

Genetic polymorphism of 17Y-STR loci in central of Iraq Population

Ban Ameen¹, Mohammed Mahdi Al-Zubaidi², Majeed Arsheed Sabbah³, Mohammed I. Nader⁴, Dhuha Salim Namaa⁵, Hala K. Ibrahim⁶, Thooalnoon Younes Al-Janabi⁷, Asia Abdul Lateef Mahdi⁸, Reem Husam Al-Tabra⁹, Miriam Jasim Shehab¹⁰, Nadhum Hussien Safir¹¹, Haider K. AL-Rubai¹² and May Ridha Jaafar¹³

¹Forensic DNA Center Research and Training Al-Nahrain University, Baghdad, IRAQ.

²Forensic DNA Center Research and Training Al-Nahrain University, Baghdad, IRAQ.

³Forensic DNA Center Research and Training Al-Nahrain University, Baghdad, IRAQ.

⁴Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, Baghdad University, Baghdad, IRAQ.

⁵Forensic DNA Center Research and Training Al-Nahrain University, Baghdad, IRAQ.

⁶Forensic DNA Center Research and Training Al-Nahrain University, Baghdad, IRAQ.

⁷Forensic DNA Center Research and Training Al-Nahrain University, Baghdad, IRAQ.

⁸Forensic DNA Center Research and Training Al-Nahrain University, Baghdad, IRAQ.

⁹Forensic DNA Center Research and Training Al-Nahrain University, Baghdad, IRAQ.

¹⁰Forensic DNA Center Research and Training Al-Nahrain University, Baghdad, IRAQ.

¹¹Forensic DNA Center Research and Training Al-Nahrain University, Baghdad, IRAQ.

¹²Forensic DNA Center Research and Training Al-Nahrain University, Baghdad, IRAQ.

¹³Forensic DNA Center Research and Training Al-Nahrain University, Baghdad, IRAQ.

²Corresponding Author: molecular_fdna@yahoo.com

ABSTRACT

In this study, a sample of 178 central of Iraq population was analyzed using 17 Y- chromosome short tandem repeat (STR) polymorphisms. A total of 155 different haplotypes were identified, among which 135 were individual specific. The high haplotype diversity (0.994) supports the usefulness of Y-STR markers in central Iraq population diversity investigation. Our results suggest a close genetic relationship between Central of Iraq and other populations of the Arabian Peninsula, and an even more pronounced similarity of Kuwaiti populations and Yemenis and Saudi Arabians.

Keywords- Haplotype, Y chromosome, population structure, Arabian Peninsula

(Gill *et al.* ,1997; Olaisen *et al.* , 1998). That same year, the Federal Bureau of Investigation (FBI) announced a core of 13 autosomal STR loci required for the National DNA Index System (NDIS), a subset of the U.S. National Combined DNA Index System (CODIS). These markers were chosen to be highly informative, easily amplified using PCR, and on different chromosomes or opposite ends of the same chromosome so that they are almost genetically unlinked (Olaisen ,1998).

A DNA analysis of markers located on the Y chromosome is valuable in certain cases, particularly in sexual assault cases, in which evidence often contains a mixture of DNA from both a female victim and a male perpetrator. The forensic community has agreed on a core set of eight Y-STR (Y-chromosome short tandem repeat) markers, named the minimal haplotype DNA Y-chromosome(unique) segment (DYS) (DYS19, DYS389 I, DYS389 II, DYS390, DYS391, DYS392, DYS393 and DYS385ab) to use in routine Y-STR analysis (Kayser *et al.* , 1997).

The routine Y-STR analysis is based on multiplex amplification of the minimal haplotype loci as well as the loci recommended by Scientific Working Group on DNA Analysis Methods (SWGDM) (DYS439 and DYS439). The amplified fragments are then size separated using CE. (Ahmadian *et al.*, 2000; Nordstrom *et al.*, 2000).

Highly variable regions within the DNA termed Short Tandem Repeats (STRs) are widely used for characterizing population structure and estimating human genetic diversity (Silva *et al.*, 2012; Peter, 2016).

Such DNA-based data also provide leads in disease susceptibility studies, paternity and individual identification. Population genetic analyses utilizing such

I. INTRODUCTION

Humans' origin and migrations is an active topic in the field of anthropology and molecular genetics (Weidenreich ,1943; Cann *et al.* , 1987). Separate genetic profiles can be constructed using standard marker sets for the autosomal (chromosomes one through 22), Y-chromosome, and mitochondrial DNA. The types of genetic marker currently used in forensic genetic practice are length polymorphisms called short tandem repeats (STRs). STRs used forensically typically have a simple or more complex tetranucleotide repeat structure. The markers are highly polymorphic, with between 15 and more than 100 alleles observed at a locus, giving these markers, taken together, high power to discriminate between individuals (Butler, 2006).

In 1997, an effort was made to standardize the nomenclature for STR alleles, based on the number of repeated motifs or overall length polymorphism size

variable markers have identified bidirectional human migration through the Middle East, linking movement through Africa, Asia and Europe (Maca-Meyer *et al.*, 2003; Kundu and Ghosh, 2015).

The Y chromosome is less variable than the other chromosomes. Many markers are thus needed to obtain a high degree of discrimination between unrelated males (Hanson and Ballantyne, 2007; Kuppureddi *et al.*, 2010).

Research in DNA technologies has helped law enforcement agencies such as the police in the investigation of crimes such as murder, attempted murder, physical assault, and sexual assault. Sexual assault such as rape is one of the most violent crimes and is a serious problem which is faced by many countries (Kebareng, 2015).

The Y-chromosome haplotype is commonly constructed using Short Tandem Repeat (STR) markers. As the Y-chromosome is subject to rapid genetic drift, haplotypes can be used to study the geographical distribution of ethnic groups (Qamar *et al.*, 2002). The Y-chromosome contains the largest non-recombining section within the human genome, providing informative haplotypes for genetic analyses of populations (Underhill and Kivisild, 2007). The main source of information about Y-STR is the website YHRD.org. In practice, the frequency of the Y-STR profile in question can be estimated by referencing databases with large numbers of Y-STR profiles and counting the number of matching profiles within the population of interest. This provides an estimate of the profile probability for the Y-haplotype. One of these databases is the Y Chromosome Haplotype Reference Database (YHRD) (Willuweit and Roewer, 2013). Following the guidelines of the International Society of Forensic Genetics (ISFG) for the publication of genetic population data, submissions to YHRD are required to consist of at least 17 Y-STR markers (including the eight-marker minimal Y-haplotype: DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS385) for 200 or more individuals from a population. As of 2015, YHRD contained more than 84,000 Y-profiles of 17 markers or greater in 572 populations.

It contains a description of the Y-STR loci used in forensic analysis, mutation rates, and the biggest global database available (more than 194,000 sampled individuals to this date). Moreover, one of the *f*-estimating methods is implemented on the website, and its estimates are freely available. The only drawback is that, since many researchers publish anonymously on the website and do not want their data to be globally known, the haplotypes in the database are not accessible. For a haplotype H, an expert can only access the number of occurrences of H in the database, by subpopulation, and the *f*-estimate mentioned earlier (Willuweit and Roewer, 2013).

II. MATERIALS AND METHODS

Collection samples

In this study, the buccal swab was collected from unrelated individual living in central of Iraq, 178 samples collection from central of Iraq Population. All samples after collected left to dry for 30 minutes and then after to dry put inside a letter envelope until they are extraction. All samples were DNA extraction in laboratory Al-Nahrain University in forensic DNA center research and training.

Genomic DNA extraction methods

DNA was extracted from buccal samples using organic extraction method (manual method); extraction done according to the manufacturer protocol, this method depended in forensic DNA laboratory Organic extraction method (Goodwin, 2016).

Estimate quantification of DNA

The quantity of DNA was determined according to methods described by quantifier Y Human male quantification kit (Robert *et al.*, 2005; Maura *et al.*, 2009).

Amplification Y chromosome STR by using AmpFSTR^R Y Filer kit.

Commercial kit AmpFSTR^R Y Filer PCR amplification kit (applied bio systems)

Allele frequency

The allele frequency of the multicopy locus DYS385a/b was analyzed as combination of both alleles (Butler, 2003).

Allele frequencies for Y- Chromosomal STR were calculated by direct counting therefore:

$$\text{Allele frequencies} = \frac{\text{Total no. of alleles}}{\text{Total no. of samples}}$$

Haplotype frequency

Haplotype frequency it means of each haplotype of the sample found with any sample size. Haplotype Frequency was calculated by using the Excel program.

Gene diversity (GD)

Gene diversity (GD) was calculated for each Y-STR according to the formula supplied by (Nei, 1987; Gusmao *et al.*, 2006):

$$HD = \frac{N}{N-1} (1 - \sum x^2)$$

Where *n* is the sample size and *x* the relative allele frequency. Gene diversity among populations occurs if there are differences in allele frequencies between those populations

Haplotype diversity (HD)

Haplotype Diversity (HD) was calculated using the same equation as calculating gene diversity using haplotype frequencies instead of allele frequencies (Gusmao *et al.*, 2006).

Discrimination Capacity (DC)

Discrimination Capacity (DC) was calculated using the following formula (Gusmao *et al.*, 2006)

Y-STR Haplotype Reference Database (YHRD)

The largest and most widely used forensic and general population genetics Y-STR database, known as the Y-STR Haplotype Reference Database (YHRD), was created by Willuweit and Roewer (2007).

III. RESULTS AND DISCUSSIONS

Samples collected for the purpose to we will work were collected (Y-STR) of the provinces of Iraq of Arab Individuals lives in included the central governorates of Iraq (Baghdad).

Allele, Genotype frequencies and Gene Diversity for Central of Iraq males (n=178)

Allele frequency of 178 central of Iraq Arab males individuals that scored for the 17 Y-STR chromosome STRs are shown in (Table 1) The most polymorphic loci were DYS385a/b with 38 total numbers of alleles. The GD value for DYS385 a/b among the **central of Iraq males** was 0.949. These loci have such high diversity because it is a multi-copy locus.

This was the highest variation of genes observed anywhere between all four subgroups investigated.

The highest GD value for a single-copy locus was 0.801 (DYS458). The lowest GD value was 0.373 (DYS392).

DYS38911, DYS458, DYS635, and DYS448 have 7 numbers of alleles respectively (Table 1) with genetic diversity of 0.715, 0.801, 0.688 and 0.586 respectively.

Loci which exhibited lower polymorphisms were DYS3891, DYS19, DYS391, DYS437 and DYS438 which have a total number of 4 alleles with genetic diversity 0.535, 0.531, 0.548, 0.448 and 0.584 respectively. DYS456, DYS393, DYS392 and R-Y-GATA-H4 have 6 total numbers of alleles with 0.676, 0.576, 0.373 and 0.623 genetic diversity.

Comparison of Gene Diversity in Iraq Arab male population with other populations (Filiz *et al.*, 2013; Jasem, 2013; Imad, 2014; Share, 2014; Serkan *et al.*, 2017; Tareq, 2017) showed that Iraq Arab male population share most of its predominant GD with Babylon in loci DYS392 (0.229), Saudi Arabia (0.354) and Iraq –Yazidis (0.325), While the highest genetic diversity in loci DYS385a/b (0.96) from Kuwaiti table (2).) Previous studies on the subject of a STR in Iraq included (AL-Zubaidi and Majeed, 2017; Al-Awadi *et al.*, 2014; Haider *et al.*, 2015; Thooalnoon *et al.*, 2016; Saja *et al.*, 2016 ; Majeed *et al.*, 2017).

Table (1): Allele, Genotype frequencies and Gene Diversity for central of Iraq males (n=178)

Allele	DYS 456	DYS 3891	DYS 390	DYS 3891 1	DYS 458	DYS 19	DYS 393	DYS 391	DYS 439	DYS 635	DYS 392	R-Y-GAT A-H4	DYS 437	DYS 438	DYS 448	Genotype	DYS3 85ab
6																11,11	0.011
7																11,14	0.034
8							0.006									11,15	0.028
9								0.062				0.006		0.247		11,16	0.011
10								0.612	0.051		0.034	0.084		0.584		11,18	0.006
11							0.028	0.270	0.466		0.787	0.522		0.118		12,12	0.022
12		0.180					0.567	0.056	0.365		0.039	0.309		0.051		12,13	0.006
13	0.045	0.640				0.056	0.315		0.101		0.051	0.067				12,14	0.028
14	0.264	0.157			0.017	0.635	0.073		0.017		0.067	0.011	0.708			12,15	0.039
15	0.478	0.022			0.140	0.247	0.011				0.022		0.219			12,16	0.011
16	0.157				0.202	0.062							0.067			12,17	0.006
17	0.051				0.174								0.006		0.006	12,18	0.017
18	0.006				0.309										0.017	12,19	0.017
19					0.124					0.067					0.213	12,20	0.017
19,20															0.006	13,13	0.011
20					0.034					0.494					0.590	13,14	0.028
21			0.006							0.213					0.152	13,15	0.045
22			0.129							0.129					0.017	13,16	0.034
23			0.506							0.067					1.000	13,17	0.062

24			0.225							0.022						13,18	0.163
25			0.135							0.006						13,19	0.090
26																13,20	0.028
27				0.022												14,14	0.011
28				0.079												14,15	0.028
29				0.348												14,16	0.051
30				0.382												14,17	0.011
31				0.112												14,18	0.011
32				0.051												14,19	0.006
33																15,15	0.006
34				0.006												15,16	0.006
																18,19	0.028
sum Freq	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000		1.0000
N	178	178	178	178	178	178	178	178	178	178	178	178	178	178	178		178
NA	6	4	5	7	7	4	6	4	5	7	6	6	4	4	7	38	
GD	0.677	0.535	0.663	0.715	0.801	0.532	0.576	0.548	0.640	0.688	0.37	0.623	0.449	0.584	0.586		0.949

*N=number of samples; NA= number of Alleles of each Loci; GD = gene Diversity

Table (2): Gene diversity central of Iraq comparison with other countries

	South of Iraq ^a	Baghda d ^b	Al-Anbar ^c	Diyala ^d	Iraq ^e	Iraq – Kurd ^f	Iraq - Yazidis ^g	Sudia - arbia ^h	Kuwaiti ⁱ	Emirate ^{s,j}	Turkey ^k
Allele	GD	GD	GD	G.D	GD	GD	GD	GD	GD	GD	GD
DYS456	0.692	0.677	0.653	0.635	—	0.637	0.525	0.664	0.698	0.678	0.520
DYS389 1	0.469	0.535	0.526	0.482	0.660	0.596	0.616	0.566	0.516	0.525	0.560
DYS390	0.598	0.663	0.572	0.647	0.657	0.708	0.732	0.663	0.533	0.707	0.700
DYS389 11	0.675	0.715	0.623	0.603	0.714	0.728	0.726	0.726	0.699	0.684	0.710
DYS458	0.856	0.801	0.794	0.830	—	0.827	0.816	0.829	0.783	0.837	0.840
DYS19	0.572	0.532	0.472	0.603	0.665	0.634	0.721	0.600	0.593	0.643	0.640
DYS393	0.575	0.576	0.519	0.639	0.514	0.585	0.634	0.651	0.638	0.641	0.580
DYS391	0.534	0.548	0.606	0.588	0.580	0.493	0.464	0.493	0.574	0.455	0.550
DYS439	0.575	0.640	0.612	0.560	0.299	0.675	0.738	0.604	0.637	0.678	0.650
DYS635	0.668	0.688	0.715	0.693	—	0.764	0.770	0.678	0.717	0.707	0.700
DYS392	0.373	0.373	0.230	0.253	0.269	0.514	0.325	0.354	0.477	0.402	0.450
R-Y-GATA-H4	0.542	0.623	0.543	0.578	—	0.658	0.667	0.481	0.559	0.590	0.720
DYS437	0.497	0.449	0.347	0.567	—	0.547	0.526	0.353	0.409	0.437	0.440
DYS438	0.553	0.584	0.595	0.593	0.526	0.698	0.660	0.508	0.611	0.643	0.700
DYS448	0.517	0.586	0.445	0.631	—	0.659	0.525	0.627	0.577	0.625	0.670
DYS385 a/b	0.939	0.949	0.898	0.933	0.839/0.65	0.958	0.952	0.921	0.957	0.949	0.950

Y-STR-haplotype and haplotype frequency

In this study 178 haplotypes each with 17 alleles were analyzed by used excel. analysis measures the Haplotype and Haplotype frequency The observed number of Haplotype and Haplotype frequency of any

individual have been tabulated in Table (4). We identified 178 different haplotype in our study sample. 155 (135, 87.1%) were unique Haplotype , 20 were (12.9%) were replicated haplotype among more individuals.

The highest haplotype were in sample 3 at frequency 0.0168 (haplotype replicated 3 times), while the haplotype number were replicated 2 times(frequency of haplotype 0.0112).

Haplotype diversity and Discrimination Capacity
DC of the 17 Y-STR Yfiler 0.870968 while HD was (0.998) in 17 Y-STR table (3)

Table (3): Discrimination Capacity and Haplotype diversity

	Y-STR haplotype
sample size	178
Number of haplotype	155
Unique haplotype	135
Repeating haplotype	20
HD	0.99835
Discrimination capacity	0.870968

Table (4): haplotype frequency in central of Iraq

ID	DYS 456	DYS 389I	DYS 390	DYS 389I	DYS 458	DYS 19	DYS38 5ab	DYS 393	DYS 391	DYS 439	DYS 635	DYS 392	GA TA H4	DYS 437	DYS 438	DYS44 8	Frequency
MB42	13	13	23	31	18	14	11,11	13	10	12	23	11	10	15	10	20	0.011236
MB194	15	14	25	31	15	15	11,14	13	11	10	23	11	12	14	11	20	0.005618
MB201	15	14	25	32	15	15	11,14	13	11	11	23	11	12	14	11	20	0.005618
MB30	16	13	24	28	17	14	11,14	12	11	12	23	15	11	15	12	19	0.005618
MB83	16	13	25	30	16	15	11,14	13	10	10	23	11	13	14	11	20	0.005618
MB190	16	13	23	28	15	14	11,14	12	11	12	23	13	12	15	12	19	0.005618
MB132	16	14	24	31	15	15	11,14	13	11	10	23	11	12	14	11	20	0.005618
MB68	15	13	24	28	18	14	11,15	12	11	12	23	14	12	15	12	19	0.011236
MB134	15	13	24	29	15	15	11,15	13	11	10	23	11	12	14	11	20	0.005618
MB91+	15	13	25	30	16	15	11,15	13	10	10	23	11	13	14	11	20	0.005618
MB170	16	13	24	31	15	16	11,15	13	11	10	23	11	12	15	11	21	0.005618
MB131	14	14	24	31	19	14	11,16	12	9	14	21	12	12	14	10	18	0.005618
MB146	15	12	24	29	18	14	11,16	12	10	11	20	11	11	14	10	21	0.005618
MB72	15	13	23	31	17	14	11,18	12	11	11	21	11	11	14	10	20	0.005618
MB135	15	14	24	31	18	15	12,12	13	10	12	21	11	12	15	10	20	0.005618
MB35	15	15	24	32	18	15	12,12	13	10	12	21	11	12	15	10	20	0.016854
MB121	15	13	24	29	15	16	12,13	13	10	11	23	13	12	14	11	19	0.005618
MB50	15	13	24	29	17	14	12,14	12	11	13	23	13	12	14	12	19	0.005618
MB78	15	14	24	29	17	14	12,14	13	10	13	23	15	12	15	12	19	0.005618
MB172	15	12	22	29	18	14	12,14	14	10	12	22	11	11	16	10	22	0.005618
MB199	16	12	23	27	16	15	12,14	15	10	11	22	11	13	17	10	21	0.005618
MB197	17	12	23	28	17	15	12,14	13	10	11	22	11	11	15	10	20	0.005618
MB167	15	12	22	28	17	16	12,15	14	11	11	21	11	12	15	10	21	0.005618
MB189	15	12	22	28	17	16	12,15	14	11	11	21	11	12	14	10	21	0.005618
MB140	15	12	22	29	15	14	12,15	11	10	12	26	14	11	15	11	19	0.005618
MB169	16	13	22	29	17	14	12,15	12	10	11	22	11	11	14	9	22	0.005618
MB102	16	13	22	30	16	14	12,15	12	10	11	21	11	11	14	9	22	0.005618
MB122	16	13	24	30	17	14	12,15	12	10	11	22	11	11	14	9	20	0.011236
MB23	15	13	23	29	16	14	12,16	12	10	11	23	11	10	15	9	21	0.011236

MB5	16	12	22	28	15	14	12,17	11	10	14	24	14	11	15	10	19	0.005618
MB75	13	13	22	29	18	14	12,18	12	10	11	21	11	11	14	11	20	0.005618
MB84	14	13	24	29	19	14	12,18	12	11	11	21	11	11	14	10	20	0.005618
MB103	15	12	25	29	18	13	12,18	12	12	11	22	11	10	14	10	20	0.005618
MB40	15	13	22	28	19	15	12,19	13	10	13	21	11	10	14	10	20	0.005618
MB44	14	13	23	30	19	14	12,19	12	11	12	21	11	11	14	10	20	0.011236
MB13	15	13	23	29	15	14	12,20	12	10	11	21	11	13	15	9	19	0.005618
MB70	15	13	23	30	15	14	12,20	12	9	12	21	11	13	15	9	19	0.011236
MB93+	14	13	24	30	16	14	13,13	12	10	11	24	11	12	16	9	21	0.005618
MB178	15	13	22	29	16	15	13,13	14	10	11	21	11	12	16	9	21	0.005618
MB52	15	12	24	27	16	14	13,14	13	10	11	22	14	11	14	11	19	0.011236
MB151	15	12	23	30	20	15	13,14	13	10	13	22	12	11	16	10	20	0.005618
MB183	15	14	25	30	17	16	13,14	14	10	12	23	13	13	14	12	19	0.005618
MB115	16	13	24	29	18	15	13,14	12	10	11	21	11	11	15	9	21	0.005618
MB108	15	12	25	29	18	15	13,15	12	10	11	21	11	11	14	9	19	0.005618
MB133	15	12	22	30	18	15	13,15	13	10	11	22	11	12	15	10	21	0.005618
MB200	15	12	25	30	18	15	13,15	12	10	11	20	11	11	14	9	19	0.005618
MB6	15	13	25	30	18	15	13,15	12	10	11	21	11	11	14	9	19	0.005618
MB96	15	13	25	30	19	15	13,15	12	10	11	21	11	11	14	9	19	0.005618
MB198	15	13	25	30	18	15	13,15	12	10	11	21	11	11	14	9	20	0.005618
MB3	15	14	23	34	17	14	13,15	12	10	11	22	11	10	15	9	20	0.005618
MB2	16	13	25	30	18	15	13,15	12	10	11	21	11	11	14	9	19	0.005618
MB100	14	14	24	30	16	14	13,16	12	10	12	22	11	11	14	9	19	0.005618
MB104	15	13	23	27	15	14	13,16	12	10	10	24	11	10	15	9	20	0.005618
MB61	15	13	23	29	15	15	13,16	12	11	11	22	11	12	14	9	20	0.005618
MB69	15	13	23	29	16	14	13,16	12	10	12	25	11	11	15	9	21	0.005618
MB168	15	13	23	29	15	15	13,16	12	10	12	21	11	13	14	9	21	0.005618
MB97	17	13	23	29	15	15	13,16	12	9	12	22	11	12	14	9	21	0.005618
MB98	13	13	24	29	17	15	13,17	12	10	12	21	11	10	15	10	18	0.005618
MB145	13	13	24	29	18	15	13,17	12	10	12	21	11	10	15	9	18	0.005618
MB176	13	13	23	29	18	14	13,17	12	10	12	21	11	11	14	10	20	0.005618
MB111	14	14	23	30	16	13	13,17	12	11	11	21	11	11	14	10	20	0.005618
MB117	14	13	23	30	17	14	13,17	12	11	11	21	12	11	14	10	20	0.005618
MB166	15	13	23	30	18	14	13,17	12	10	11	21	11	11	14	10	20	0.005618
MB193	15	13	23	30	16	14	13,17	12	10	14	23	11	12	15	9	21	0.005618
MB81	16	12	22	28	15	14	13,17	11	10	13	24	14	12	14	10	19	0.005618
MB12	17	13	23	29	15	15	13,17	12	9	12	22	11	12	14	9	21	0.011236
MB85	17	13	24	29	14	15	13,17	12	9	12	22	11	12	14	9	21	0.005618
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MB179	14	13	23	30	18	14	13,18	12	10	11	21	11	11	14	10	20	0.005618

MB33	14	13	23	30	19	14	13,18	12	10	11	21	12	11	14	10	20	0.011236
MB101	14	13	23	30	19	14	13,18	12	11	13	21	11	11	14	10	20	0.005618
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MB28+	14	12	22	29	16	16	16,16	14	10	12	20	10	12	16	10	20	0.005618
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MB159	15	13	22	29	16	15	17,19	12	10	13	21	11	12	16	9	21	0.005618
MB156	15	13	25	30	16	14	18,19	13	10	13	20	11	12	14	10	20	0.016854
MB17	15	14	24	32	16	14	18,19	13	10	12	20	11	12	14	10	20	0.005618
MB142	15	14	24	31	16	15	18,19	13	10	12	20	11	12	14	10	20	0.005618

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