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Genetic diversity analysis in soybean (*Glycine max* (L.) Merrill.) using SSR markers

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Abstract
Genetic diversity of 40 soybean (*Glycine max* (L.) Merrill.) genotypes were carried out using 20 SSR primers. A total of 179 alleles were detected and 28 primers, out of 34 primers showed polymorphic results with 40 genotypes. The highest PIC value of 0.90 was recorded in Satt316 whereas lowest of 0.43 was recorded in Soy satt 160. The dendrogram analysis depicted that the 40 genotypes were grouped in 2 different clusters i.e. A and B. The similarity matrix revealed the diversity among genotypes. The present work on genetic diversity among soybean genotypes from Gujarat could provide useful information for efficient utilization of these materials in future breeding programme.

Keywords: soybean, SSR, genetic diversity, primers

Introduction
Soybean (*Glycine max* (L.) Merrill.) is one of the world's most important economic legume crops. It is the second most important oil seed crop in India, next to Indian mustard. A number of cultivars have been released in India from different soybean breeding centers for growing under different agroclimatic conditions by introduction, selection, mutation and hybridization of elite cultivars and germplasm through systematic breeding and evaluation programmes (Chauhan *et al*., 2015) [1]. Genetic relationships among accessions are helpful for designing future breeding efforts for yield, quality and pest resistance improvement (Tantasawat *et al*., 2011) [2]. The genetic diversity and relationship can be assessed by the differences in morphological and agronomic traits, pedigree information, geographic origins, isozymes, and DNA markers. However, some factors affect these methods, e.g. the influence of environmental factors on morphological and agronomic traits, uncertain or incomplete data and possible errors in the pedigree information and origins of accessions, and the limitation of data provided by isozymes. Among different DNA markers, restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNAs (RAPDs), amplified fragment length polymorphisms (AFLPs), single nucleotide polymorphisms (SNPs) and microsatellites or simple sequence repeats (SSRs) have been used extensively in soybeans, each with its own advantages and limitations. SSRs have been shown to produce the highest polymorphisms compared to RFLPs, AFLPs and RAPDs, and have much greater ability to identify unique alleles in elite and parental soybean germplasm than other marker systems (Tantasawat *et al*., 2011) [2]. The level of polymorphism of SSRs is related to a number of factors including the repeat number and motif. Simple sequence repeat (SSR) polymorphism is positively correlated with number of repeat units (Song *et al*., 2010) [3].
Keeping view the above facts, the present study have been carried out for identification and genetic diversity analysis of 40 soybean genotypes released from different breeding centers in India.

Materials and Methods
The study was conducted at Plant Biotechnology Laboratory, Department of Genetics and Plant Breeding, B.A. College of Agriculture, Anand Agricultural University, Anand.

DNA isolation
Few healthy seeds were sown and allowed to grow for three weeks in the pots. For the proper growth and emergence of healthy seedlings, these pots were watered regularly and proper light and temperature conditions were maintained. Total genomic DNA extraction from leaves of three weeks old seedlings were carried out by Cetyl trimethyl ammonium bromide (CTAB) method (Saghai *et al*., 1984) [4]. 300 mg tissue samples were homogenized in liquid nitrogen prior mixing with 800µl of extraction buffer and were incubated for 1 hour at 65º C in water

bath. Later on Chloroform: isoamyl alcohol (24:1) was added in the tubes and centrifuged at 4ºC for 15 minutes at 12,000 rpm. The supernatant was collected and washed 1- 2 times with Chloroform: isoamyl alcohol (24:1) and kept for precipitation with absolute alcohol for overnight. The samples were centrifuged at 5,000 rpm for 15 min to get DNA in pellet form. The pellets were further washed with 70% alcohol and kept for drying and the quality was confirmed through Nanodrop N.D. 1000 software (ver 3.7.1).

Simple Sequence Repeats

A total of 34 SSR markers were used (Tantasawat *et al*., 2011) [2] (Ghosh *et al*., 2014) [5] out of which 28 different markers showed polymorphic results (Table 2). Analysis was performed in 25µl reaction volume containing 50ng genomic DNA, 10X polymerase buffer, 25mM dNTPs, 0.5 µl of each primer (10pmol), 1 unit of Taq DNA polymerase using Applied Biosystem Thermal cyclers. The cycling conditions were: 1 cycle of 94°C for 7min followed by 40 cycles of 45 sec each of 94°C, 48°C, 72°C and finally 1 cycle of 72°C for 5min. Total of 10 µl aliquots of the amplification products loaded in 3% (w/v) agarose gel for electrophoresis in 1X TBE buffer and stained with Ethidium bromide and documented using SYNGENE GENESNAP G-BOX gel documentation system. These photographs were used to score the DNA bands for analysis.

Data scoring and clustering

The bands appearing without ambiguity were scored as 1(present) or 0(absent) for each primer. Coefficients of similarity were calculated as Jaccard's similarity coefficient by SIMQUAL subroutine in SIMILARITY routine. The matrix of similarity was clustered using UPGMA algorithim under Sequential Agglomerative Hierarchial Nestinf (SHAN) module using NTSYSpc version 2.2 (Rohlf, 2000) [6]. The polymorphism information content (PIC), a measure of the allelic diversity at a locus, was determined as $PIC=1-\Sigma Pi^2$ where Pi is the frequency of the i^{th} allele in the examined test lines. The cophenetic correlation analysis was carried out using COPH function of NTSYS pc. and dendrogram constructed based on similarity coefficients (Anderson *et al*., 1993) [7].

Results and Discussion

A total of 179 alleles were detected and 28 primers, out of 34 primers showed polymorphic results with 40 genotypes. The highest PIC value of 0.90 was recorded in Satt316 whereas lowest of 0.43 was recorded in Soy satt 160 (Table 2). The dendrogram analysis depicted that the 40 genotypes were grouped in 2 different clusters *i.e.* A and B. The genotypes *viz*., GS-3, JS-335, PK-472, JS-9305, GSoy-1, GSoy-2, JS-75-46, JS-79-170, PK-805, JS-81-607, PK-746, JS-9752, HIMSO-5506, DS-89-4, DS-89-18, MACS-303, NRC-37, NRC-7 and JS-9560 clustered together in group A, whereas remaining genotypes were grouped in B cluster (Figure 1). The present result is in accordance with the findings of Tantasawat *et al*. (2011) [2] where they study the genetic diversity of 25 soybean varieties through eleven SSR primers. They suggested that SSR markers are efficient for measuring genetic diversity and relatedness as well as identifying varieties of soybean. Similar worked was carried out by Ghosh *et al*. (2014) [5], where 32 soybean genotypes were used to find out the genetic diversity among them through 10 SSR primers. The similarity coefficient ranged from 0.0 to 0.63 where, maximum similarity was recorded between VLS-20 and EC-93601. The results showed a clear distinction between the genotypes. Chauhan *et al*., (2015) [1] studied the molecular characterization and genetic diversity assessment in soybean varieties using SSR markers and they concluded that genetic similarity coefficient for the largely numbers of pairs was in the range of 0.1- 0.4 signifying high diversity between the chosen genotypes.

The similarity distance can be used to plan breeding programme because higher the genetic distance the better will be the variability. To plan an efficient breeding programme it is crucial to be aware of the latent genetic diversity and relatedness in the base population. Therefore utilizing diverse genotypes can offer a chance to isolate superior segregants.

The present work on genetic diversity among soybean genotypes from Gujarat could provide useful information for efficient utilization of these materials in future breeding programme.

Table 1: List of soybean genotypes

Sr. No.	Name of genotypes	Sr. No.	Name of genotypes
1	GS-3	21	EC-93601
2	JS-335	22.	MO-32
3	PK-472	23.	HIMSO-5506
4	JS-9305	24.	J-158
5	MACS-58	25.	DS-89-18
6	G.SOY-1	26.	AGS-51
7	G.SOY-2	27.	AGS-13
8	JS-75-46	28.	J-301
9	JS-79-190	29.	DS-83-12-2
10	JS-81-607	30.	AMR-SEL-KH-06
11	CAT-148	31.	TAX-34251
12	AGS-156	32.	AGS-109
13	CAT-746	33.	MACS-303
14	PK-805	34.	CAT-195
15	PK-746	35.	J-563
16	IC(SH)-8744	36.	DS-87-4
17	JS-79-4-1	37.	NRC-37
18	PI-283327	38.	NRC-7
19	EC-95278	39.	JS-9752
20	VLS-20	40.	JS-9560

Table 2: List of SSR primers used in the present study

Sr. No.	Primer name	Sequence (Forward)	Sequence (Sequence)	PIC
1.	Soy satt 001	TGT GCA ATG ATA GTA CAT AGA TAT	GTG CTG ATT GAA CTA TTT GTA GT	0.56
2.	Soy satt 005	TAT ATC CTA GAG AAG AAC TAA AAA A	GTC GAT TAG GCT TGA AAT AAT AC	0.79
3.	Soy satt 148	TTA AGG ATT AAT TGA GAC AAA ATC A	CTA AAG CAT CAC AAA ACA GAG C	0.81
4.	Soy satt 160	ACA TCA AAA GTT TAT AAC GTG TAG	CTC CCA CAC AGT TTT CAT ATA AT	0.43
5.	Soy satt 171	TTG AGG GCT CCC ACA CAG TT	CAA AAG TTT ATA ACG TGT AGA TTA A	0.49
6.	Soy satt 173	CCG GTC CAA TCT TTA TTC AAA C	CCA AGC GAA ATC ACC TCC TCT	0.83
7.	Soy satt 185	CAT ATG AAT AGG TAA GTT GCA CT	TGT CAC TAT AAA TGG TAC CTA TTA	0.83
8.	Soy satt 285	GCG ACA TAT TGC ATT AAA AAC ATA CTT	GCG GAC TAA TTC TAT TTT ACA CCA ACA AC	0.85

9.	Soy satt 307	GCG CTG GCC TTT AGA AC	GCG TTG TAG GAA ATT TGA GTA GTA AG	0.77
10.	Soy satt 309	GCG CCT TCA AAT TGG CGT CTT	GCG CCT TAA ATA AAA CCC GAA ACT	0.52
11.	Soy satt 409	CCT TAG ACC ATG AAT GTC TCG AAG ATA	CTT AAG GAC ACG TGG AAG ATG ACT AC	0.60
12.	Satt359	GCG AGA AAA TAA TCC TGC TCA AG	GCG TTT AAG TCC AAT AAC AAA GAT AAC	0.50
13.	Satt149	TTG CAC ATT CTT TTT GGT AAA CAG TCA TAA	GTT GGA GGC CAT AGT CAC ATT AAT CTT AGA	0.66
14.	Satt276	GCG GAC GGT AAG GAC TAT TTA TGA TA	GCG TCA GAT GAA AAA AAA TAA GAT AC	0.84
15.	Satt135	CAC GGA TTT TAA ATC ATT ATT ACA T	TTC CAA TAC CTC CCA ACT AAC	0.83
16.	Satt367	GCG GAT ATG CCA CTT CTC TCG TGA C	GCG GAA TAG TTG CCA AAC AAT AAT C	0.71
17.	Satt125	CAA ATA AAA ACA TAT ACC TCT TGT	TGC CTT ACT CTA CTC TGT TTC	0.70
18.	Satt365	TGC TCC CCT CTG CCT TTT TTT CTA TTT T	AAG GAT GAG TTT GAT AAA CAT GAA TGA AGA A	0.80
19.	Satt557	GCG GGA TCC ACC ATG TAA TAT GTG	GCG CAC TAA CCC TTT ATT GAA	0.82
20.	Satt245	AAC GGG AGT AGG ACA TTT TAT T	GCG CCT CCT GAA TTT CAA AGA ATG AAG A	0.74
21.	Satt316	GTG AGA AAC TAG CCA AGA ATA GA	CAA TTG TTT CCA AAT GAC ACT	0.90
22.	Satt354	GCG AAA ATG GAC ACC AAA AGT AGT TA	GCG ATG CAC ATC AAT TAG AAT ATA CAA	0.69
23.	Satt319	CAA CTC AGT AGG GGT CAA TAA CAA	TGA AAT AGG GAA AAT AAG GGA ACA	0.77
24.	Satt545	CAA TGC CAT TCC ATA TTT GTT	CAA TTG CCC TAG TTT TGA TAG	0.81
25.	Satt126	GCT TGG TAG CTG TAG GAA	ATA AAA CAA ATT CGC TGA TAT	0.50
26.	Satt249	GCG GCA AAT TGT TAT TGT GAG AC	GGC CAG TGT TGA GGG ATT TAGA	0.78
27.	Sat_356	GCG CCG GAA AAA TGT GAG AAA TCA TAA AA	GCG TTG CAT GAC TAT CAT TCA ATC AAA AAT	0.83

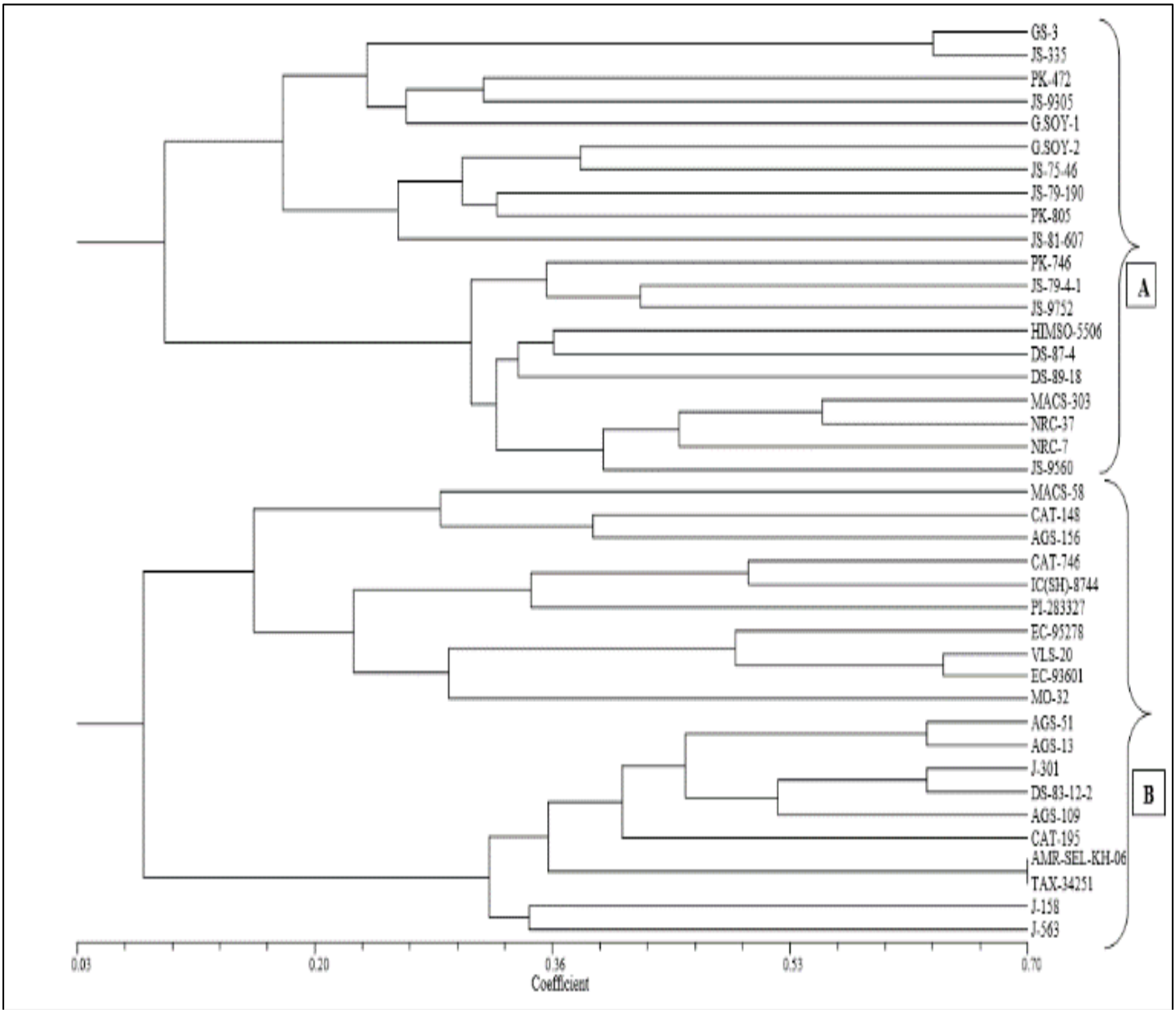

Fig 1: Dendrogram showing genetic diversity between 40 genotypes

Table 3: Similarity matrix of 40 soybean genotypes for SSR primers

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
1	1																																							
2	0.63	1																																						
3	0.19	0.27	1																																					
4	0.23	0.27	0.31	1																																				
5	0.19	0.24	0.24	0.28	1																																			
6	0.22	0.26	0.18	0.27	0.31	1																																		
7	0.10	0.18	0.18	0.18	0.15	0.38	1																																	
8	0.11	0.15	0.19	0.19	0.16	0.25	0.38	1																																
9	0.11	0.21	0.22	0.15	0.15	0.15	0.29	0.30	1																															
10	0.13	0.14	0.15	0.12	0.15	0.23	0.34	0.32	0.28	1																														
11	0.05	0.09	0.10	0.07	0.07	0.07	0.10	0.16	0.22	0.15	1																													
12	0.02	0.07	0.07	0.04	0.09	0.04	0.09	0.18	0.24	0.14	0.39	1																												
13	0.02	0.07	0.05	0.05	0.07	0.09	0.10	0.13	0.10	0.12	0.29	0.31	1																											
14	0.00	0.05	0.05	0.02	0.05	0.10	0.16	0.17	0.10	0.18	0.23	0.25	0.33	1																										
15	0.02	0.04	0.07	0.05	0.07	0.09	0.07	0.10	0.13	0.21	0.32	0.31	0.44	0.38	1																										
16	0.02	0.07	0.05	0.02	0.07	0.10	0.05	0.08	0.10	0.15	0.26	0.28	0.36	0.34	0.45	1																								
17	0.02	0.05	0.07	0.05	0.05	0.07	0.07	0.13	0.13	0.10	0.29	0.28	0.32	0.30	0.55	0.26	1																							
18	0.08	0.10	0.10	0.10	0.10	0.10	0.07	0.17	0.16	0.10	0.26	0.25	0.22	0.27	0.42	0.26	0.48	1																						
19	0.10	0.12	0.10	0.10	0.18	0.09	0.05	0.13	0.13	0.09	0.32	0.42	0.39	0.29	0.39	0.41	0.32	0.38	1																					
20	0.00	0.02	0.02	0.02	0.08	0.13	0.11	0.21	0.14	0.16	0.21	0.30	0.35	0.29	0.45	0.32	0.37	0.38	0.40	1																				
21	0.00	0.00	0.06	0.03	0.03	0.03	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.03	0.03	0.03	0.03	0.03	0.00	1																			
22	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.03	0.00	0.00	0.03	0.03	0.03	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.26	1																		
23	0.05	0.08	0.08	0.05	0.02	0.02	0.00	0.03	0.00	0.00	0.03	0.02	0.08	0.03	0.00	0.00	0.03	0.00	0.02	0.00	0.30	0.39	1																	
24	0.03	0.08	0.02	0.05	0.05	0.13	0.08	0.05	0.03	0.02	0.03	0.02	0.02	0.11	0.02	0.03	0.05	0.03	0.00	0.06	0.15	0.19	0.36	1																
25	0.03	0.05	0.03	0.05	0.03	0.11	0.05	0.06	0.03	0.03	0.03	0.00	0.03	0.09	0.03	0.03	0.03	0.03	0.00	0.06	0.17	0.15	0.29	0.50	1															
26	0.03	0.05	0.02	0.05	0.05	0.08	0.02	0.00	0.03	0.02	0.03	0.02	0.02	0.03	0.00	0.00	0.00	0.00	0.00	0.03	0.07	0.10	0.19	0.31	0.38	1														
27	0.03	0.05	0.02	0.02	0.00	0.02	0.00	0.03	0.08	0.05	0.08	0.13	0.00	0.03	0.02	0.02	0.00	0.03	0.02	0.03	0.11	0.18	0.22	0.26	0.23	0.44	1													
28	0.06	0.11	0.00	0.03	0.03	0.05	0.00	0.00	0.03	0.02	0.08	0.08	0.05	0.05	0.03	0.03	0.00	0.00	0.03	0.00	0.07	0.15	0.19	0.28	0.21	0.28	0.46	1												
29	0.06	0.08	0.03	0.03	0.05	0.05	0.00	0.00	0.03	0.02	0.08	0.05	0.03	0.00	0.03	0.03	0.00	0.00	0.03	0.00	0.07	0.15	0.16	0.19	0.17	0.32	0.52	0.64	1											
30	0.03	0.03	0.06	0.06	0.06	0.03	0.00	0.00	0.03	0.00	0.06	0.13	0.00	0.03	0.03	0.00	0.00	0.00	0.03	0.00	0.05	0.09	0.11	0.11	0.12	0.07	0.29	0.26	0.32	1										
31	0.00	0.03	0.00	0.00	0.03	0.00	0.05	0.03	0.05	0.00	0.00	0.08	0.05	0.06	0.03	0.00	0.03	0.03	0.05	0.03	0.08	0.03	0.06	0.09	0.06	0.09	0.09	0.13	0.13	0.17	1									
32	0.05	0.05	0.02	0.05	0.11	0.08	0.00	0.00	0.00	0.02	0.08	0.02	0.03	0.00	0.05	0.03	0.00	0.00	0.05	0.03	0.00	0.00	0.03	0.12	0.09	0.12	0.08	0.19	0.16	0.15	0.33	1								
33	0.08	0.07	0.02	0.05	0.08	0.07	0.00	0.00	0.02	0.05	0.11	0.07	0.02	0.03	0.05	0.02	0.00	0.00	0.05	0.03	0.00	0.00	0.03	0.11	0.06	0.11	0.11	0.27	0.15	0.15	0.23	0.63	1							
34	0.08	0.07	0.00	0.02	0.05	0.05	0.02	0.03	0.05	0.02	0.05	0.07	0.05	0.03	0.05	0.02	0.02	0.03	0.08	0.03	0.00	0.00	0.05	0.05	0.03	0.15	0.08	0.23	0.19	0.07	0.37	0.44	0.60	1						
35	0.05	0.49	0.00	0.02	0.05	0.05	0.02	0.03	0.03	0.00	0.05	0.08	0.05	0.03	0.05	0.03	0.03	0.03	0.08	0.03	0.00	0.03	0.09	0.06	0.03	0.09	0.08	0.19	0.19	0.11	0.38	0.52	0.44	0.63	1					
36	0.03	0.02	0.02	0.00	0.02	0.02	0.00	0.00	0.03	0.02	0.08	0.05	0.02	0.03	0.02	0.03	0.00	0.00	0.02	0.00	0.00	0.03	0.03	0.06	0.00	0.06	0.11	0.19	0.16	0.07	0.24	0.36	0.34	0.30	0.46	1				
37	0.03	0.02	0.02	0.00	0.02	0.02	0.00	0.00	0.02	0.02	0.08	0.02	0.02	0.00	0.02	0.02	0.00	0.00	0.02	0.00	0.00	0.03	0.03	0.03	0.00	0.11	0.11	0.15	0.23	0.03	0.28	0.34	0.29	0.33	0.50	0.70	1			
38	0.05	0.05	0.00	0.02	0.05	0.05	0.02	0.03	0.02	0.00	0.02	0.05	0.05	0.03	0.05	0.02	0.02	0.03	0.08	0.03	0.00	0.00	0.03	0.03	0.06	0.11	0.08	0.12	0.12	0.07	0.42	0.39	0.33	0.48	0.56	0.30	0.38	1		
39	0.03	0.02	0.00	0.00	0.08	0.05	0.02	0.03	0.03	0.00	0.03	0.05	0.05	0.03	0.05	0.03	0.03	0.03	0.08	0.06	0.00	0.00	0.00	0.03	0.06	0.06	0.03	0.09	0.06	0.03	0.29	0.41	0.34	0.34	0.46	0.36	0.34	0.50	1	
40	0.00	0.00	0.03	0.00	0.00	0.00	0.03	0.03	0.05	0.00	0.00	0.05	0.03	0.05	0.05	0.03	0.05	0.05	0.05	0.03	0.04	0.00	0.00	0.06	0.03	0.06	0.12	0.16	0.13	0.07	0.35	0.32	0.23	0.27	0.37	0.32	0.31	0.36	0.37	1

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