

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).



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