Biocontainment Strategies for Genetically-Modified Yeasts

Shamlan M. S. Reshamwala, PhD

PI, iGEM 2023 Team ICT-Mumbai
Institute of Chemical Technology, Mumbai

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Strategy 1: Nutritional auxotrophy

Auxotrophic markers have been traditionally used to clone genes in *Saccharomyces cerevisiae*

- *ura3*
- *trp1*
- ...

Auxotrophies can also be used as a biocontainment strategy

Nutritional auxotrophies can be circumvented by scavenging metabolites from the environment

Strategy 2: Engineering dependency on an orthogonal molecule – conditional expression of essential genes

Cells can be engineered to express an essential gene under control of a promoter that is induced by a molecule that is not expected to occur in the natural environment

For example, histone genes have been placed under control of an estradiol-inducible promoter*

Inactivating mutations may give rise to escape mutants that are no longer ‘addicted’

* *Proc Natl Acad Sci USA* 112(6):1803–1808 (2015)

Strategy 3: Engineering dependency on an orthogonal molecule – conditional stability of essential proteins

Here, essential proteins are engineered to be dependent on small molecule ligands for correct folding and activity.

In *S. cerevisiae*, a destabilizing domain degron, which can be stabilized by estradiol, has been added to essential proteins, leading to estradiol-dependent growth[†]

[†] bioRxiv 2022.11.24.517818

Strategy 4: Engineering sensitivity to a commonly occurring molecule

Cells can be engineered to be highly sensitive to molecules ubiquitous in the environment

For example, *S. cerevisiae* lacking both native fluoride exporter genes (*fex1/2*) is highly sensitive to fluoride[‡]

‡ *Nat Commun* 11(1):5459 (2020)

Strategy 5: Synthetic auxotrophy

Non-canonical amino acids (ncAA), xeno-nucleic acids (XNA) and non-natural nitrogen bases-dependent synthetic auxotrophies are excellent biocontainment strategies

Orthogonal translation systems, comprising of aminoacyl-tRNA synthetases that incorporate ncAA at repurposed stop codons, have been reported in *S. cerevisiae*[§]

[§] *ACS Synth Biol* 11(7):2284–2299 (2022)

Strategy 6: Kill switches

Kill switches are genetic circuits which, when expressed or repressed, lead to cell death

Cell death can be triggered by expression of nucleases[¶] or toxin-antitoxin systems[‖]

[¶] *Yeast* 22(3):203–212 (2005)
[‖] *Appl Environ Microbiol* 66(12):5524–5526 (2000)

Implementing appropriate safeguards to prevent escape

Escape frequency has to be determined for any biocontainment strategy implemented in GM organisms

Escape frequency is a function of genetic drift, environmental supplementation, horizontal gene transfer and evolutionary processes

According to NIH guidelines,** escape frequency should be less than 1 in 10^8

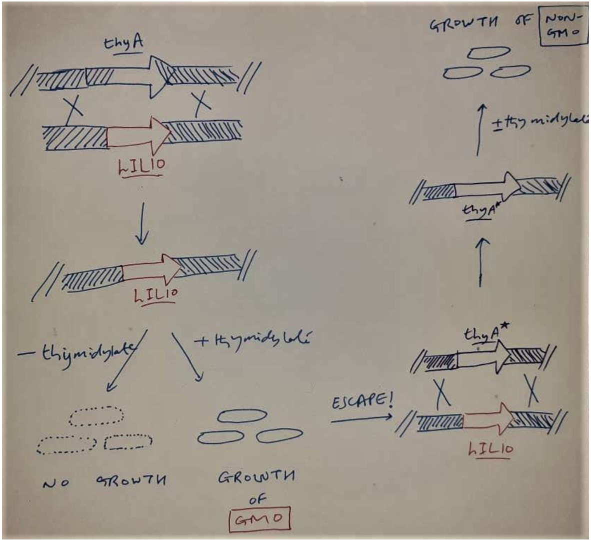
** *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules*, April 2019

Use of multiple biocontainment strategies can reduce escape frequencies

A double mutation in *Saccharomyces boulardii* in the *THI6* and *BTS1* genes, causing thiamine auxotrophy and increased sensitivity to cold, has been recently reported[††]

[††] *Front Bioeng Biotechnol* 11:1136095 (2023)

An elegant biocontainment strategy used in *Lactococcus lactis*‡‡



‡‡ *Nat Biotechnol* 21(7):785–789 (2003)

Thank you!