MeX pipeline for analysis of mobile genetic elements in cancer genome

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ABSTRACT

Background: Mobile genetic elements (MGEs) comprise a major portion of the human genome and are essential for genetic diversity. These elements are known to have the capability to induce mutations in the human genome. To date, there are several MGE insertions which have been reported to be associated with cancer. We aim to use genome next-generation sequencing data and appropriate bioinformatics tools to accurately identify the insertion sites of MGEs in the human genome.

Results: Herein, we introduce the MeX pipeline for the localization and annotation of MGEs in paired-end sequencing data. It requires the reference genome sequence, MGE sequences and paired-end sequencing reads. We evaluated MeX on high depth (>75×) Illumina HiSeq data produced at the Broad Institute (NA12878) against human genome 38-built (including only chromosome 1, 2 and 3) and Alu elements. We could identify 78 reference and 1 non-reference Alu insertions in the NA12878 sample. Upon annotation, it was found that the non-reference Alu element was in the 3' UTR region of the RNF2 gene. Out of 78 reference insertions, 42 were in the intronic region, 7 in the upstream region, 5 in the downstream region, 1 in the 3' UTR region and the rest were not associated with any gene. MeX showed high performance for the identification and annotation of MGEs in genome samples.

Conclusion: This study showed that MeX is a robust and powerful tool for the identification and annotation of MGE insertions. It may also serve as a valuable tool to study the phenotypic changes resulting from transpositional events in cancer genomics.

Keywords: Transposon, paired-end sequencing, cancer, target-site duplication, variant annotation

INTRODUCTION

MGEs, also known as transposons or jumping genes, are the DNA segments that can change their location within the genome. These MGEs are present in all the organisms, prokaryotes as well as eukaryotes and they make up a large fraction of the human genome that include at least 45% of the whole genome [Pace et al. 2007]. They are known to have a significant role in genome organization and evolution [Makalowski et al. 2019]. Active transposons are also known to cause mutagenic effects that cause several genetic disorders, like cancer [Jang et al. 2019]. A previous study conducted in our lab has shown that the human genes associated with cancer has significantly higher number of MGEs as compared to the genes that are not

associated with cancer [Preeti et al. 2021]. We have also presented an efficient computational protocol for MGE insertion site analysis. ELAN, the suite of tools uses standard techniques to identify different MGEs and their distribution on the genome [Rawal et al. 2011]. Previously, in our lab we have also developed efficient platform for vaccine target identification [Rawal et al. 2021] and generated comprehensive maps of molecules that are implicated in human associated diseases [Jagannadham et al. 2016].

Recent advancement in high-throughput sequencing technologies have given opportunities to understand the functionality of transposable elements and their role in cancer. However, due to the repetitive nature of these DNA segments, their identification remains a challenging area of computational biology. Despite of the complexity, there are many tools available for the identification of MGEs, like, RetroSeq [Keane et al. 2013], Tangram [Wu et al. 2014], TraFiC [Tubio et al. 2014], TranspoSeq [Helman et al. 2014], Tea [Lee et al. 2012], TEMP [Zhuang et al. 2014], ngs_te_mapper [Linheiro et al. 2012], PopoolationTE [Kofler et al. 2012], iMGEins [Bae et al 2018], and MGERT [Guliave et al. 2019]. There is no single MGE detection method that can be concluded as best. Nelson et al. 2017 applied a consensus approach, where multiple methods are integrated in a pipeline for the identification.

Being a mutagenic agent, transposons play a role in cancer development. A very important application of such detection methods could be to find the impact of MGE insertion in the human genome and its consequences on cancer development. Here, in this study we generated a framework, named MeX, for MGE insertion identification and their characterization to determine its correlation to cancer.

METHODOLOGY

1. Input Requirement

MeX requires three input files, sequencing reads in fastq format, human reference genome (hg18 or hg19) in fasta format and reference MGEs in fasta format. This pipeline takes paired-end sequencing data as sample input. To evaluate MeX, we used high depth ($>75\times$) Illumina HiSeq data produced at the Broad Institute from the 1000 Genomes Project. Whole genome sequencing of (CEU) Utah residents with ancestry from Northern and Western Europe. This dataset was downloaded from NCBI SRA under accession number [SRR622461](#). The complete human reference genome can be downloaded from [UCSC browser](#) for required genome assembly. We extracted the repeat database from Repbase [Bao et al. 2015]. To assess the efficiency of the pipeline a section of the human genome, (including chromosome 1, 2 and 3) and the repeat database (including all the Alu elements) was used.

2. MeX Implementation

a. Preprocessing of reads

Fastq file preprocessing is an essential step to obtain clean data for downstream processing. The pipeline utilizes the fastp tool [Chen *et al.* 2018] for preprocessing. To filter out low quality reads, we performed filtering of reads, pruning of reads, adapter trimming, polyG/polyX tail trimming and UMI preprocessing using a parallel processing approach. It automatically searches for the adapter region in the sequence reads and trim with even only one base in the tail. If fastp identified overlapping regions in paired-end reads, it was automatically corrected, only if one pair had high quality ($Q>30$) and another pair had low quality ($Q<15$). This step also removed polyG and polyX from read tails as these are common artifacts in Illumina sequencing. While UMI preprocessing, it reduced background noise and improved sensitivity for ultra-low frequency mutation detection.

b. Quality Control Check

The second step provided a quality control check that was performed with the help of the FastQC tool. It ensured that there were no problems or biases in the preprocessed data. It provided a summary of the total number of sequences processed, lengths of the shortest or longest sequence reads, overall percentage of GC content, sequence quality per base, quality score per sequence, overall N content, degree of duplication, etc. All these parameters were evaluated and results were represented as pass or fail.

c. MGE Identification

MGE target site identification was performed using `ngs_te_mapper2` [Bergman, 2012]. The identification process is detailed as follows:

i. Raw sequence read mapping with MGE database

This step takes two input files, including sequencing reads (in fastq) and MGE database (in fasta). First, all the reads were aligned to the MGE database. After alignment, those reads were extracted whose match includes the start or end of the query MGE. If a read had two or more matches to different MGEs then the best match was selected. The best match was categorized based on the length of the matching sequence and the quality of the matched sequence. The quality was decided based on the number of gaps and mismatches along with the lower number of blocks. Those read matches with more than 1 block, mismatch, or gap for every 20 bp of target and query sequences were rejected.

ii. Selected reads mapping with reference genome

Those reads that had aligned with either at the start or end of MGEs, were mapped to reference the human genome. We then selected for mapped reads with one or fewer mismatches in 20 for both the read and reference genome sequences. Reads were retained if a match to the reference genome or TE was included at the beginning or end of the read. These sequences also had to match the reference TE start/end exactly where the genomic region begins or vice versa.

iii. Identification of target site duplication (TSD)

To find TSDs, mapped reads that had distances between the start coordinate of one read and the end coordinate of the next read were found sequentially in the genome that overlapped by less than or equal to 20 bp. This overlap distance is defined as TSD. The identified MGEs are then reported in bed format with information regarding chromosome number, start and end position, identified transposon family, and sense of the identified strand.

3. Variant Calling

The non-reference MGE insertions were then called as variants in Variant Call File (VCF) format. This file comprises information of chromosomal number, genomic location, transposon family, and supporting reads. All this information is then used for annotation of the identified MGE insertion.

4. Annotation

The VCF annotation was performed using the Ensembl Variant Effect Predictor (VEP) [McLaren et al. 2016]. This annotation step will predict multiple features of the identified non-reference MGE. MeX provides user-defined options for annotation with respect to GRCh37 and GRCh38 assembly. It provides all the information regarding, gene, transcript ID, predicted consequence, nucleotide change at coding region, amino acid change at protein level, exon/intron number, and prediction of impact on protein function using in silico tools like SIFT [Ng et al. 2003] and Polyphen [Adzhubei et al. 2010]. MGE at a single position were initially annotated with respect to all different transcripts for the given gene. However, it was further filtered to only canonical transcript annotation.

RESULTS

1. MeX implementation

a. Preprocessing

Figure 2 represents the general summary of this step. 31.30 M reads were removed to enhance the overall quality of the sample. The base calling accuracy, measured by Phred quality score (represented as Q score), was increased from 84.45% to 96.41% at Q score 20 and from 80.60% to 93.16% at Q score 30. There was no significant difference in GC content. The overall rate of duplication is 5.34% and 98.59% of reads are with unknown length of insert size (see Figure 3 and 4). Figure 5 and 6 shows the difference in the comparison of read quality and base content before and after preprocessing.

b. Quality check

All the quality parameters of read 1 and read 2 after the preprocessing are represented in Figure 7 and 8. All the parameters passed the qualification criteria, except for per sequence GC content and sequence length distribution, which showed medium qualification. Overall the sample was qualified for further evaluation.

c. MGE Identification

Using the MeX pipeline we could identify 78 reference and 1 non-reference Alu elements in the NA12878 sample in the first three chromosomes (see **Table S1** and **Table S2**).

2. Variant Calling and Annotation

All the identified MGEs were called in BED file format and these transposons were converted to VCF files/formats. These transposons were then annotated. The reference elements were identified in 55 different genes (see **Table S3**). Out of these 55 reference elements, 42 are in the intronic region, 7 in the upstream region, 5 in the downstream region, and 1 in the 3' UTR region. No reference transposon was identified in the exonic region. The non-reference element was identified in the *RNF2* gene (see **Table 3**). The *RNF2* gene element was annotated to be in the 3' UTR region.

CONCLUSION

MGEs play an important role in genomic instability. Recent studies have shown that MGE insertion can cause mutagenic changes to the human genome that leads to phenotypic changes like cancer. This suggests the importance of the dynamics of MGEs in the human genome. MeX was developed to accurately identify the breakpoints of inserted MGEs and predict the possible changes that will be caused by the inserted MGE

at protein level. MeX has a valuable feature of annotating the identified MGEs that makes it better than the other available MGE identification tools.

Contribution of Authors

This study was conducted under the overall guidance of KR, who contributed in protocol, critical evaluation of data and manuscript. The pipeline was designed, constructed and validated by PP and RS. Manuscript writing was done by PP and KR. All the authors are responsible for the content of the manuscript.

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Figure 1. Overview of MeX pipeline

Figure 2. Summary of Preprocessing Step

General	
fastp version:	0.20.1 (https://github.com/OpenGene/fastp)
sequencing:	paired end (101 cycles + 101 cycles)
mean length before filtering:	101bp, 101bp
mean length after filtering:	100bp, 100bp
duplication rate:	5.344108%
Insert size peak:	31
Before filtering	
total reads:	179.965424 M
total bases:	18.176508 G
Q20 bases:	15.349548 G (84.447181%)
Q30 bases:	14.650156 G (80.599400%)
GC content:	39.894825%
After filtering	
total reads:	148.665340 M
total bases:	14.949997 G
Q20 bases:	14.412864 G (96.407135%)
Q30 bases:	13.927247 G (93.158865%)
GC content:	39.349110%
Filtering result	
reads passed filters:	148.665340 M (82.607724%)
reads with low quality:	30.797632 M (17.113083%)
reads with too many N:	502.452000 K (0.279194%)
reads too short:	0 (0.000000%)
Adapters	
Adapter or bad ligation of read1	
The input has little adapter percentage (~0.376446%), probably it's trimmed before.	
Sequence	Occurrences
all adapter sequences	770722
Adapter or bad ligation of read2	
The input has little adapter percentage (~0.376446%), probably it's trimmed before.	
Sequence	Occurrences
all adapter sequences	770722

Figure 3. Rate of duplication estimated by Fastp in raw reads. The duplication rate is the fraction of mapped reads where any 2 reads share the same 5' and 3' coordinates. *x*-axis represents the percentage of reads and the GC ratio and *y*-axis represents level of duplication (unit unknown)

Figure 4. Estimation of insert size by Fastp in raw reads. The insert size is the number of base pairs sequenced from a DNA fragment. *x*-axis represents the percentage of reads and *y*-axis represents insert size (in bp).

Figure 5. Comparison of read quality before and after preprocessing

Figure 6: Comparison of base content before and after filtering

Figure 7. Quality parameters of SRR622461_1

Figure 8. Quality parameters of SRR622461_2

Figure 9:

a) Read Mapping with MGE library

b) Shortlisted read mapping with reference genome

c) Identification of Target-Site Duplication (TSD)

Figure 10:

Statistics

If the workflow has been executed in cluster/cloud, runtimes include the waiting time in the queue.

Figure 11:

a)	b)
General statistics	General statistics
Lines of input read	117
Variants processed	117
Variants filtered out	0
Novel / existing variants	-
Overlapped genes	98
Overlapped transcripts	904
Overlapped regulatory features	16

Lines of input read	1
Variants processed	1
Variants filtered out	0
Novel / existing variants	-
Overlapped genes	1
Overlapped transcripts	4
Overlapped regulatory features	0

Figure 12:

Figure 13:

Table 1: Software dependencies required to run each component of the MeX pipeline

Software	Fastp	FASTQC	ngs_te_mapper	VEP	Version Used in this Study
Linux	✓	✓	✓	✓	18.04
Conda	✓	✓	✓	✓	4.9.2
Git	✓	✓	✓	✓	2.17.1
Perl			✓	✓	5.26.2
Java		✓			11.0.8
C++	✓				11
RepeatMasker (Smit et al. 2013)			✓		4.0.7
BEDTools (Quinlan and Hall 2010)			✓		2.29.2
SAMTools (Li et al. 2009)			✓		1.9
BCFTools (Li et al. 2009)			✓		1.9
BWA (Li 2013)			✓		0.7.17

Table 2: Data dependencies required for each component of MeX pipeline

Data files	Fastp	FASTQC	ngs_te_mapper	VEP
Reference genome (fasta)			✓	
Reference MGE sequences (fasta)			✓	
*Annotated Reference MGE sequences (GFF3)			✓	

Unaligned reads (single-end fastq)	✓	✓	✓	
Unaligned reads (paired-end fastq)	✓	✓	✓	
Identified MGE (VCF)				✓

Table 1: Identified non- reference MGEs BED format

Chromosome	Start Position	End Position	Information	ID	Strand Sense
chr1	185100356	185100367	AluYGibF9 -11 0.33 3 3 6	.	+

Table 2: Identified non- reference MGEs VCF format without annotation

Chromosome	Start	End	Consequence	Strand	Information
chr1	185100356	185100367	INS	+	FAMILY=AluYGibF9;SUPPORTIN G_READS=6;TYPE=non-reference;

Table 3: Identified non- reference MGEs VCF format with annotation

Location	SYMBOL	Feature	Consequence	IMPACT	VARIANT_CLASS	HGVSc	HGVSp	EXON	BIOTYPE
chr1:185100356-185100367	<i>RNF2</i>	NM_007212.4	3_prime_UTR_variant, feature_elongation	MODIFIER	insertion	-	-	7/7	protein_coding
chr1:185100356-185100367	<i>RNF2</i>	XM_005245413.3	3_prime_UTR_variant, feature_elongation	MODIFIER	insertion	-	-	7/7	protein_coding
chr1:185100356-185100367	<i>RNF2</i>	XM_011509851.3	3_prime_UTR_variant, feature_elongation	MODIFIER	insertion	-	-	7/7	protein_coding
chr1:185100356-185100367	<i>RNF2</i>	XM_011509852.2	3_prime_UTR_variant, feature_elongation	MODIFIER	insertion	-	-	7/7	protein_coding