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Applications Note

Sequence analysis

# Cas-analyzer: an online tool for assessing genome editing results using NGS data

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## Abstract

**Summary:** Genome editing with programmable nucleases has been widely adopted in research and medicine. Next generation sequencing (NGS) platforms are now widely used for measuring the frequencies of mutations induced by CRISPR-Cas9 and other programmable nucleases. Here, we present an online tool, Cas-Analyzer, a JavaScript-based implementation for NGS data analysis. Because Cas-Analyzer is completely used at a client-side web browser on-the-fly, there is no need to upload very large NGS datasets to a server, a time-consuming step in genome editing analysis. Currently, Cas-Analyzer supports various programmable nucleases, including single nucleases and paired nucleases.

**Availability and Implementation:** Free access at http://www.rgenome.net/cas-analyzer/.

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**Supplementary information:** Supplementary data are available at *Bioinformatics* online.

## 1 Introduction

Programmable nucleases such as zinc finger nucleases (ZFNs), transcription-activator-like effector nucleases (TALENs), and RNA-guided endonucleases derived from CRISPR-Cas9/Cpf1 systems, which are adaptive immune responses in bacteria and archaea, are widely used for genome editing in many research fields including biology, biotechnology, agriculture, and medical science (Kim and Kim, 2014). The type II Cas9 nuclease from *Streptococcus pyogenes* (SpCas9) was the first CRISPR nuclease used for genome editing (Cho *et al.*, 2013; Cong *et al.*, 2013; Jinek *et al.*, 2013; Mali *et al.*, 2013); since that time, various orthogonal Cas9 nucleases such as StCas9 (Cong *et al.*, 2013), NmCas9 (Hou *et al.*, 2013) and SaCas9 (Ran *et al.*, 2015) have been developed. Recently, putative type V Cpf1 nucleases from *Acidominococcus* and *Lachnospiraceae* were reported to mediate efficient genome editing in human cells (Kim *et al.*, 2016a; Zetsche *et al.*, 2015) and mice (Hur *et al.*, 2016; Kim *et al.*, 2016b). Moreover, dimeric CRISPR nucleases such as RNA-guided nickases (Cho *et al.*, 2014; Ran *et al.*, 2013) and RNA-guided FokI nucleases (Tsai *et al.*, 2014), or biochemical improvement of wild-type SpCas9 (Kleinstiver *et al.*, 2016; Slaymaker *et al.*, 2016) have been developed for genome editing to reduce off-target effects.

Programmable nucleases introduce DNA double-strand breaks at user-defined target sites in the genome, ultimately inducing targeted gene knockout or knock-in via the cell's own repair systems [error-prone non-homologous end joining or homology-directed repair (HDR) in the presence of a DNA template, respectively]. The induced mutation rates in cells can be estimated in a straightforward manner by using Surveyor nuclease (Perez *et al.*, 2008), the T7 endonuclease I (T7E1) assay (Kim *et al.*, 2009), polyacrylamide gel electrophoresis (Zhu *et al.*, 2014) or droplet digital PCR (Nelson *et al.*, 2016). However, these methods do not allow analysis of mutant sequences and are limited by relatively poor sensitivity. Recently we and other groups have used targeted deep sequencing to detect programmable nuclease-induced mutations with high sensitivity and precision and to analyze mutation patterns (Baek *et al.*, 2016).

However, analysis of next generation sequencing (NGS) data is difficult for many researchers. Although a few web-based tools such as

CRISPR-GA (Güell *et al.*, 2014), AGEseq (Xue and Tsai, 2015) and CRISPResso (Pinello *et al.*, 2016) are available, they are inconvenient to use because their web interfaces require a very long time to upload large data files (Supplementary Material S1). AGEseq and CRISPResso also support a command-line interface, but they are not accessible to researchers who are not familiar with bioinformatics. To address this issue, we present a web-based tool, Cas-Analyzer that is constructed with a JavaScript-based algorithm; thus, it wholly runs on the client-side so that large amounts of sequencing data do not need to be uploaded to the server. Thanks to the improvements in the newest JavaScript engines in the most recent web browsers (Supplementary Table S1), this tool works in a reasonable time. Currently, Cas-Analyzer supports a variety of nucleases, including single nucleases (SpCas9, StCas9, NmCas9, SaCas9, CjCas9 and AsCpf1/LbCpf1) and paired nucleases (ZFNs, TALENs, Cas9 nickases and dCas9-FokI nucleases).

## 2 Implementation

### 2.1 File loading

To use Cas-Analyzer, deep sequencing data are needed, which can be obtained by amplifying the target locus of genome edited cells (Supplementary Material S2) followed by NGS. The format of the raw output data is usually Fastq or gzip-compressed, and both data types are acceptable to Cas-Analyzer (Fig. 1A). For the compressed files, we used a JavaScript library 'pako' (http://nodeca.github.io/pako/), which is slightly modified to support blocked gzip files. If users upload paired-end sequencing data, Cas-Analyzer first merges paired-end reads by the JavaScript port of Fastq-join, a part of ea-utils (https://code.google.com/archive/p/ea-utils/).

### 2.2 Data analysis

Cas-Analyzer analyzes the uploaded data and calculates mutation frequencies in three steps (Fig. 1B–D): (i) Cas-Analyzer first finds the cleavage point in the reference sequence for the selected nuclease. Using the given comparison range (R) parameter, Cas-Analyzer defines 12nt of indicator sequences on both sides of the given reference sequence and then selects the valid sequences, which contain both indicators with up to a 1-nt mismatch, from the uploaded data. (ii) For the selected sequences, Cas-Analyzer then counts the recurrent frequency of each sequence and excludes the sequences below the given minimum frequency (n). (iii) Cas-Analyzer finally classifies the filtered sequences into three different groups: 'insertion', 'deletion' or 'WT or substitution' based on comparing the sequence length with the length of the given reference sequence. Optionally, if a WT marker range (r) is given, the short sequence around the cleavage point will be used as the marker of wild-type. If this marker exists in the query sequence, it will always be classified into the 'WT or substitution' group regardless of its length. Additionally, if the donor DNA sequence for HDR is given, Cas-Analyzer defines an HDR indicator (>8nt) by comparing the donor sequence with the reference sequence and classifies all query sequences that have the HDR indicator into the 'HDR' category.

### 2.3 Sequence alignment

For user convenience, after data analysis is complete, the results (a relatively small amount of data) are aligned to the reference sequence by using a JavaScript ported EMBOSS Needle (Rice *et al.*, 2000). The aligned results are categorized by mutation type and sorted in descending order by count. In addition, the position and size of insertions or deletions are depicted as interactive graphs on the results web page (Fig. 1E and F).

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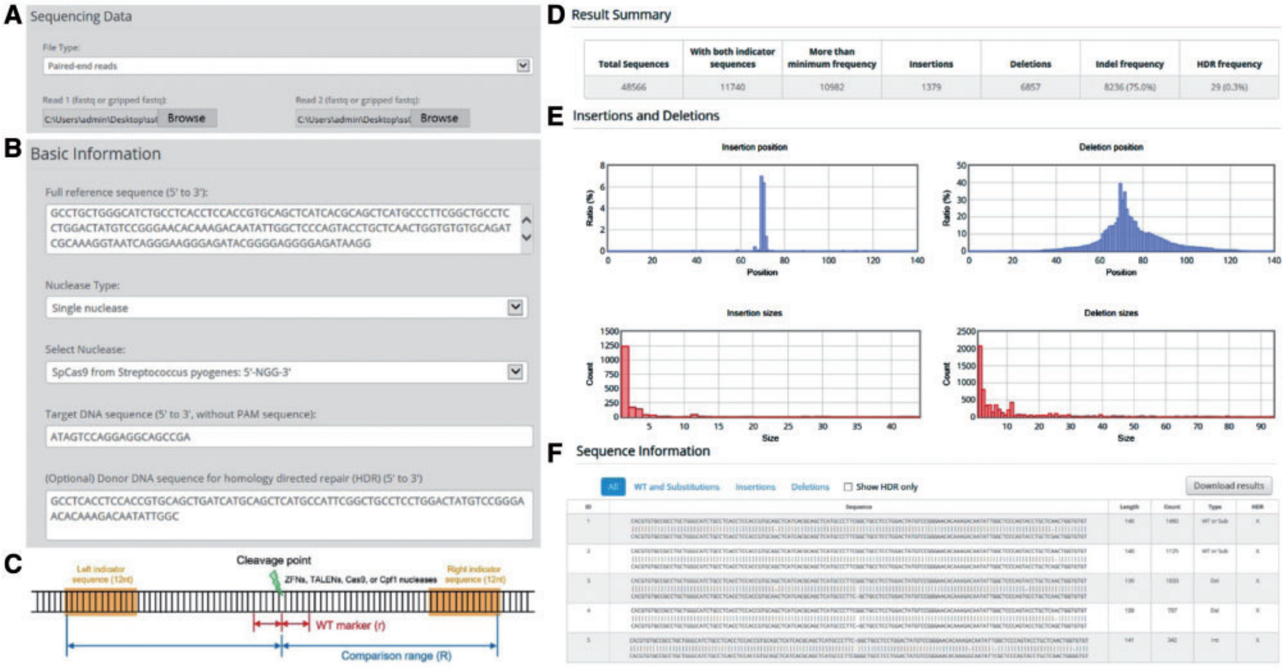

File Type: Paired-end reads

Read 1 (fastq or gzipped fastq): C:\Users\admin\Desktop\ss(

Read 2 (fastq or gzipped fastq): C:\Users\admin\Desktop\ss(

Full reference sequence (5' to 3'):
GCCTGCTGGGCATCTGCCTCACCTCCACCGTGCAGCTCATCACGCAGCTCATGCCCTTCGGCTGCCTC
CTGGACTATGTCCGGGAACACAAAGACAATATTGGCTCCCAGTACCTGCTCAACTGGTGTGTGCAGAT
CGCAAAGGTAATCAGGGAAGGGAGATACGGGGAGGGGAGATAAGG

Nuclease Type: Single nuclease

Select Nuclease: SpCas9 from Streptococcus pyogenes: 5'-NGG-3'

Target DNA sequence (5' to 3', without PAM sequence): ATAGTCCAGGAGGCAGCCGA

(Optional) Donor DNA sequence for homology directed repair (HDR) (5' to 3')
GCCTCACCTCCACCGTGCAGCTGATCATGCAGCTCATGCCATTCGGCTGCCTCCTGGACTATGTCCGGGA
ACACAAAGACAATATTGGC

| Total Sequences | With both indicator sequences | More than minimum frequency | Insertions | Deletions | Indel frequency | HDR frequency |
|---|---|---|---|---|---|---|
| 48566 | 11740 | 10982 | 1379 | 6857 | 8236 (75.0%) | 29 (0.3%) |

| ID | Sequence | Length | Count | Type | HDR |
|---|---|---|---|---|---|
| 1 | [illegible] | 140 | 1482 | WT or Sub | X |
| 2 | [illegible] | 140 | 1125 | WT or Sub | X |
| 3 | [illegible] | 139 | 1033 | Del | X |
| 4 | [illegible] | 139 | 797 | Del | X |
| 5 | [illegible] | 141 | 342 | Ins | X |

**Fig. 1.** Overview of Cas-Analyzer. **(A)** Uploading NGS data files. Single-end reads, paired-end reads, or already merged sequencing data are allowed. **(B)** Basic information about the query sequences are required for using Cas-Analyzer. **(C)** Indicators used in the analysis step. **(D)** The results are summarized as a table that includes the mutation count and frequency. **(E)** Insertions and deletions are also visualized as graphs. **(F)** All filtered sequences from the input data are aligned with the reference sequence

*Conflict of Interest*: none declared.

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