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CENTER FOR ADVANCED BIOENERGY AND BIOPRODUCTS INNOVATION

CRISPR-COPIES: An in silico Platform for Discovery of Neutral Integration Sites for CRISPR/Cas-Facilitated Gene Integration

Background/Objective

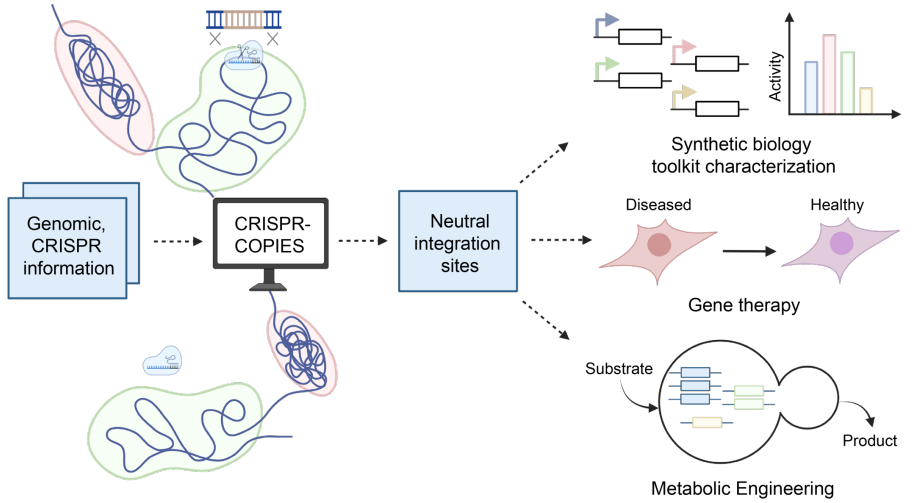
The CRISPR/Cas system is a powerful tool for genome editing that allows precise and targeted edits. However, selecting a suitable location on a chromosome to integrate genes is challenging. This process involves manual screening of sites for multiple criteria such as CRISPR/Cas-mediated factors, genetic stability and gene expression, followed by extensive characterization efforts that are time and resource intensive. To address these challenges, we developed CRISPR-COPIES, a **CO**mputational **P**ipeline for the **I**dentification of CRISPR/Cas-facilitated int**E**gration **S**ites.

Approach

CRISPR-COPIES was built using design rules, various on-target models, and ScaNN, a model for approximate nearest neighbor search, to design pooled gRNA library for screening intragenic sites with high on-target and low off-target activity. The tool identified neutral integration sites in three diverse species: *Saccharomyces cerevisiae*, *Cupriavidus necator*, and HEK293T cell line, which were validated *in vivo*. These sites were utilized to engineer a yeast strain with increased 5-aminolevulinic acid production, a valuable chemical with applications in agriculture and the food industry. Additionally, a user-friendly web interface for CRISPR-COPIES was developed.

Results

CRISPR-COPIES identifies genome-wide neutral integration sites for any organism and CRISPR/Cas system, typically within two to three minutes for most bacterial and fungal genomes. The characterized sites outperform previously reported sites in terms of transgene expression or stability.



Overview and application of CRISPR-COPIES in the field of biotechnology.

Significance/Impacts

CRISPR-COPIES is a publicly available tool (biofoundry.web.illinois.edu/copies/) that simplifies and accelerates strain construction to produce valuable chemicals and to engineer bioenergy crops for higher yields, pest resistance, and/or environmental resilience.

Boob, et al. 2024. "CRISPR-COPIES: An *in silico* Platform for Discovery of Neutral Integration Sites for CRISPR/Cas-Facilitated Gene Integration." *Nucleic Acids Research*. DOI: 10.1093/nar/gkae062.