

Comparative analysis of human chromosomal DNA digestion with restriction endonucleases in vitro and in silico

Category: [Study of Genome DNA](#)

Hits: 33722

[Abdurashitov M.A.](#), Tomilov V.N., Chernukhin V.A., Gonchar D. A., Degtyarev S. Kh.

Translated from "Medical genetics" V.6, No 8, pp 29-36, 2007

Theoretical analysis of human chromosomal DNA cleavage at 15 nucleotide sequences, which are the recognition sites of various restriction endonucleases, has been carried out. Distribution diagrams of calculated DNA fragments have been constructed based on earlier proposed method of mammalian genomes digestion in silico. A similar study of human Alu- and LINE1-repeats nucleotide sequences, which are present in informational databases, has been performed and corresponding diagrams of DNA fragments distribution have been plotted. Distribution diagrams of chromosomal DNA digestion, which results in formation of low molecular weight DNA fragments, correspond to those for Alu-repeats; whereas the digestion, which results in formation of large molecular weight DNA fragments - are similar to those for LINE-repeats. All theoretical data have been compared to experimental patterns of human genomic DNA cleavages with respective restriction endonucleases and a good correspondence for the most of DNA diagrams has been observed.

INTRODUCTION

With the completion of the "Human Genome Project" in 2002, more than 96% of the euchromatic part of human genome was determined [1]. Human DNA primary structure data are still being updated and corrected, and the portion of the known and verified sequence is growing. Based on the known DNA sequences of human and some other mammalian genomes we have proposed a simple method to perform restriction enzymes analysis *in silico* [2]. This method allows calculating of the distribution diagrams for DNA fragments, produced by cleavage of the euchromatic part of chromosomal DNA at short nucleotide sequences, which are the restriction enzymes recognition sites. Comparison of such diagrams with experimentally observed patterns of rat, mouse and human genomic DNAs cleavage with several REs has shown correspondence of *in vitro* and *in silico* restriction patterns. In this work we studied human genomic DNA cleavage by a wide spectrum of REs.

The goal of our work was to i) plot DNA fragments distribution diagrams for human genomic DNA cleavage at 15 recognition sites, including 4-, 5- and 6-nucleotides sequences, ii) develop a method of

analysis of nucleotide sequences of human Alu- and LINE1-repeats from corresponding databases, iii) construct distribution diagrams for Alu- and LINE1-repeats digestion at selected 15 recognition sites, iv) carry out human chromosomal DNA digestion with corresponding restriction endonucleases and v) compare theoretical and experimental data on human DNA cleavage.

MATERIALS AND METHODS

Isolation of genomic DNA from human blood cells. DNA was isolated according to [3] with some modifications as indicated below. Each donor blood sample was placed in a tube and immediately mixed with 6% EDTA solution in ratio 9 to 1. Five ml of solution containing 0.9% NaCl and 3% gelatin was added to 5 ml of mixture in each tube and mixed by gently inverting the tube several times. The tube was then left at 37 °C for 30 min. Leukocyte-rich supernatant fraction (top layer) was collected and centrifuged (30 min at 1000 g and 20 °C). The supernatant was discarded and the cell precipitate was thoroughly resuspended, by inverting the tube, in 2 ml of washing buffer (0.1 M Tris-HCl, pH 7.5; 0.1 M NaCl; 30 mM MgCl₂). The cell suspension was again centrifuged for 10 min, at 7200 g and 20 °C, and the supernatant was removed by gentle aspiration.

The obtained pellet of washed cells was carefully resuspended in 2 ml of lysis buffer (Tris-HCl 50 mM, pH 8.0; 10 mM EDTA; 0.1 M NaCl; 0.5% SDS; 0.01 mg/ml proteinase K; 0.01 mg/ml RNase A) and then incubated at 37 °C overnight (18 h).

4 M NaCl was added to the suspension to a final concentration of 0.1 M. Further, 2 ml of phenol (saturated with 10mM Tris HCl pH 8.0) was added. The final mixture was incubated for 10 min at room temperature, with constant agitation, and then centrifuged at 11000 g for 2 min at 20°C. The upper aqueous phase was transferred into a cone flask with a pipette using a scissored tip. Total of 2 ml of solution, composed of equal volumes of phenol (saturated with 10mM Tris HCl, pH 8.0) and chloroform/isoamyl alcohol (in proportion 24:1), was then added. Extraction and centrifugation was then performed in the way previously described, after which, 2 ml of chloroform/isoamyl alcohol (24:1) was added to the aqueous phase. The procedure was followed by further extraction and centrifugation in the same way.

Then aqueous phase was placed into 50 ml glass and 4 M NaCl was added to a final concentration of 0.1 M, the mixture was then cooled on ice. Isopropanol, cooled to 0°C, was added dropwise with slow stirring. DNA precipitate was spooled on a glass rod, washed in 70% ethanol, and gently dried with sterile air (3-6 min). DNA precipitate was left to dissolve in 1-1.5 ml of sterile TE buffer (10mM Tris HCl pH 7.0; 1mM EDTA) for 24 hours at 4°C.

Hydrolysis of chromosomal DNA. The following restriction endonucleases from SibEnzyme Ltd., were used (recognition sites are given in brackets):

1. AluI (5'- AGCT-3 '), 2. AsuHPI (5'-GGTGA-3' and 5'-TCACC-3 '), 3. Bpu10I (5'-CCTNAGC-3' and 5'-GCTNAGG-3'), 4. BstDEI (5'-CTNAG-3 '), 5. Bst2UI (5'-CCWGG-3'), 6. BstSCI (5'-CCNGG-3 '), 7. BstMAI (5'-GTCTC-3' and 5'-GAGAC-3'), 8. HinfI (5'-GANTC-3 '), 9. BssECI (5'-CCNNGG-3'), 10. FauNDI (5'-CATATG-3 '), 11. XbaI (5'-TCTAGA-3'), 12. MroXI (5'-GAANNNTTC-3 '), 13. KpnI (5'-GGTACC-3'), 14. Msp20I (5'-TGGCCA-3 '), 15. AspA2I (5'-CCTAGG-3').

Hydrolysis reactions were performed for 3 hours, at optimal temperature, in 40 µl of the reaction mixture

containing 6 µg of DNA, SE-buffers, as recommended by the manufacturer, and 3 µl of restriction enzyme.

Before hydrolysis reaction, all DNA preparations were treated with RNase A (0.1 mg/ml) for 10 min, at room temperature, and then dialyzed in the DispoDialyzer MWCO 50,000 tubes (Sigma, USA) against TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA), 100 times the volume of a single DNA preparation, at 4 °C for 20 hours.

Electrophoresis. Electrophoresis in 8% polyacrylamide gel was used to separate DNA fragments with lengths of 40 to 500 bp (6 µg of hydrolyzed DNA was loaded on gel in each run); 1.5% of low melting point agarose ("Sigma", USA) was used to separate DNA fragments in the range of 200-2000 bp. Electrophoresis in 1% agarose "Type I-A, Low EEO" ("Sigma", USA) was used to separate DNA fragments of higher molecular weight. Three µg of hydrolyzed DNA was loaded on agarose gel in each run. Tris-acetate buffer was used for electrophoresis in all cases. After electrophoresis DNA bands were stained with ethidium bromide and photographed in UV light.

Nucleotide sequences of DNA repeats. Human DNA sequence was obtained from the the following resource: <ftp://ftp.ensembl.org/pub/> (Updated June 2, 2006). Sequences of Alu- and LINE1-repeats were obtained from [the human genome nucleotide sequences database](#) (version of March 2006), using Table Browser service on the site. Alu-repeats consisted of 1193407 sequences with total length of ~ 350 million bp. Selection of LINE1-repeats included 927,393 sequences with total length of ~ 510 million bp.

Consensus sequences of Alu-repeat subfamilies were downloaded from [the database Repbase Update](#) [4].

Construction of theoretical diagrams of DNA fragments distribution. Theoretical diagrams of the relationship of total fragment mass to their length (in bp) were constructed by the method described in [2]. Vertical distribution diagrams were plotted as previously described [5].

The analysis of repetitive sequences families and the construction of fragments distribution diagrams were carried out based on the method described earlier [2] with some modifications. A search for the respective restriction endonuclease recognition site was performed in each repetitive sequence and lengths of the generated DNA fragments were determined. End fragments on both sides of the sequence were not considered. Then we calculated the total number of DNA fragments with a given length in the range of 1-6000 bp, as well as the total bp number for all fragments of this length within the selection. The calculations were carried out using standard AMD 64 class personal computer.

RESULTS AND DISCUSSION

Comparison of the experimental and calculated data for chromosomal DNA digestions.

Previously, we have constructed diagrams of chromosomal DNA fragments distribution after cleavage at the recognition sites of the following restriction enzymes: HaeIII (recognition site GGCC), MspI (CCGG), Kzo9I (GATC) and Bst2UI (CCWGG). A good correlation has been observed between the calculated data and the actual experimental results for rat, mouse and human DNA hydrolysis with these enzymes [2]. In the present work we have constructed diagrams of human chromosomal DNA fragments distribution after DNA cleavage with a wider range of recognition sites, including 6-nucleotide sequences. According to the method proposed earlier [5], the distribution diagrams were plotted using arctangentoidal scale, which simulated DNA fragments distribution in agarose gel electrophoresis. The

diagrams were selected based on the presence of peaks of 5.5 and more million base pairs, which is a threshold value for visualization of respective bands in the analysis of DNA cleavage products by electrophoresis in agarose gel [5]. Figures 1 and 6 show selected diagrams of DNA cleavage for nine 4-5 nucleotides sequences, which are recognition sites of restriction enzymes AluI, AsuHPI, Bst2UI, BstSCI, BstDEI, BssECI, BstMAI and HinfI. Additionally, Fig. 1 displays a diagram for 6 bp recognition site of restriction enzyme Bpu10I, due to a presence of one 5.5 million bp peak. Fig. 1 also presents experimental results on human DNA hydrolysis with corresponding restriction endonucleases.

Fig. 1. Comparison of electrophoregrams (8% PAAG) with calculated distribution diagrams, visualized DNA fragments at peak values, which can be on electrophoregrams, are shown. "s" - fragments, which are probably result of satellite DNA cleavage. M - DNA fragment lengths marker pUC19/Mspl. The lengths of DNA fragments of this marker are shown at left in the bottom row.

Comparing the diagrams we can conclude that DNA fragment 50 bp, which is generated by human DNA digestion at recognition site of BssECI, gives the highest peak. A corresponding band can be clearly seen in the gel photograph, along with fragments of 156-159 bp and 207-208 bp, which are represented by smaller peaks in the diagram. Fragments of 49-50 bp, 67-68 bp, 84-86 bp, 115-119 bp and 165-168 bp, which are produced by DNA hydrolysis with restriction enzyme Bst2UI, are also visible and were discussed earlier [2]. BstSCI restriction enzyme, which has a similar recognition site, shows the same pattern of hydrolysis products distribution. However, it should be noted, that there is a shift of DNA bands for BstSCI products, compared with the data on DNA cleavage with Bst2UI. Abnormal mobility of BstSCI DNA fragments in polyacrylamide gel, was confirmed separately (data not shown), and is probably due to long 5'-protruded ends. Analysis of the remaining diagrams revealed single peaks with lengths of 79 bp, 157-159 bp and 135-136 bp, which are visible on the gel photographs for DNA hydrolysis with HinfI, AsuHPI and Bpu10I restriction enzymes, respectively. The DNA hydrolysis with BstDEI results in the appearance of bands, which correspond to 48 bp and 116-118 bp fragments. The picture of the DNA hydrolysis with HinfI restriction enzyme shows fragments of 171 and 342 bp, which are products of α -satellite DNA cleavage [6]. Satellite DNA fragment, 342 bp in length, is also seen after DNA splitting with restriction enzyme AsuHPI, while the 171 bp fragment is seen after hydrolysis with restriction enzyme BstDEI. Clear and distinctive pattern is observed after DNA cleavage with restriction endonuclease BstMAI. In this case we can also see correlation between experimental

data and the theoretical diagram, which provides a set of fragments with lengths of 158-159 bp, 166-167 bp, 189-190 bp and 197 bp. Theoretical DNA cleavage at the AluI recognition site AGCT produces three peaks with the lengths of 32, 49 and 60 bp and corresponding bands are also visible on the gel picture of the actual DNA hydrolysis with this enzyme.

Thus, a comparison of gel photographs and plotted diagrams reveals a good correspondence between the experimental and theoretical data for DNA cleavage.

Fig. 2. Comparison of electrophoregrams (1.5%) with calculated distribution diagrams. Lengths of fragments with peak values, which can be determined at electrophoregrams are shown. "sat" - fragments, which are probably result of satellite DNA cleavage. M - DNA fragment lengths marker SE 1 kb DNA Ladder. The lengths of fragments of this marker are shown at left in the bottom row.

Figures 2 and 7 present DNA hydrolysis patterns and corresponding diagrams for DNA cleavage with restriction endonucleases AspA2I, FauNDI, KpnI, Msp20I, MroXI and XbaI. Gel photographs demonstrate clearly visible bands after DNA cleavage with the above mentioned enzymes. Meanwhile, as seen in Figures 2 and 4, DNA fragment peaks, which correspond to these bands, are indeed present in the diagrams, although the value of these peaks does not exceed 4 million bp. In particular, these photographs show bands for fragments with the following lengths: 636 bp (AspA2I enzyme hydrolysis), 852 bp and 420 bp (Msp20I enzyme hydrolysis), bp 1205, 1561 bp and 1791 bp, (KpnI enzyme hydrolysis), 1153 bp (FauNDI enzyme hydrolysis), 1368 bp (XbaI enzyme hydrolysis) and 1301 bp (MroXI enzyme hydrolysis).

Fig. 3. Comparison of the distribution diagrams calculated for whole genomic DNA cleavage and Alu-repeats set cleavage at recognition sites of AluI (AGCT) and Bst2UI (CCWGG). The abscissa shows the DNA fragment lengths (bp) whereas ordinate shows total number of nucleotides for all fragments of this size.

Previously [5], we have discussed the influence of two factors on the visualization of the DNA fragments in experiment. The first factor is the existence of DNA fragments cluster of similar lengths, which differ in size by more than 2 nucleotides. This difference results in an overlap of the corresponding bands in the gel photographs. The overlap may allow the visualisation of even smaller peaks. This could be the reason for the 636 bp fragment appearance in AspA2I restriction enzyme hydrolysis, since the analysis of the diagram data shows that the sum total of all 631-638 bp DNA fragments exceeds 7 million bp. For KpnI the sum total of obtained DNA fragments 1559-1569 bp and 1199-1214 bp exceeds 5.5 million bp in each case. The 1800 bp band appearance can be attributed primarily to the satellite DNA hydrolysis, rather than the presence of the 1791 bp fragment, since the sum total is not significant enough for this fragment visualization. The same overlap effect of the adjacent bands may explain the visualization of the fragments with following lengths: 1) fragments 852 bp (total length for nearby fragments is approximately 9 million bp) and 420 bp (~ 5.7 million bp) in the hydrolysis Msp20I restriction endonuclease. 2) 1153 bp (~ 7.5 million bp) in the hydrolysis FauNDI restriction endonuclease. 3) 1368 bp (~ 8.5 million) in the hydrolysis of XbaI restriction endonuclease. 4) 1301 bp (~ 9.8 million bp) in the hydrolysis of restriction endonuclease MroXI.

Thus, observable experimental band may correspond to an individual fragment of a certain length (such as 50 bp fragment produced by the hydrolysis of DNA with BssECI restriction enzyme), or to a cluster of fragments with similar length. Formation of these clusters is common in the case of human DNA fragments visualization in agarose gels. A similar phenomenon was previously described for rat genomic DNA cleavage [5].

Another factor, which makes it difficult to visualize DNA fragments in the experiment, is the presence of the DNA spot in the experimental photographs. This spot corresponds to the basic curve of the theoretical diagrams, and is produced by cleavage of nonrepetative DNA [5]. Such basic curve can be seen on the diagram of DNA cleavage at CTNAG site, and the corresponding spot is observed on the gel photograph of DNA hydrolysis with BstDEI. The DNA spot conceals the 342 bp fragment, produced by satellite DNA hydrolysis, whereas the fragment is clearly visible in the gel photographs of the DNA hydrolysis with restriction enzymes AsuHPI and HinfI (Fig. 1).

Fig. 4. Comparison of the distribution diagrams calculated for whole genomic DNA cleavage and LINE1-repeats set cleavage at recognition sites of KpnI (GGTACC) and Msp20I (TGGCCA). The abscissa shows the DNA fragment lengths (bp) whereas ordinate shows total number of nucleotides for all fragments of this size.

Patterns of DNA hydrolysis by restriction endonucleases MroXI and XbaI (except for 1301 and 1368 bp fragments, discussed above) do not correspond to DNA cleavage diagrams. This is probably due to the specifics of the α -satellite DNA hydrolysis with these enzymes. Previously, it has been shown [7] that aside from the typical bands of 171 bp (monomer) and 342 bp (dimer), the satellite DNA cleavage produces fragments even greater than 2000 bp in length. This can be explained by the presence of oligomeric α -satellite repeats, which vary in primary structure, and has been described for DNA hydrolysis with restriction enzyme XbaI [7]. In our experiments we observed such phenomenon in DNA cleavage with restriction endonucleases KpnI, MroXI and XbaI. However, the absence of nucleotide sequences of multisatellite DNAs in the informational databases did not allow us to confirm the obtained experimental data by the calculations.

Analysis of Alu-family and LINE1-repeats in genomic DNA The presence of peaks in the DNA fragments distribution diagrams and corresponding bands in the gel-electrophoresis is probably associated with the existence of the so-called repeats (repetitive elements) in eukaryotic DNAs. Two groups of DNA repeats, which are most frequently found in the human genome are: the Alu-repeats [8, 9] and the repetition family LINE1 (L1-repeats, [10, 11]). [The database of human genome nucleotide sequences](#) provides two corresponding sets of sequences. Based on the number of copies of DNA repeats in the human genome [1] it can be assumed that the presence of a peak in distribution diagrams is associated either with Alu or LINE1-repeats. Previously, we have shown that distribution diagrams of rat genomic DNA cleavage at restriction endonuclease recognition sites correspond very well to digestion patterns of consensus DNA sequence of rat LINE1 repeat [5]. There is no such consensus DNA sequence of human LINE1 repeats in the informational databases. Because of this, we have carried out an analysis of LINE1 repeats primary structure of more than 900,000 DNA sequences from [database](#), as described in "Materials and Methods". Based on this analysis, we constructed diagrams of the LINE1 fragments distribution, after cleavage at restriction endonuclease recognition sequences. Similar diagrams were constructed for Alu-repeats. Figure 3 displays DNA fragments

distribution diagrams for Alu-repeats cleavage at sites AGCT (AluI) and CCWGG (Bst2UI), while Figure 4 shows LINE1-repeats digestion at sites GGTACC (KpnI) and TGGCCA (Msp20I).

Fig. 5. AluI sites (AGCT) distribution among Alu repeats subfamilies.

Bottom characters "*" indicate common nucleotides for all sequences. AluI recognition sites are in grey. AluI-fragments, which are visible in experiment, are shown by arrows and their lengths are given.

Comparison of diagrams in Figures 1 and 3 shows similarity in the peaks distribution for cleavage of genomic DNA and Alu repeats at sites AGCT and CCWGG. Similarity is also observed for cleavage of genomic DNA and LINE1 repeats at DNA sequences GGTACC and TGGCCA. A value of any peak in the repeats distribution diagram is the height of that peak in genomic distribution diagram minus the corresponding value on the basic curve. The basic curve is a result of remaining genomic DNA cleavage. Fragment distribution in the basic curve depends on a number of parameters, such as GC-composition of genomic DNA, enzyme's recognition sequence, the frequency of certain dinucleotides sequences in the genome. These parameters were discussed earlier [5].

Diagrams of the genomic DNA and Alu - LINE1-repeats cleavage, like those shown in Fig. 3 and 4, have been constructed for recognition sequences of other restriction endonuclease, also used in this work. Similar to restriction enzymes AluI, Bst2UI, KpnI and Msp20I, peaks in diagrams of genomic DNA splitting coincide with peaks of either Alu-repeats or LINE1-repeats cleavage. Table 1 summarizes the results, where each of the analyzed peaks in the diagram of genomic DNA splitting is referenced to a corresponding peak from Alu- or LINE1-repeats digestion diagrams.

Data in Table 1 shows that the large fragments (more than 200 bp) in genomic DNA distribution diagrams are formed from LINE1-repeats. This is probably because the length of LINE1-repeats is greater than 6,000 bp, while the length of Alu-repeats is less than 300 bp. Hence the formation of large fragments from Alu repeats is impossible. Earlier, we have studied the rat genomic DNA cleavage and we have found that almost all fragments larger than 200 bp correspond to the DNA fragments, observed in the cleavage of consensus DNA sequence of rat LINE1 repeats [5].

However, as Table 1 shows, all peaks with length less than 200 bp are formed exclusively from Alu-repeats. Absence of short fragments produced by LINE1 repeats cleavage may be explained in the following way. Most of LINE1 fragments in the human genome are shorter than the original complete LINE1 fragment [1, 11]. Only 15% of all L1-repeats in human genome are about 6000 bp length [11]. Even L1-repeats fragments, which can be seen in the experiment, are represented by low peaks in the diagrams (no more than 4 million bp). These fragments are visualised in agarose gels due to fragments cluster formations. LINE1 repeats do not have internal DNA repeats and upon cleavage of LINE1 repeats, low molecular weight DNA fragments are produced in the same quantities as large fragments.

This excludes visualization of low molecular weight fragments due to a proportional decrease in total number of nucleotides. Besides, realization of the DNA fragments clustering effect, as it occurs in agarose gel, is impossible for low molecular weight DNA fragments in acrylamide gels. Meanwhile, visualization of small DNA fragments, produced by Alu-repeats cleavage, is possible due to a larger number of such repeats, compared to a number of LINE1 repeats.

Special attention should be devoted to the diagram of genomic DNA cleavage at sequence AGCT, a recognition site of AluI restriction endonuclease. It is well known that Alu-repeats contain one AluI site, which is common for all Alu subfamilies [9]. Our work has demonstrated the existence of three distinct fragment peaks 60, 49 and 32 bp in length. These fragments are present in the diagrams of both genomic DNA and Alu repeats cleavage at nucleotide sequence AGCT (Fig.1 and 3). We can also observe bands, which correspond to these DNA fragments in chromosomal DNA hydrolysis by restriction endonuclease AluI, which has the recognition sequence AGCT. We carried out additional analysis of some subfamilies of Alu-repeats (Fig. 5). Multiple alignment of nucleotide sequences of Alu repeats shows that some subfamilies also have a second AluI site. Cleavage of these repeats may be the reason for the existence of 32, 49 and 60 bp fragments. In particular, 60 bp DNA fragment is formed after cleavage of subfamily AluJo; 49 bp DNA fragment is formed from subfamily AluY; and 32 bp - from subfamily AluSc. AluJo and AluY-repeats are present in the human genome in large quantities of about 145,000 and 128,000 copies, respectively [12]. AluSc-repeat is represented significantly lower [12] in quantities of about 49,000 copies. This value is not enough to visualize 32 bp fragment in experiments and a real number of Alu repeats copies containing this fragment is discussed separately [7].

Thus, the presence of peaks in the distribution diagrams is associated with large quantities of DNA repeats in eukaryotic genomes. The cleavage of both the LINE1 and Alu-repeats makes a major contribution to the pattern of human DNA hydrolysis, whereas satellite DNA cleavage provides extra, sometimes brighter, bands in some gel photographs. However, observed peaks in the fragment distribution diagrams of rat genomic DNA cleavage coincide with peaks in similar diagrams of consensus rat LINE1 repeat digestion only [5]. This difference between human and rat diagrams is probably due to a presence of B1-repeats (an analogue of Alu-repeats) in the rat genomic DNA [13], in quantities about 7 times less than Alu-repeats in the human genome [1]. Additionally, the relative amount of LINE1 repeats in rat genome (~23%) is higher than in human genome (~17%) [8, 13].

The proposed method of restriction enzymes analysis may be used in DNA diagnostics. It does not require complex and expensive equipment and can be performed in ordinary medical laboratories equipped for standard RFLP (Restriction Fragments Lengths Polymorphism) analysis. Results of the earlier study on DNA digestion with restriction enzymes HaeIII and Kzo9I [2], as well as the current study of 15 restriction endonucleases, provide 17 unique DNA cleavage patterns. Beyond any doubts, the list of restriction enzymes used in human genome studies will be extended in subsequent works. Another study of interest is a comparative investigation of patterns of DNA cleavage with restriction enzymes, which differ in sensitivity to methylated DNA. And finally, the most promising direction of investigation is the study of methylation status of LINE1, Alu-repeats and satellite DNA. This study might be performed based on DNA cleavage with the new methyl-directed site-specific DNA endonucleases, recently discovered at SibEnzyme [14-17].

The authors thank Nesterov AE for assistance in DNA samples preparation.

Restriction endonuclease	Peak values of the fragments in figures	Family repeat		
		L1	Alu	α -satellite
AluI	60		+	
	49		+	
	32		+	
AsuHPI	342			+
	171			+
	157-159		+	
Bpu10I	135-136		+	
BstDEI	171			+
	131-135		+	
	116-118		+	
	48		+	
BstSCI	165-168		+	
	115-119		+	
	84, 86		+	
	67-68		+	
	49-50		+	
Bst2UI	165-168		+	
	115-119		+	
	84, 86		+	
	67-68		+	
	49-50		+	
Hinfl	342			+
	171			+
	79		+	
BssECI	207-208		+	
	156-159		+	
	50		+	
BstMAI	197-200		+	
	189-190		+	
	166-167		+	

	158-159		+	
FauNDI	1153	+		
XbaI	1368	+		
	846	+		
MroXI	1301	+		
KpnI	1791	+		
	1561	+		
	1205	+		
Msp20I	852	+		
	420	+		
AspA2I	636	+		

Table 1. A presence of DNA fragments with peak value in DNA repeats families

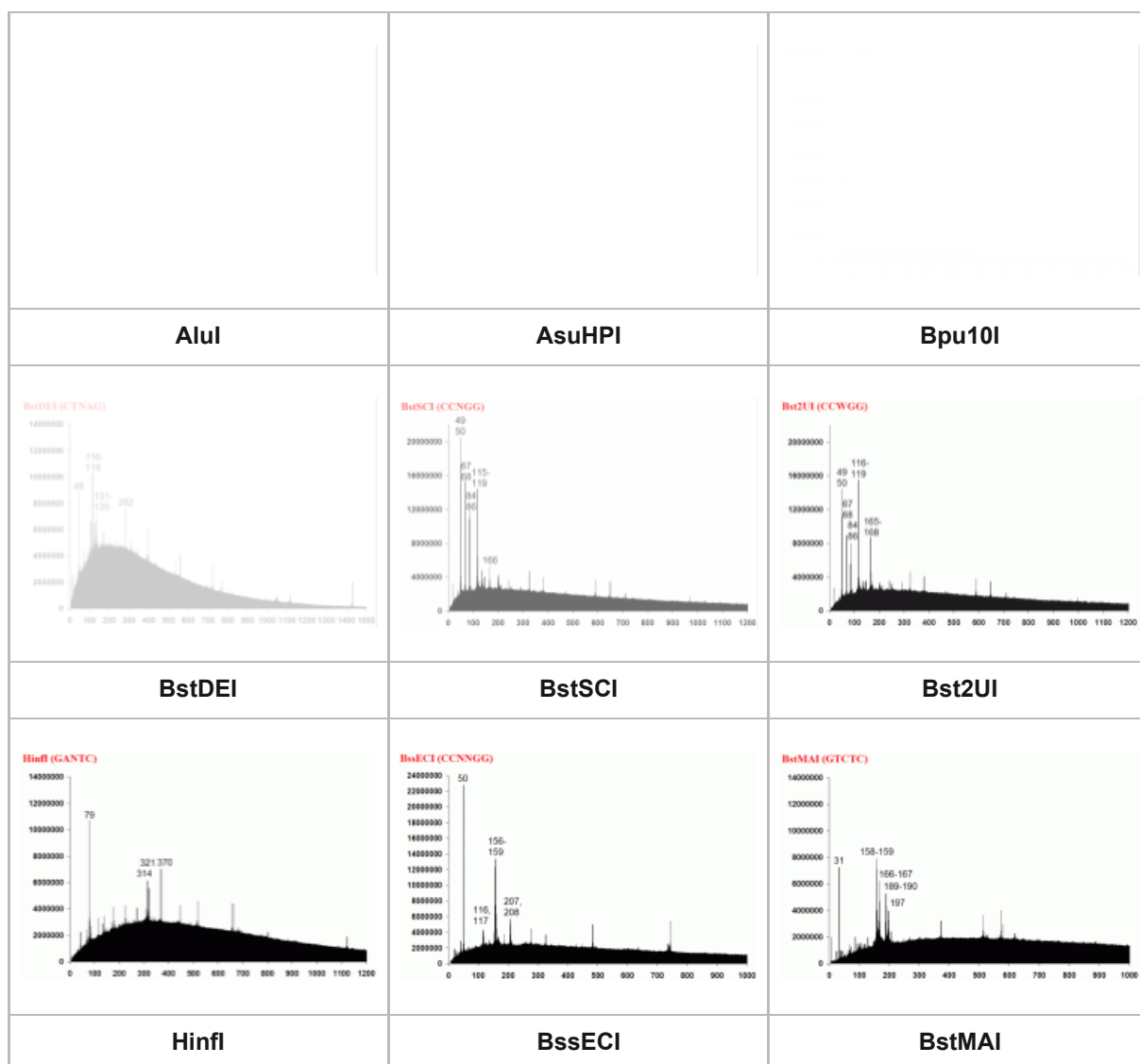


Fig. 6. DNA fragments distribution diagrams (total bp) depending on the fragment size (bp) for restriction endonucleases recognition sites.

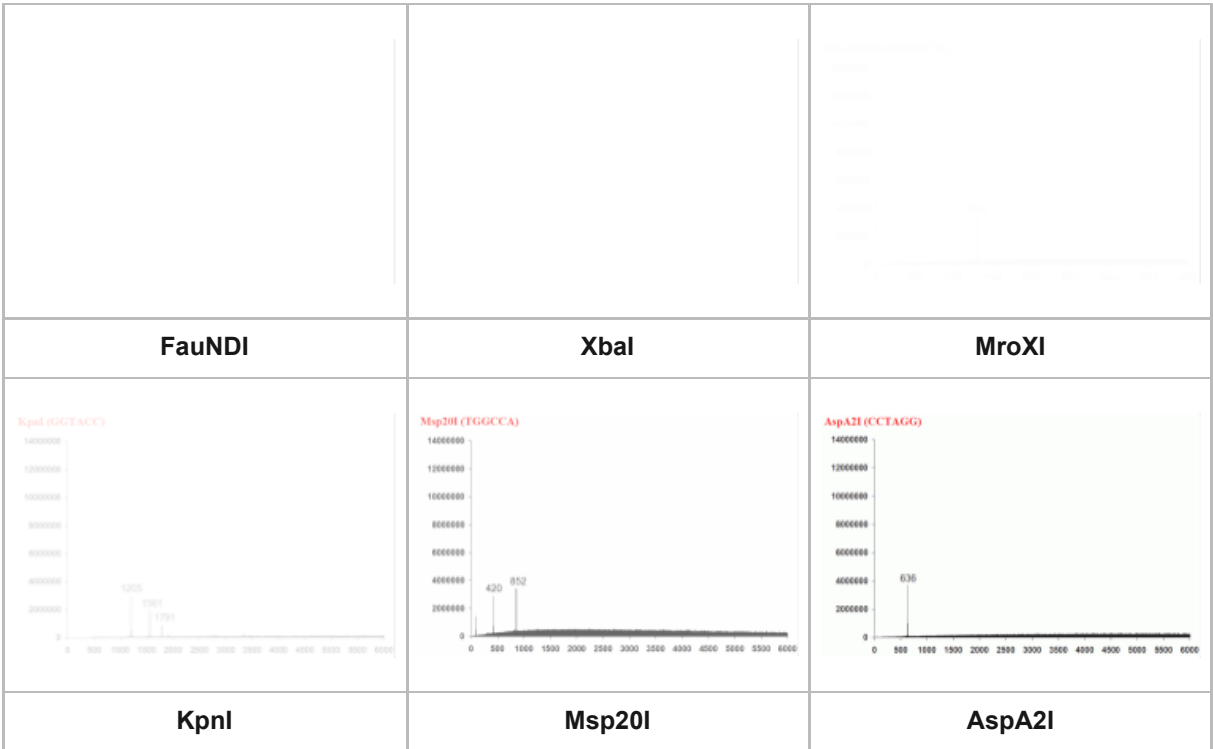


Fig. 7. DNA fragments distribution diagrams (total bp) depending on the fragment size (bp) for restriction endonucleases recognition sites.

REFERENCES

1. International Human Genome Sequencing Consortium. Initial sequencing and analysis of the human genome. // Nature. - 2001. - Vol. 409. - P. 860-921.
2. Abdurashitov M.A., Tomilov V.N., Chernukhin V.A., Gonchar D.A. , Degtyarev S.Kh. Mammalian chromosomal DNA digestion with restriction endonucleases *in silico*. // Ovchinnikov bulletin of biotechnology and physical and chemical biology. - 2006. - V. 2 N 3. - P. 29-38 (Russian). ([online version](#))
3. Lindblom B., Holmlund G. Rapid DNA purification for restriction fragment length polymorphism analysis. // Gene Anal. Tech. - 1988. - Vol. 5. - P. 97-101.
4. Jurka J., Kapitonov V. V., Pavlicek A., Klonowski P., Kohany O., Walichiewicz J. Repbase Update, a database of eukaryotic repetitive elements. // Cytogenic and Genome Research. - 2005. - Vol. 110. - P. 462-467.
5. Chernukhin V.A., Abdurashitov M.A., Tomilov V.N., Gonchar D.A. , Degtyarev S.Kh. Comparative restriction analysis of rat chromosomal DNA *in vitro* and *in silico*. // Ovchinnikov bulletin of

- biotechnology and physical and chemical biology(Russian). - 2006. - V. 2 N 3 - P. 39-46. ([online version](#))
6. Choo K. H., Vissel B., Nagy A., Earle E., Kalitsis P. A survey of the genomic distribution of alpha satellite DNA on all the human chromosomes, and derivation of a new consensus sequence. // Nucleic Acids Res. - 1991. - Vol. 19. - P. 1179-1182.
 7. Thompson J. D., Sylvester J. E., Gonzalez I. L., Costanzi C. C., Gillespie D. Definition of a second dimeric subfamily of human alpha satellite DNA. // Nucleic Acids Res. - 1989. - Vol. 17. - P. 2769-2782.
 8. Hitrinskaya I.Yu., Stepanov V.A., Puzyrev V.P. Alu-repeats in the human genome. // Molecular biology. - 2003. - V. 37 N 3. - P. 382-391.
 9. Houck C. M., Rinehart F. P., Schmid C. W. A ubiquitous family of repeated DNA sequences in the human genome. // J. Mol. Biol. - 1979. - Vol. 132. - P. 289-306.
 10. Ostertag E. M., Kazazian H. H. Jr. Biology of mammalian L1 retrotransposons. // Annu. Rev. Genet. - 2001. - Vol. 35. - P. 501-538.
 11. Szak S. T., Pickeral O. K., Makalowski W., Boguski M. S., Landsman D., Boeke J. D. Molecular archeology of L1 insertions in the human genome. // Genome Biology. - 2002. - Vol. 3. - research0052.1-research0052.18.
 12. Grover D., Kannan K., Brahmachari S. K., Mukerji M. ALU-ring elements in the primate genomes. // Genetica. - 2005. - Vol. 124. - P. 273-289.
 13. Rat Genome Sequencing Project Consortium. Genome sequence of the Brown Norway rat yields insights into mammalian evolution. // Nature. - 2004. - Vol. 428. - P. 493-521.
 14. Chmuzh E.V., Kashirina J.G., Tomilova J.E., Mezentzeva N.V., Dedkov V.S., Gonchar D.A., Abdurashitov M.A., Degtyarev S.Kh. Restriction endonuclease Bis I from Bacillus subtilis T30 recognizes methylated sequence 5'-G(m5C)↓NGC-3'. // Biotechnologia (Russian). - 2005. - N 3. - P. 22-26. ([online version](#))
 15. Chernukhin V.A., Najakshina T.N., Abdurashitov M.A., Tomilova J.E., Mezentzeva N.V., Dedkov V.S., Mikhnenkova N.A., Gonchar D.A., Degtyarev S. Kh. A novel restriction endonuclease Glal recognizes methylated sequence 5'-G(m5C)^GC-3'. // Biotechnologia (Russian). - 2006. - N 4. - P. 31-35. ([online version](#))
 16. Chernukhin V.A., Chmuzh E.V., Tomilova J.E., Nayakshina T.N., Gonchar D.A., Dedkov V.S., Degtyarev S.Kh. A novel site-specific endonuclease Glul recognizes methylated DNA sequence 5'-G(5mC)^NG(5mC)-3'/3'-(5mC)GN^(5mC)G. // ([online version](#))
 17. Chernukhin V.A., Tomilova J.E., Chmuzh E.V., Sokolova O.O., Dedkov V.S., Degtyarev S.Kh. Site-specific endonuclease Bisl recognizes DNA sequence 5'-G(5mC)N^GC-3' and cleaves it producing 3' sticky ends. // ([online version](#))