

THE 67<sup>TH</sup> INTERNATIONAL



# OPEN READINGS

CONFERENCE FOR STUDENTS OF PHYSICS AND NATURAL SCIENCES

**BOOK OF  
ABSTRACTS** | **2024**



Vilnius  
University

VILNIUS UNIVERSITY PRESS

Editors:

Martynas Keršys  
Rimantas Naina  
Vincentas Adomaitis  
Emilijus Maskvytis

Cover and Interior Design:

Goda Grybauskaitė

Vilnius University Press  
9 Saulėtekio Av., III Building, LT-10222 Vilnius  
info@leidykla.vu.lt, [www.leidykla.vu.lt/en/](http://www.leidykla.vu.lt/en/)  
[www.knygynas.vu.lt](http://www.knygynas.vu.lt), [www.journals.vu.lt](http://www.journals.vu.lt)

Bibliographic information is available  
on the Lithuanian Integral Library Information System (LIBIS) portal [www.ibiblioteka.lt](http://www.ibiblioteka.lt)  
ISBN 978-609-07-1051-7 (PDF)

© Vilnius University, 2024

## EXPLORING THE IS200/IS605 FAMILY TRANSPOSABLE ELEMENTS FOR NOVEL GENOME EDITING TOOLS

Gytis Druteika<sup>1</sup>, Tautvydas Karvelis<sup>1</sup>

<sup>1</sup>Vilnius University, Life Sciences Center  
[gytis.druteika@gmc.vu.lt](mailto:gytis.druteika@gmc.vu.lt)

Mobile genetic elements (MGE), such as transposons, bacteriophages, plasmids, and insertion sequences (IS), were historically called 'junk DNA' of the prokaryotic and eukaryotic genomes. Nowadays, the significance of MGEs is well understood since they are the driving force of evolution, allowing organisms to rapidly adapt to changing environmental conditions [1]. One of the most widely distributed groups of insertion sequences belongs to the IS200/IS605 family. Structurally, these transposons are not very complex, as the IS200/IS605 family sequences only encode proteins required for transposition: TnpA and TnpB. TnpA is a transposase responsible for the mobility of IS, while TnpB turned out to be an evolutionary ancestor of CRISPR-Cas effector nucleases, which are widely used in gene editing experiments [2, 3]. However, the large size of commonly used Cas9 and Cas12 effectors complicates their delivery to the target cells. Recently, the transposon-encoded TnpB was applied as a programmable tool for gene editing in human cell cultures. Considering its miniature size, compared to Cas9 or Cas12 nucleases, TnpB has promising properties for broad application in various gene editing assays [4, 5]. Nevertheless, due to the high expectations for efficiency, specificity, and the requirement for nucleases to target a wide range of genes, there is a great need for discovering novel programmable DNA-targeting proteins. Since TnpB-encoding insertion sequences are extremely widespread, this study aimed to characterize a set of TnpB homologs. Bioinformatic analysis and sequence alignments enabled us to select diverse representatives from several groups of TnpB homologs. By using biochemical assays, we determined the requirements for the TAM sequence, which is recognized by the TnpB protein, and for the guide RNA. This study contributes to current knowledge about diverse TnpB proteins and will be valuable for further characterization of these potential gene editing tools.

- 
- [1] Krupovic, M., Shmakov, S., Makarova, K. S., Forterre, P. & Koonin, E. V. Recent Mobility of Casposons, Self-Synthesizing Transposons at the Origin of the CRISPR-Cas Immunity. *Genome Biol Evol* 8, 375–386 (2016).
  - [2] Sadler, M., R. Mormile, M. & L. Frank, R. Characterization of the IS200/IS605 Insertion Sequence Family in *Halanaerobium Hydrogeniformans*. *Genes (Basel)* 11, (2020).
  - [3] Kapitonov, V. V., Makarova, K. S. & Koonin, E. V. ISC, a Novel Group of Bacterial and Archaeal DNA Transposons That Encode Cas9 Homologs. *J. Bacteriol.* 198, 797–807 (2016).
  - [4] Karvelis, T. et al. Transposon-associated TnpB is a programmable RNA-guided DNA endonuclease. *Nature* 599, 692–696 (2021).
  - [5] Altae-Tran, H. et al. The widespread IS200/IS605 transposon family encodes diverse programmable RNA-guided endonucleases. *Science* 374, 57–65 (2021).