# Identifying Novel Membrane Proteins for Bioproduction

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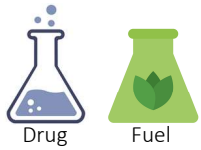
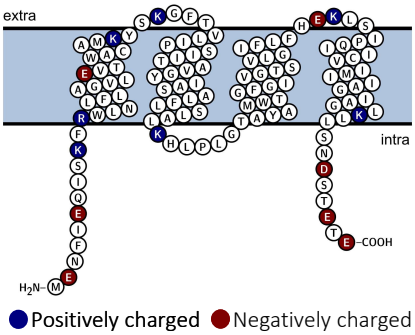
## Small Multidrug Resistance transporters (SMRs)

- Model microbes are used for bioproduction of drug-like molecules and fuels, but these target molecules can be toxic to cells.
- Using membrane proteins that confer resistance to toxic compounds can make bioproduction more efficient.




- SMRs are membrane proteins that function to expel drug-like molecules from cells and contribute to bacterial antibiotic resistance.
- A potential SMR was found in non-model anaerobic fungus *Neocallimastix californiae.* **We aim to determine if the SMR can function in the model organisms *Escherichia coli* and yeast, *Saccharomyces cerevisiae*.**

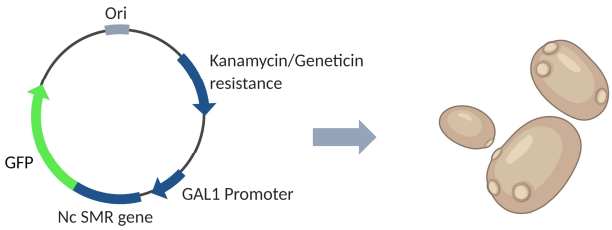



Predicted topology of the *N. californiae* SMR.

- 4 transmembrane helices
- charged loops
- glutamate in the first transmembrane helix

## The fungal SMR is introduced into model organisms

### Yeast transformation

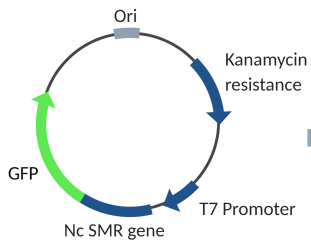



The *N. californiae* SMR gene was cloned on a pITy plasmid vector and tagged with GFP.



The plasmid was transformed into yeast strains BJ5465 and BJ5464.

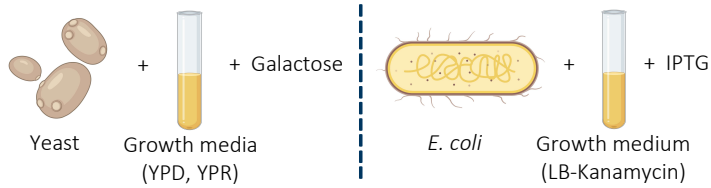
### *E. coli* transformation




The *N. californiae* SMR gene was cloned on a pWaldo plasmid vector and tagged with GFP.

The plasmid was transformed into *E. coli* strain DH5α and then BL21(DE3).

### Inducing gene expression




Growth in media was monitored using optical density at 600 nm ($OD_{600}$).

## The fungal SMR gene can be expressed in model organisms

SDS polyacrylamide gel electrophoresis (SDS-PAGE) confirmed the presence of the SMR by detecting the GFP tag fused to the SMR gene.

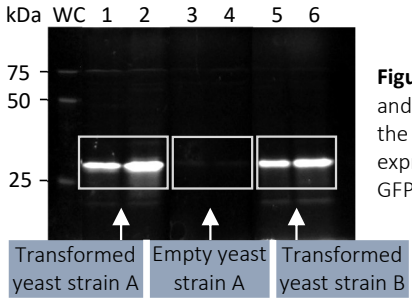



**Figure 1.** Yeast strains BJ5465 (A) and BJ5464 (B) transformed with the Nc SMR show SMR gene expression via SDS-PAGE in-gel GFP fluorescence.

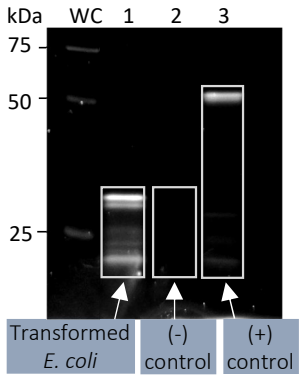
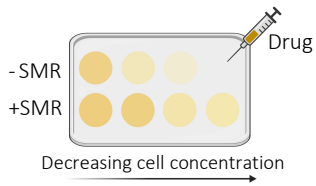



**Figure 2.** *E. coli* strain BL21(DE3) transformed with the Nc SMR shows SMR gene expression via SDS-PAGE in-gel GFP fluorescence.

WC=WesternC Protein Standard

## Conclusions and future work

- **We expressed the *N. californiae* SMR in the model organisms *S. cerevisiae* and *E. coli*.**
- We are testing whether the SMR increases the tolerance of *S. cerevisiae* and *E. coli* to drug-like compounds to determine its function.




- A functional SMR could be applied to engineer model microbes for improved bioproduction by increasing tolerance to toxic products.

## Acknowledgements

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