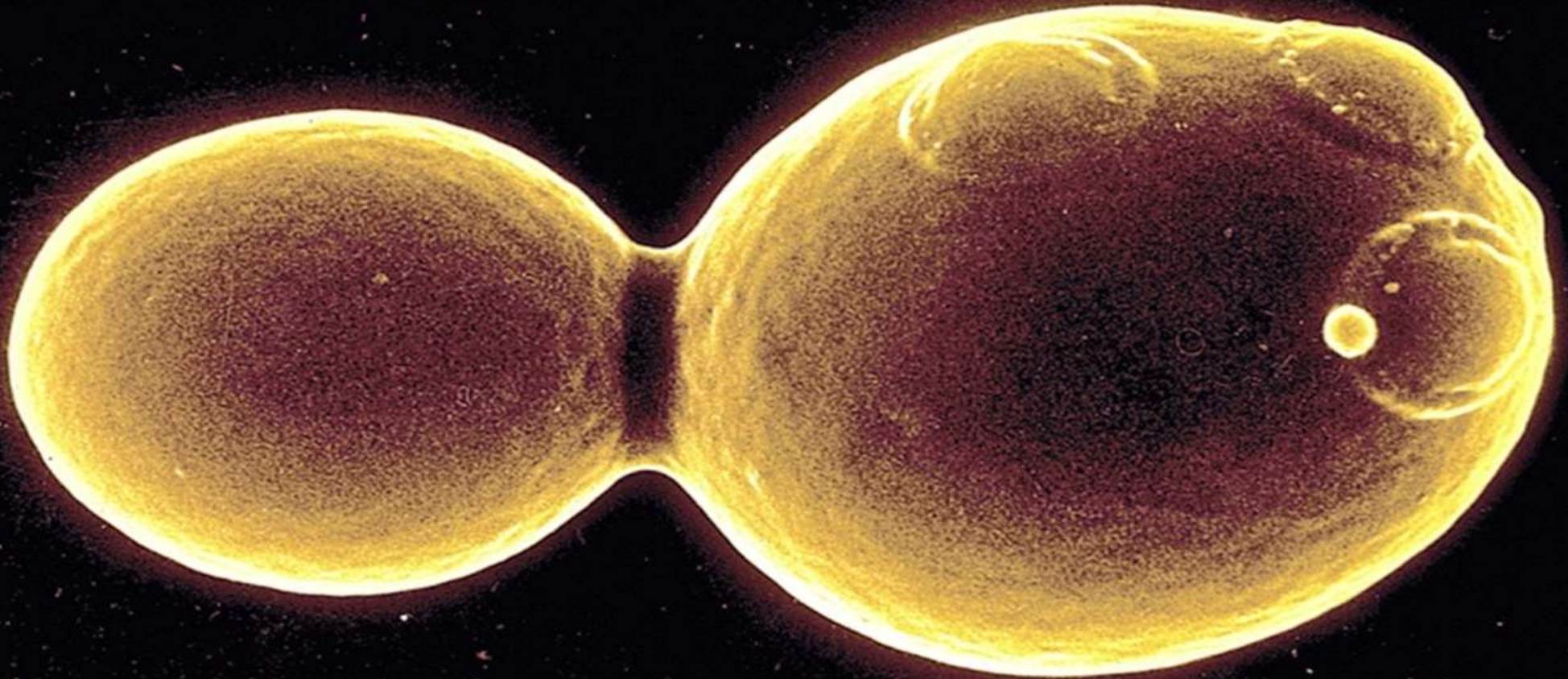


More for Less:

Metabolic Engineering of Yeast for Ethanol Production



Nicholas Memmer
College of Agriculture
Dept. of Microbiology

Overview

- 1) The Ethanol Industry
- 2) Yeast and Yeast Metabolism
- 3) Metabolic Engineering of Yeast

Economics of Ethanol: Industry Statistics

- 13.3 Billion Gallons produced in 2013*
 - 86,000 Jobs
 - \$44 Billion Contributed to the GDP
 - \$8 Billion in Taxes Paid
 - \$36 Billion Spent on Raw Materials

* Renewable Fuels Association 2014 Industry Outlook

Economics of Ethanol: Government Involvement

- Foreign Oil and Energy Independence
- Energy Policy Act of 1992
 - “Promote the development and use... of domestic replacement fuels in lieu of petroleum motor fuels”
- Energy Policy Act of 2005
 - The RFS
 - 7.5 Billion gallons of blended fuels
- Energy Independence and Security Act (2007)
 - 36 Billion Gallons by 2022

Economics of Ethanol: Agriculture

- Corn
 - \$36 Billion spent in 2013
 - The Midwest
 - 1997-2006 Corn was grown at a loss
 - Corn payments in 2012 were 82% lower than in 2006

Economics of Ethanol: Related Industries

- Animal Feed
- Fermentation Chemicals
 - \$100 Billion Industry*
 - \$400 Billion by 2030*

Ethanol as a Biofuel

- E10, E15, E85
- Engine Benefits
- Carbon Footprint and “Green Energy”



<http://www.carsbikesbacon.com/img/14312.o.jpg>



<https://carlsvanrentals.files.wordpress.com/2011/07/e85-fuel.jpg>

Takeaway:
Ethanol is a big, profitable industry.



Industrial Production of Ethanol

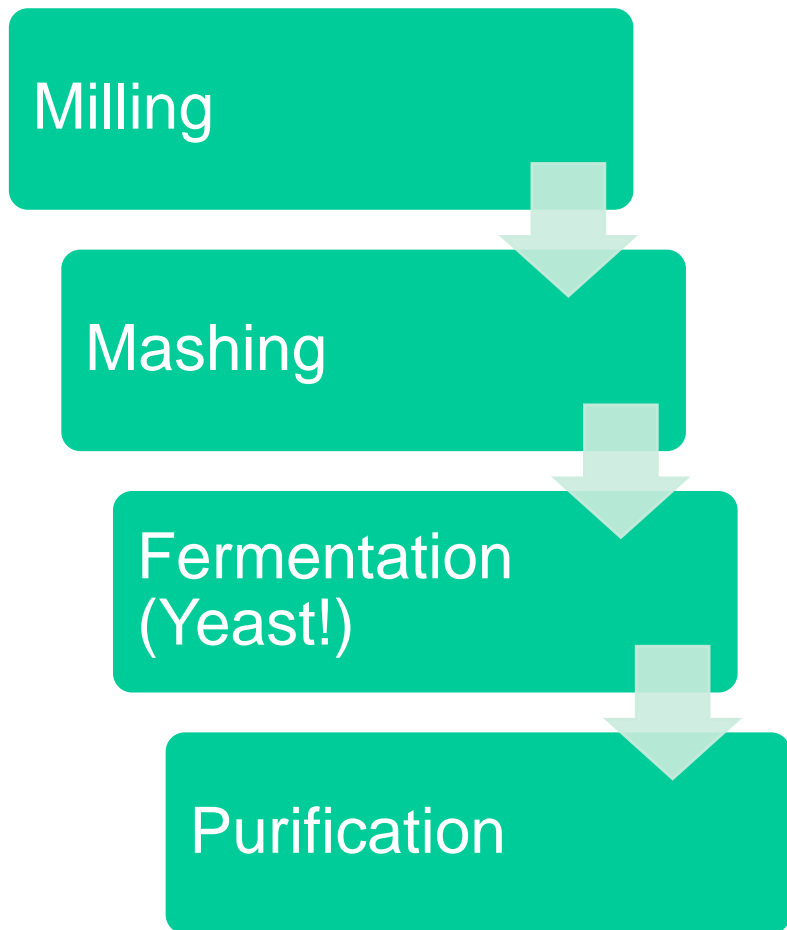
- Feedstocks
 - “Sugar” (It’s corn...)
 - Cellulose
- Dry Milling
- Wet Milling
- The Role of Yeast
 - Biological catalyst



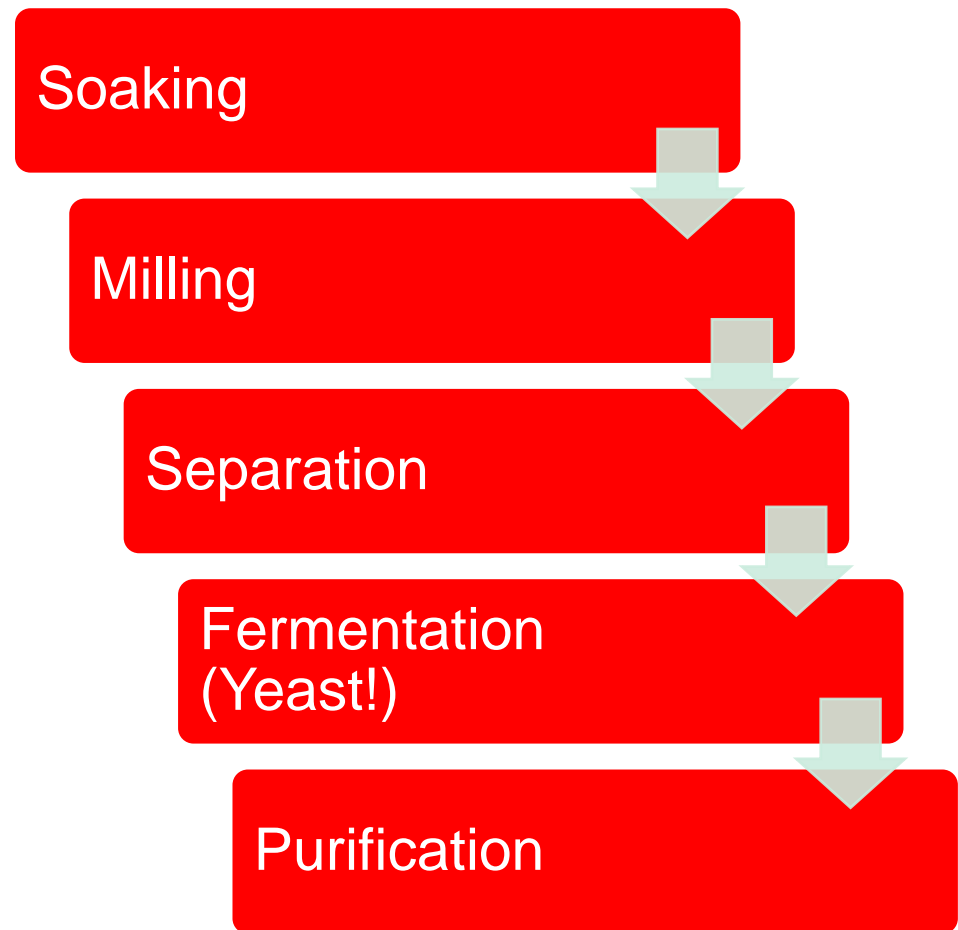
<http://www.theblaze.com/wp-content/uploads/2012/02/EthanolPlant.jpg>

Production Paradigms

Dry Milling



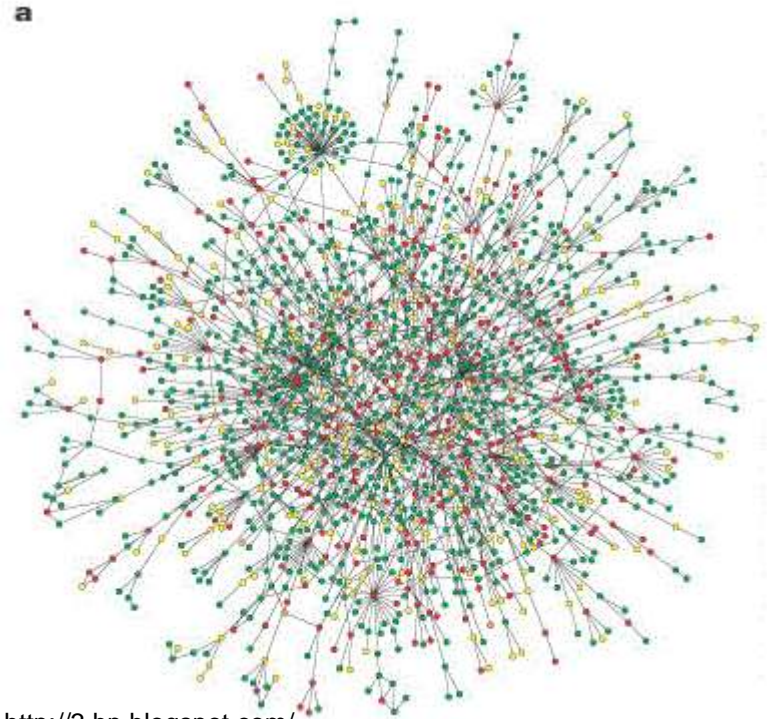
Wet Milling



Takeaway:
**You want ethanol, you're
going to need yeast**

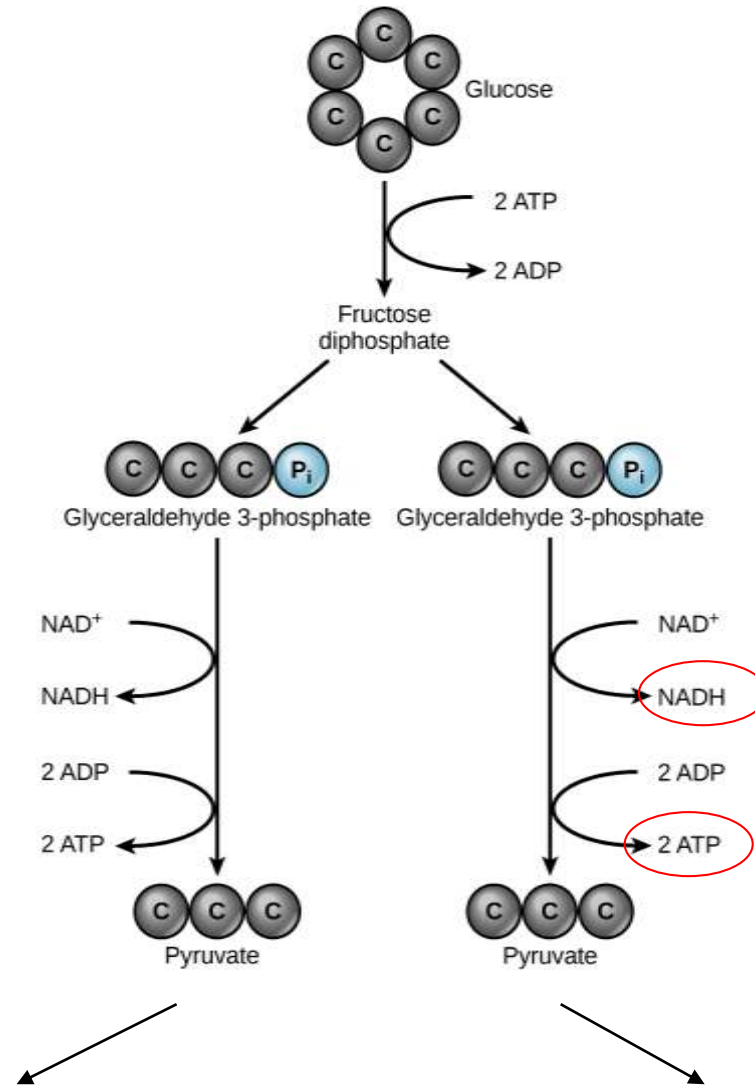
Yeast

- Single-celled fungi
- “Brewers yeast”
- Well understood
 - Genetics
 - Proteomics
 - Lots of tools



<http://3.bp.blogspot.com/--muYzxHcolo/TWUeRJ5ij0I/AAAAAAAAACbi/WaEAVcjhWgU/s1600/yeast+proteome.GIF>

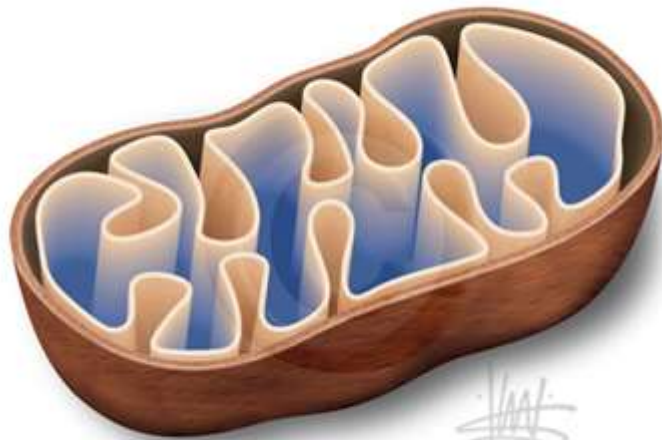
Yeast Metabolism



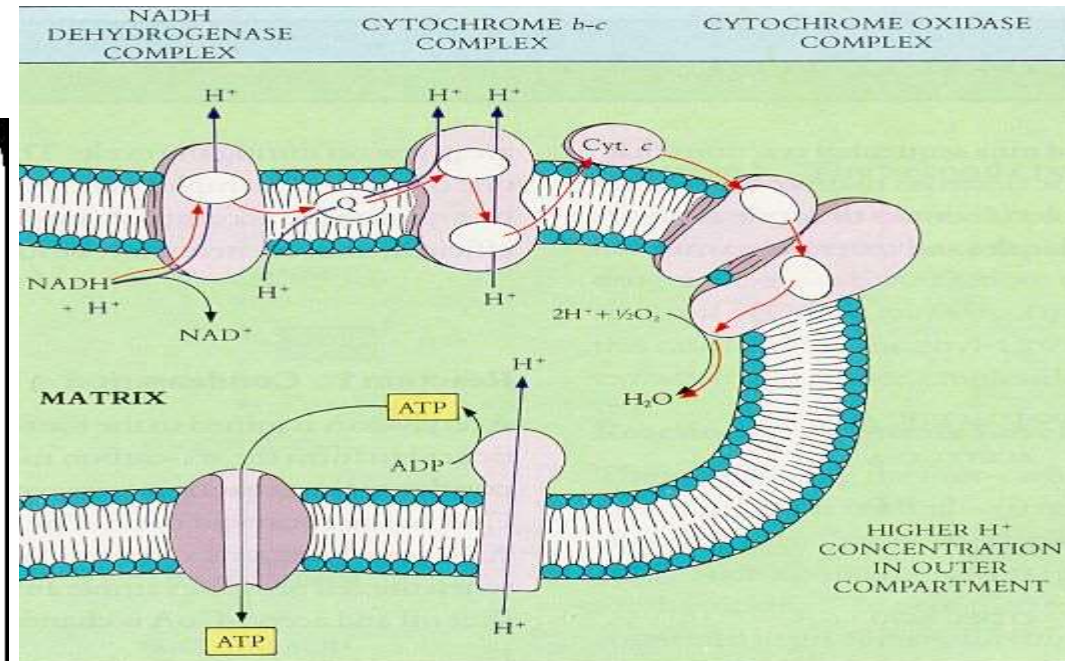
Is there oxygen?

Aerobic Respiration

AKA “The mitochondria is the powerhouse of the cell!”



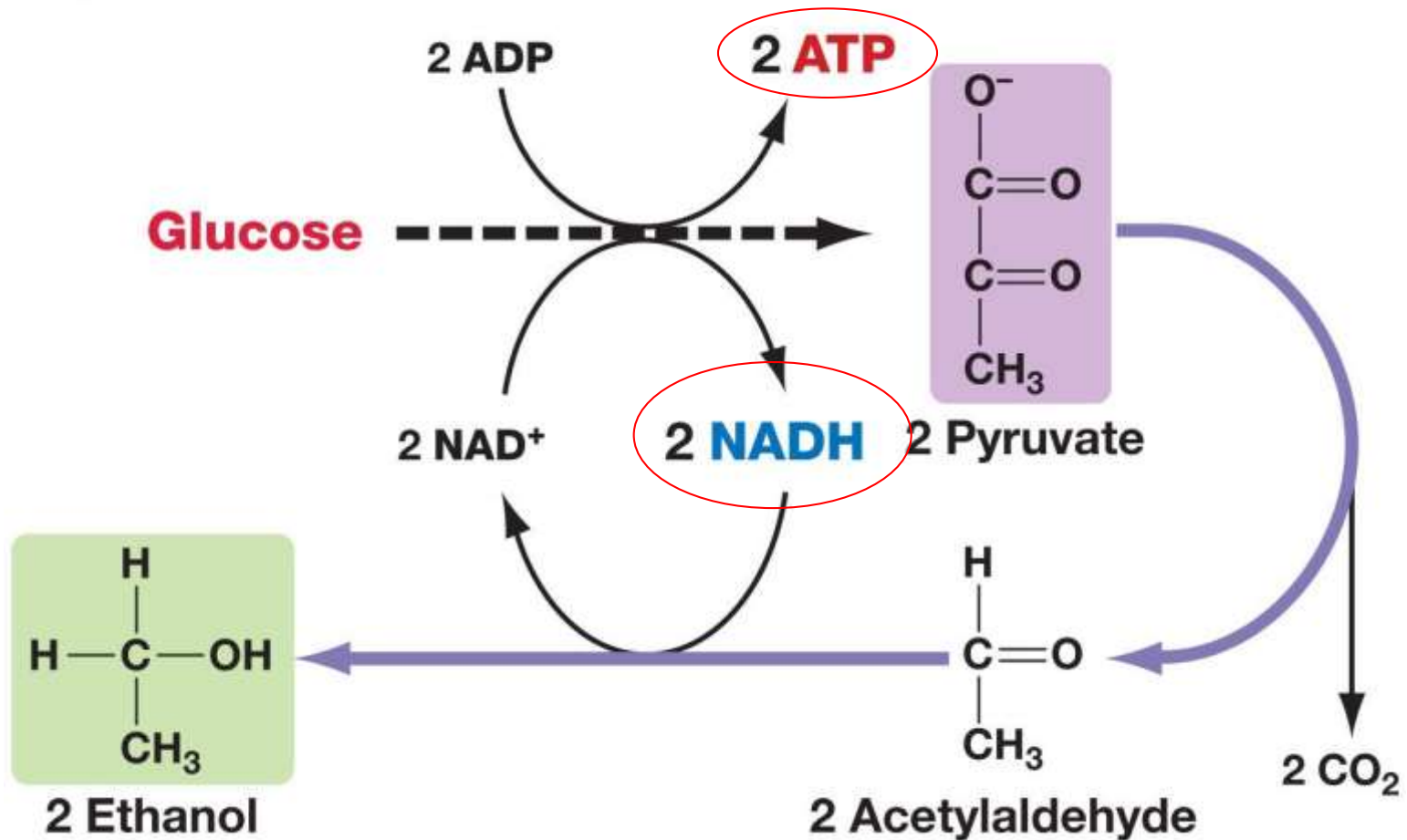
<http://penrules.com/portfolio/illustration/mitochondria.html>



<http://www.bing.com/images/search?q=electron+transport+chain&view=detailv2&&id=36F5502FAB9EEE5090C1D80E22D191AE944E877A&selectedIndex=29&ccid=Ja1ZYikQ&simid=608009203198265056&thid=JN.aVKxKC8pEsxFcHMr5HbZHg&ajaxhist=0>

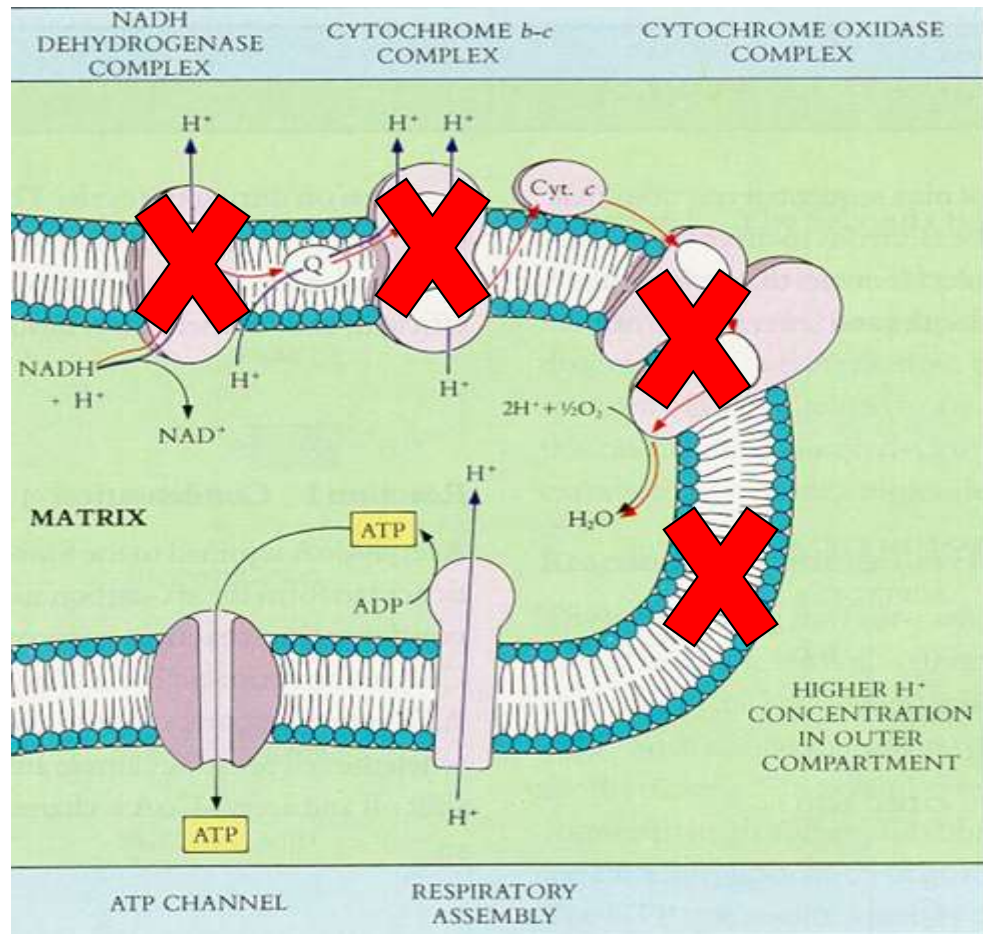
Alcoholic Fermentation

(b) Alcohol fermentation occurs in yeast.

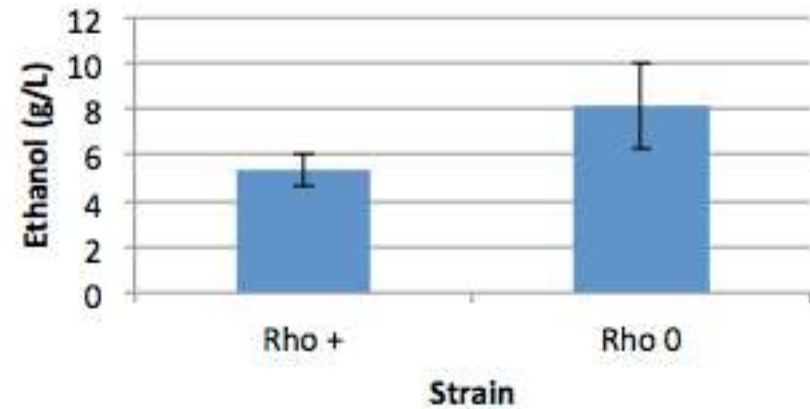


© 2011 Pearson Education, Inc.

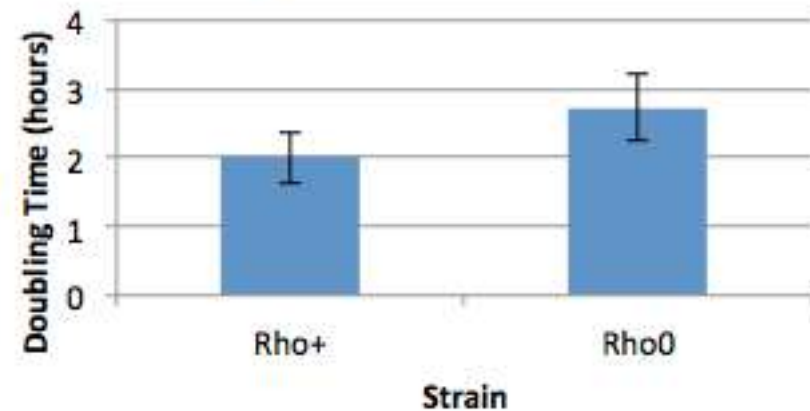
The Rho-Zero State



EtOH Concentration

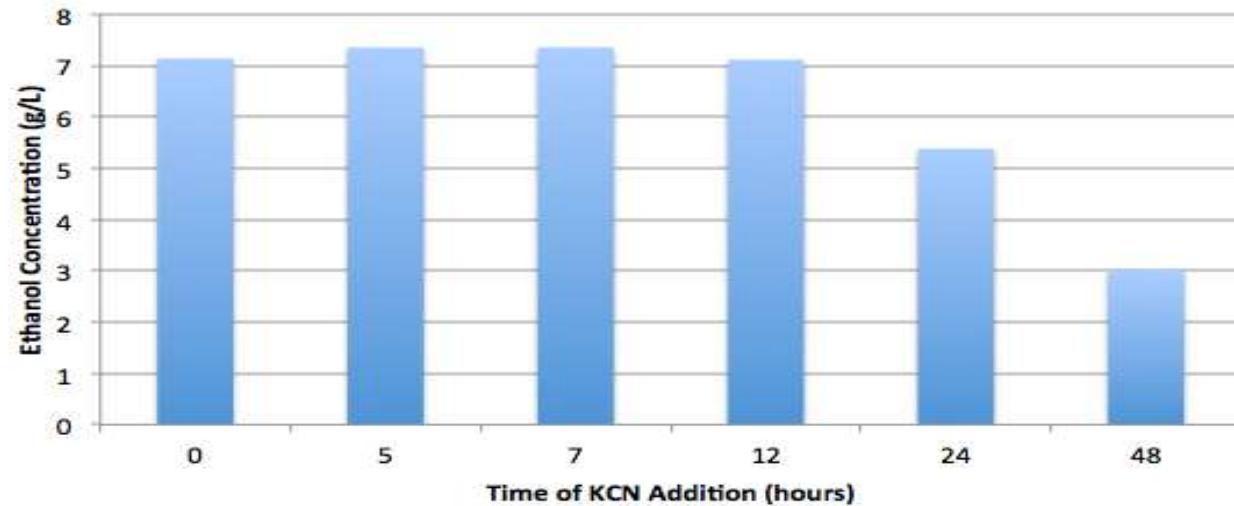


Average Doubling Time

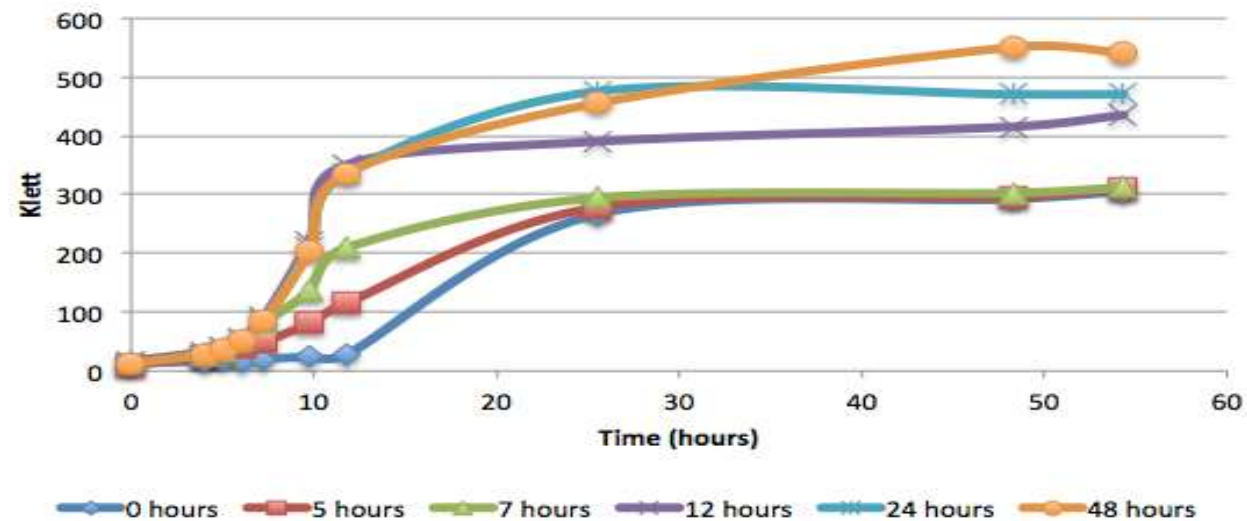


The Rho-Zero State

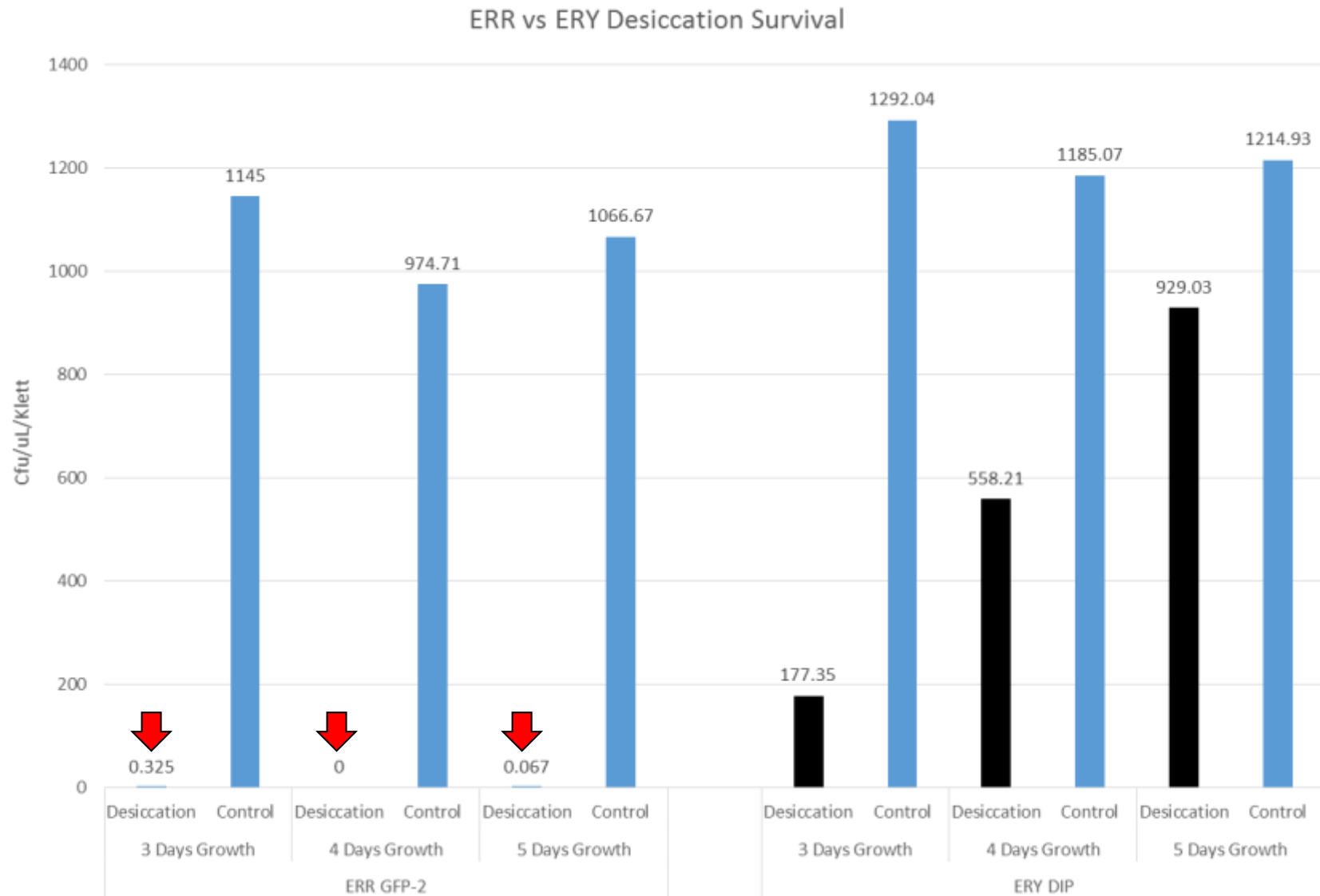
Ethanol Concentration After 52 Hour Fermentation



KCN Growth Curve



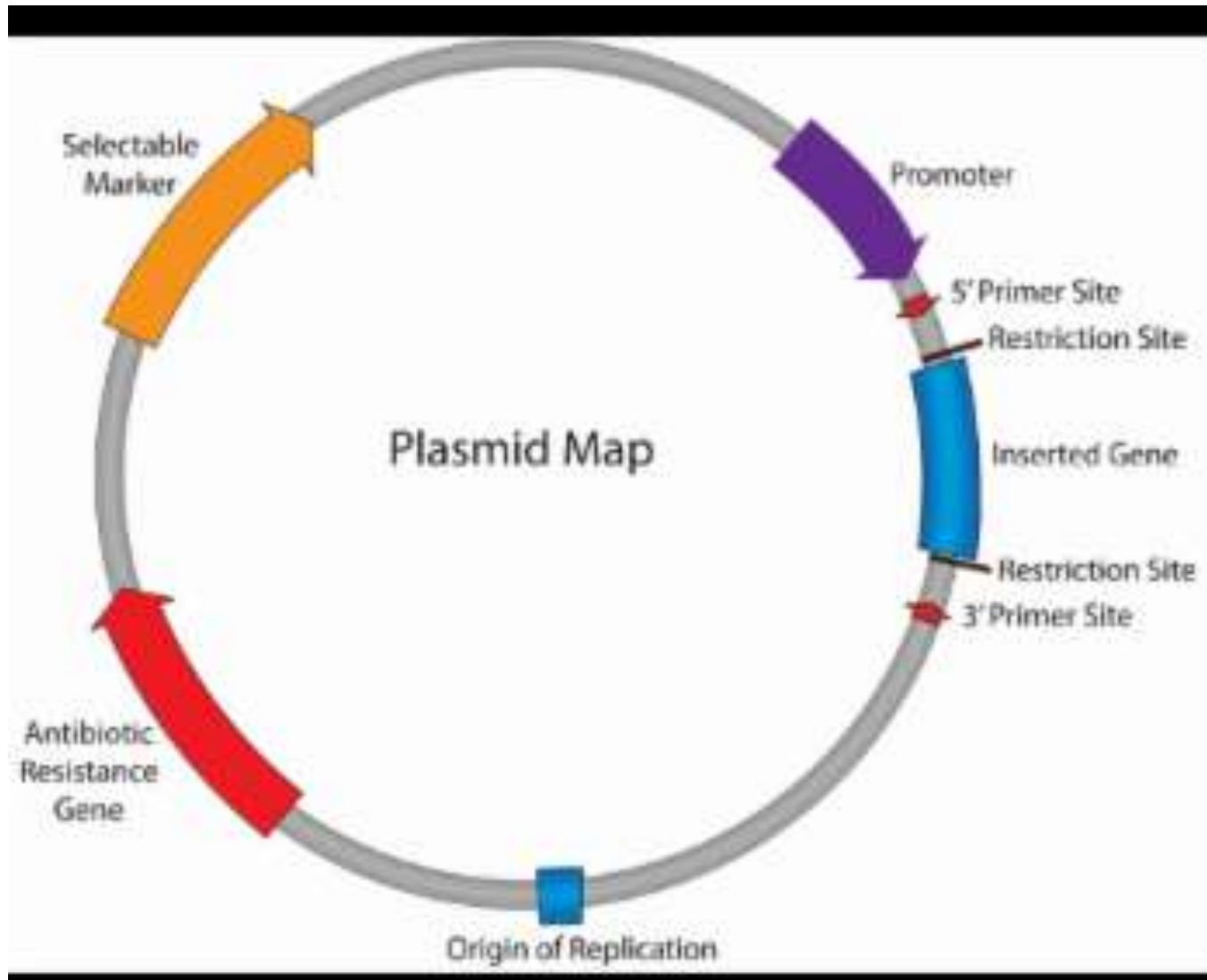
Rho-Zero Desiccation Tolerance



Takeaways:

1. Yeast can live with or without oxygen
2. Yeast living without oxygen produce ethanol
3. Yeast in the rho-zero state produce more ethanol, but grow slowly and don't survive desiccation.

Metabolic Engineering of Yeast



Inducing the Rho-Zero State

“...**dominant inducible interfering transgene that will render yeast respiratory deficient during the production phase of commercial fermentations.**”

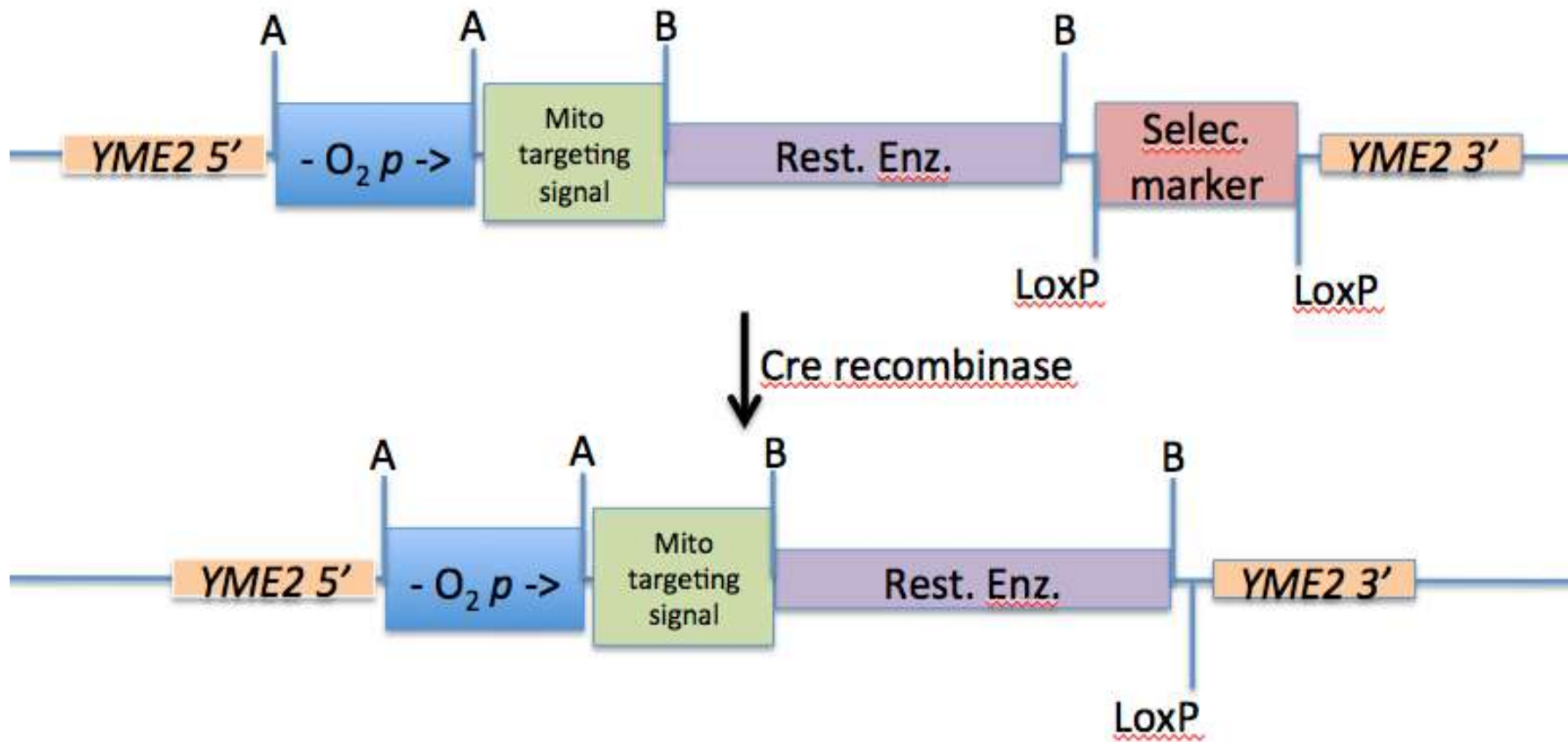


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“...using a gene to mess with mitochondria once yeast start fermenting”

The Transgene



Takeaways:

1. A plasmid containing a “toxic gene” will be introduced
2. Lack of oxygen will lead to expression of the toxic gene
3. The toxic gene will alter the gene expression in the mitochondria
4. Altered gene expression will lead to the rho-zero state, increasing ethanol production

Conclusions and Implications

The system proposed here, if successful, will be broadly applicable to current and future ethanol production models. Implementation of this system will save ethanol producers money and improve production efficiency.

Acknowledgements

Thanks to Dr. Peter Thorsness for involving me in this research

Thanks to Rachel Watson, my undergraduate advisor, for making sure I got my USPs done

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