north-west side of Aravalli ranges. These zones are not an official administrative unit but well-known cultural-historical regions in Rajasthan. India as well as Rajasthan had always been and continued to be a region of many ethnicities. In ancient time, India has long fascinated the western people with Rajasthan offered a gateway to them. Aryans, Iranians, Turco-Afgans, Graeco-Macedonians and Tibetano-Burmans were absorbed into the ethnic admixture from abroad. These ethnic groups nurtured their distinctive customs in varied topography of Rajasthan. Additional genetic diversity is added by the socio-cultural mosaic and the social profile of the state. Numerous castes are widely dispersed and distinguished by several levels of hierarchy at the regional.

"Microsatellites" as the name suggested, are short or micro stretches of DNA comprising of 1 to 6 base pairs that are repeated in tandem, and hence known as Short Tandem Repeats (STRs). The incredible micro length, polymorphic nature and immense capacity to amplify minute traces of DNA that serves as templates, make short tandem repeat (STR) markers indispensable workhorse that widely used in forensic and paternity testing as well as in population genetics studies.[2] Human identification testing has considerably benefited by utilizing these current core loci.[3] Allele frequency data from a substantial number of unrelated healthy members of a community must be used to provide accurate results from bio-statistical analysis of DNA profiles.[2] Cavalli-Sforza and Edwar (1964), identified that comparing gene frequencies for one or two loci is unreliable since each allele has a unique distribution in the population.[4] The genetic correlation only becomes evident if a significant number of loci are analyzed. Moreover, the genetic variation between populations is far lower than intra-population variation at the gene level.[5-8] Basically, humans look diverse on the outside, but at the gene level, populations are not very different from each other. However, if a large number of loci are analyzed even minor variations can be detected with sufficient accuracy.[5]

STR typing became standard practice in the majority of forensic laboratories, resulting in a compilation of STR databases across several nations. Different nations created their own STR databases with varying number of STR loci. Nevertheless, working group of CODIS core loci believes that number of selective loci must be expanded in order to strengthen the discrimination power which support in personal identification and facilitate global data sharing. Forensic DNA databases have steadily expanded due to their benefits for the entire forensic community.

Forensic DNA analysis and human genetic applications are made more feasible with the PowerPlex Fusion 6C system(PPF 6C) a 27-locus multiplex.[9] The PPF 6C system, a six-dye kit can amplify 27 loci, including 20 autosomal loci suggested in expanded CODIS set (CSF1PO, FGA, TH01, TPOX, vWA, D1S1656, D2S1338, D2S441, D3S1358, D5S818, D7S820, D8S1179, D10S1248, D12S391, D13S317, D16S539, D18S51, D19S433, D21S11, and D22S1045), three more autosomal STRs (Penta E, Penta D, and SE33) to enhance the power of discrimination, two sex chromosome markers (Amelogenin and DYS391), and two rapidly mutating Y-STRs (DYS570 and DYS576).[10]

There have been several studies conducted to screen 23 autosomal STRs, in different Indian populations.[11-14] According to all of these research, the 23 autosomal STR loci set is suitable for forensic identification and familial testing among the diverse populations. However, not even a single study has specifically focused on the Hadoti population. Therefore, the aim of the present study is to develop a population-specific autosomal STR database for the unrepresented south-eastern region of Rajasthan i.e. Hadoti, using the PowerPlex-Fusion 6C (PPF 6C) system by eliminating Amelogenin, DYS391, DYS570

and DYS576 loci. The study further seeks to evaluate the forensic efficiency of the selected 23 STR markers by estimating allele frequencies and key forensic parameters. The resulting database is expected to provide a reliable reference for forensic casework, improve the accuracy of individual identification, and strengthen statistical interpretation in this regional population.

MATERIAL AND METHOD

Area of Present Study

Rajasthan lied in the north-western part of India, between 23°3′ to 30°12′ North latitudes and 69°39′ to 78°18′ East longitudes. The study area i.e. Hadoti, is located in the south-eastern part of Rajasthan, between 24°25′ to 25°51′ North latitudes and 75°15′ to 76°45′ East longitudes. It lies between the Malwa Plateau to the east, the Aravali Range to the west, and the Marwar Plateau to the southwest, bordering the state of Madhya Pradesh. The total geographical area of this region is 14481.6 square kilometers. This region includes the entire Kota, Bundi, Baran and Jhalawar district.

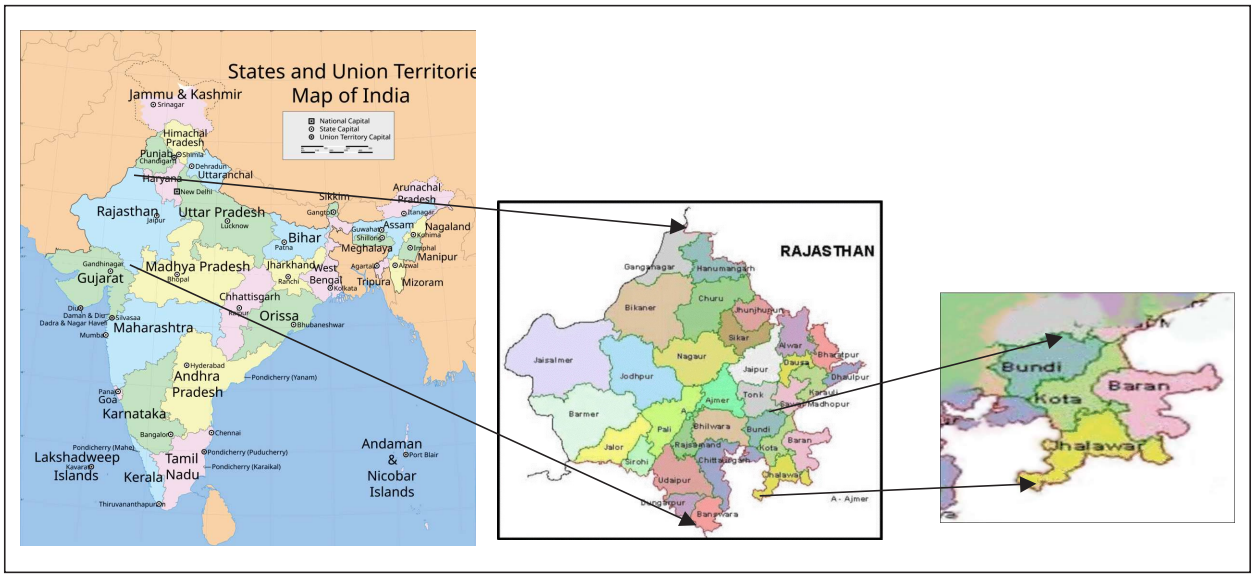

Figure 1: Location map of Hadoti region

Samples Size

Randomly, 423 blood samples and their relevant data were collected from presumably healthy and unrelated individuals (194 females and 229 males), belonging to Hadoti region of Rajasthan. The study was performed in accordance with the Institutional Ethical Committee of Poornima University, Jaipur, Rajasthan, India, vide letter no. ECINEW/INST/2024/4214 dated 18.10.2025. Written, informed consent was obtained from all participants prior to sampling. All the blood samples were transported to the laboratory for analysis.

Laboratory Analysis

Genomic DNA extraction

For DNA extraction, one punch of bloodstains on FTA Classic Cards (Whattman) was sufficient. A 1.2 mm punch from FTA card contains around 5-20 ng of genomic DNA.[15] DNA was directly amplifed from a 1.2 mm FTA card punch without quantification, according to the protocols in the PowerPlex Fusion 6C manual, using Eppendorf Mastercycler Nexus X2. DNA fragments were separated and analyzed by using an Applied Biosystems 3500 Genetic Analyzer (Thermo Fisher Scientific), provided with the internal lane standard WEN ILS 500 and allelic ladder in PowerPlex Fusion 6C system kit. Genotype determination and allele calling for 23 autos omal loci were carried out using GeneMapper ID-X software version 1.6 (Thermo Fisher Scientific).

Statistical Analysis

The population and forensic genetic parameters such as matching probability (MP), paternity index (PI), discrimination capacity (PD), power of exclusion (PE), polymorphism information content (PIC), observed (Hobs) and expected (Hexp) heterozygosities,[16] were calculated

using STRAF 2.2.2, an online tool for STR data analysis. Hardy-Weinberg Equilibrium (HWE) was also determined via STRAF. Both the conventional significance threshold *p*-value of 0.05 and an adjusted *p*-value of 0.002, derived through Bonferroni correction, were then used to assess the obtained results.

RESULTS

Allele frequencies and forensic performance

The allele frequencies and forensic parameters were calculated for the Hadoti's population of Rajasthan as given in Table 1.

The study of autosomal STRs enhanced our understanding of genetic diversity and population dynamics in the Hadoti region of south- eastern Rajasthan. A total of **300** alleles were studied with an average of **13** alleles per locus in the group. Average frequency of 0.111 (D3S1358), 0.143 (TH01), **0.063** (D21S11), 0.077 (D2S441), 0.067 (D18S51), 0.050 (Penta E), 0.111 (D5S818), 0.083 (D2S1338), 0.111 (D13S317), 0.1 (D7S820), 0.125 (D16S539), 0.125 (D22S1045), 0.111 (CSF1PO), 0.083 (Penta D), **0.090** (vWA), 0.023 (SE 33), 0.1 (D10S1248), **0.090** (D8S1179), **0.067** (D12S391), 0.071 (D19S433), 0.059 (D1S1656), 0.143 (TPOX) and 0.059 (FGA) were noted. A maximum frequency of 0.387 at D2S441 and a minimum frequency of 0.001 at various loci were detected across 23 loci. A maximum of 42 alleles were found at SE 33, followed by 20 at Penta E and a minimum of 7 alleles at TH01 and TPOX. In this study, the presence of specific two alleles for the majority of the STRs - D3S1358 (15 and 16), TH01 (6 and 9), D1S1656 (11 and 16), D2S1338 (18 and 19), D2S441 (10 and 11), D21S11 (29 and 30), D18S51 (14 and 15), D19S433 (13 and 14), Penta E (11 and 16), D5S818 (11 and 12), D13S317 (11 and 12), D16S539 (11 and 12), D7S820 (8 and 11), D12S391 (17 and 18), D10S1248 (14 and 15), D22S1045 (11 and 15), CSF1PO (11 and 12), Penta D (9 and 11), vWA (16 and 17), SE 33 (18 and 19), D8S1179 (10 and 14), TPOX (8 and 11) and FGA (23 and 24) were also noted (Figure 1).

By analyzing the most common (MCA) and least common alleles (LCA),(Table 2) it was shown that allele 11 at D2S441 has occurred 387 times, followed by the 15th allele of D22S1045, which has been seen 385 times. In several of the loci, there are single alleles only appeared once. Particularly, allele 20.3 in D1S1656, allele 15 in D13S317, allele 6 in Penta E, allele 15 in D16S539, alleles 27 in D2S1338, allele 16 in CSF1PO, allele 10.3 in TH01, allele 12 in vWA, allele 6 in D7S820, allele 18 in D8S1179, allele 19.3 in D12S391 and allele 12 in D22S1045 were identified once.

Table 2: MCA and LCA of Hadoti population of Rajasthan

Locus	MCA		LCA
	Allele	Frequency	Allele
D3S1358	16	0.324	13, 20
D1S1656	16	0.169	20.3
D2S441	11	0.387	9, 13.3, 17
D10S1248	14	0.264	10,19
D13S317	12	0.262	15
Penta E	11	0.135	6
D16S539	11	0.344	15
D18S51	14	0.307	10, 22, 24
D2S1338	18	0.171	27
CSF1PO	12	0.358	16
Penta D	11	0.243	16, 17
TH01	9	0.281	10.3
vWA	17	0.281	12
D21S11	30	0.219	26, 28.2, 29.1
D7S820	8	0.245	6
D5S818	11	0.340	7, 8
TPOX	11	0.371	7, 13
D8S1179	14	0.199	18
D12S391	18	0.273	19.3
D19S433	13	0.278	11.2, 17.2
D22S1045	15	0.385	12
SE 33	19	0.99	9, 14.3, 15.2, 16.2, 17.2, 28, 29, 30, 34.2
FGA	24	0.200	20.3, 25.2

The highest polymorphic information content (PIC) value of 0.943 was found at the SE 33 locus, followed by 0.908 at Penta E, 0.873 at D1S1656, and 0.861 at D2S1338 locus. SE 33 had the greatest value of expected heterozygosity (Hexp) (0.947), tailed by Penta E (0.916), and D1S1656 (0.885). Heterozygosity (Hobs) was measured between 0.719 (CSF1PO) to 0.953 (SE 33). In terms of discriminating capacity, the values of 0.866 (TPOX) and 0.992 (SE 33) were discovered to be the lowest and highest, respectively. TPOX was shown to have the highest matching probability among the sampled population (0.134). Power of exclusion was measured between 0.458 (CSF1PO) to 0.904 (SE 33). In SE 33, the total paternity index reached a peak of 10.575 suggesting that the STR loci are highly informative for kinship testing in the studied population. There was no significant departure from the Hardy-Weinberg equilibrium ($p>0.05$), after Bonferroni corrections.

Table 1: Observed allele frequencies and Forensic parameters in Hadoti population of Rajasthan (n=423)

Allele	CSF1PO	D10S124	D12S391	D13S317	D16S539	D18S51	D19S433	D1S1656	D21S11	D22S1045	D2S1338	D2S441	D3S1358	D5S818	D7S820	D8S1179	FGA	Penta D	Penta E	SE33	TH01	TPOX	vWA
5																			0.056				
6															0.001			0.004	0.001		0.268		
7				0.007										0.001	0.028			0.005	0.057		0.149	0.004	
8	0.005			0.193	0.074			0.039				0.002		0.001	0.245	0.007		0.020	0.005	0.005	0.148	0.349	
9	0.034			0.098	0.154			0.004				0.001		0.030	0.067	0.002		0.232	0.012	0.001	0.281	0.138	
9.1															0.001								
9.3																					0.144		
10	0.191	0.001		0.085	0.095	0.001		0.019		0.006		0.307		0.122	0.221	0.187		0.162	0.053		0.007	0.092	0.002
10.3																					0.002		
11	0.311	0.015		0.243	0.344	0.018	0.006	0.158		0.280		0.387		0.340	0.236	0.064		0.243	0.135	0.002		0.371	
11.1														0.001									
11.2							0.001																
11.3												0.054											
12	0.358	0.018		0.262	0.191	0.071	0.084	0.102		0.004		0.079		0.298	0.175	0.099		0.128	0.112	0.008		0.043	0.001
12.2							0.007																
12.3												0.006											
13	0.077	0.145		0.082	0.124	0.143	0.278	0.122				0.024	0.001	0.194	0.022	0.155		0.134	0.096	0.014		0.004	0.005
13.2							0.020																
13.3												0.001											
14	0.015	0.264		0.028	0.017	0.307	0.266	0.099		0.063		0.115	0.044	0.013	0.002	0.199		0.053	0.071	0.014			0.112
14.2							0.066													0.007			
14.3																				0.001			

Allele	CSF1PO	D10S124	D12S391	D13S317	D16S539	D18S51	D19S433	D1S1656	D21S11	D22S1045	D2S1338	D2S441	D3S1358	D5S818	D7S820	D8S1179	FGA	Penta D	Penta E	SE33	TH01	TPOX	vWA
15	0.007	0.257	0.008	0.001	0.001	0.169	0.131	0.148		0.385		0.019	.0304			0.182		0.015	0.095	0.020			0.069
15.2							0.052													0.001			
15.3								0.019												0.002			
16	0.001	0.228	0.009			0.136	0.056	0.169		0.189	0.008	0.004	0.324			0.085		0.002	0.113	0.040			0.255
16.2							0.025													0.001			
16.3								0.032												0.002			
16.4																		0.004					
17		0.064	0.112			0.080	0.007	0.039		0.063	0.053	0.001	0.228			0.019		0.002	0.072	0.064			0.281
17.2							0.001													0.001			
17.3			0.007					0.025															
18		0.007	0.273			0.033		0.007		0.011	0.171		0.093			0.001	0.006		0.043	0.084			0.165
18.3			0.011					0.015															
19		0.001	0.142			0.017					0.143		0.005				0.032		0.045	0.099			0.085
19.3			0.001					0.002															
20			0.089			0.012					0.121		0.001				0.126		0.012	0.074			0.019
20.2																	0.002						
20.3								0.001									0.001						
21			0.109			0.007					0.060						0.108		0.012	0.039			0.005
21.1																				0.004			
21.2																	0.006			0.012			
22			0.116			0.001					0.082						0.143		0.005	0.008			
22.1																				0.002			

vWA																						
TPOX																						
TH01																						
SE33	0.021	0.006	0.017		0.019		0.060		0.048		0.050	0.001	0.047	0.001		0.076		0.001	0.060		0.035	
Penta E		0.004																				
Penta D																						
FGA	0.009	0.170		0.200	0.002	0.123	0.001	0.051		0.014		0.005										
D8S1179																						
D7S820																						
D5S818																						
D3S1358																						
D2S441																						
D2S1338		0.191		0.086		0.061		0.018		0.005												
D22S1045																						
D21S11								0.001		0.005		0.158	0.001	0.204	0.001	0.008	0.005	0.219	0.021	0.033	0.100	0.004
D1S1656																						
D19S433																						
D18S51		0.004		0.001																		
D16S539																						
D13S317																						
D12S391		0.065		0.035		0.017		0.006														
D10S124																						
CSF1PO																						
Allele	22.2	23	23.2	24	24.2	25	25.2	26	26.2	27	27.2	28	28.2	29	29.1	29.2	29.3	30	30.2	31	31.2	32

Allele	CSF1PO	D10S124	D12S391	D13S317	D16S539	D18S51	D19S433	D1S1656	D21S11	D22S1045	D2S1338	D2S441	D3S1358	D5S818	D7S820	D8S1179	FGA	Penta D	Penta E	SE33	TH01	TPOX	vWA
32.2									0.187											0.026			
33																				0.002			
33.2									0.044											0.019			
34.2									0.008											0.001			
Nall	9	10	15	9	8	15	14	17	16	8	12	13	8	9	10	11	17	12	20	42	7	7	11
PIC	0.686	0.754	0.839	0.784	0.764	0.804	0.794	0.873	0.816	0.685	0.861	0.693	0.695	0.698	0.769	0.828	0.849	0.800	0.908	0.943	0.750	0.662	0.776
PM	0.117	0.080	0.037	0.055	0.071	0.054	0.055	0.026	0.049	0.119	0.030	0.114	0.117	0.113	0.072	0.044	0.035	0.056	0.016	0.008	0.082	0.134	0.072
PD	0.883	0.920	0.963	0.941	0.929	0.946	0.945	0.974	0.951	0.881	0.970	0.886	0.883	0.887	0.928	0.956	0.965	0.944	0.984	0.992	0.918	0.866	0.928
PE	0.458	0.589	0.637	0.513	0.567	0.683	0.584	0.688	0.702	0.465	0.711	0.473	0.485	0.505	0.593	0.697	0.730	0.651	0.773	0.904	0.563	0.469	0.660
TPI	1.777	2.431	2.783	2.014	2.299	3.205	2.403	3.254	3.411	1.808	3.525	1.839	1.888	1.977	2.459	3.357	3.777	2.897	4.500	10.575	2.274	1.823	2.979
Hobs	0.719	0.794	0.820	0.752	0.783	0.844	0.792	0.846	0.853	0.723	0.858	0.728	0.735	0.747	0.797	0.851	0.868	0.827	0.889	0.953	0.780	0.726	0.832
Hexp	0.732	0.788	0.855	0.811	0.792	0.826	0.817	0.885	0.838	0.730	0.875	0.734	0.741	0.743	0.800	0.848	0.865	0.824	0.916	0.947	0.785	0.712	0.804
P-value	0.989	0.170	0.213	0.380	0.600	0.565	0.835	0.155	0.935	0.247	0.379	0.187	0.150	0.499	0.892	0.498	0.642	0.373	0.021	0.013	0.554	0.005	0.412

PIC – Polymorphism Information content, **PM –** Probability of matching, **PD –** Power of Discrimination, **PE –** Power of Exclusion, **TPI –** Total Paternity Index, **Hobs –** Observed heterozygosity, **Hexp –** Expected heterozygosity, **Nall –** Number of alleles

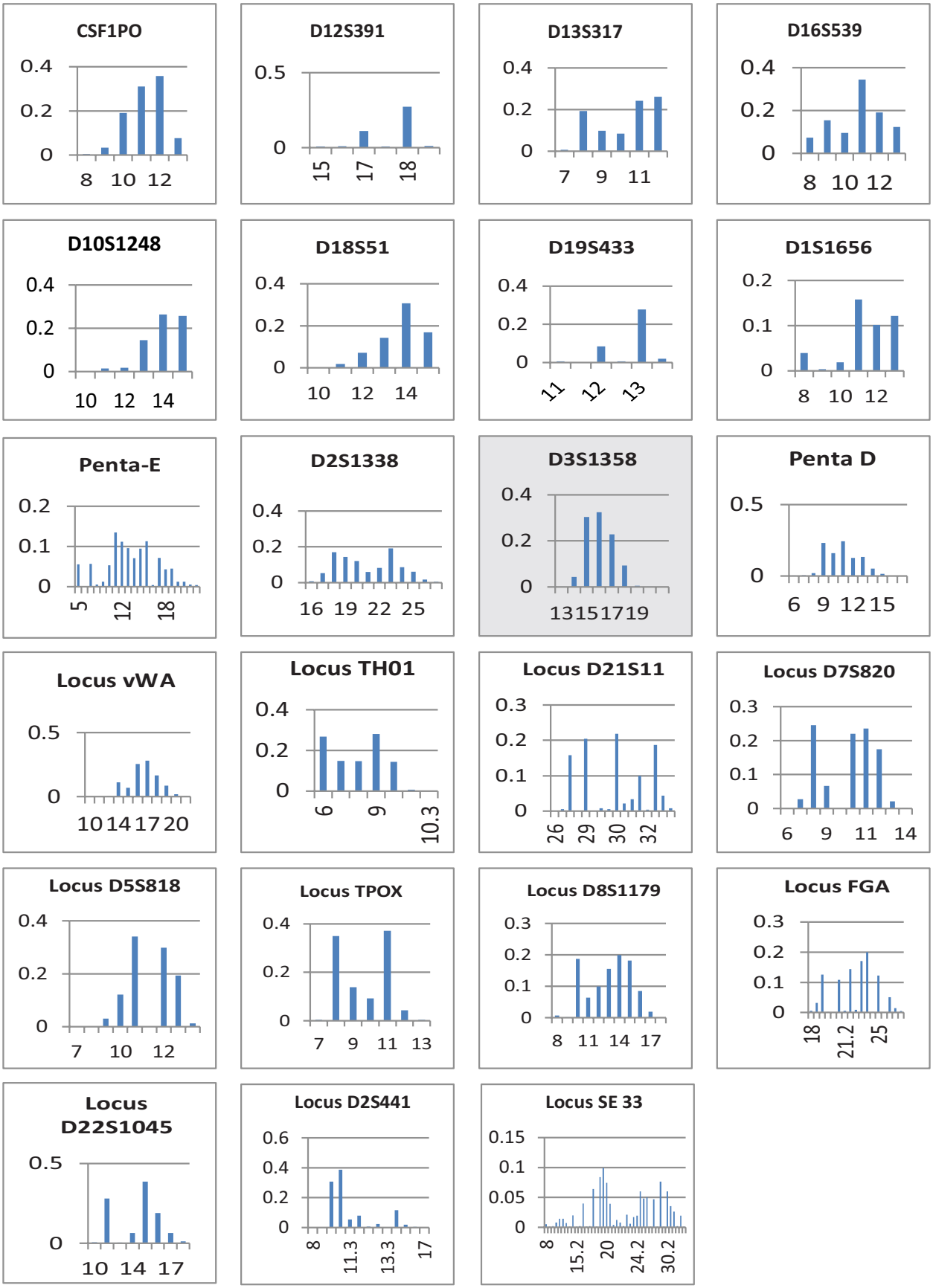

Figure 1: Display of obtained allele frequencies at 23 studied loci

DISCUSSION

There is sufficient anthropological and historical data to demonstrate that people belonged to a variety of ethnic backgrounds, cultures and languages have lived in India for a very long time and enriched the current gene pool of the subcontinent.[17,18] The gene pool of a population is the genetic constitution of its composing individuals, who received them from their parents and this gene pool of a population can be represented by a range of gene frequencies. Numerous studies conducted in different parts of the world over the past century have revealed the frequency of genes or alleles based on a number of standard phenotypic traits, for example, blood groups, serum proteins, red cell enzymes, human leukocyte antigens and immunoglobulin profiles.[18,19] Every system shows different ethnic and geographical distribution. The populations majorly studied were from North India (Himachal Pradesh, Uttar Pradesh, Punjab), western India (Rajasthan, Gujarat, Maharashtra), southern India (Andhra Pradesh) and eastern India (Orissa, West Bengal, Assam).

In this study, allele frequencies in the Hadoti population of Rajasthan ranged from 0.001 (many alleles) to 0.387 (D2S441) which closely aligns with another genetic study conducted by Srivastava *et al.,* (2015), on the central Indian population by utilizing 15 autosomal STR loci with 582 healthy individuals, ranging from as low as 0.001 up to 0.381.[20] Similarly, Dev *et al.* (2022) worked on genomic history and forensic features of Gurjars from western Uttar Pradesh and National Capital Region Delhi with 215 people, and observed frequencies ranging from 0.002 to 0.384, which is again near to our results.[14] A study on Genetic Diversity of Autosomal STR Markers in the Brahmin Population of Rajasthan and Haryana by Sharma *et al.*(2023) reported that the allele 11 was the most common at D2S441, with a frequency of 0.452 which corresponds well with our findings in which allele 11 of D2S441 is most prevalent with highest frequency of 0.387.[21] We have reported, SE 33 has the highest polymorphism information content (PIC) score of 0.943 and the highest discrimination capacity value of 0.992, which is in close agreement with another similar study, by Sahoo *et al.,* (2020). He conducted a 21-autosomal-STR study in Odisha, India with 508 healthy and unrelated people, and reported SE33 as the most polymorphic and discriminatory locus while TPOX at the lower end of PIC across loci.[22] Similarly, Dixit *et al.,* (2020) evaluated genetic and forensic features of Madhya Pradesh population with 374 individuals using 23 autosomal STRs and revealed that the most polymorphic and discriminatory STR loci in the studied population was SE 33 with values of 0.94 and 0.990, respectively.[23] Srivastava *et al.* (2020) conducted a genomic study of the Muslim population from Telangana (India) with 184 people utilizing 23 autosomal STRs and discovered that SE 33 had the highest PIC and PD with reported values of 0.941 and 0.987.[12] A study conducted by Nath *et al.,* (2021) with 15 autosomal STRs, with an aim to determine the genetic diversity and forensic parameters for Tripuri population, reported 158 alleles with the average 10.53 alleles per locus which are reasonably more close to our findings i.e. **13** alleles per locus.[24] An average around 12–13 alleles per locus is plausible, especially in highly diverse populations. In our study, allele 19 (0.99) at the SE 33 locus had the highest frequency and it was also the most common allele in the five states i.e. Odisha, Madhya Pradesh, Telangana, Himachal Pradesh and Rajasthan.[25] Similar to our study, most common allele at THO1 (allele 9), D8S1179 (allele 14), CSF1PO (allele 12), Penta E (allele 11) and D16S539 (allele 11) was also prevalent in four predominant population groups of central India.[26] Also, the five most prevalent alleles (30 at D21S11, 12 at D13S317, 11 at D16S539, 12 at CSF1PO, 17 at vWA) were noted in a study related to genomic diversity of the muslim population in Telangana (India) inferred from 23 autosomal STRs by Srivastava *et al.* (2020).[12] In our investigation, locus SE 33 revealed 42 alleles and TPOX and TH01 show minimum number of alleles i.e 7 which is very similar observations in many studies nationally and worldwide utilizing Poweplex Fusion 6C system kit for amplification. The results of our investigation revealed that all of the analyzed loci exhibited a high degree of genetic polymorphism, with observed heterozygosity (Hobs) ranging from 0.719 (CSF1PO) to 0.953 (SE 33) in the studied population. Srivastava *et al.* (2020) examined the Muslim population of Telangana, India for Genetic Polymorphism of 23 Autosomal STR loci and discovered that observed heterozygosity (Hobs) varied from 0.663 (D22S1045) to 0.924 (SE 33) in the analyzed

population, which is remarkably similar to our findings.[12]

The statistical evaluation of allelic frequency of autosomal STRs helps to derive various forensic parameters such as power of discrimination, match probability and polymorphic information content, which enhances the efficiency of human identification. Moreover, such data reduces false inclusions or exclusions, making forensic investigations more precise, robust, and scientifically valid especially when India begins to develop a systematic infrastructure for Forensic DNA analysis. The observed large scale DNA dataset did not capture micro genetic substructure. This indicates that pooling data across diverse large scale populations may obscure local allele frequency patterns. Especially, regional data is more relevant in India where caste, tribe and community- base endogamy affects genetic patterns. Therefore, the inclusion and expansion of region specific datasets is essential to improve the accuracy and representativeness of forensic and population genetic analyses.[27,28,29] Therefore, genetic data from such unrepresented regional population like Hadoti is of immense importance in forensics because it strengthens the accuracy and reliability of DNA profiling at regional level. Regional databases ensures quicker identification in criminal cases, missing persons tracking and disaster victim identification by minimizing errors that arise when using data from genetically distinct groups.

CONCLUSIONS

This is the first study, to the best of our knowledge, that makes use of the 23 STRs included in the PowerPlex Fusion 6C System in Hadoti populations. In summary, the high diversity observed for all 23 loci supports the usefulness of the studied markers for forensic analysis in Hadoti region of Rajasthan, including familial relationship and human identification. The locus SE33 among all the studied loci was found to be most discriminatory and polymorphic in the investigated population. Moreover, it is speculated that the data collected for this study would increase the India's DNA databank at regional level.

Acknowledgments

The authors are grateful to all subjects for their contribution and participation in this study.

Funding

This study received no funding from public, commercial, or non profit organizations.

Availability of data and materials

Data and materials would be made available upon request.

DECLARATIONS

Ethics approval and consent to participate

Ethical clearance for this study was obtained from the Institutional Ethical Committee of Poornima University, Jaipur, Rajasthan, India, vide letter no. ECINEW/INST/2024/4214 dated 18.10.2025. This study was approved in accordance with the Helsinki Declaration. Prior to the data collection, informed written consent was obtained from the participants.

Consent for publication

Participants were made aware that this research would be published.

Competing interests

No competing interest

REFERENCES

1. Khan M. Haryana. In The Territories and States of India 2024 2024 Feb 22 (pp. 140-147). Routledge.
2. Butler J.M., Hill C.R. Biology and genetics of new autosomal STR loci useful for forensic DNA analysis. Forensic Science Review. 2012 Jan 4; 24(1): 15.
3. Butler J.M. Short tandem repeat typing technologies used in human identity testing. Biotechniques. 2007 Oct 1; 43(4): Sii-v.
4. Cavalli-Sforza L.L., Barrai I., Edwards A.W. Analysis of human evolution under random genetic drift. InCold Spring Harbor symposia on quantitative biology 1964 Jan 1 (Vol. 29, pp. 9-20). Cold Spring Harbor Laboratory Press.
5. Bhasin M.K. Morphology to molecular anthropology: Castes and tribes of India. Int J Hum Genet. 2009 Sep 1; 9(3-4): 145-230.
6. Lewontin R.C. The apportionment of human diversity. Evol. Biol. 1972; 6: 381–398. (doi:10.1007/978- 1-4684-9063-3_14)

7. Nei M., Roychoudhury A.K. Genic variation within and between the three major races of man, Caucasoids, Negroids, and Mongoloids. American journal of human genetics. 1974 Jul; 26(4): 421.
8. Nei M. Estimation of average heterozygosity and genetic distance from a small number of individuals. genetics. 1978 Jul 20; 89(3): 583-90.
9. Promega Corporation. *PowerPlex® Fusion 6C System* [Product information]. Promega; (cited n.d.). Available from https://www.promega.com/products/forensic-dna-analysis-ce/str-amplification/powerplex-fusion-6c-system
10. Boavida A., Bogas V., Sampaio L., Gouveia N., Porto M.J., Corte-Real F. PowerPlex® fusion 6C system: internal validation study. Forensic sciences research. 2018 Jun; 3(2): 130-7.
11. Dixit S., Shrivastava P., Kumawat R.K., Kaitholia K., Dash H.R., Sharma H., Choubey G. Forensic genetic analysis of population of Madhya Pradesh with PowerPlex Fusion 6C™ multiplex system. International journal of legal medicine. 2019 May 1; 133(3): 803-5.
12. Srivastava V., Surekha Rani H., Kumawat R., Chaubey G., Shrivastava P. Genomic diversity of the Muslim population from Telangana (India) inferred from 23 autosomal STRs. Annals of Human Biology. 2020 Nov 16; 47(7-8): 652-8.
13. Srivastava A., Nath S., Das K.K., Kumar A., Kushwaha P., Kumar A., Srivastav K.V., Bhasney V., Rana M., Dixit S., Kumawat RK. Forensic characterization and genomic diversity of Assam population viewed from 23 autosomal STRs. International journal of legal medicine. 2021 Nov; 135(6): 2307-8.
14. Dev K., Kesharwani L., Kushwaha P., Kumar A., Srivastav K.V., Bhasney V., Kumawat R.K., Dixit S., Srivastava A., Chaubey G., Shrivastava P. Molecular characterization of 23 Y chromosomal STR markers in the Gurjar population of National Capital Region (NCR), India. International Journal of Legal Medicine. 2022 Mar; 136(2): 555-6.
15. Alaeddini R. Forensic implications of PCR inhibition—a review. Forensic Science International: Genetics. 2012 May 1; 6(3): 297-305.
16. Gouy A., Zieger M. STRAF—a convenient online tool for STR data evaluation in forensic genetics. Forensic Science International: Genetics. 2017 Sep 1; 30: 148-51.
17. Bhasin M.K., Walter H., Danker-Hopfe H. People of India: an investigation of biological variability in ecological, ethno-economic, and linguistic groups. (No Title). 1994.
18. Papiha S.S. Genetic variation in India. Human biology. 1996 Oct 1: 607-28.
19. Bhasin M.K., Walter H. Genetics of castes and tribes of India. Kamla-Raj Enterprises; 2001.
20. Shrivastava P., Jain T., Trivedi V.B. Genetic polymorphism study at 15 autosomal locus in central Indian population. Springerplus. 2015 Sep 30; 4(1): 566.
21. Sharma S., Sahajpal V., Singh A., Yadav R., Thakur M., Bhandari D., Ranga S., Kadian L., Yadav C. Genetic diversity of autosomal STR markers in the brahmin population of Rajasthan and Haryana: significance in population and forensic genetics. Современные технологии в медицине. 2023; 15(1 (eng)): 63-72.
22. Sahoo S., Samal R., Behera S., Swain A.K., Biswas S., Shrivastava P., Kumawat R.K., Chaubey G., Dash H.R. Genomic portrait of Odisha, India drawn by using 21 autosomal STR markers. International journal of legal medicine. 2020 Sep; 134(5): 1671-3.
23. Dixit S., Dubey I.P., Kumawat R.K., Tripathi I.P., Shrivastava D., Panda M., Shrivastava P. Genetic Polymorphism of 15 Autosomal STR loci in Population of Madhya Pradesh. Medico-Legal Update. 2020 Oct 1; 20(4).
24. Nath S., Kumawat R.K., Badiye A., Srivastava A., Dash H.R., Dixit S., Kaitholia K., Chaubey G., Shrivastava P. Genetic diversity and forensic characterization of 15 autosomal microsatellite markers in the North-east Indian Tripuri population. International Journal of Legal Medicine. 2021 Sep; 135(5): 1797-8.
25. Dixit S., Shrivastava P., Dash H.R., Kaitholia K., Sahajpal V., Sahoo S., Srivastava V., Rani H.S., Mishra A., Choudhary S.K., Thekkatavan A. Assessment of significance and forensic relevance of SE33 (ACTBP2) locus in five Indian populations. Gene Reports. 2021 Sep 1; 24: 101293.
26. Sarkar N., Kashyap V.K. Genetic diversity at two pentanucleotide STR and thirteen tetranucleotide STR loci by multiplex PCR in four predominant population groups of central India. Forensic science international. 2002 Aug 28; 128(3): 196-201.
27. Kashyap V.K., Sitalaximi T., Chattopadhyay P., Trivedi R. DNA profiling technologies in forensic analysis. International Journal of Human Genetics. 2004 Mar 1; 4(1): 11-30.

28. Kumar P, Singh S, & Kashyap VK. Genetic variation at 15 autosomal STR loci in four populations of India. Forensic Science International: Genetics. (2012);6(2):e71–e73. https://doi.org/10.1016/j.fsigen.2011.03.001

29. Singh S., Trivedi R., & Kashyap V.K. Forensic evaluation of 21 autosomal STR loci in Indian populations. *BMC Research Notes.* (2018); *11*(1): 32. https://doi.org/10.1186/s13104-018-3125-0