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Evaluation of non-specific CRISPR/Cas9 activity in a yeast model

Andrey R. Shumega [1], Elena I. Stepchenkova [2], Sergei G. Inge-Vechtomov [2]

[1] Saint Petersburg State University, Saint Petersburg, Russia;
[2] Vavilov Institute of General Genetics, Saint Petersburg, Russia

CRISPR/Cas9-based genome editing systems are widely used for genetic modification of different organisms. One of the drawbacks of CRISPR/Cas9 methods is the non-specific activity of Cas9, which can lead to accumulation of unwanted mutations in the edited genome [1]. Therefore, the development of *in vivo* models for high-throughput analysis of factors influencing the frequency of mutagenesis associated with the use of CRISPR/Cas9 technologies is a relevant task. Yeast *Saccharomyces cerevisiae* is a convenient object for developing such models [2].

Here we represent a yeast *in vivo* model that allows us to evaluate the effects of nucleotide sequence of the protospacer adjacent motif (PAM) and the guide RNA (gRNA) on the efficiency of binding between the gRNA/Cas9 complex and the target sequence in the genome. Since the Cas9 activity is lethal in cells lacking a donor sequence for homologous repair of double-strand breaks caused by this endonuclease, in the proposed test-system, the reduced efficiency of transformation by a plasmid encoding Cas9 and various gRNA variants reflects the efficiency of recognition of the target gene by the gRNA/Cas9 complex.

To study the influence of different PAM variants, with a consensus of NGG, on CRISPR/Cas9 activity, we obtained four isogenic strains that differ in their PAM sequence (AGG, TGG, CGG, GGG) in the codon 202 of the chromosomal copy of the reporter gene *URA3*. To evaluate the effect of incomplete matching between gRNA and the target site sequences, we propose using a series of plasmids based on the pML107 vector, encoding Cas9 and one of the 20 possible gRNA variants with single nucleotide substitutions at each of the 20 positions. The results obtained so far indicate the potential of the proposed approach.

Keywords: *Saccharomyces cerevisiae*; CRISPR/Cas9; PAM.

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AUTHORS' INFO

Andrey R. Shumega, Saint Petersburg State University, Saint Petersburg, Russia; ORCID: 0000-0001-7880-4138; eLibrary SPIN: 9332-1552; e-mail: shumega84@mail.ru

Elena I. Stepchenkova, PhD, Head, Laboratory of Mutagenesis and Genetic Toxicology; Assistant, Department of Genetics and Biotechnology; Vavilov Institute of General Genetics, Saint Petersburg, Russia; ORCID: 0000-0002-5854-8701; eLibrary SPIN: 9121-7483; e-mail: stepchenkova@gmail.com

Sergei G. Inge-Vechtomov, Cand. Sci (Biol.), PhD, Professor, Academician of RAS; Vavilov Institute of General Genetics, Saint Petersburg, Russia; ORCID: 0000-0002-2832-6825; eLibrary SPIN: 3743-7626; e-mail: ingevechtomov@gmail.com