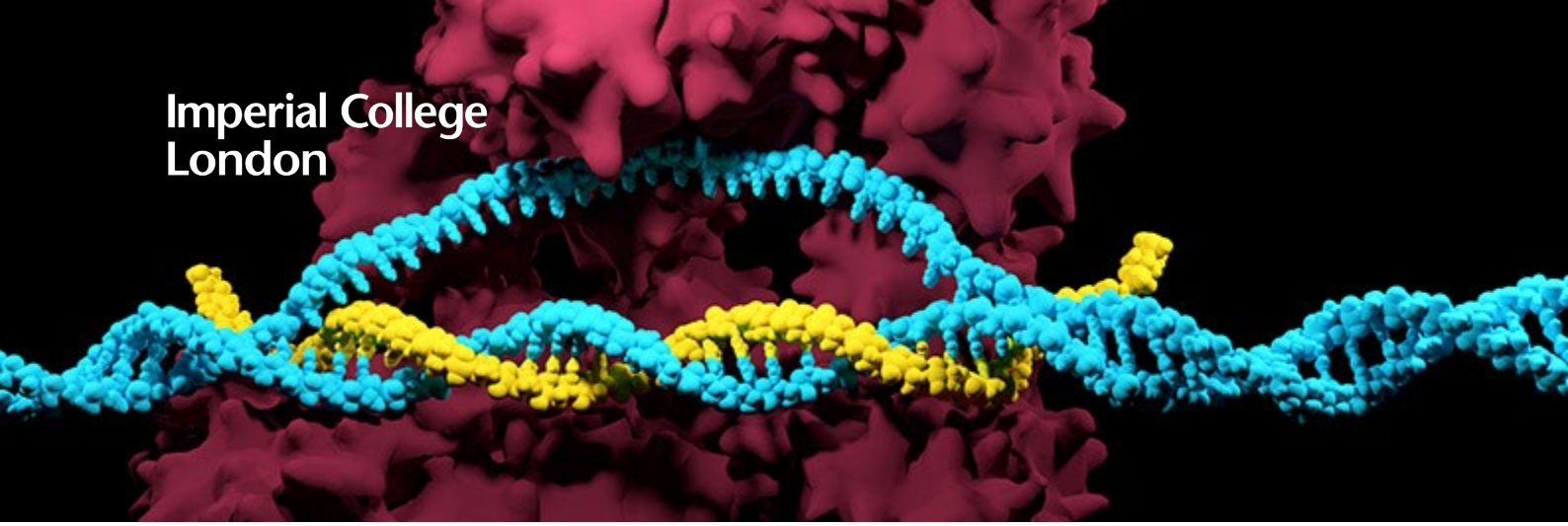

Imperial College London

An Inducible, multiplexed CRISPR system

A CRISPR-based platform for inducibly up- and down-regulating up to 24 genes in the yeast, Saccharomyces cerevisiae.

Proposed use

This innovation addresses concerns related to potential disruptions in gene expression during the transformation process, particularly for genes critical to growth or essential processes. The proposed approach is anticipated to empower researchers to target genes within the Saccharomyces cerevisiae genome without undesirable effects on their expression during transformation and handling without an inducer. This breakthrough opens avenues for studying essential genes and combinations thereof, potentially uncovering synthetic lethality relationships.

Problem addressed

Slow genetic manipulation and low multiplexity are significant limitations in current metabolic engineering methods. These limitations hinder the ability to efficiently design and optimize microbial strains for various biotechnological applications, including the production of biofuels, chemicals, pharmaceuticals, and other valuable compounds.

Technology overview

This is a novel reversible array repression technique that enables the encoding of multiple guide RNAs (gRNAs) on a single transcript with precise control over their expression. The utilization of mutTetR ensures robust repression, effectively silencing the array when in the off state. Conversely, the activation of TetA leads to strong expression, facilitating transcription of the entire array in the on state. This dynamic regulation results in a significant fold-change in the expression of target genes. The strategic silencing of the array when not in use is expected to streamline the process of transforming CRISPRai constructs, promoting better growth in the absence of the inducer.

Intellectual property information

Patent: WO 2023/148491

Paper: https://www.nature.com/articles/s41467-022-32603-7

Inventor information

Dr. Rodrigo Ledesma-Amaro Reader in Synthetic Biology, Faculty of Engineering, Department of Bioengineering

Benefits

- Allow inducibility with no leakiness for the first time
- Inducible up- and down-regulation of up to 24 genes
- Quick and easy design and assembly of gRNA arrays
- Optimised for low burden
- Entire system delivered on single construct, compatible with all common lab strains
- Available with 10 selectable markers

Diana Yin

Industry Partnerships and Commercialisation Executive - Engineering

Email: d.yin@imperial.ac.uk

Technology reference: 11009