Genetically Modified Yeast Breaks Down Gluten in Beer

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About Celiac Disease

•Autoimmune disorder that affects the digestive system

•Severe reaction when exposed to gluten, a protein found in wheat, rye, and barley

•Antigenicity of gluten due to proline and glutamine rich peptides LQLQPFPQPQLPYPQPQLPYPQPQLPYPQPQPF [3]

•Peptides processed by a Celiac's immune system

•Damages villi in the small intestine and interferes with absorption of nutrients from food [2]

Introduction

The purpose of our experimentation was to engineer a strain of yeast capable of secreting an enzyme that would break down gluten during the fermentation process of brewing beer. Our system is composed of the following:

•*KumaMax:* Kumamolisin-As_N291D, G319S, D358G, D368H (2011 UW iGEM) with codons altered for expression in yeast
- •Maximal activity at pH of 4 and would work well in the pH range of 5.2-5.5 found in beer
- •Shown to cleave the peptide sequence PQPQLP (common motif in gluten) [5]

•*Mating factor alpha* (MF-α) secretion tag [1]
- •Tag's sequence was placed directly upstream of KumaMax
- •Signal sequence is cleaved in the golgi before protein export [5]
- •The DNA synthesized by IDT, but we were also able to extract it from the yeast genome by PCR

•*Expression vectors*
- •pCM189: centromeric yeast plasmid, low copy number, tet-off promoter
- •pCM190: episomal yeast plasmid, high copy number, tet-off promoter
- •Integrative plasmid constructed by adding the resistance gene for geneticin (Kan) to pCM189/MF-alpha/Kuma-max.

Materials and Methods

•All DNA was synthesized by IDT.

•Sequence for KumaMax obtained courtesy of the 2011 University of Washington iGEM team

•Sequence for MF-α was taken from the NCBI database.

•Plasmids provided by CSU's Argueso lab

•Media components borrowed from CSU Microbiology Department

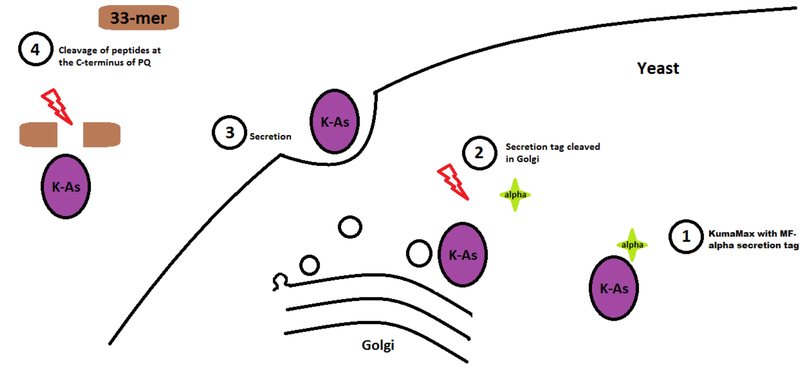
•Gel extraction and Miniprep kits purchased from Qiagen.

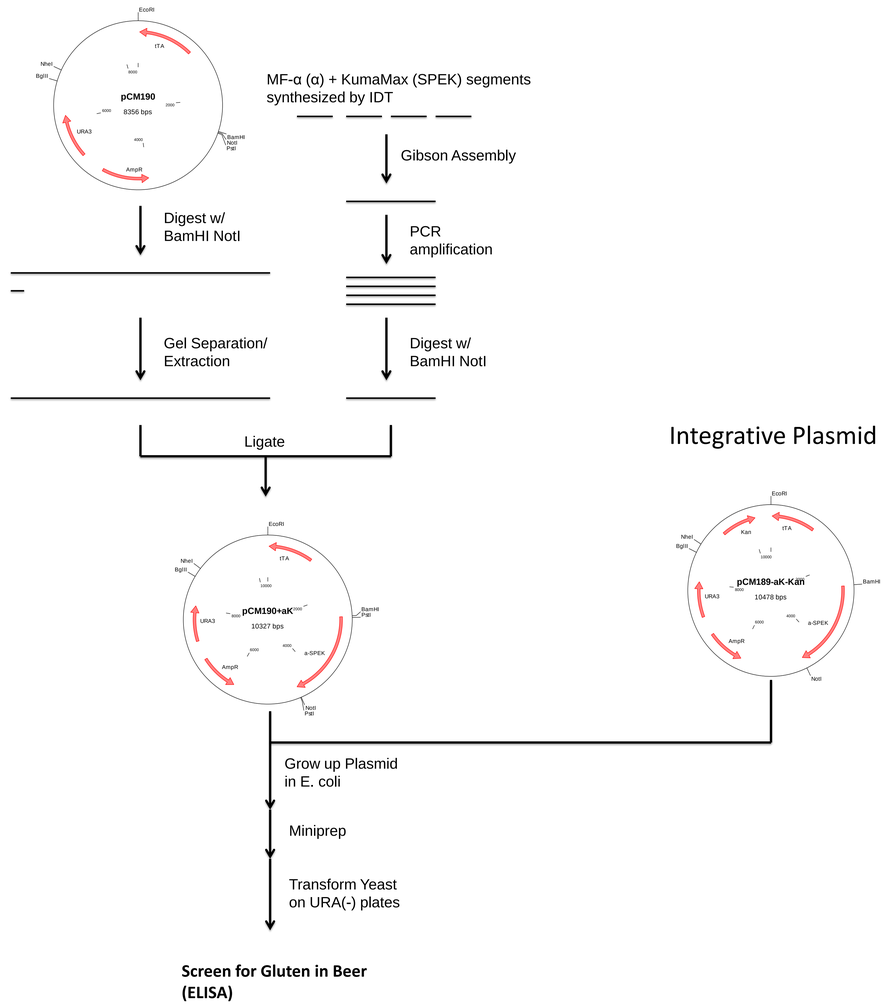
•Restriction enzymes purchased from New England Biolabs or borrowed from CSU's Willusz lab

•ELISA test donated from R-biopharm

•Peptide with fluorophore and quencher from Biomatik

•Other chemicals, reagents and materials purchased from the CSU chemical stockroom

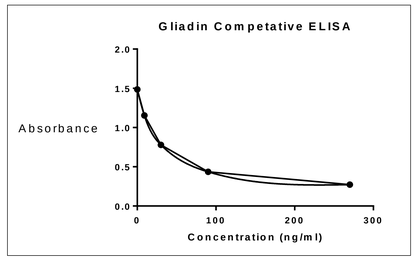




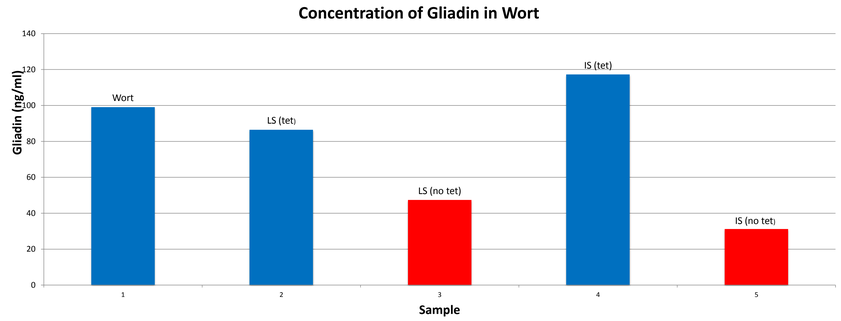
Results

Competitive ELISA

Standard Curve:



Sample	Absorbance	Concentration (ng/ml)
control wort	0.41	99.05109
lab strain (tetracycline)	0.447	86.44935
lab strain (no tet)	0.633	47.31686
industrial strain (tet)	0.366	117.1983
industrial strain (no tet)	0.768	31.20483



Discussion

•ELISA demonstrated that the engineered yeast were capable of breaking down gluten during fermentation

•*Further work will include:*

•optimization of the enzyme

•completion of an integrative plasmid

•Insertion of integrative plasmid into brewing yeast

•Shift the focus from proof of concept in the laboratory to a potentially marketable gluten-free beer

Acknowledgements/Sponsors

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1. Anthony J. Brake, James P. Merryweather, Doris G. Coit, Ulrike A. Heberlein, Frank R. Masiarz, Guy T. Mullenbach, Mickey S. Urdea, Pablo Valenzuela, and Philip J. Barr. (1984). α-factor-directed synthesis and secretion of mature foreign proteins in saccharomyces cerevisiae. *Biochemistry*, *81*(August 1984), 4642-4646.
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