

## MicroBiome Study

### Samples:

Manufacturer	Harvest Year	Style	Varietal	Region	Other
Briess	2015	Pilsen	Merit 57	Wyoming	Manitowoc Facility, June 2016
Briess	2015	Pilsen	Copeland	Canada	Manitowoc Facility, June 2016
Briess	2016	Pilsen	Merit 57	Wyoming	Manitowoc Facility, Dec 2016
Briess	2016	Pilsen	Synergy	Wyoming	Chilton Facility, Dec 2016
Blacklands Malt	2016	Pale	Endeavor	Vega, Texas	Harvested May 2016. Malted ____
Weyermann	2016	Barke Pilsner	Barke	Agrargesellschaft Prießnitz, Germany	Harvested July-Aug 2016, Haßfurt facility Nov 2016
Patagonia	2015		Sebastian	X Region de Los Lagos, Chile	Malted May 31st, 2016, Lot 162233002021

### Analyses:

#### Malt Microbiome Analysis:

[Samples of raw grain for sequencing]

- 1) Briess 2015 Wyoming Pilsen Malt; t=10min, t=24hr
- 2) Briess 2015 Canada Pilsen Malt; t=10min, t=24hr
- 3) Patagonia Pale Malt; t=10min, t=24hr
- 4) Blackland's 2-row Malt; t=10min, t=24hr

#### Wort Microbiome Analysis:

*[Benchtop wort analysis for sequencing at ideal temperature w/ pH, control, TA, gravity]*

- 1) Briess 2015 Wyoming Pilsen Malt; t=0, t=24, t=48
- 2) Briess 2015 Canada Pilsen Malt; t=0, t=24, t=48
- 3) Patagonia Pale Malt; t=0, t=24, t=48
- 4) Blackland's 2-row Malt; t=0, t=24, t=48
- 5) Control; t=0, t=24, t=48

#### Wort NMR Analysis:

[Benchtop wort analysis using NMR for organic acid content and production]

- 1) Briess 2015 Wyoming Pilsen Malt; t=0, t=24, t=48
- 2) Briess 2015 Canada Pilsen Malt; t=0, t=24, t=48
- 3) Patagonia Pale Malt; t=0, t=24, t=48
- 4) Blackland's 2-row Malt; t=0, t=24, t=48
- 5) Control; t=0, t=24, t=48

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### Sample Preparation:

- 1) A nutrient-rich starter will be prepared and inoculated with the chosen grain and let stir (700rpm) at 43C for 24 hours.
- 2) Liquid Malt Extract: CBW (brand) Pilsen Light SG 1.035
- 3) Calcium Carbonate:
- 4) Nutrient BSG (Brewers Supply Group) Yeastex 82 Lot# 13200253668
- 5) Grain: Hamilton Beach, custom grind deluxe, Milled in coffee grinder 3 seconds, percolator setting
- 6) Water: De-ionized, autoclaved, 75F when mixed.
- 7) Ager plates made for pre-inoculated wort as a control to show if anything is alive. (Copeland had a little grain added to it already before we thought of this). All clear except Copeland.

Nutrient Rich Buffered Starter (NRBS) Preparation												
Sample	LME (283g)	CaCO <sub>3</sub> (10g)	Nutrient (1.5g)	Water (2000mL)	Grain Amount (20g)	Gravity	pH (pre-innoc)	Temperature	Time in	Time Out	Final Gravity	Final pH
2015 Briess Merit 57	290	10	1.5	1800	20+/0.5g	1.045	5.71	43	2:49pm	2:03pm	1.043	4.44
2015 Briess Copeland	290	10	1.5	1800	20+/0.5g	1.046	5.69	43	2:49pm	2:01pm	1.045	4.44
2016 Blacklands Endeavor	285	10	1.5	1800	20+/0.5g	1.044	5.74	43	2:49pm	2:02pm	1.043	4.47
2015 Patagonia Sebastian	284	10	1.5	1800	20+/0.5g	1.044	5.71	43	2:49pm	2:04pm	1.043	4.54
2016 Briess Merit 57	290	10	1.5	1800	20+/0.5g	1.045	5.89	43	12:00pm	12:00pm	1.043	4.09
2016 Briess Synergy	290	10	1.5	1800	20+/0.5g	1.045	5.89	43	12:00pm	12:00pm	1.043	4.07
2016 Weyerhamer Pilsner	290	10	1.5	1800	20+/0.5g	1.045	5.89	43	12:00pm	12:00pm	1.044	4.16

- 6) Use 25mL of NRBS for each grain and QS to 2000mL with acidified wort. Let stir at 43C for 48 hours. (NOTE: time point data taken after inoculation)
- 7) Lactic Acid \_\_\_\_\_
- 8) Liquid Malt Extract\_\_\_1132g QS 8L, pH to 4.63
- 9) Nutrient: none

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Wort Sample Preparation									
Sample	LME (10oz)	LA	Water (2000 mL)	NRBS (25mL)	Time in	Time Out	T=5min (SG, pH, TA)	T=24	T=48
2015 Briess Merit 57	283g x 4 = 1132g	4.63	2000	25	2:14pm		1.045; 4.71; 0.40	1.044; 3.63; 0.90	1.044; 3.49; 1.08
2015 Briess Copeland	283g x 4 = 1132g	4.63	2000	25	2:14pm		1.046; 4.76; 0.43	1.045; 3.59; 0.93	1.044; 3.51; 1.09
2016 Blacklands Endeavor	283g x 4 = 1132g	4.63	2000	25	2:14pm		1.046; 4.72; 0.40	1.045; 3.64; 0.95	1.044; 3.54; 1.07
2015 Patagonia Sebastian	283g x 4 = 1132g	4.63	2000	25	2:14pm		1.045; 4.72; 0.42	1.044; 3.61; 0.90	1.044; 3.48; 1.06
2016 Briess Merit 57	290g	4.6	2000	25	12:30pm	1:00pm	1.048; 4.55; 0.26	1.047; 3.59; 0.64	1.042; 3.46; 0.70
2016 Briess Synergy	290g	4.6	2000	25	12:30pm	1:00pm	1.048; 4.55; 0.27	1.042; 3.46; 0.61	1.034; 3.16; 1.01
2016 Weyermann Pilsner	290g	4.6	2000	25	12:30pm	1:00pm	1.048; 4.55; 0.26	1.042; 3.46; 0.61	1.041; 3.44; 0.92

(Note: LA=lactic acid; LME=liquid malt extract; NRBS=nutrient-rich buffered starter; TA=titratable acidity)