Data in Brief

Genome-wide mapping of hot spots of DNA double-strand breaks in human cells as a tool for epigenetic studies and cancer genomics

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Hot spots of DNA double-strand breaks (DSBs) are associated with coordinated expression of genes in chromosomal domains (Tchurikov et al., 2011 [1]; 2013). These 50–150-kb DNA domains (denoted “forum domains”) can be visualized by separation of undigested chromosomal DNA in pulsed-field agarose gels (Tchurikov et al., 1988; 1992) and used for genome-wide mapping of the DSBs that produce them. Recently, we described nine hot spots of DSBs in human rDNA genes and observed that, in rDNA units, the hot spots coincide with CTCF binding sites and H3K4me3 marks (Tchurikov et al., 2014), suggesting a role for DSBs in active transcription.

Here we have used Illumina sequencing to map DSBs in chromosomes of human HEK293T cells, and describe in detail the experimental design and bioinformatics analysis of the data deposited in the Gene Expression Omnibus with accession number GSE53811 and associated with the study published in DNA Research (Kravatsky et al., 2015). Our data indicate that H3K4me3 marks often coincide with hot spots of DSBs in HEK293T cells and that the mapping of these hot spots is important for cancer genomic studies.

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1. Direct link to deposited data

http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE53811.

2. Experimental design, materials and methods

2.1. DNA preparation

The steps of the procedure are shown schematically in Fig. 1A. To reduce non-specific hydrodynamic breakage, DNA samples were isolated from cells embedded in 0.5% low-melt agarose as described previously [3,7–9]. About 6 million HEK293T cells in 2 mL of culture medium were pelleted by centrifugation at 2000 rpm in a Minispin centrifuge (Eppendorf), resuspended in 0.3 mL of the same medium, gently mixed at 42 °C with an equal volume of a solution containing 1% low-melt agarose L (LKB) in PBS, and stored at 4 °C for 3 months. Each DNA–agarose plug usually contained about 15 μg of DNA, corresponding to about 1 million cells.

To test the quality of isolated DNA, fractionation in pulsed-field gels was performed as described previously [1,3,4]. Portions of the original
**2.2. Rapid amplification of forum domains termini (RAFT) procedure**

The steps of the procedure are shown schematically in Fig. 1B. About 1.5 μg of isolated DNA (see above) was ligated with 70 ng of double-stranded oligonucleotide (25-bp long 5′-phosphorylated 5′-pCCCTGT CAGTATAAGGAATTCGGG 3′ oligonucleotide annealed to a 26-bp long 5′ biotinylated 5′-CCGAATTCTCCTTATACTGCAGGGG 3′ oligonucleotide) in 150 μL of a solution containing 67 mM Tris–HCl (pH 8.4), 6 mM MgCl₂, 10 mM 2-mercaptoethanol, 16.6 mM ammonium sulfate, 6.7 μM EDTA, 5 μg/mL BSA, 1 mM dNTPs, 1 μg of primer corresponding to Sau3A adaptor (5′ CCAGAAGCTTAAGCGGCCGCAAAC 3′), 1 μg of primer corresponding to biotinylated oligonucleotide (5′-CGGAATTCTCCTTATACTGCAGGGG 3′), and 1 U of Taq polymerase was performed using a Mastercycler Personal thermal cycler (Eppendorf). Amplification conditions were 90 °C for melting, 65 °C for annealing, and 72 °C for extension, for 1 min each. The final DNA sample contained the amplified genome-wide preparation of DNA fragments delimited by a base at a particular DSB and the nearest Sau3A site.

**2.3. Library preparation**

Libraries were prepared according to Illumina’s instructions accompanying the DNA Sample Kit (Part # 0801-0303). Briefly, DNA was end-repaired using a combination of T4 DNA polymerase, Escherichia coli DNA Pol I large fragment (Klenow polymerase), and T4 polynucleotide kinase. The blunt, phosphorylated ends were treated with Klenow fragment and dATP to yield a protruding 3′-A base for ligation of Illumina’s adapters, which have a single T-base overhang at the 3′ end. After adapter ligation, DNA was PCR amplified with Illumina primers for 15 cycles. Library fragments of ~200–400 bp and ~400–1200 bp were isolated as bands from an agarose gel, and were sequenced on the Genome Analyzer Ix and MiSeq, respectively, following the manufacturer’s protocols.

**2.4. Data processing**

Fig. 2 shows the bioinformatics pipeline used. The standard Illumina analysis pipeline using their phiX control software was used for basic calling. At the first step of processing, quality control was performed using FastQC [10]. Next, reads were trimmed for RAFT primer sequences by use of Cutadapt v. 1.3 [11]. Some options were common for both datasets:

- minimum-length = 30 – trimmed-only – quality-base = 33 – quality-cutoff = 3 -n 2

The option “-trimmed-only” was used to remove from trimmed files all reads that did not have RAFT primers. This option setting ensures that after removal of primers the data set consists of reads of sufficient length to have contained RAFT primers before removal. The following options were applied to remove 5′ attached RAFT primers from reads:

- g CCAGAATTCTCCTTATACTGCAGGGG
- g CGGAATTCTCCTTATACTGCAGGGG

Cutadapt was used in the paired-end mode for the paired-end Illumina GA Ix dataset and in the single-end mode for the single-end MiSeq dataset. At the next step, the trimmed files from both sequencing machines were merged.
Performances were using Genome Track Analyzer [6].

The genometric analysis of both datasets was performed using bowtie v.1 with the following command line arguments. The downloaded raw reads for Rep1, Rep2, and Signal from Encode accession wgEncodeEH000953 were aligned to the same genome hg19/GRCh37p10 by use of bowtie v.1 with the following command line arguments. The resulting SGR file contains the DSBs with one-nucleotide resolution and their coverage.

To prepare the H3K4me3 mark dataset, the following steps were performed. The downloaded raw reads for Rep1, Rep2, and Signal from Encode accession wgEncodeEH000953 were aligned to the same genome hg19/GRCh37p10 by use of bowtie v.1 with the following command line options: --best -m 1 -chunkmbs 1024. Peak calling was performed using the MACS2 [14] peak caller with the options callpeak --size hs to set the correct genome size. Peak summits obtained from MACS2 were used for further analysis. The genometric analysis of both datasets was performed using Genome Track Analyzer [6].

3. Discussion

The RAFT procedure includes several steps in which very long DNA molecules are manipulated in solution—from the elution of DNA with the ligation of biotinylated oligonucleotides (steps 2–5 in Fig. 1B). Although only a gentle mixing of solution was performed, a random fragmentation of forum domains cannot be excluded during these steps. Nevertheless, our previous data strongly demonstrate that the level of this random hydrodynamic fragmentation of DNA molecules in the conditions used is much lower than the non-random fragmentation detected at the hot spots of DSBs [5].

The data on the distribution of hot spots of DSBs in the human genome could be used for the study of chromosomal breakage associated with regulation of gene expression and different genomic rearrangements (translocations, inversions, and deletions).

We studied the positional and ordering correlations between DSBs and H3K4me3 marks in the chromosomes of human HEK293T cells using Genome Track Analyzer [6]. The H3K4me3 mark is a well-known promoter-specific histone modification that is associated with transcription and active genes. This epigenetic mark selectively directs global TFIID recruitment to active genes, some of which are also p53 targets [15]. The summary of correlations is shown in Table 1 and demonstrates strong positional correlations between DSBs and H3K4me3 peak summits for all chromosomes of H293T cells. Such correlations support the hypothesis regarding the relationships between DSBs and coordinated gene expression [2].

Interesting questions arise from the ordering correlations, which are significant not only for chromosomes 2, 3, 18, and 19 and show that in these chromosomes H3K4me3 peak summits often precede DSBs. In future work we plan to analyze the significant correlation pairs for these chromosomes in different genome browsers and automatic annotation systems to reveal the possible biological meaning of these correlations. The strong correlation between H3K4me3 marks and hot spots of DSBs has been described in human rDNA units, suggesting an important role for DNA breaks in actively transcribed genes [5].

Fig. 3 shows one example of DSB mapping important for cancer genomic studies. The BAM file was used in locating hot spots of DSBs inside the TMPRSS2 and ERG genes located on the minus strand of chr21 at a distance about 3 Mb. These genes were selected because recurrent gene fusions between TMPRSS2 and ET3 family genes occur at high frequency in prostate cancer [16]. We detected several regions in the TMPRSS2 and ERG genes that are enriched with DSBs. Deletion, rather than translocation, is reported to be the main mechanism for TMPRSS2-ERG gene fusion (81 vs. 19%) [16]. Detected hot spots of DSBs (Fig. 3) could be involved in such genomic rearrangements. It has been shown that the regions possessing hot spots of DSBs in human rDNA genes often form contacts with other genomic regions also possessing hot spots of DSBs, and it has been suggested that this fact could explain the origin of Robertsonian translocations [5]. It is known that regions of the same chromosome make 3D contacts more often than between different chromosomes [17]. TMPRSS2 and ERG genes are located very close to each other on chr21, providing a potential for contacts between their regions possessing DSB hot spots. Currently, we are performing 4C (circular chromosome conformation capture) experiments in order to study genomic contacts between these genes, to uncover the possible mechanism of this and some other cancerogenic gene fusions.

Our data suggest that hot spots of DSBs are associated with various epigenetic mechanisms of gene regulation and with the formation of 3D chromosomal structures, both of which are conserved in different cell types, with dramatic consequences for genomic integrity should they go awry [25]. Hence, data on the distribution of DSB hot spots in the human genome provide a new tool for studies of cancer genomics and genomic features associated with the regulation of gene expression.
Table 1
Correlation of the data on mapping of DSBs and H3K4me3 marks in HEK293T cells.

| Chromosome | \(z\)   | Correlation  | \(p\)     | \(z_p\)   | Ordering | \(p_z\)  |
|------------|---------|--------------|-----------|-----------|----------|----------|
| chr1       | 11.142  | strong corr  | 0.0000    | -1.6450   |          | 0.1000   |
| chr2       | 3.218   | strong corr  | 0.0013    | -3.0600   | 2⇒1     | 0.0022   |
| chr3       | 6.001   | strong corr  | 0.0000    | -2.0400   | 2⇒1     | 0.0414   |
| chr4       | 3.501   | strong corr  | 0.0005    | -1.0700   |          | 0.2845   |
| chr5       | 4.925   | strong corr  | 0.0000    | 0.6430    |          | 0.5205   |
| chr6       | 5.856   | strong corr  | 0.0000    | 1.3680    |          | 0.1712   |
| chr7       | 5.461   | strong corr  | 0.0000    | -1.7190   |          | 0.0857   |
| chr8       | 3.864   | strong corr  | 0.0001    | -0.6400   |          | 0.5224   |
| chr9       | 5.439   | strong corr  | 0.0000    | 0.2440    |          | 0.8075   |
| chr10      | 3.818   | strong corr  | 0.0001    | -0.2390   |          | 0.8110   |
| chr11      | 4.857   | strong corr  | 0.0000    | 0.2150    |          | 0.8295   |
| chr12      | 4.160   | strong corr  | 0.0000    | -0.7700   |          | 0.4411   |
| chr13      | 2.395   | corr         | 0.0166    | 0.1390    |          | 0.8897   |
| chr14      | 3.096   | strong corr  | 0.0020    | -0.4870   |          | 0.6260   |
| chr15      | 2.989   | strong corr  | 0.0028    | -1.7060   |          | 0.0881   |
| chr16      | 5.115   | strong corr  | 0.0000    | -0.7840   |          | 0.4328   |
| chr17      | 2.701   | strong corr  | 0.0069    | -0.1660   |          | 0.8685   |
| chr18      | 2.222   | corr         | 0.0263    | -2.3430   | 2⇒1     | 0.0191   |
| chr19      | 5.534   | strong corr  | 0.0000    | -2.8680   | 2⇒1     | 0.0041   |
| chr20      | 3.995   | strong corr  | 0.0001    | 1.0110    |          | 0.3122   |
| chr21      | 2.748   | strong corr  | 0.0060    | -1.2790   |          | 0.2008   |
| chr22      | 2.651   | strong corr  | 0.0080    | -0.3760   |          | 0.7066   |
| chrX       | 3.085   | strong corr  | 0.0020    | -0.6310   |          | 0.5282   |
| Genome-wide| 29.000  | strong corr  | 0.0000    | -3.8930   | 2⇒1     | 0.0001   |

\(z\) and \(z_p\) are calculated by Genome Track Analyzer [6] and characterize the positional and ordering correlations between DSBs and H3K4me3 peak summits. The 1% significance thresholds for \(|z|\) and \(|z_p|\) in the case of random correlations correspond to 2.58, while 5% significance thresholds correspond to 1.96. The negative values of \(z_p\) indicate that H3K4me3 mark peak summits precede DSBs for some chromosomes (2, 3, 18, 19). The corresponding \(p\)-values were calculated using Gaussian statistics. All data have number of pairs of the nearest neighbors (NN) exceeding 50 to ensure the applicability of Gaussian statistics.

### Color and values legend

| \(z\)-value | Meaning               |
|-------------|-----------------------|
| \(|z| < 1.8\) | Insignificant, no correlation |
| 1.8 ≤ \(|z| < 1.96\) | Fuzzy correlation |
| 1.96 ≤ \(|z| < 2.58\) | Significant correlation, \(p < 0.05\) |
| \(|z| ≥ 2.58\) | Strong correlation, \(p < 0.01\) |
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