Removal of Endogenous Esterase Expression in *S.cerevisiae*

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2014 Transfer-to-Excellence Research Experiences for Undergraduates Program (TTE REU Program)

Abstract

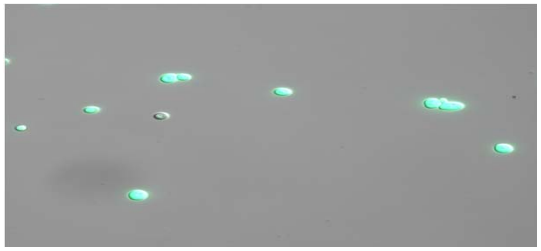
Yeast, *Saccharomyces cerevisiae*, has a potential to be engineered as a reactor for proteins and chemicals. We are studying the role of esterases in its cells. To do this we attempted to create a yeast strain with no expression of its six esterases. We used the new genetic engineering technologies of Golden Gate and CRISPR/Cas9 to remove, or "knock-out", esterase expression.

Motivation

- Industrial production of chemicals, drugs and fuel is carbon intensive and bad for the Earth
- Many advanced chemical reactions happen in living cells
- Living cell have the capacity to be clean, self-replicating factories that run on sugar instead of fossil fuels

Application

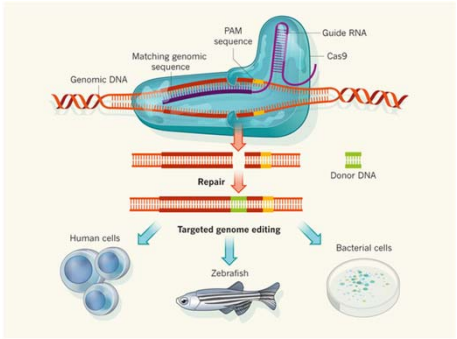
- Fluorescein diacetate(FDA) is a compound which fluoresces and changes from non-polar to polar when reacted upon by an esterase, this means it glows and stays where reacted in the cell.
- We used FDA to show background activity before and after esterase expression removal("knockout")



Process

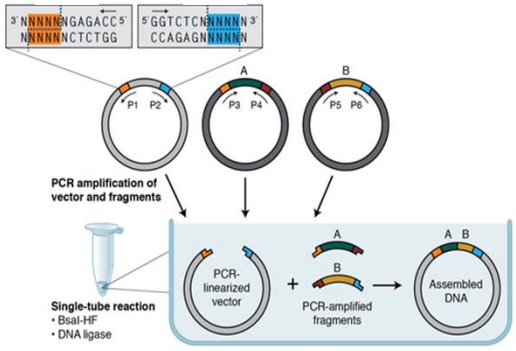
CRISPR/Cas9

- Natural prokaryotic defense systems against foreign DNA to create precise cuts in a genome
- With these cuts and appropriate repair DNA, inserts to a genome can be made as well

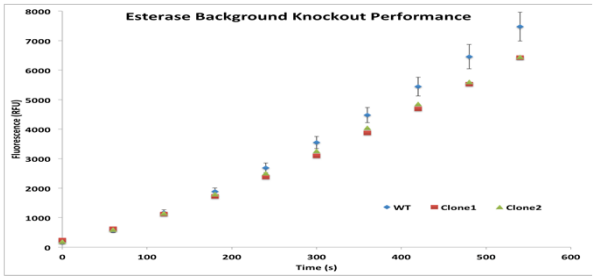


Golden Gate Assembly

- Allows orderly assembly of multiple fragments.
- Reusable parts for modular design
- A simultaneous digestion and ligation reaction(One Pot)

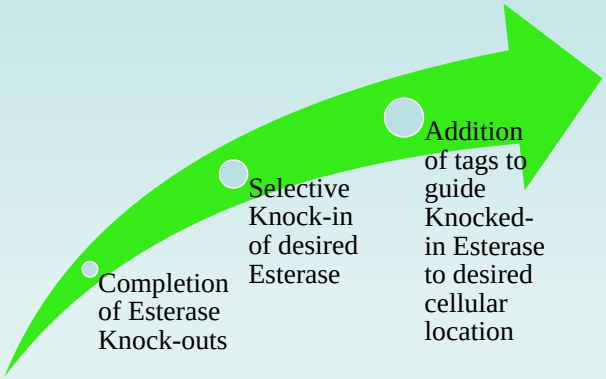


Results



- "Knocked-out" two of the six esterases
- This graph shows a difference in FDA reactions between our mutant clone and the wild-type yeast, indicating less esterases in mutant cells
- This has not been confirmed by colony PCR

Future Work



Acknowledgements

C. T.-K. would like to thank Bernardo Cervantes, Zach Russ, John Dueber and the members of the Dueberlab for sharing their wonderful workplace and interesting skills. Thanks to Lili Caughman, Lea Marlor, Sharnia Artis, and Shaila Kotadia for all their time and effort. Thanks to Priya Shah, Kelly Pernell, Leilt Seblega, and Keith Schoon for their encouragement and support. Thanks to Laurie Jean Vogler with whom all things are possible. Thanks to Synberc, E^{3}S, EBI, UC Berkeley, and the NSF for providing place and means to conduct this research.

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Support Information
This work was funded by National Science Foundation Awards ECCS-0939514 & EEC-1157089.