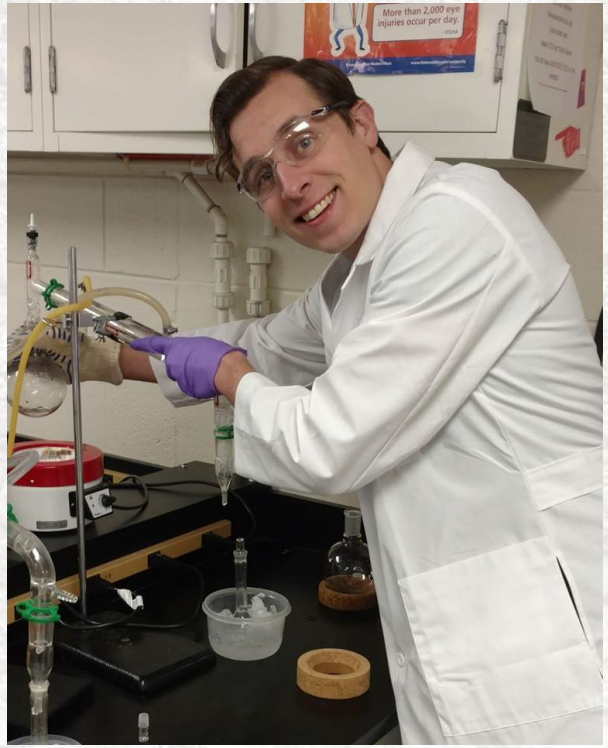
Demystifying Diastaticus

An Exposé of Everyone's Favorite Explosive Yeast



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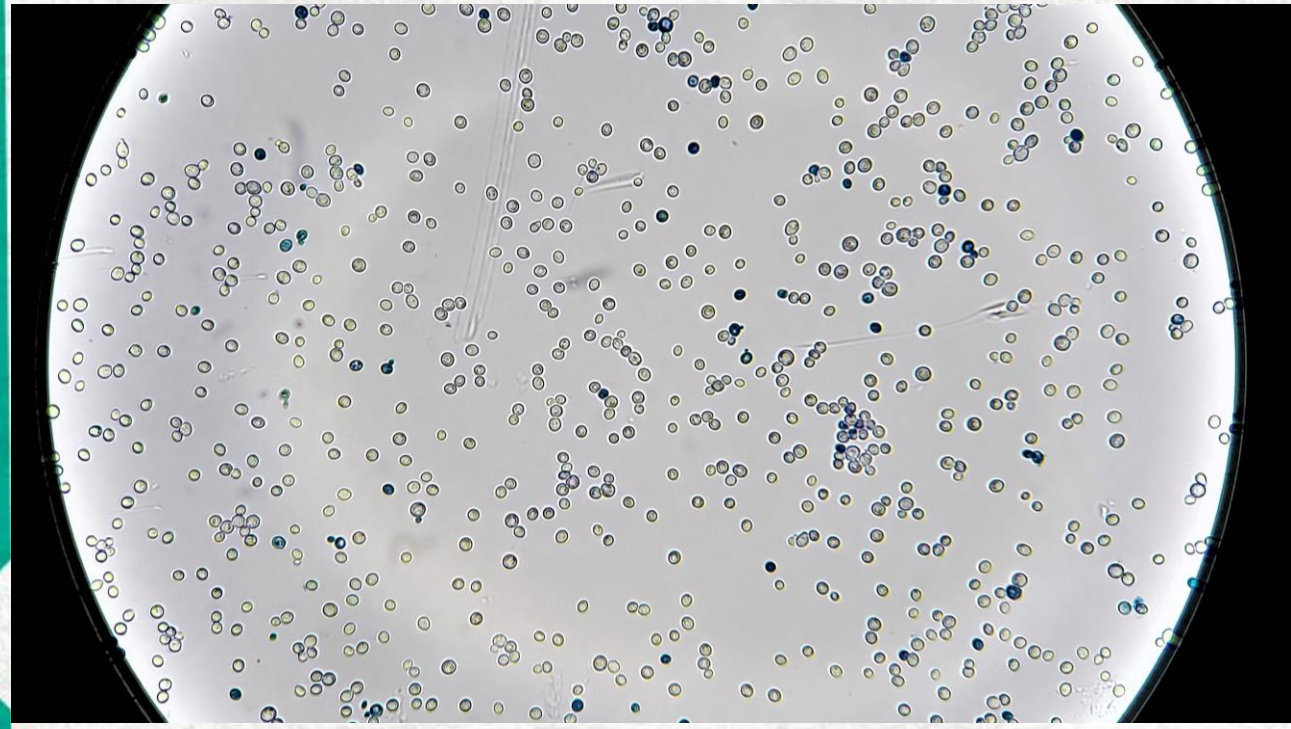
What is Saccharomyces cerevisiae var. diastaticus?



- Saccharomyces = Sugar fungus
- cerevisiae = from beer
- diastaticus = separation (of starch)

When Yeast Goes Variant

Variant: taxonomic designation below species and subspecies

GROUP V: More than 6° were fermented, *i.e.*, the final attenuation was below that of secondary yeast (*Brettanomyces*)

Species	Final attenuation	Degrees fermented
Schizosaccharomyces octosporus	1006·4	8·2
Schizosacch. pombé	1006·1	8·5
Schizosacch. pombé var. *mellacei*	1006·1	8·5
Schizosacch. pombé strain *liquefaciens*	1006·1	8·5
Schizosacch. formosensis ..	1006·3	8·3
Schizosacch. sautawensis ..	1006·3	8·3
Schizosacch. japonicus ..	1004·0	10·6
Schizosacch. taito	1006·2	8·4
NEWLY ISOLATED ORGANISMS		
(1) *Saccharomyces* sp. ..	1004·2	10·4
(2) *Streptococcus* sp. in synergism with *S. cerevisiae*	1006·9	7·7
Streptococcus sp. in synergism with *Br. bruxellensis* var. *non-membranaefaciens*	1002·7	11·9
(3) *Lactobacillus* sp. in synergism with *S. cerevisiae*	1006·4	8·2
Lactobacillus sp. in synergism with *Br. bruxellensis* var. *non-membranaefaciens*	1002·6	12·0

Super Attenuation of Beer

- Andrews & Gilliland
- Describe a “novel species” in 1952
- Later genomic research (~1985) reclassified as S. cerevisiae variant

What makes diastaticus different?

Starch degradation

Maltotriose

Maltotetraose

Dextrin

Starch

It's in their Genes

STA1

STA2

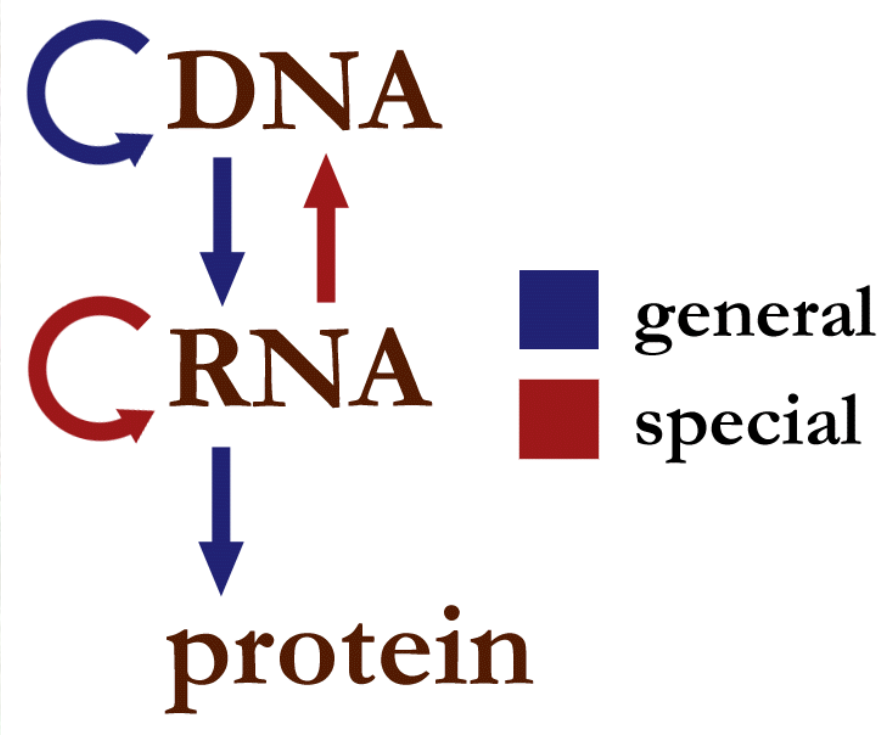
STA3

Molecular Machines

Extracellular Glucoamlyase

The Central Dogma:

Information transfer within cells



How it works:

- Signal within cell activates DNA (gene)
- DNA transcribed into mRNA
- RNA converted to protein by Ribosome

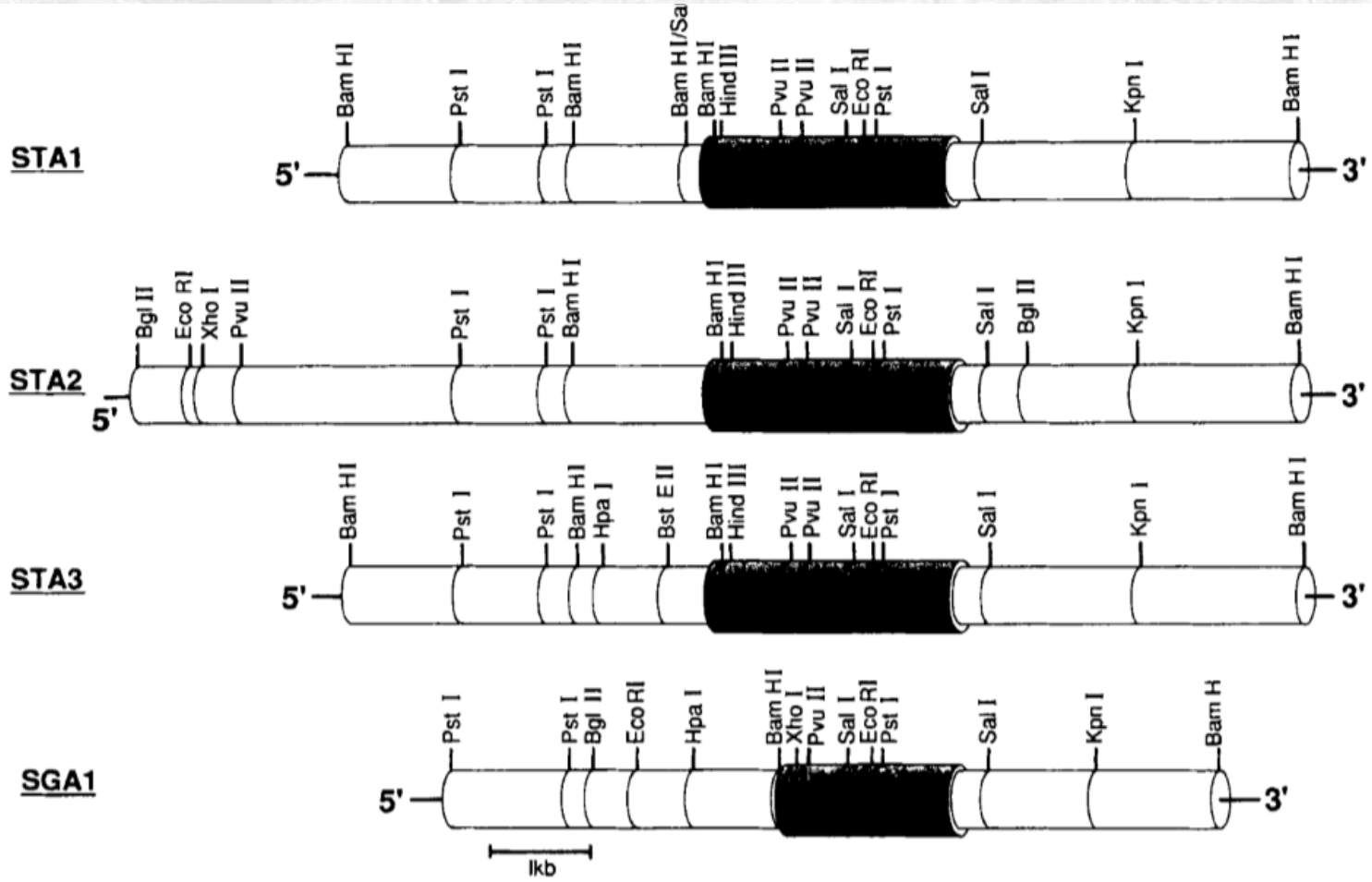


FIGURE 2. Restriction endonuclease maps of the *STA* and *SGA* genes from *Saccharomyces*. The structural genes encoding the glucoamylases are highlighted. The restriction maps of *STA1*, *STA2*, and *STA3* are identical.[30,31] The middle and 3′ regions of *SGA1* are identical to the corresponding regions of the *STA* genes.[29,50]

STA Gene family is highly homologous

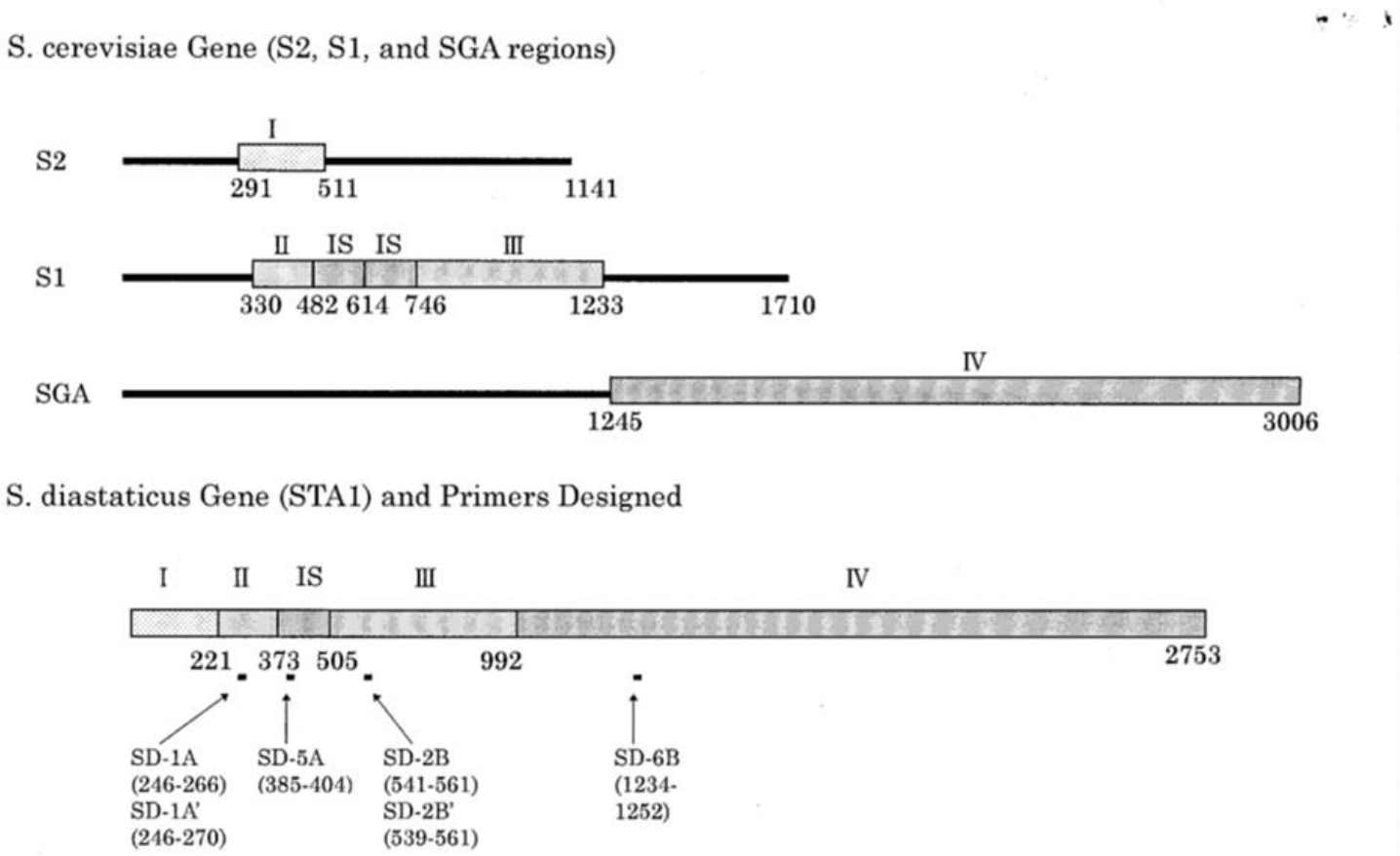
The Glucoamylase Multigene Family in Saccharomyces cerevisiae var. diastaticus: An overview. Pretorius et al. 2008.

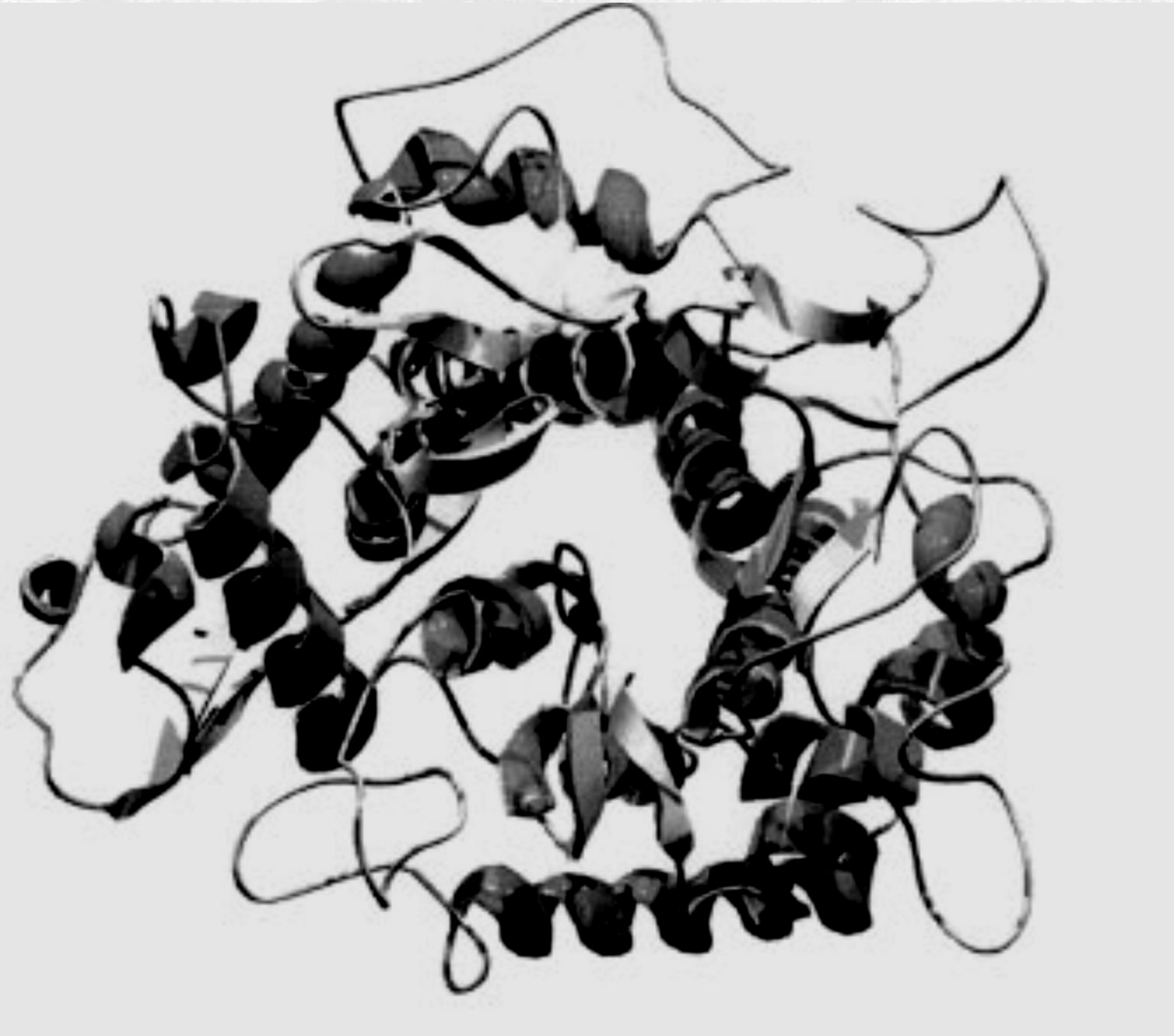
DEX allelic to STA genes

Allelism within the DEX and STA gene families in Saccharomyces diastaticus. Erratt JA, Nasim A. 1986

Rapid Methods for Detecting Saccharomyces diastaticus, a Beer Spoilage Yeast, Using the Polymerase Chain Reaction

H. Yamauchi, H. Yamamoto, Y. Shibano, N. Amaya, and T. Saeki, JASBC 1998

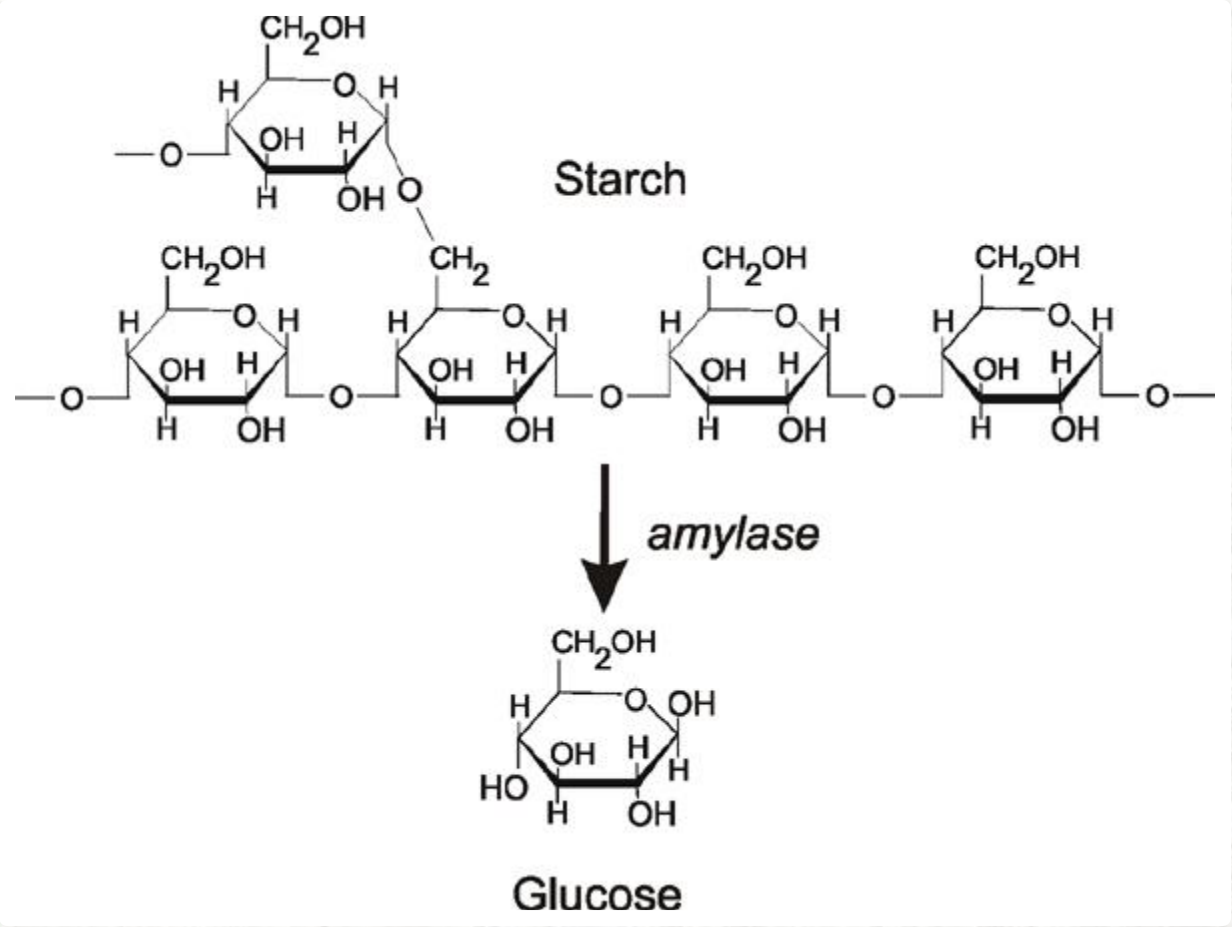




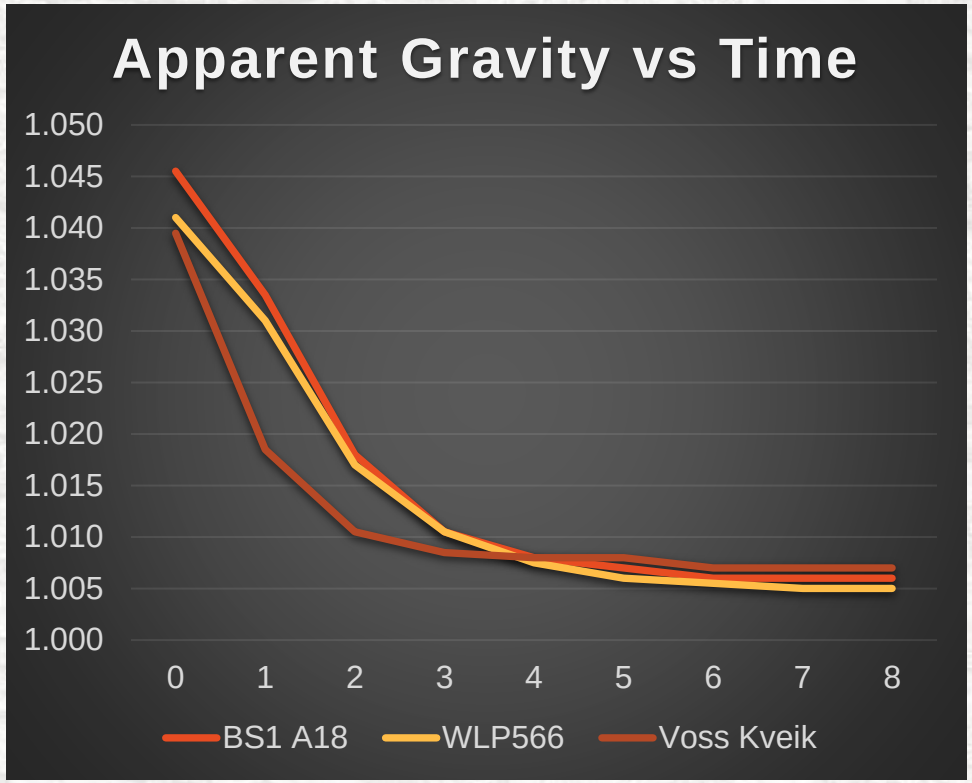
Structural analysis of glucoamylase encoded by the *STA1* gene of *Saccharomyces cerevisiae* (var. *diastaticus*)

Adams et al, Yeast 2004; **21**: 379–388.

Enzymatic Action!



Brewing with diastaticus

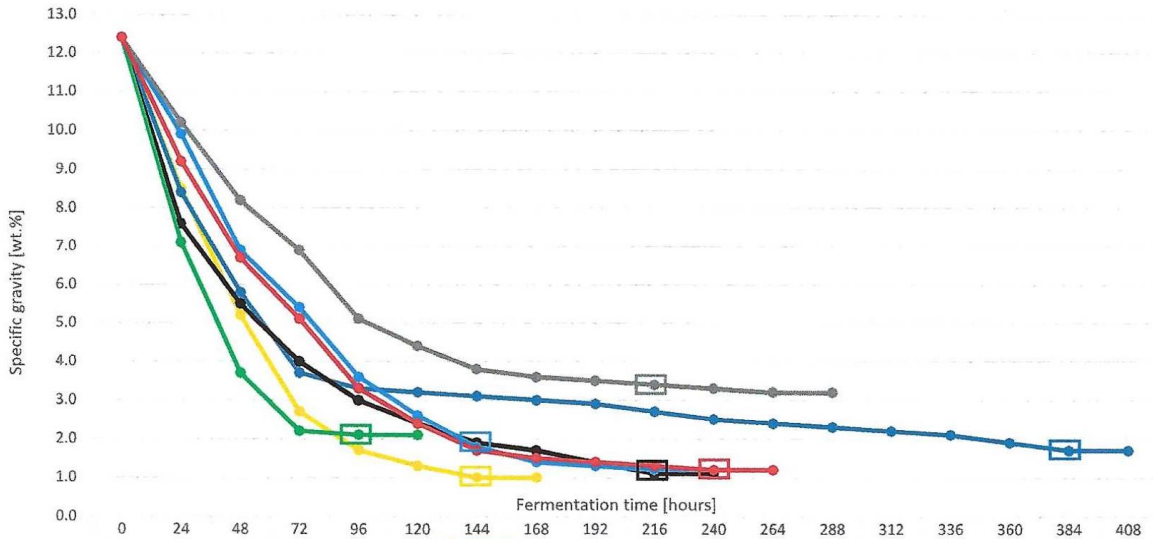


Strains:

- Brewing Science Institute A-18 London Ale III (STA1-)
- White Labs WLP566 Belgian Saison II (STA1+)
- Voss Kveik (STA1-)

STA1 positive yeast took longest to reach terminal gravity, lower FG than STA1 negative yeast!

Not all diastaticus Fermentations are the same



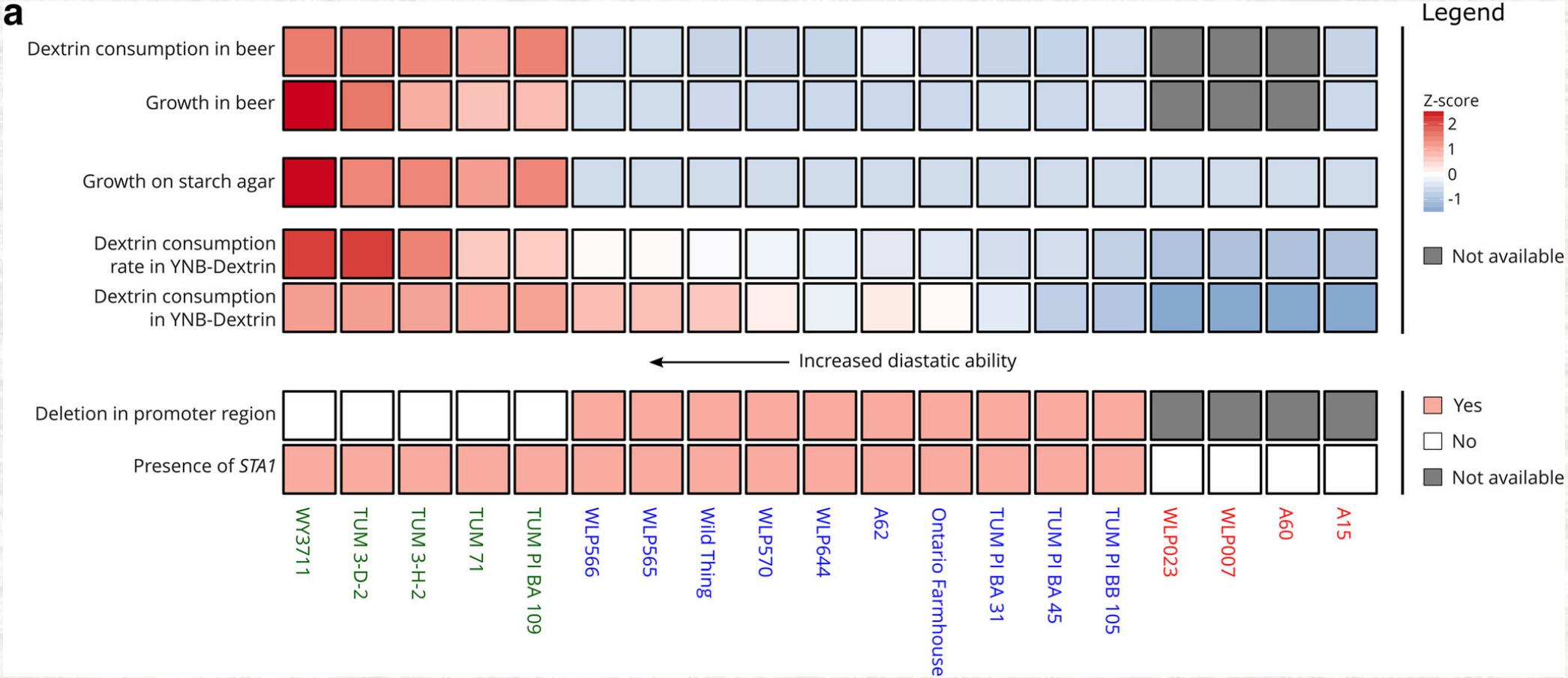
	0	24	48	72	96	120	144	168	192	216	240	264	288	312	336	360	384	408
DSM 70487	12.4	8.5	5.2	2.7	1.7	1.3	1.0	1.0										
TUM 1-H-7	12.4	8.4	5.8	3.7	3.3	3.2	3.1	3.0	2.9	2.7	2.5	2.4	2.3	2.2	2.1	1.9	1.7	1.7
TUM PI BB 121	12.4	7.6	5.5	4.0	3.0	2.4	1.9	1.7	1.4	1.1	1.1							
TUM PI BA 124	12.4	7.1	3.7	2.2	2.1	2.1												
TUM 71	12.4	9.9	6.9	5.4	3.6	2.6	1.8	1.4	1.3	1.2	1.2							
TUM 3-H-2	12.4	10.2	8.2	6.9	5.1	4.4	3.8	3.6	3.5	3.4	3.3	3.2	3.2					
TUM 3-D-2	12.4	9.2	6.7	5.1	3.3	2.4	1.7	1.5	1.4	1.3	1.2	1.2						

Figure 6. Drop in specific gravity measured in a single reference vessel compared with the average in final gravity (marked with box) measured in triplicate for the tested yeast strains DSM 70487, TUM 1-H-7, TUM PI BB 121, TUM PI BA 124, TUM 71, TUM 3-H-2 and TUM 3-D-2; confidence level 95%.

Saccharomyces cerevisiae variety diastaticus friend or foe?—spoilage potential and brewing ability of different Saccharomyces cerevisiae variety diastaticus yeast isolates by genetic, phenotypic and physiological characterization.

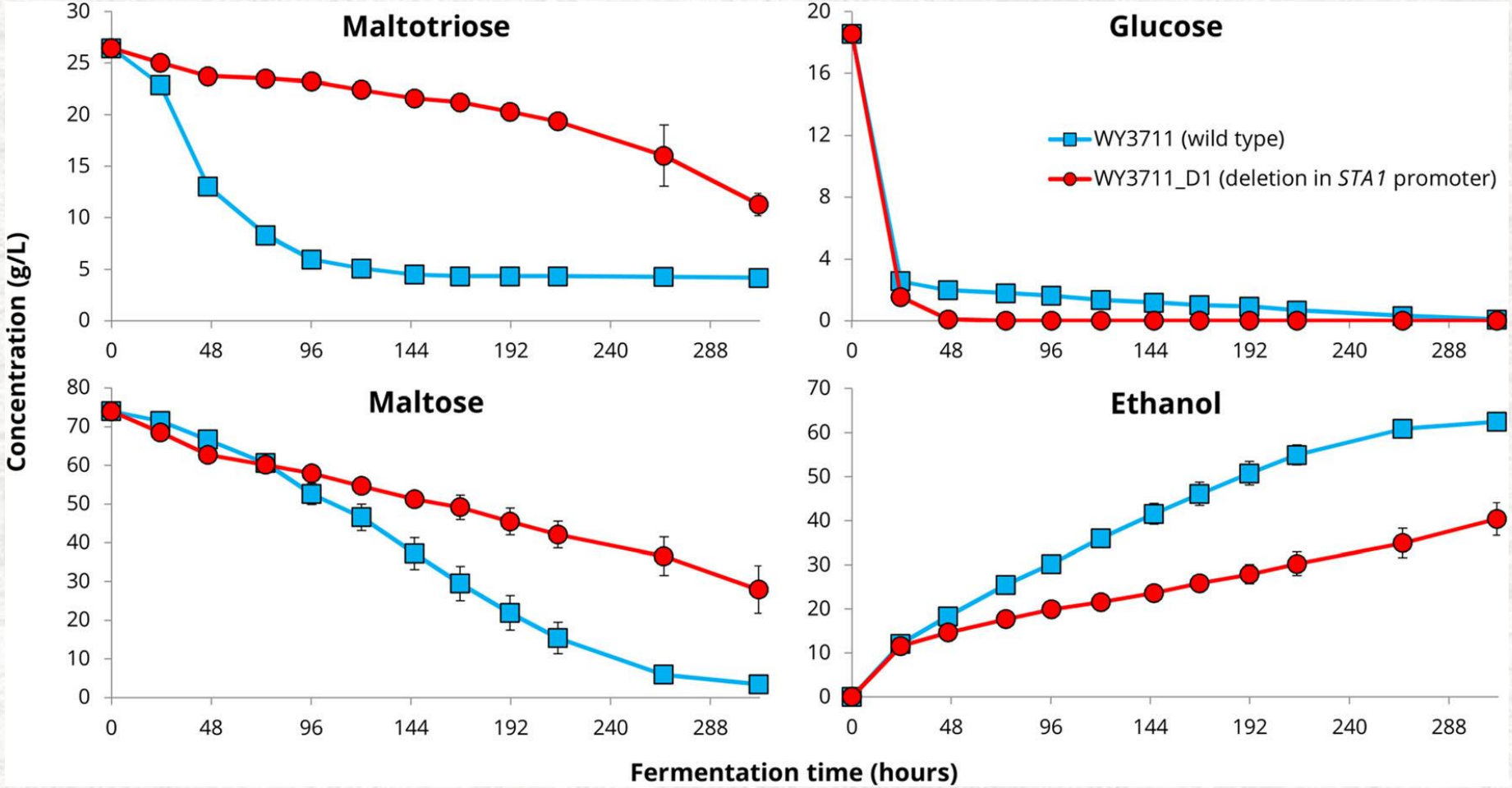
Tim Meier-Dörnberg et al, 2018

Differences in Fermentation Related to Different Gene Expression



A deletion in the STA1 promoter determines maltotriose and starch utilization in STA1+ Saccharomyces cerevisiae strains. Krogerus et al 2019

Differences in Fermentation Related to Different Gene Expression



A deletion in the STA1 promoter determines maltotriose and starch utilization in STA1+ Saccharomyces cerevisiae strains. Krogerus et al 2019

Diastaticus as a Contaminant

Locations, Effects, & Detection

What happens when diastaticus is present?

Potential Impacts

Over-attenuation

- Final gravity 1.004 or less
- Flavor imbalance
- Package failure
  - Bottle bombs or Can shrapnel = SAFTEY ISSUE!!!

Phenolic Off Flavor

- Not all strains POF+

Sediment/haze

Where does contamination occur?

- Brewhouse
- Pipework
- Pitching yeast
- Fermentation cellar
- Packaging lines

Incidence of *Saccharomyces cerevisiae* var. *diastaticus* in the Beverage Industry: Cases of Contamination, 2008–2017

Tim Meier-Dörnberg et al 2017

Table 3. Number of findings of *S. cerevisiae* var. *diastaticus* and type of contamination per year from 2008 to June 2017

	Finding		Type of contamination and number of positive findings[a]	
Year	Negative	Positive	Primary	Secondary
2008	0	1	0	1
2009	0	1	0	1
2010	4	4	2	2
2011	11	4	1	3
2012	4	5	1	4
2013	7	4	1	3
2014	3	3	0	3
2015	10	17	5	12
2016	18	19	7	12
01–06/2017	7	4	1	3
Total	64	62	18	44
	126		62	

[a] Primary contamination is in the brewhouse, fermentation cellar, and storage cellar, and secondary contamination is in the bottling hall.

What is the level of risk?

TABLE III

Determination of the Minimal Tolerable Level of *S. diastaticus* in a 12-oz. Bottle of Beer

Cells Per Bottle	No. of Bottles Inoculated	Bottles Surviving Test [a]
2,200	4	0
250	4	0
200	8	0
100	8	0
50	4	0
20	8	0
10	8	0
5	4	1
4	6	6
	Total 54	

[a] No precipitation after at least six weeks at 29°C.

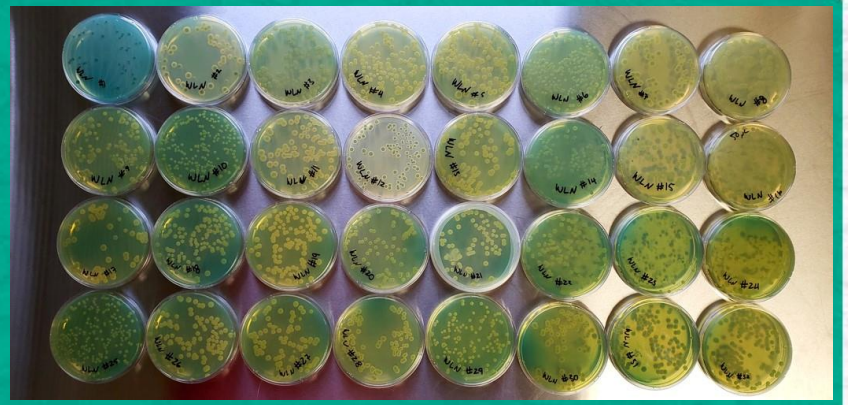
Low Cell numbers can still cause problems!

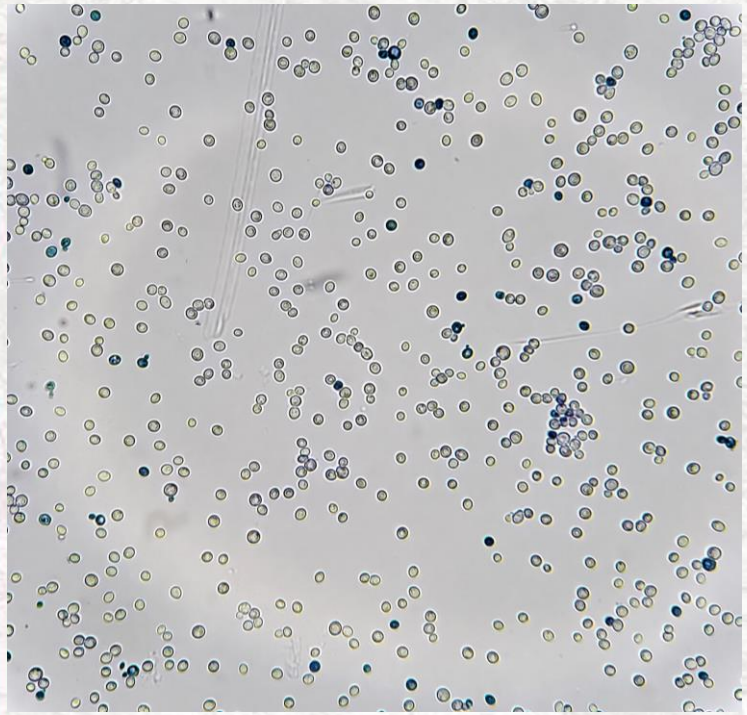
Best practice: Zero Tolerance

The Viability of Minimal Numbers of Saccharomyces diastaticus in Beer, Robert P. Greenspan (1966)

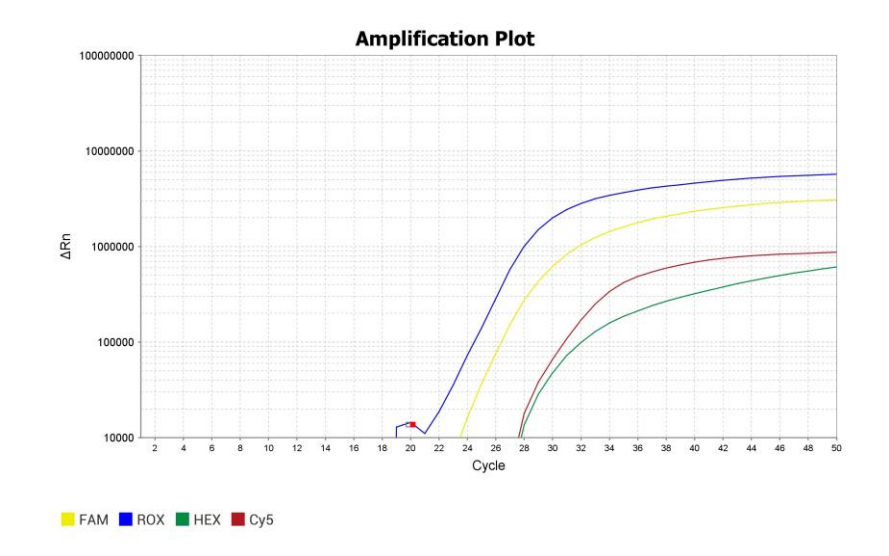
Methods of Detection

Total Plate Counts

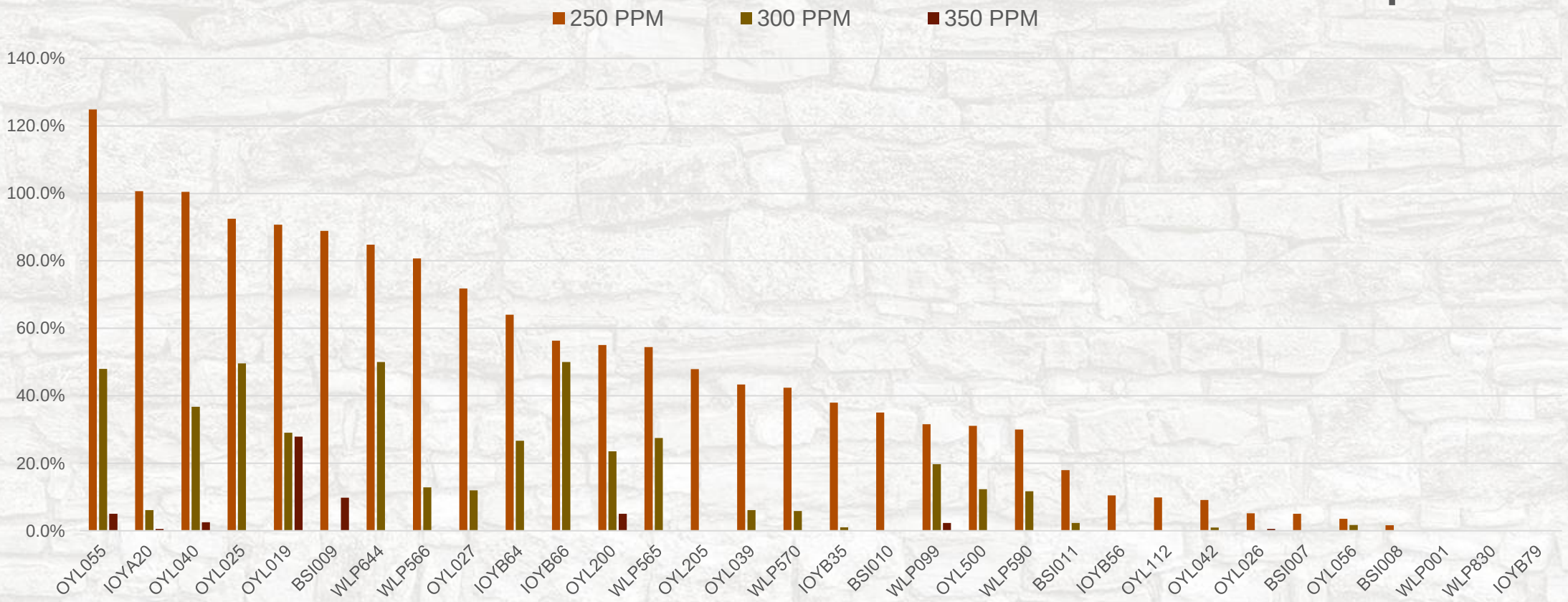




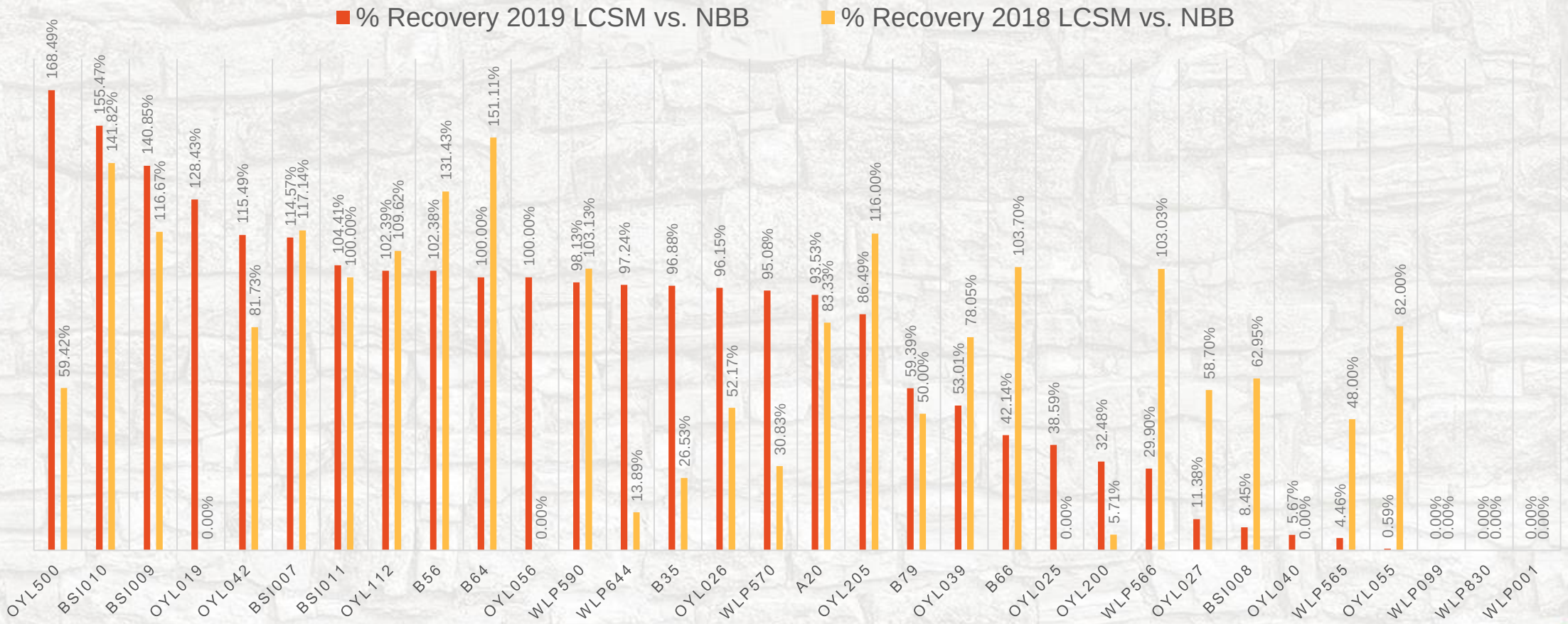
Polymerase Chain Reaction



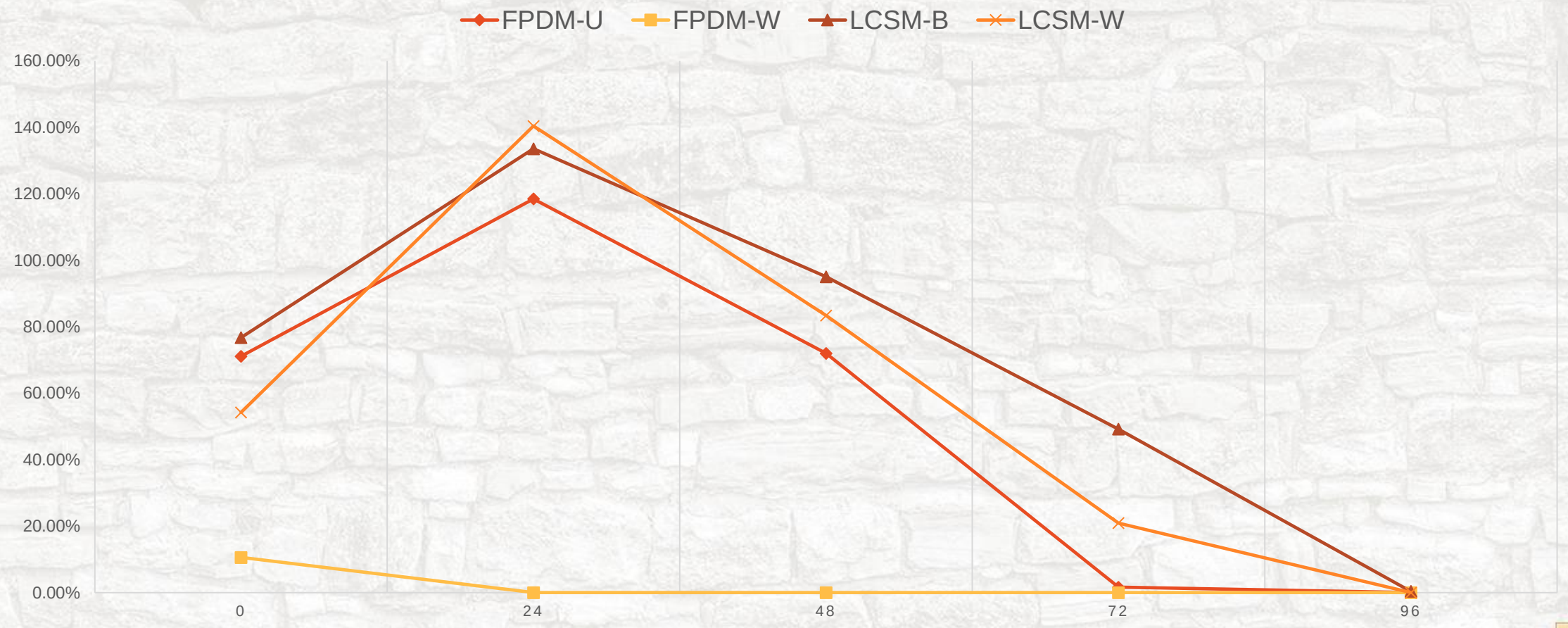
% RECOVERY NBB VS. YPD + $CuSO_4$



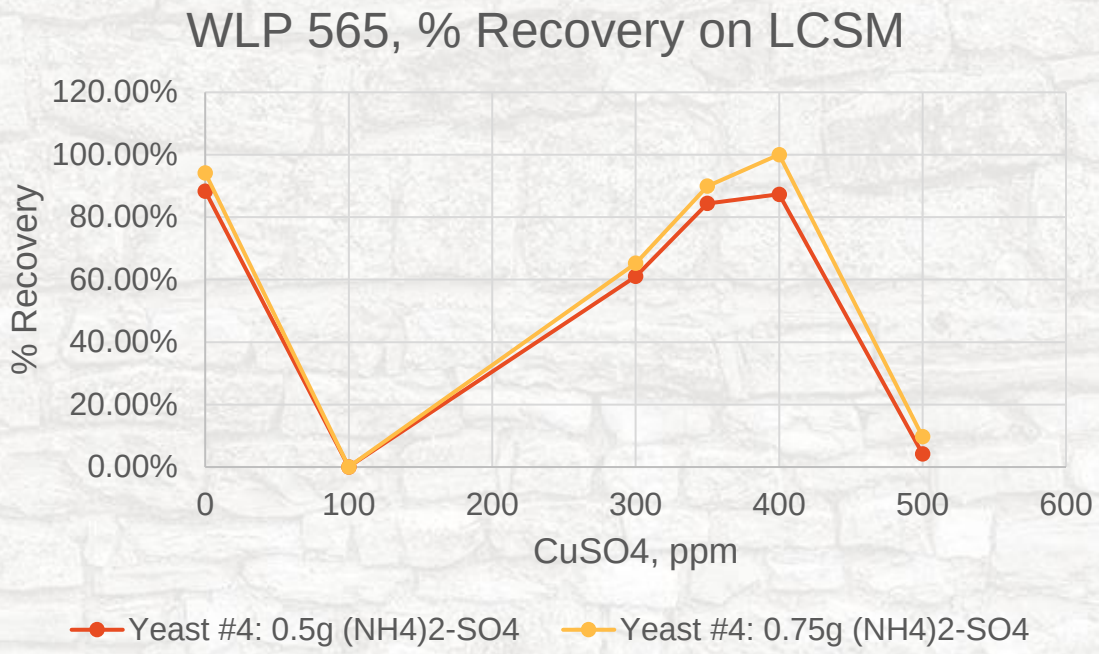
LCSM: 2018 VS 2019

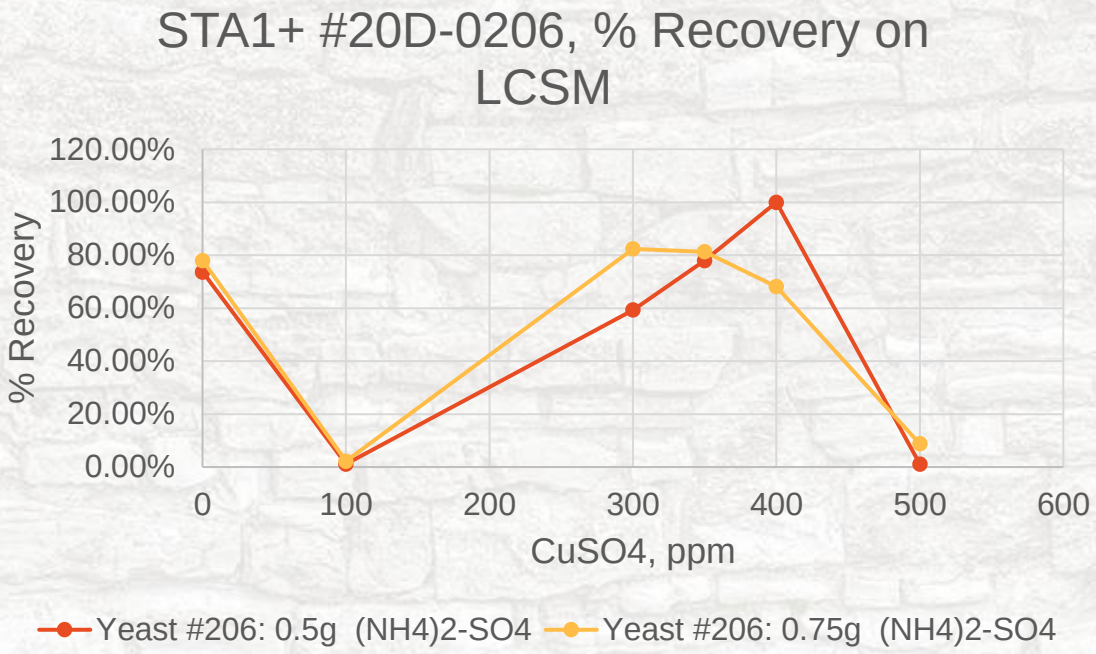


IOYB64, % RECOVERY VS NBB

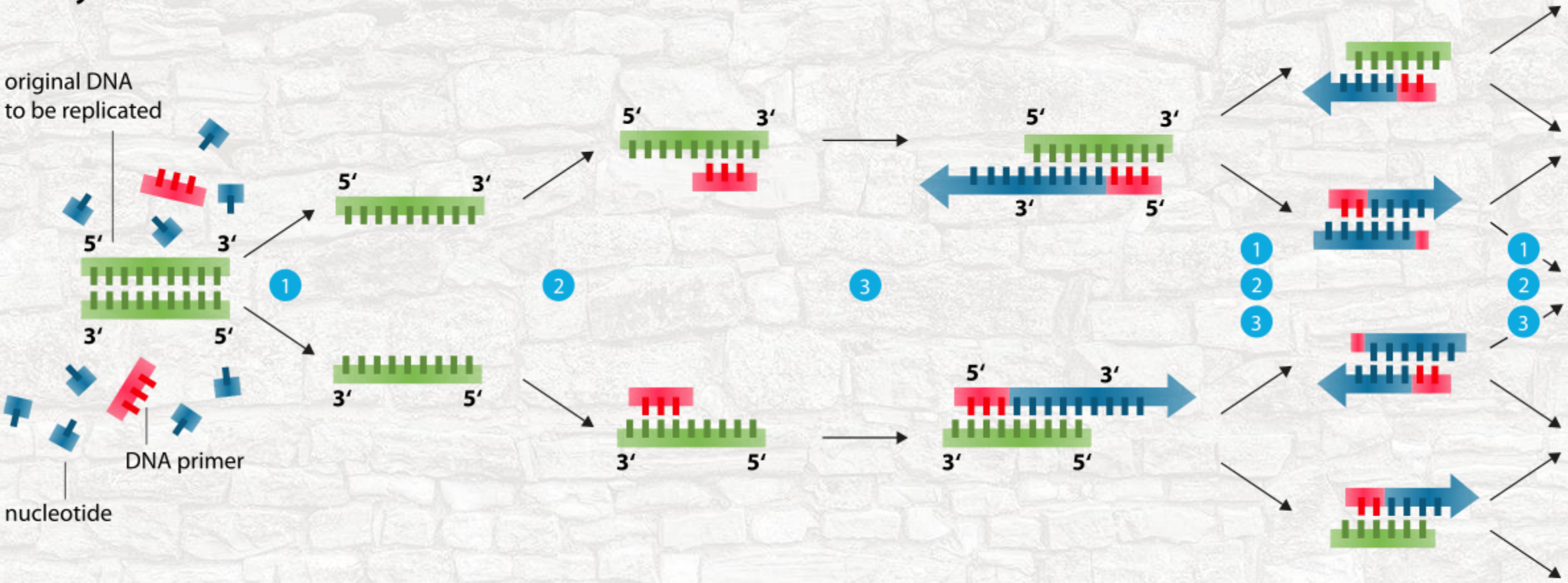


Optimizing LCSM For Diastaticus





Polymerase chain reaction - PCR



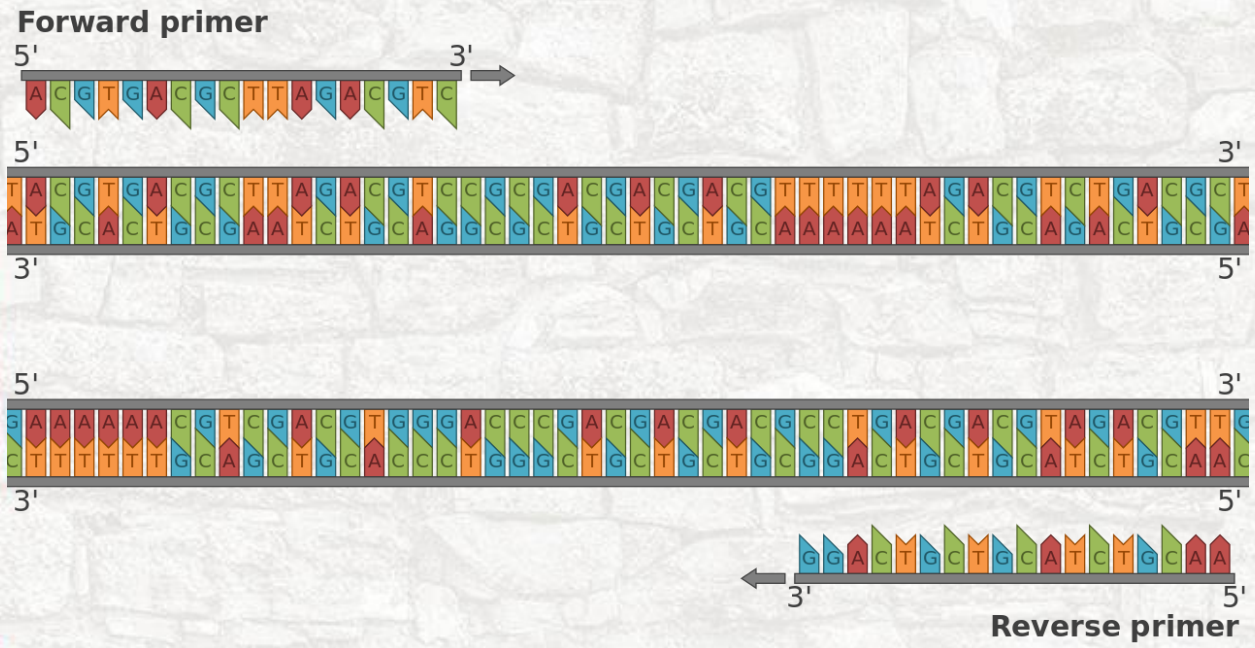
1 **Denaturation** at 94-96°C

2 **Annealing** at ~68°C

3 **Elongation** at ca. 72 °C

- Enzoklop - CC BY-SA 3.0, https://commons.wikimedia.org/w/index.php?curid=32003643

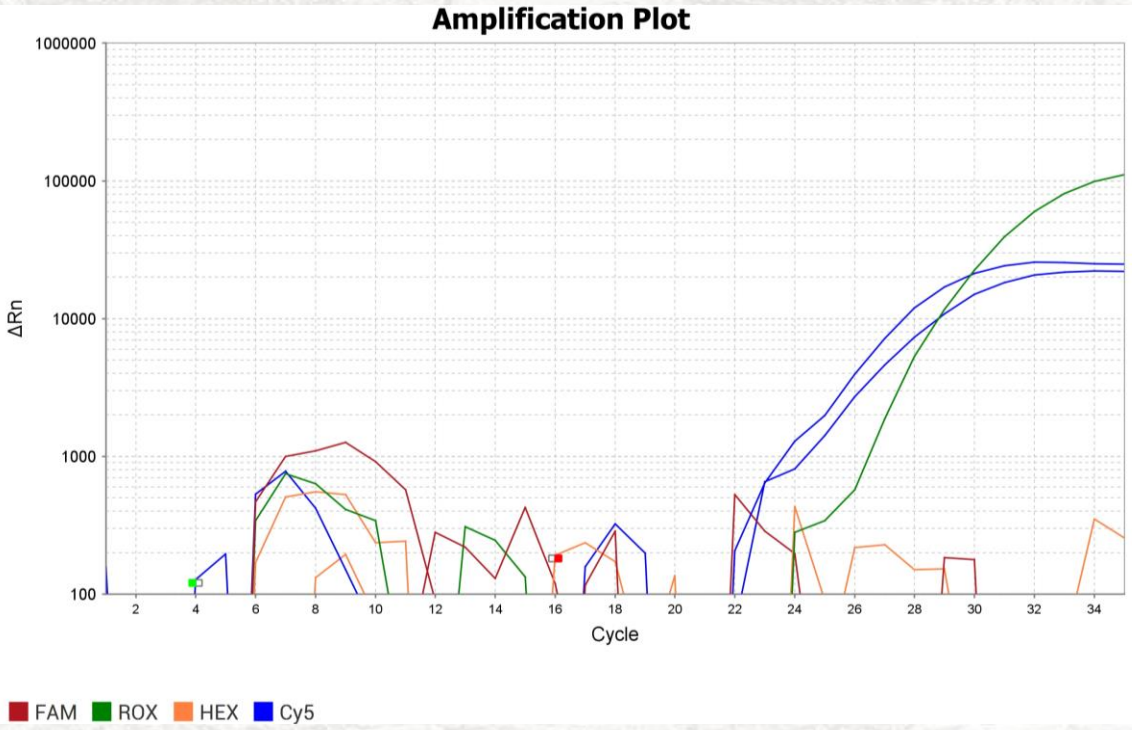
A Primer on Primers



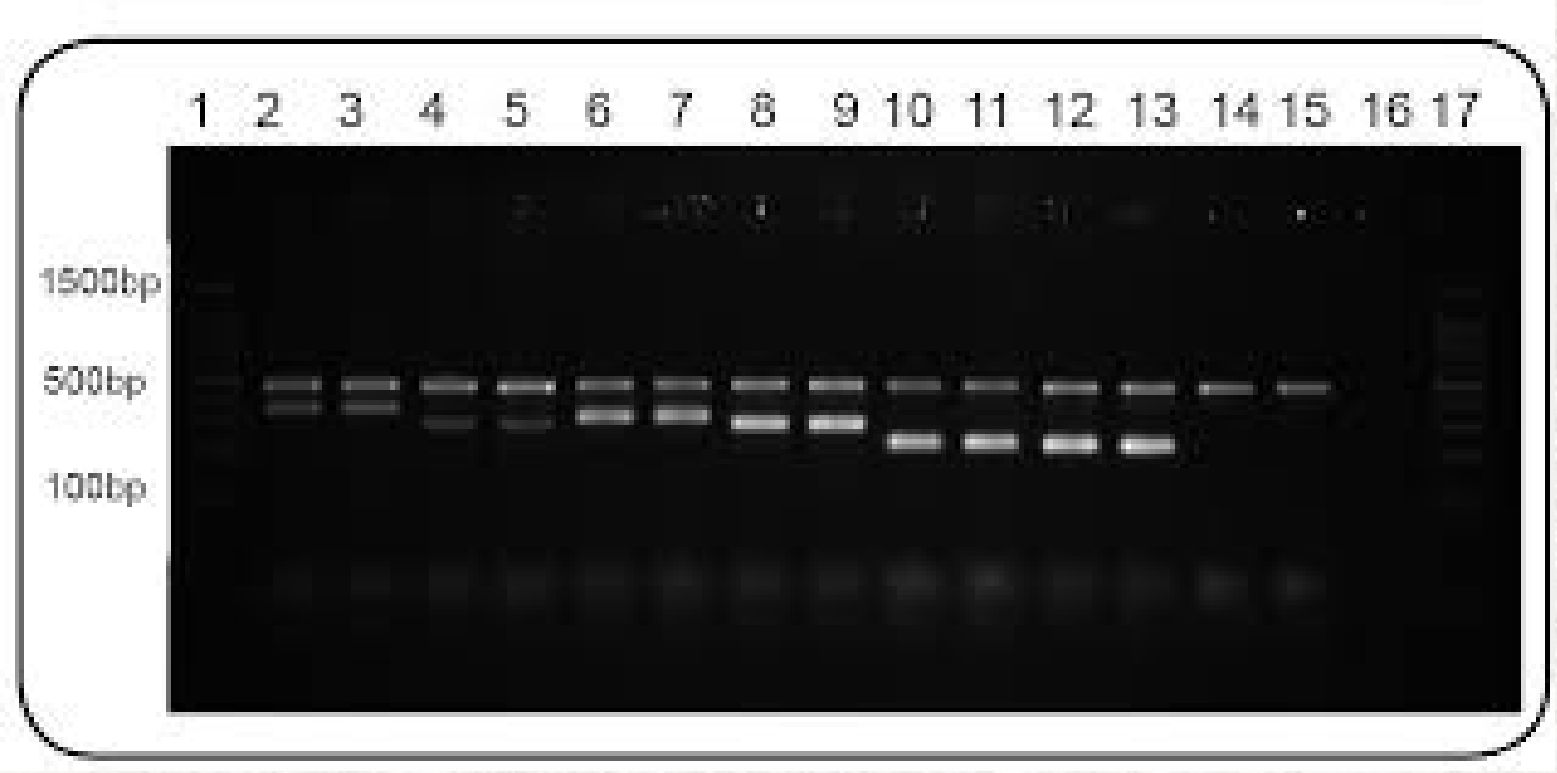
- Short, single-stranded nucleic acid utilized in the initiation of DNA synthesis
- Both Forward and Reverse Needed to Amplify Gene of interest

- By Zephyris - CC BY-SA 3.0, https://commons.wikimedia.org/w/index.php?curid=26794032

Real Time PCR



- DNA amplification detected by fluorescent dyes
- Can be visualized in Real Time!



End Point Detection

Thank you!

- Team BDAS
- Chuck Skypeck and BA Staff
- Justin Levaugn
- Jess Caudill
- David Bryant
- Lance Shaner & Laura Burns
- Karen Fortmann & Kara Taylor
- Spencer Weeks
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