
We need a bigger boat: Development of a starch fermentation assay as a tool for quality assurance
MASTER BREWERS
EST 1887
ASSOCIATION OF THE AMERICAS
UNITED WE BREW

We need a compliment to *STA1* PCR

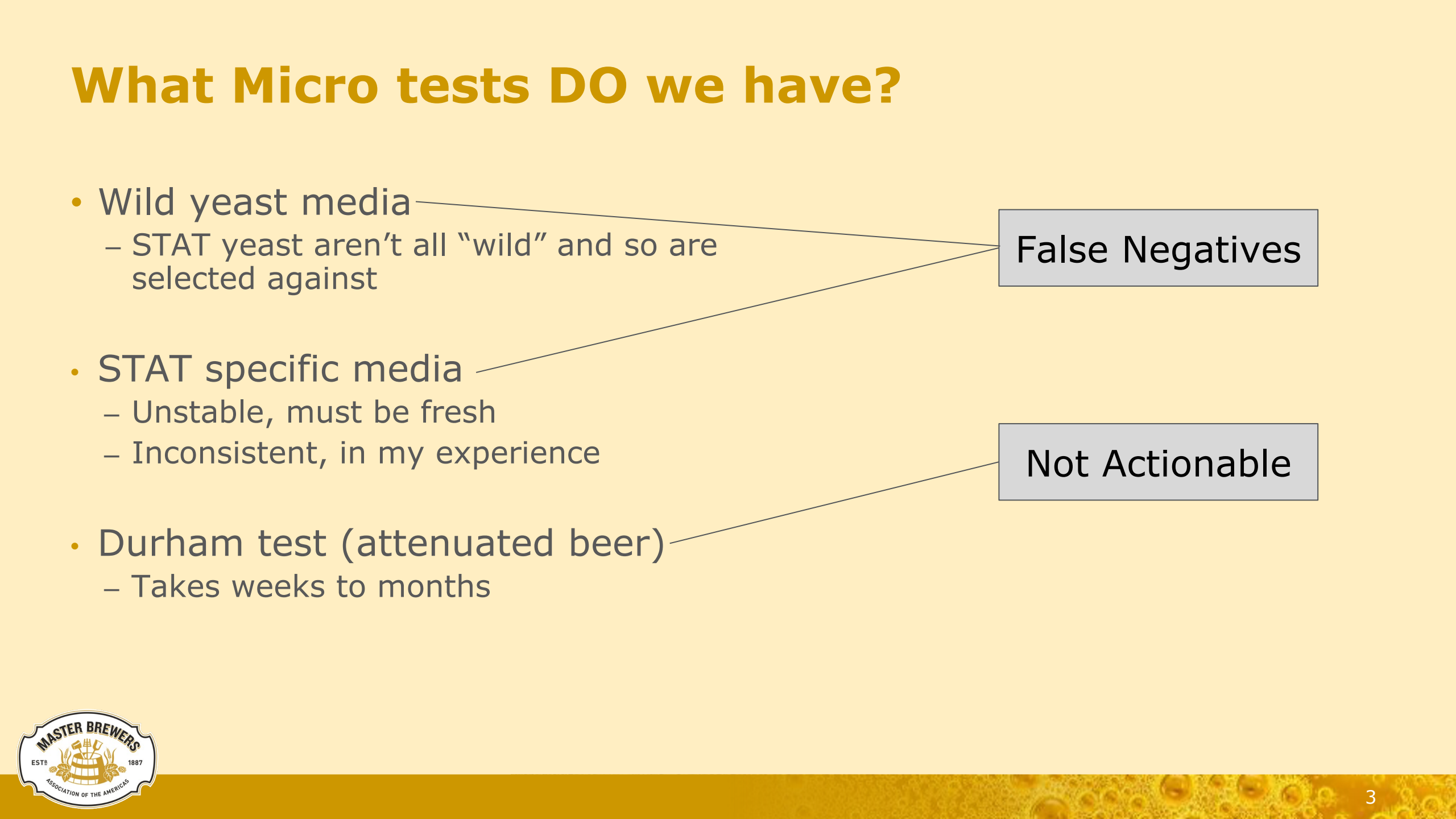
- Limitations to PCR
  - Swamping
  - Lack of controls
  - Sensitivity vs. Specificity
- Too much reliance on one test
  - PCR positive, but low risk strains.
  - PCR Negative but diastatic strains?
- 50+ commercial *STA1*+ strains
  - They don't all function the same
- PCR cost for small breweries.

Life will find a way.

-Michael Crichton
Jurassic Park - 1990

What Micro tests DO we have?

- Wild yeast media
  - STAT yeast aren't all "wild" and so are selected against
- STAT specific media
  - Unstable, must be fresh
  - Inconsistent, in my experience
- Durham test (attenuated beer)
  - Takes weeks to months



Goals for this test:

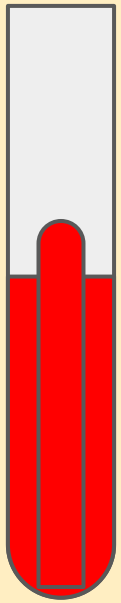
- Functional
  - Can “X” ferment beer starch?
- Quick
  - Days not weeks
- Compatible with my workflow
- Stable
- Robust
- Sensitive
- Non-selective
- Cheap

Keep it simple, Stupid

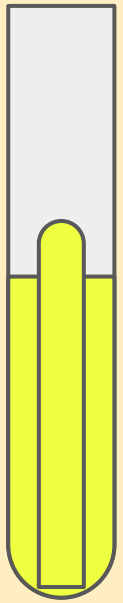
-Kelly Johnson

Inspiration: Bacterial Carbohydrate Fermentation

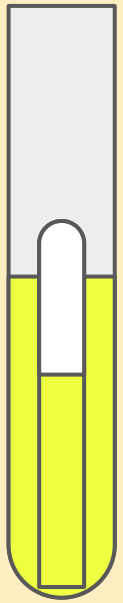
- Rich broth
- Test carbohydrate
- pH indicator
- Gas trap



No Growth
No Fermentation
No Gas



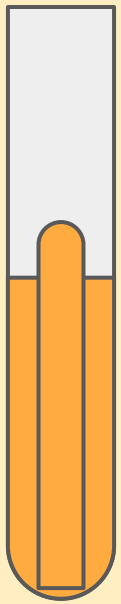
Growth
Fermentation
No Gas



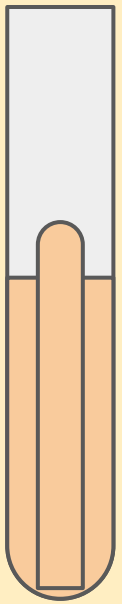
Growth
Fermentation
Gas

Yeast Extract, Peptone, Maltodextrin (YPM)

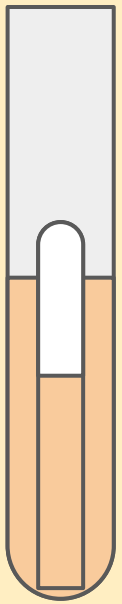
- Yeast Extract (1%)
- Bacto Peptone (2%)
- Maltodextrin (4%)
- pH adjust to 5.5



No Growth



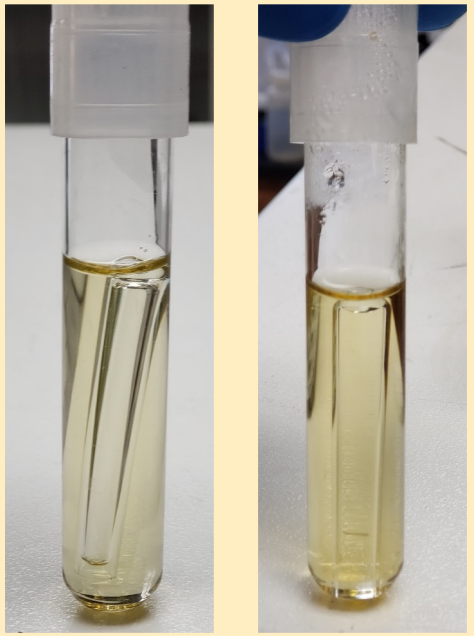
Light Growth
No Gas



Growth
Gas

YPM Preparation

- Kimax test tubes
- Durham tubes
- Reusable caps
- Cost per use <$0.10
- Reuse tubes and caps
- Make up a rack at a time.



Controls at 2-3 days

Sta1-

WLP001

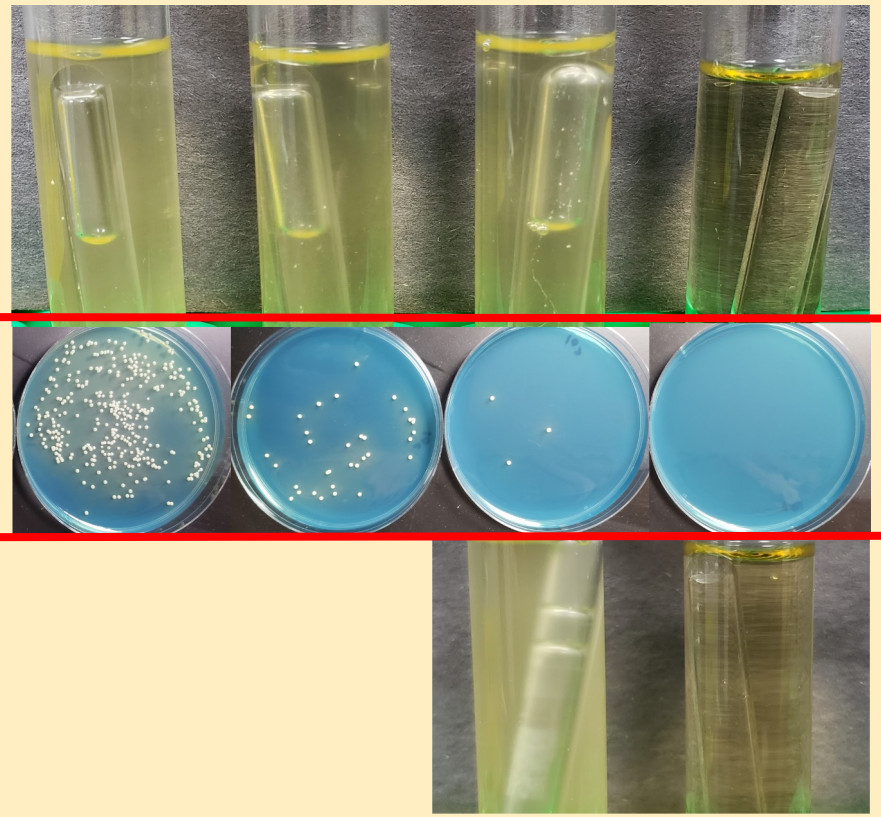
Sta1+

WY3711

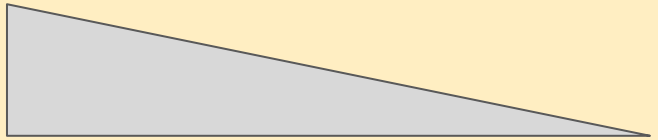
Inhibitor

WY3711
+ EDTA

Sensitivity Test: STAT yeast serial dilution



WY3711 serial dilution



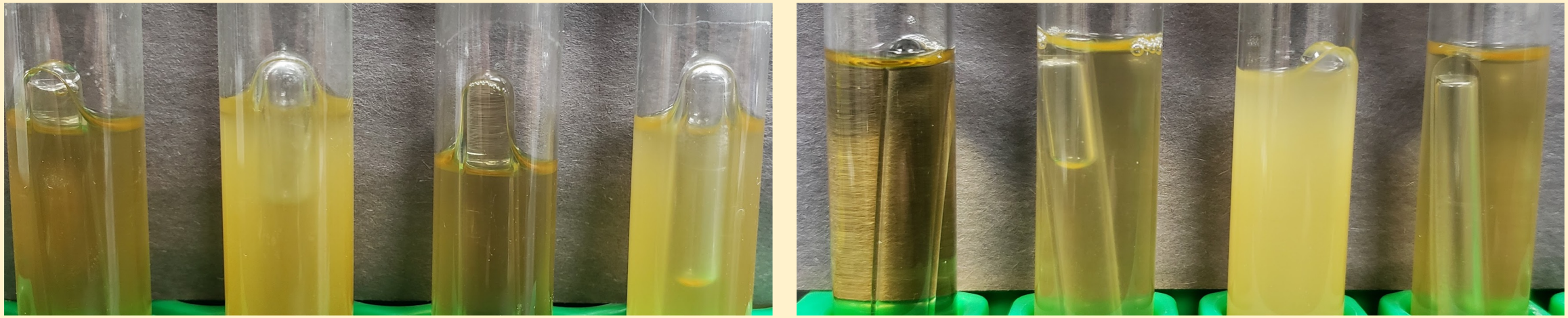
WLN plating for cfu

Same as above in a clean tank sample (containing ~10^3 WLP001)

- Single digit cfu sensitivity even in high background samples at 2-5 days.

Stability Test:

Cold stored RT 2 weeks Autoclaved 2x Autoclaved 4x



Neg 3711 Neg 3711 Neg 3711 Neg 3711

WLP830 WLP001

*

- Test is shelf stable without altering results

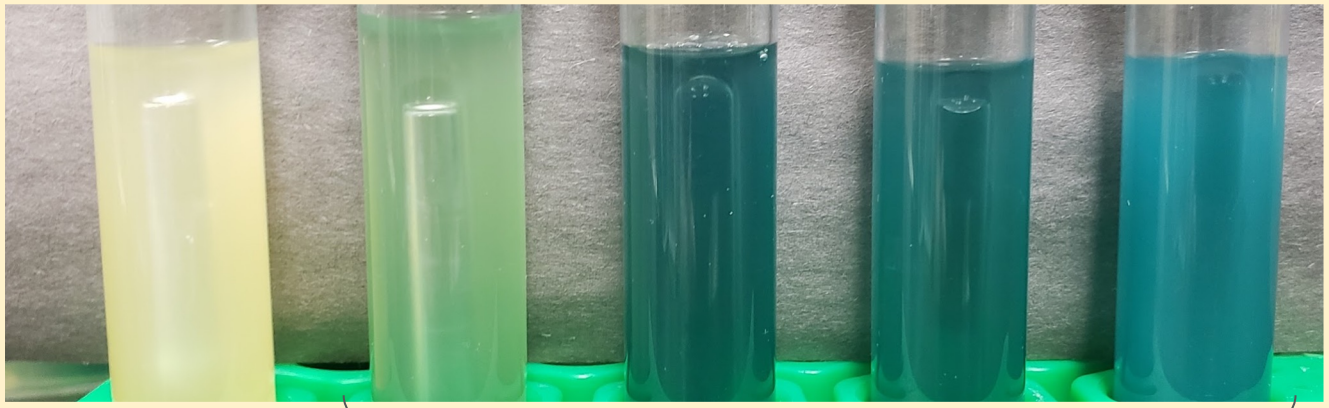
Consistent Results

Strain	Description	STA1	UAS	Days +	OYL	Prom.	This
026 / 3711	French Saison	+	+	2	+++	+++	+++
042 / 566	Belgian Saison II	+	-	8	++*	++	+
027 / 565	Belgian Saison I	+	-	5	++	++	++
019 / 570	Belgian Golden Ale	+	-	-	+	+	-
200 / 644	Brux-Like Sacc Trois	+	-	-	+	+	-

- Consistent with Krogerus et al. (Promoter study) and Burns et al. (OYL study)

Compatibility with other methods: pH indicators

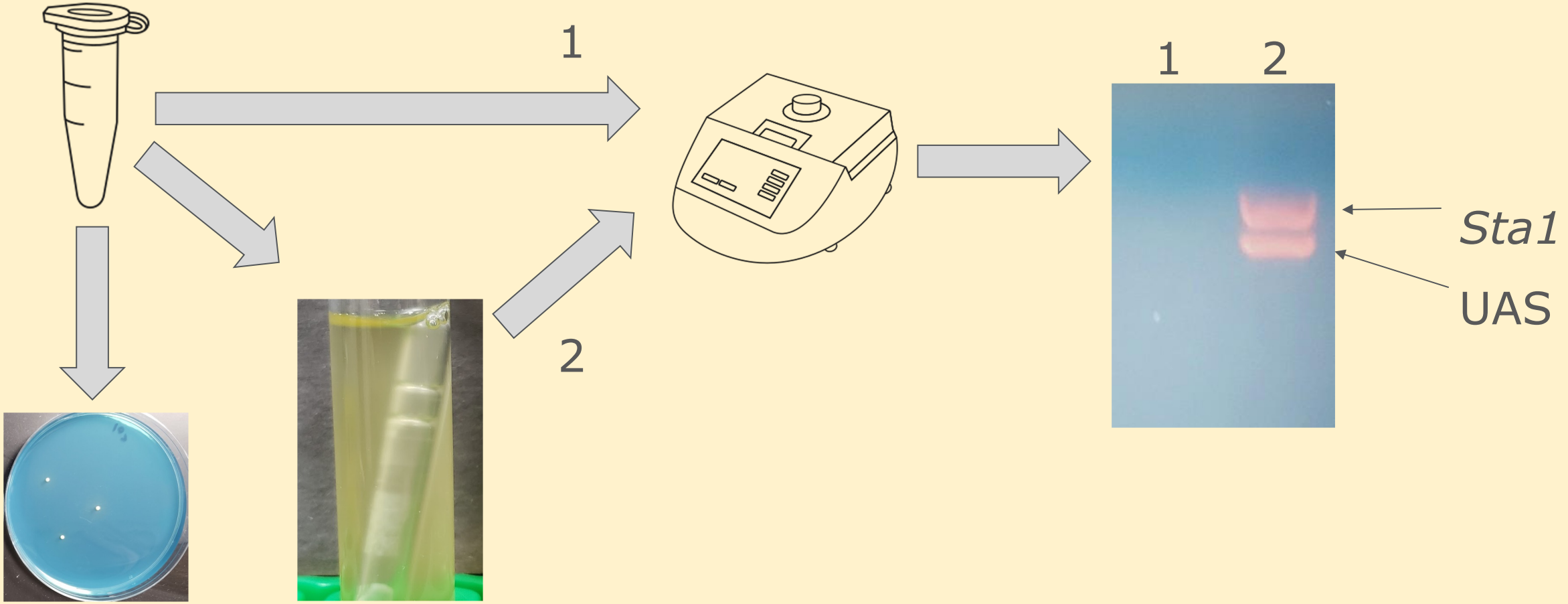
WY3711 BBT 0.1mL	WY3711 BBT 0.1mL	WLP830 mid 0.1mL	WLP300 BBT 100mL	OYL402 BBT 100mL



\+ Bromocresol Green

- YPM is compatible with pH indicators, but color change timeline is longer than gas production

YPM as a PCR Enrichment Broth



- YPM enrichment is compatible with Platinum Direct PCR Universal Kit - Thermo

Fail Points and Tips:

- Adding too much fermentable sample
  - 0.1 mL of early to mid ferm. sample
  - 1.0 mL of terminal samples
  - 100mL of BBT (centrifuged)
- Hop creep
  - Potential for further study
- Non-culturable microbes

False Positive

False Negative

Tips:

- Cheap peptone
  - Poor growth, slower results (not different)
- Durham tubes vs. 6mm test tubes

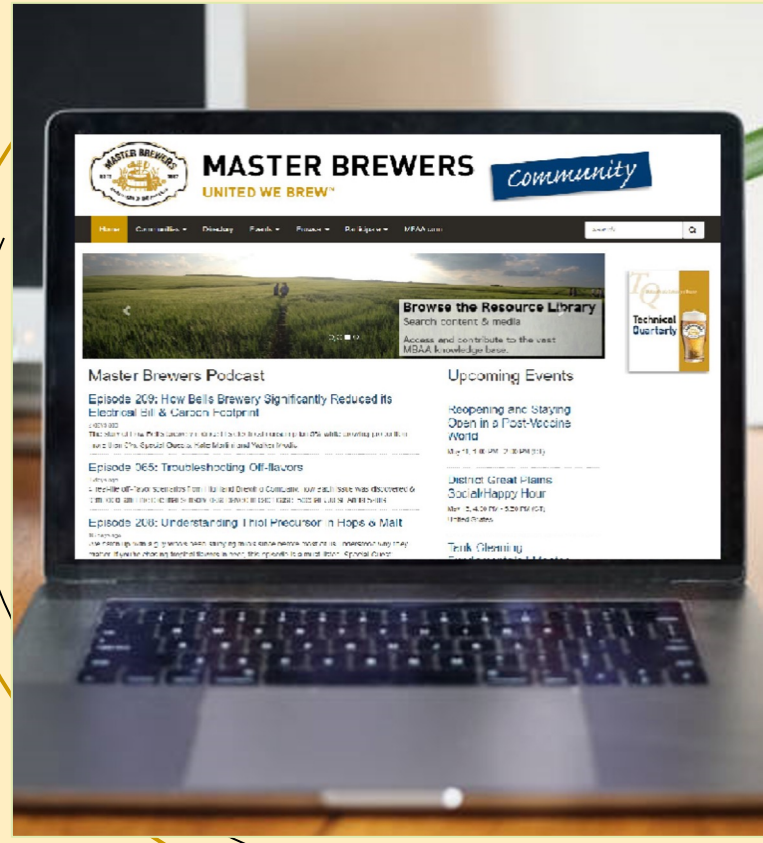
Future Work:

- Hammer out own hop creep inconsistencies
  - Useful screening tool?
- Continue STAT library comparison
  - Are all strains consistent?
- Starch staining/pH indicator studies.
- HPLC of breakdown products during starch fermentation.

Resources

- Laura T. Burns, Christine D. Sislak, Nathan L. Gibbon, Nicole R. Saylor, Marete R. Seymour, Lance M. Shaner & Patrick A. Gibney. Aug 2020. Improved Functional Assays and Risk Assessment for STA1+ Strains of Saccharomyces cerevisiae. JASBC. pp 167-180

- Kristoffer Krogerus, Frederico Magalhães, Joosu Kuivanen, Brian Gibson. July 2019. A deletion in the STA1 promoter determines maltotriose and starch utilization in STA1+ Saccharomyces cerevisiae strains. Applied Microbiology and Biotechnology.



Q&A