KOnezumi: a web application for automating gene disruption strategies to generate knockout mice

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Applications Note

Genome analysis

KOnezumi: a web application for automating gene disruption strategies to generate knockout mice

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Abstract

Summary: Although gene editing using the CRISPR/Cas9 system enables the rapid generation of knockout mice, constructing an optimal gene disruption strategy is still labourious. Here, we propose KOnezumi, a simple and user-friendly web application, for use in automating the design of knockout strategies for multiple genes. Users only need to input gene symbols, and then KOnezumi returns target exons, gRNA candidates to delete the target exons, genotyping PCR primers, nucleotide sequences of the target exons and coding sequences of expected deletion products. KOnezumi enables users to easily and rapidly apply a rational strategy to accelerate the generation of KO mice using the CRISPR/Cas9 system.

Availability and implementation: This web application is freely available at http://www.md.tsukuba.ac.jp/LabAnimalResCNT/KOanimals/konezumi.html.

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

1 Introduction

Knockout (KO) mouse generation represents a critical tool to investigate the functions of a target gene *in vivo*. Recently, the CRISPR/Cas9 system has become a widely used approach to generate KO mice because of its simplicity and applicability. It enables the generation of approximately half of founder mice that have biallelic mutations at the target locus, indicating that it is possible to establish KO mice within a few months (Mashiko *et al.*, 2013; Mizuno *et al.*, 2014). Therefore, screening the functions of multiple genes *in vivo* using CRISPR KO mice is now feasible.

However, constructing a gene target strategy is still a time consuming and labourious process. Although many software and web tools are available that assist in specific tasks, including identifying target exons, designing gRNAs to disrupt genes of interest and selecting genotyping PCR primers, users need to use each tool separately, and some of the tools require programming skills. These limitations can be bottlenecks to designing construct strategies, especially when aiming to target multiple genes.

Here, we present KOnezumi, a web tool that automates gene disruption designs. Users only need to input gene symbols, and then KOnezumi rapidly outputs all required information to generate KO mice.

2 Materials and methods

2.1 Overview

KOnezumi accepts multiple inputs of the MGI gene symbol (Fig. 1A). After submitting inputs, KOnezumi instantly returns target exons, candidates of gRNAs, PCR primers, nucleotide sequences of target exons and sequences of deleted transcripts (Fig. 1B–F). Figure 1B shows a general gene disruption strategy, including schema of gene structures, deletion sizes and PCR product sizes. KOnezumi also outputs gRNA and PCR primer candidates to remove target exons and to check genotypes, respectively (Fig. 1C and D). Furthermore, KOnezumi provides nucleotide sequences of target exons and deleted transcripts (Fig. 1E and F).

Fig. 1. Screen captures from the KOnezumi website. (**A**) Top page. The server accepts single or multiple MGI gene symbols as inputs. (**B–F**) Typical output of KOnezumi. (B) Overview of target gene locus and gene information. (C) A list of CRISPR/Cas9 gRNA candidates. (D) A list of genotyping PCR primer candidates. (E) Nucleotide sequences of target exons. (F) Protein-coding sequences of deletion transcripts. Frameshift codons, PTC and the last exon are highlighted as green, red and light purple, respectively. (Color version of this figure is available at *Bioinformatics* online.)

All data and tools used in KOnezumi are listed in the Supplementary Methods.

2.2 Designable genes and target exons

In total, 12 374 genes are currently designable. These genes contain critical exons, which the International Mouse Phenotyping Consortium (IMPC) defined as common exons to all transcript variants, and the deletion of these exons will disrupt more than fifty percent of the protein-coding sequence by frameshift mutation (Skarnes *et al.*, 2011).

2.3 CRISPR gRNA candidates

KOnezumi outputs gRNA candidates located within 200 bp of each flanking sequence of target exons, which are named gRNA-L (left) and gRNA-R (right). The applicable Cas9 enzyme originates from S. pyogenes (PAM: NGG). A schematic explanation of gRNA allocation is provided in the Supplementary Figure S1.

Each CRISPR target site was scored by MIT specificity (Hsu *et al.*, 2013) and predicted cutting efficiency (Doench *et al.*, 2016; Moreno-Mateos *et al.*, 2015) using CRISPOR (Concordet and Haeussler, 2018). The gRNA candidates are ranked by their high specificity and efficiency based on their scores.

2.4 Genotyping PCR primer candidates

PCR primers are allocated to the left genomic region of the gRNA-L (Primer-L; left), the deleted region (Primer-M; middle) and the right region of the gRNA-R (Primer-R; right). Gel electrophoresis will display a larger deletion band than wild-type band. An explanation of primer allocation is in the Supplementary Figure S1.

We utilized Primer3 (Koressaar and Remm, 2007; Untergasser *et al.*, 2012) with default parameters to design primers. Next, we mapped every candidate primer to the mm9 genome using Bowtie (Langmead *et al.*, 2009), allowing 0, 1 or 2 mismatches or gaps in the alignment and counting the numbers of mapped sequences to score their sequence specificity.

2.5 Nucleotide sequences of target exons and protein-coding sequences (CDSs) of deletion transcripts

The goal of gene deletion is to confirm that target sequences are successfully removed and that deleted transcripts are not translated into proteins. KOnezumi outputs sequences of target exons and CDSs of expected deletion products. The sequences of the target exons can be applicable to confirm removal by DNA sequencing. Furthermore, when deleted transcripts will acquire premature termination codon (PTC), KOnezumi reports the distance between PTC and last exon–exon junction. Users can expect the aberrant transcripts will be degraded by Nonsense-mediated mRNA decay (NMD) when the PTC will locate more than 50–55 nucleotides upstream of the last exon–exon junction (Maquat, 2004).

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