Using MGIEasy PCR Free library construction technology and DNBSEQ™ high-throughput sequencing technology to evaluate the editing efficiency of CRISPR/Cas9 gene editing and genome CRISPR/Cas9 library

Highlight

Simple and automated workflow

- Library construction can be completed within 3.9 hours. Compatible with automatic sample preparation systems to provide an automated workflow

No amplification error accumulation

- WGS PCR-free library preparation in combination with sequencing by the MGI DNBSEQ™ platform has no amplification error accumulation, resulting in better genome fidelity

Compatible with Multiple Species Types

- Compatible with human, animals, plants, bacteria, fungi etc., e.g. human (blood, saliva, fresh tissue), mice, rice, E.coli and metagenomics

Excellent variant performance

- The variant detection performance of FS PCR-free is similar to Covaris PCR-free, and better than traditional PCR, especially InDels

Introduction

In recent years, the CRISPR-Cas9 gene editing system and its derived single-base gene editing system have been widely used in scientific research and clinical treatment, however the huge difference in efficiency of the CRISPR system at different gene editing sites hinder its application of the system. therefore it is particularly important to verify the editing efficiency of different guide RNAs (gRNAs) at gene editing targets before experiments. There are many factors that affect the efficiency of gRNA editing, including the secondary structure of the gRNA itself, the delivery efficiency of the delivery system, the looseness of gRNA binding to the editing target site, the level of epigenetic modification of the editing target, the secondary structure, the mutation repair mode of the target cell and the probability of missing the target and so on. Sanger sequencing can detect the editing efficiency of candidate gRNAs with low throughput and "low depth". However, high-throughput sequencing technology is needed if you want to accurately and meticulously study the types and proportions of genotypes at editing sites and obtain more data, in addition, the genome-wide CRISPR/Cas9 library screening technology has been widely used. This technology can cover almost all known genes. It can be enriched forward or backward with specific screening markers, and then the gRNA region is amplified by PCR to get the enriched candidate gRNA data set, so as to screen for functional target genes, such as drug-resistant genes of cancer. Genome CRISPR/Cas9 high-throughput screening technology cannot do without the help of high-throughput sequencing technology.

The Lars Institute of Regenerative Medicine, BGI Institute of Life Sciences, Xiang Xi and others, relying on MGIEasy PCR free library and DNBSEQ sequencing platform, established a set of TRAP-seq platform for high-throughput detection of CRISPR/Cas9 gene editing efficiency, which realized the rapid production and research of large-scale gRNA gene editing map and efficiency in cells, and constructed the efficiency database of CRISPR/Cas9, single base editing (ABE7.10, CBE4) system in human cells, and through the deep machine learning of big data, the efficiency and genotype product prediction models of the three editors have been established, making the development and application of the CRISPR gene editing system faster and more accurate. In-depth and meticulous research on the edited genotype of CRISPR editing target sites by using MGIEasy

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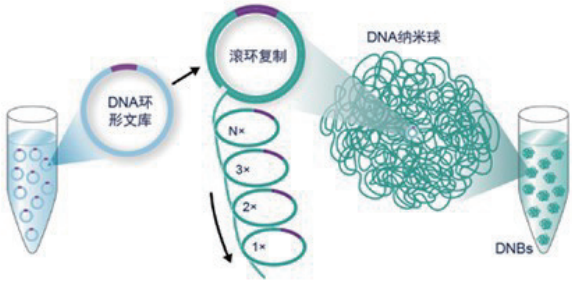
PCR Free library preparation and high-throughput sequencing strategy has become an indispensable technology platform in the CRISPR research field.

MGI provides packages such as MGIEasy PCR-Free series library preparation reagents and DNBSEQ™ high-throughput sequencing platform, etc. to provide users with a full range of products and support.



Massively parallel quantification of CRISPR editing in cells by TRAP-seq enables better design of Cas9, ABE, CBE gRNAs of high efficiency and accuracy

The lentivirus-based TRAP-seq system

The lentivirus-based system has four main features: (1) A human U6 promoter; (2) Golden-Gate Assembly (GGA) based cloning with a lac Z marker for precise and efficient insertion of an expression cassette; (3) A green fluorescent protein (GFP) marker for measuring viral titer and real-time tracking of viral delivery; (4) A puromycin selection gene for enrichment of stably transduced cells.

Artificial synthesized oligo sequence contains a gRNA expression cassette (20bp spacer + 82bp scaffold) and a 37bp surrogate target site, flanked by a 31bp GGA cloning site and PCR handles. （Fig.2）

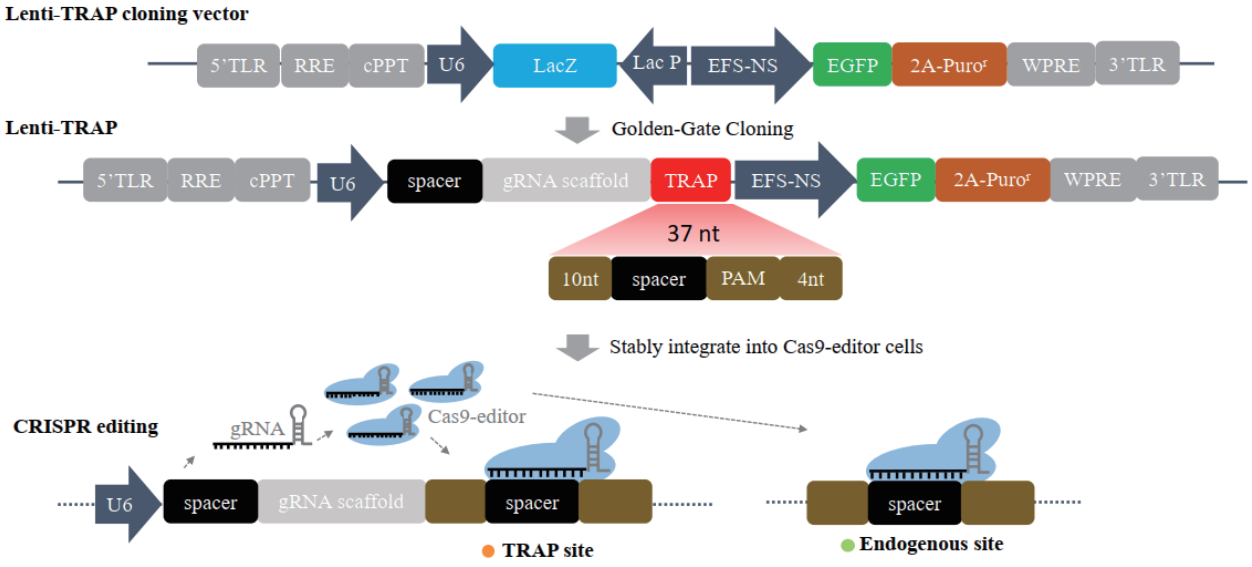


Figure 1. Schematic illustration of the lentivirus-based system

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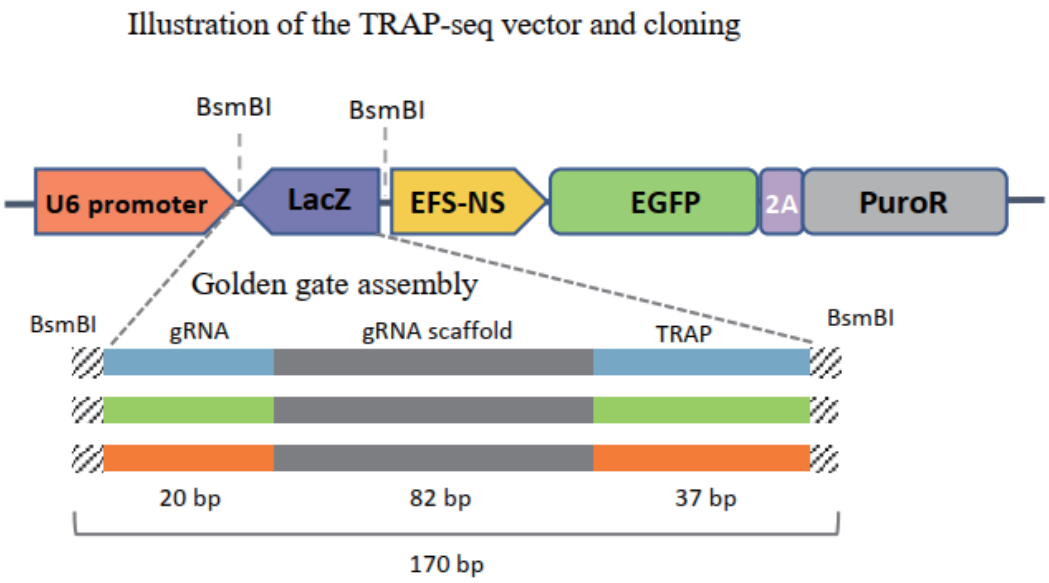

Figure 2. Schematic illustration of the TRAP-seq vector and cloning

Generation of 12K TRAP-seq lentiviral library

12K TRAP oligos were generated by microarray synthesis. The oligos were amplified with PCR and cloned into the lentivirus-based TRAP-seq vector system. Transformation was then carried out using home-made chemically competent DH5a cells. HEK293T cells were used for lentivirus package. As the pLenti-TRAP-seq vector expresses a EGFP gene, the functional titer of lentivirus prep was assayed by FCM.

12K TRAP-seq library lentivirus transduction, cell harvest and PCR amplicons for DNBSEQ

HEK293T-SpCas9, -ABE7.10 and -CBE4 cells and wild HEK293T cells were cultured for the 12K TRAP-seq lentivirus library transduction. Cells were harvested and stored in -20 ℃ freezer for further genomic DNA extraction. The procedures were showed in Fig. 3.

The genomic DNA was purified and subjected to PCR for amplification of the TRAP DNA with PCR handles. PCR-free library was prepared using MGIEasy FS PCR-free DNA library Prep kit following the manufacturer's instruction. All the samples were subjected to pair-ended 150 bp deep-sequencing on DNBSEQ platform.

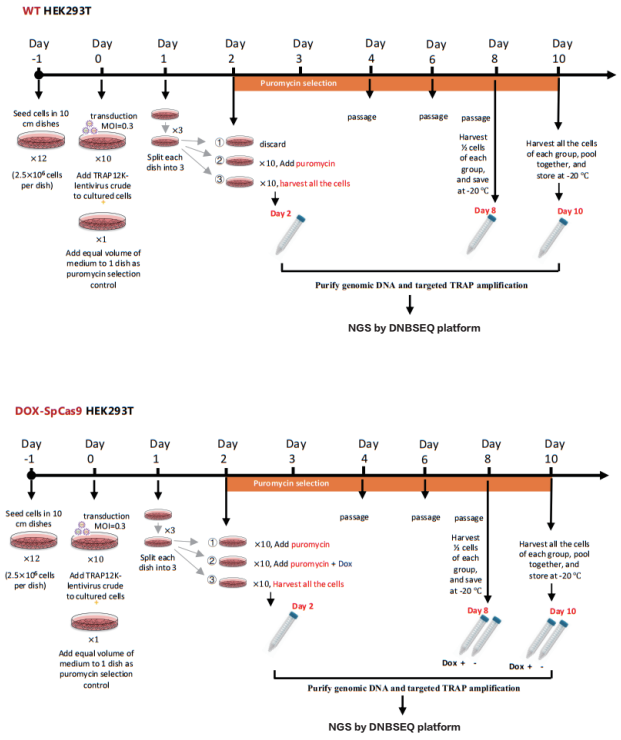

Figure 3. Schematic illustration of the experiment of cell culture and harvest

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Data Analysis of gene editing efficiency, outcomes, and character

These results support the notion that SpCas9 expression level and cultivation time affect gene editing efficiency. As expected, significantly higher editing efficiencies were achieved with high SpCas9 expression level and long cultivation time. （Fig.4a、Fig.4b、Fig.4c）

The optimal GC content and deltaG energy for gene editing is [50-70%] GC and [-5; -1] KJ/mol, respectively. Gene editing efficiency was affected by characterization of target's upstream and downstream sequences.

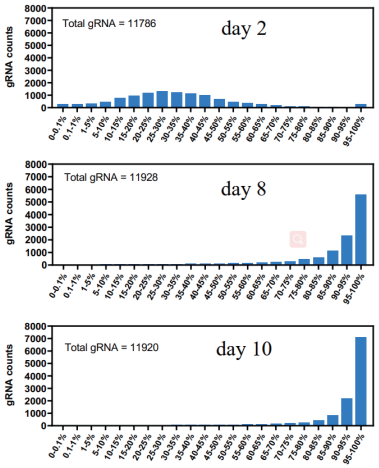

Figure 4a. Gene editing efficiency in Dox-induced HEK293T-SpCas9 cells with different cultivation time

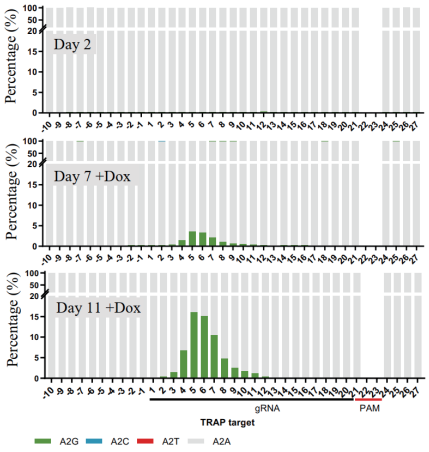

Figure 4b. Gene editing efficiency in Dox-induced HEK293T-ABE cells with different cultivation time

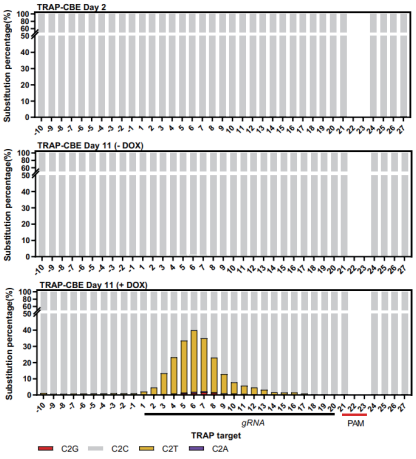

Figure 4c. Gene editing efficiency in Dox-induced HEK293T-CBE cells with different cultivation time

An improved machine learning model to predict SpCas9 efficiency

To further streamline the prediction of SpCas9 efficiency and the identification of nucleotide features important for gRNA activity, 80% of the 12K TRAP-seq gRNA efficiency data was used to train the GNL-Scorer - a machine learning algorism that previously developed based on the Bayesian Ridge regression (BRR) model and 2485 features. 20% of the 12K TRAP-seq gRNA efficiency data was used for validation. The accuracy of predicting the over gene editing efficiency is approximately 70%, better than other algorisms (Fig. 5).

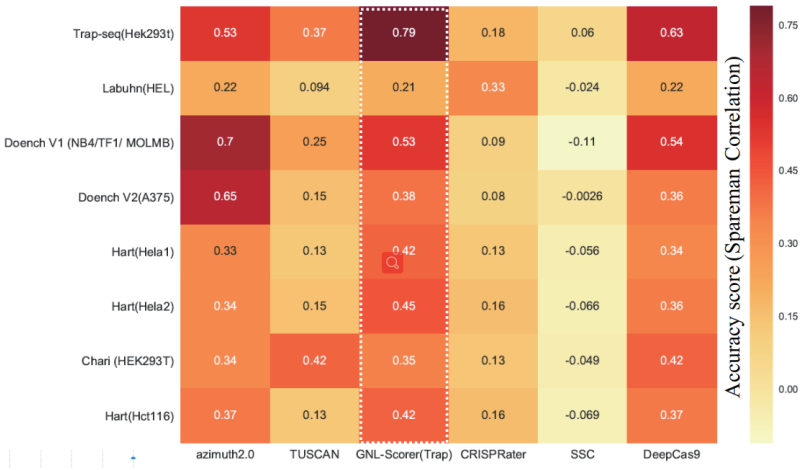

Figure 5. Comparison of SpCas9 gRNA efficacy predictions in a regression schema for various datasets and prediction models

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Conclusion

In the current application note, we introduced MGIEasy PCR Free library construction technology and MGI DNBSEQ™ high-throughput sequencing technology in gene editing database construction. The library construction method based on PCR free has better SNP and InDel accuracy; Combined with MGI DNBSEQ series genetic sequencer, it can achieve accurate detection of Crispr-Cas9 gene editing effects. High-throughput sequencing technology and lentiviral gene editing reporting system can quickly and large-scale study the efficiency and results of gRNA gene editing in vivo, so as to establish a complete gRNA gene editing database to provide for the development and application of the Crispr-Cas9 gene editing system guide.

Order information

Product Name	Configuration	Part Number
Genetic Sequencer DNBSEQ-G50RS*	/	900-000354-00
Genetic Sequencer DNBSEQ-G400RS*	/	900-000170-00
Genetic Sequencer DNBSEQ-T7RS*	/	900-000128-00
MGIEasy PCR-Free Library Prep Set	16 RXN	1000013452
MGIEasy PCR-Free Library Prep Set	96 RXN	1000013453
MGIEasy FS PCR-Free Library Prep Set	16 RXN	1000013454
MGIEasy FS PCR-Free Library Prep Set	96 RXN	1000013455

Reference

1. Xi Xiang, Kunli Qu, Xue Liang, Xiaoguang Pan, etc. Massively parallel quantification of CRISPR editing in cells by TRAP-seq enables better design of Cas9, ABE, CBE gRNAs of high efficiency and accuracy.
bioRxiv 2020.05.20.103614; doi: https://doi.org/10.1101/2020.05.20.103614

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