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🌐 **Protocols for assembly of a serine integrase-based platform for functional validation of genetic switch controllers in eukaryotic cells**

📖 [PLOS One](#)

✓ Peer-reviewed method

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Marco A. de Oliveira^{1,2}, Lilian H. Florentino^{1,2,3}, Thais T. Sales^{1,2,3}, Rayane N. Lima^{2,3}, Luciana R. C. Barros⁴, Cintia G. Limia⁵, Mariana S. M. Almeida^{2,3}, Maria L. Robledo⁵, Leila M. G. Barros^{2,3}, Eduardo O. Melo^{2,3}, Daniela M. Bittencourt^{2,3}, Stevens K. Rehen^{6,7}, Martín . Bonamino^{8,9}, Elibio Rech^{2,3}

¹Department of Cell Biology, Institute of Biological Science, University of Brasília, Brasília, Distrito Federal, Brazil;

²National Institute of Science and Technology in Synthetic Biology (INCT BioSyn), Brasília, Distrito Federal, Brazil;

³Embrapa Genetic Resources and Biotechnology, Brasília, Distrito Federal, Brazil;

⁴Center for Translational Research in Oncology, Instituto do Câncer do Estado de São Paulo, Hospital das Clínicas da Faculdade de Medicina de Universidade de São Paulo, São Paulo, São Paulo, Brazil;

⁵Molecular Carcinogenesis Program, Research Coordination, National Cancer Institute (INCA), Rio de Janeiro, Rio de Janeiro, Brazil;

⁶D'Or Institute for Research and Education (IDOR), Rio de Janeiro, Rio de Janeiro, Brazil;

⁷Institute of Biomedical Sciences, Federal University of Rio de Janeiro, Rio de Janeiro, Rio de Janeiro, Brazil;

⁸Cell and Gene Therapy Program, Research Coordination, National Cancer Institute (INCA), Rio de Janeiro, Rio de Janeiro, Brazil;

⁹Vice-Presidency of Research and Biological Collections (VPPCB), FIOCRUZ – Oswaldo Cruz Foundation Institute, Rio de Janeiro, Rio de Janeiro, Brazil

Elibio Rech: corresponding author: elibio.rech@embrapa.br;

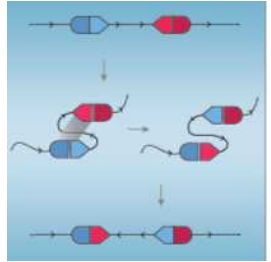
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Marco Oliveira

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Protocol status: Working

We use this collection and it's working

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Keywords: genetic switch controllers in eukaryotic cell, gene editors in eukaryote, serine integrase, important biotechnological value as genetic editing tool, gene editor, genetic editing tool, functional screening of serine, genetic switch controller, genetic material into the genome, based genetic switch, use in prokaryotic cell, genetic switch, complex multifunctional circuits in eukaryotic cell, specific recombinase, gene expression, prokaryotic cell, genome, gene expression by directional inversion, eukaryotic cell, bacteriophage, genetic regulation, endogenous molecular machinery, integrase, platform for the assembly, complex organism, dna sequence, dna sequences in different way, eukaryote, important biotechnological value, genetic material, dna sequence orientation, regulatory component, insertion, platform for functional validation, dna delivery

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Spotlight Video

The video below is a supplement with extra context and tips, as part of the protocols.io Spotlight series, featuring conversations with protocol authors.

<https://www.youtube.com/embed/AvrPg2hVg0?si=OEUw0BfM2ZyyPnCF>

Abstract

This is a collection of protocols for assembly of a serine integrase-based platform for functional validation of genetic switch controllers in eukaryotic cells in human, animal and plants. Serine integrases (Ints) are a family of site-specific recombinases (SSRs) encoded by some bacteriophages to integrate their genetic material into the genome of a host. Their ability to rearrange DNA sequences in different ways including inversion, excision, or insertion with no help from endogenous molecular machinery, confers important biotechnological value as genetic editing tools with high host plasticity. Despite advances in their use in prokaryotic cells, only a few Ints are currently used as gene editors in eukaryotes, partly due to the functional loss and cytotoxicity presented by some candidates in more complex organisms. To help expand the number of Ints available for the assembly of more complex multifunctional circuits in eukaryotic cells, this protocol describes a platform for the assembly and functional screening of serine-integrase-based genetic switches designed to control gene expression by directional inversions of DNA sequence orientation. The system consists of two sets of plasmids, an effector module and a reporter module, both sets assembled with regulatory components (as promoter and terminator regions) appropriate for expression in mammals, including humans, and plants. The complete method involves plasmid design, DNA delivery, testing and both molecular and phenotypical assessment of results. This platform presents a suitable workflow for the identification and functional validation of new tools for the genetic regulation and reprogramming of organisms with importance in different fields, from medical applications to crop enhancement, as shown by the initial results obtained. This protocol can be completed in 4 weeks for mammalian cells or up to 8 weeks for plant cells, considering cell culture or plant growth time.

Attachments



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83KB

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Attachments



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Files

 SEARCH

Protocol

NAME

Protocol for assembly of a serine integrase-based platform for functional validation of genetic switch controllers in eukaryotic cells-Human

VERSION 1

CREATED BY



Marco Oliveira
 INCT BioSyn

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Protocol

NAME

Protocol for assembly of a serine integrase-based platform for functional validation of genetic switch controllers in eukaryotic cells-Animal

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 INCT BioSyn

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Protocol

NAME

Protocol for assembly of a serine integrase-based platform for functional validation of genetic switch controllers in eukaryotic cells-Plant

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MOLECULAR ANALYSES

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