Yeast Management and QAQC for Small Breweries and Brewpubs

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Overview

- Laboratory Set-up
- Yeast storage
- Propagation
- Quality Assurance
- Quality Control
- Cost Benefit Analysis

Note

- Many of these processes contain similar or identical steps
- You might see pictures used multiple times throughout this presentation
- This is to show the relatedness between processes
  - Aseptic technique

Laboratory Set-Up

Our meager laboratory

Laboratory Set-Up

Laboratory Tools

- Autoclave
  - Used a pressure-cooker as a less expensive option

Laboratory Set-Up

Laboratory Tools

Petri Dishes and Vials

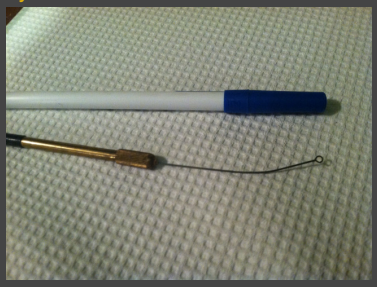
Laboratory Set-Up

Laboratory Tools

Parafilm

Laboratory Set-Up

Laboratory Tools



Inoculation Loop

Laboratory Tools

Laboratory Set-Up

Bunsen Burner

Laboratory Tools

Laboratory Set-Up

Fire Extinguisher

Item	Supplier	Price	Product Code	Need	Use
Butane	Cole Parmer	$43.00	EW-36138-90		Fuel for bunsen burner - case of 12 canisters
Purple nitrile exam gloves	Cole Parmer	$20.50	EW-86313-21		Box of 100
ParaFilm	Cynmar	$19.50	12031081		Versatile wrap for inoculated media to prevent contamination
Sterile Cotton Tipped Applicators	Cynmar	$11.50	HUM13917		Swabing for QAQC
Agar Powder	Cynmar	$59.00	CIM89103		Gelatinizing wort
Autoclave Tape	Cole Parmer	$4.25	EW-10703-58		Indicates specific temperature achived during autoclaving/pressure cooking
Test Tube Brushes	Cole Parmer	$34.00	EW-84550-52		Scrubbing glass plates, Erlenmeyer flasks, and vites
Glass Beaker - 400mL	Cynmar	$44.75	11533053		Mixing media - case of 12
Lab Tape	Cole Parmer	$5.25	EW-85209-32		Labeling, taping down LabMat
LabMat	Cole Parmer	$40.90	18050298	X	Absorbant mat for table top to minimize spillage area and to keep tabletop clean
Erlenmeyer Flask Set	Cynmar	$17.50	11520148		Yeast propagation
Portable Bunsen Burner	Cole Parmer	$50.50	SI-36310-47		Heating, boiling, mixing media
Pressure Cooker	Amazon	$94.95			Sterilizing glassware, O2/CO2 stones and zwickles under pressure
Inoculating Loop	Cole Parmer	$21.00	SI-14203-28		Inoculating yeast propagates and transfer of yeast from media to media
Loop handle	Cole Parmer	$19.00	SI-14203-34		Holds innoculating loop in a heat-resistant brass insulated sleave
5 Pack of Petri Dishes	Cynmar	$35.00	20699		Autoclavable glass for holding media $7 for 1pk/10
Lactobacilli MRS Broth	Cole Parmer	$66.00	EW-14202-24		Selective media for Lactobacilli, Pediococcus
Lyn's Cupic Sulfate Medium (LCSM)	White Labs	$65.00	TK3701		Selective media for Wild Yeast
Plastic Pipet 25mL	Cynmar	$6.00	15023993		To draw samples from Carboy - 1pk/10
Pipette Pump 25mL	Cynmar	$8.40	15024576		To operate 25mL pipet
Plastic Pipet 5mL	Cynmar	$4.67	15023990		Measuring and adding cupric sulfate for wld yeast medium
Pipet pump 10mL	Cynmar	$8.14	15024574		To operate 5mL pipet
	Total	**$678.81**			

Cleaning Glass

Laboratory Set-Up

Yeast Storage

- Making media plates with wort
- Plating yeast
- Transferring yeast from plate to slant
- Management

Making Media Plates With Wort

- Benefits
  - Yeast storage
  - Nutrients for yeast growth
  - Easily obtainable
  - Sterile
- Timing – 1 hr active over 4 hrs
  - Same day use
  - Wort media plates may be stored in the refrigerator for up to 2 months

Making Media Plates With Wort

- Collecting media to store yeast
  - Use clean glass
  - Light colored, low hopped, low gravity wort

Making Media Plates With Wort

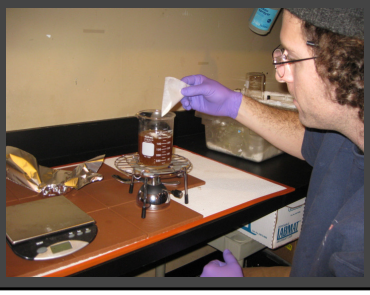
Heat wort gently while weighing 3.0g agar per 100mL wort

Making Media Plates With Wort

Yeast Storage

Add agar to wort

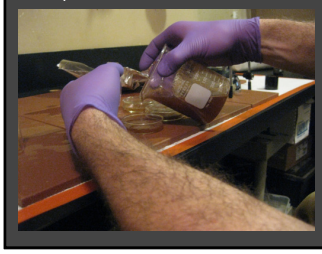
Boil gently for 5 minutes or until agar has completely solublized

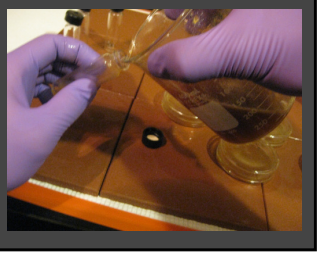


Making Media Plates With Wort

Yeast Storage

Pour plates and vials





Making Media Plates With Wort

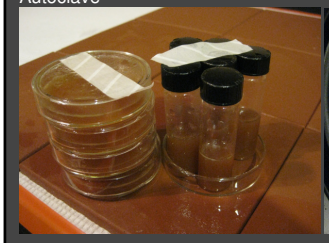
Yeast Storage

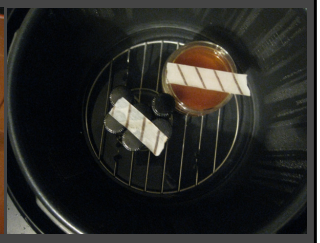
Autoclave Tape

Making Media Plates With Wort

Yeast Storage

Autoclave





Making Media Plates With Wort

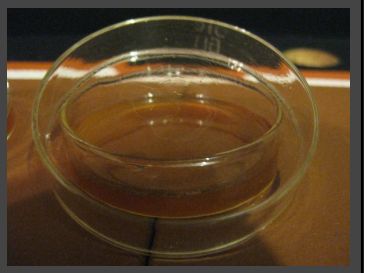
Yeast Storage

Allow media to solidify

Making Media Plates With Wort

Yeast Storage

Ready for use, or stored at 4° c/39° F in a clean container for 2 months max



Plating Yeast

Yeast Storage

Spray gloves with isopropyl alcohol

Plating Yeast

Yeast Storage

Taking primary Yeast sample

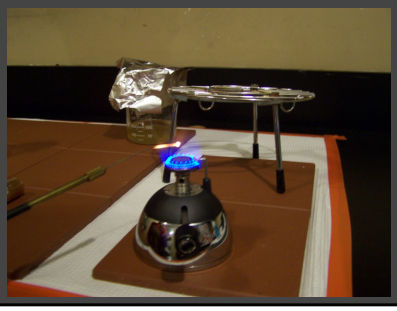
Plating Yeast

Draw off trub and yeast at the bottom of the cone prior to acquiring sample

Assemble fermenter with clean parts to help direct yeast

Plating Yeast

Flame loop

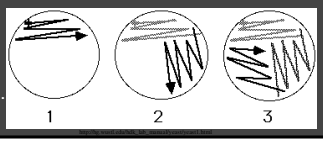


Plating Yeast

Hold loop under a stream of yeast, only a little will do

Plating Yeast

- Open the media plate just slightly, insert the loop and make a zigzag pattern towards yourself.
- Flame loop, then again draw zigzags starting in the area of the previous streak
- Repeat one more time
- You may not see the yeast on the media, but don't worry, it is.



Plating Yeast

Yeast Storage

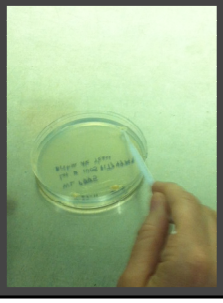
From a yeast provider

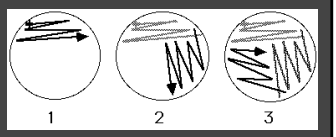
Plating Yeast

Yeast Storage

Plating Yeast

Yeast Storage





Plating Yeast

Yeast Storage

Wrap in parafilm and let sit upside down, at room temperature

Plating Yeast

Yeast Storage

- Growth will be visible in 2 days or less
- Try to identify single cell colonies

Plating Yeast

Yeast Storage

•Repeat?
•Use the single cell colony to transfer to a slant for storage, or to begin propagation.

Plating Yeast

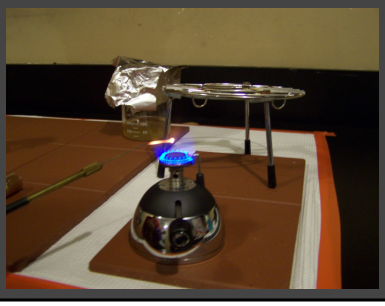
Yeast Storage

Storage

Transferring Yeast to a Slant

Yeast Storage

Flame the inoculation loop



Transferring Yeast to a Slant

Yeast Storage

- Remove the cap from the vial
- Flame the top of the vial

Transferring Yeast to a Slant

Yeast Storage

Use the inoculation loop to make a light *brush stroke* through the colony

Transferring Yeast to a Slant

Yeast Storage

Insert the inoculation loop into the vial and make a zigzag pattern towards you

Transferring Yeast to a Slant

Yeast Storage

- Flame the top of the slant
- Cap the slant and wrap in Parafilm
- Allow to sit at room temperature for 2 days
  - Store in a clean container at 4° c/39° F
- Manage slants in storage

Yeast Propagation

- Why?
  - To keep yeast healthy
  - Predictable fermentations
  - Contamination
- Timing
  - Begin propagation 7 days before first pitch into fermenting vessel

Yeast Propagation

- Steps
  1. Acquiring wort to begin propagation – 40 min
  2. Beginning propagation – 5 min
  3. Stepping up a propagation – 45 min
  4. Final yeast propagation outside of a fermenter – 45 min
  5. Sensory analysis – 5 min
  6. Pitch!

Acquiring Wort

Spray gloves with isopropyl alcohol

Acquiring Wort

- Autoclave
  - ~50mL Erlenmeyer, top covered with foil
  - Oxygen stone
  - Zwickle

Acquiring Wort

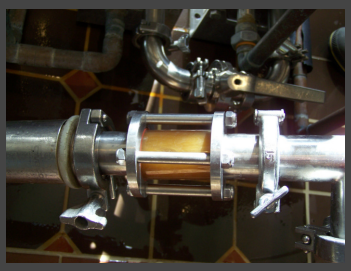
•Gather
- –Bunsen Burner
- –isopropyl alcohol
- –autoclaved Erlenmeyer

set on a clean surface close to the port from which you will draw wort

leave aluminum foil on top of Erlenmeyer

Acquiring Wort

Begin transfer of wort to fermentation tank, start oxygenation



Acquiring Wort

- Remove foil
- Flame top of Erlenmeyer

Acquiring Wort

Draw sample wort ~10mL into Erlenmeyer flask

Acquiring Wort

- Flame top of Erlenmeyer
- Replace foil
- Return to lab

Beginning Propagation

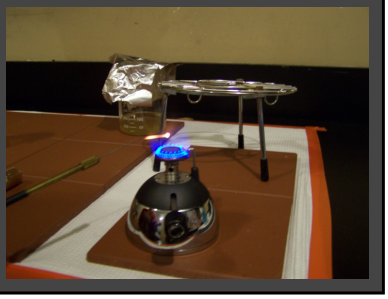
Spray gloves with isopropyl alcohol

Beginning Propagation

- Remove the foil
- Flame the top of the Erlenmeyer flask

Beginning Propagation

Flame the inoculation loop

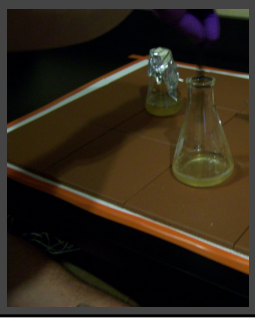


Beginning Propagation

With the loop brush the surface of the media lightly, picking up the single cell colony

Beginning Propagation

Insert the inoculation loop with yeast into the wort and shake the loop so the yeast becomes released



Beginning Propagation

- Flame the top of the flask
- Replace foil

Beginning Propagation

Cover the flask entirely with aluminum foil and ferment 1-2 days

Stepping Up A Propagation

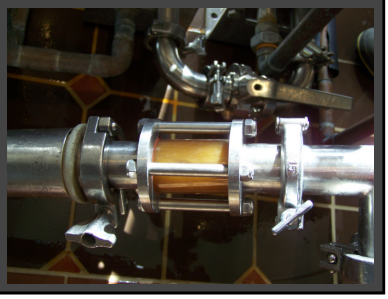
- Autoclave
  - 250mL Erlenmeyer, top covered with foil
  - Oxygen stone
  - Zwickle

Stepping Up A Propagation

- Gather
  - Bunsen Burner
  - Isopropyl alcohol
  - Autoclaved Erlenmeyer
- Leave aluminum foil on top of Erlenmeyer

Stepping Up A Propagation

Begin transfer of wort to fermentation tank, start oxygenation



Stepping Up A Propagation

- Remove foil
- Flame the top of Erlenmeyer flask

Stepping Up A Propagation

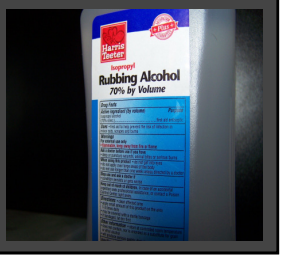
Draw sample wort ~90mL into Erlenmeyer flask

Stepping Up A Propagation

- Flame the top of Erlenmeyer flask
- Replace foil
- Return to lab

Stepping Up A Propagation

Spray gloves with isopropyl alcohol



Stepping Up A Propagation

- Remove foil
- Flame both Erlenmeyer flasks
  - Fresh wort sample
  - Initial propagate

Stepping Up A Propagation

Yeast Propagation

- Add the 10mL yeast propagate to 90mL wort
- Flame the top of the Erlenmeyer holding 100mL propagate
- Replace foil
- Agitate the mixture to homogenize the sample

Stepping Up A Propagation

Yeast Propagation

Cover Erlenmeyer with aluminum foil - ferment for 1-2 days, until krausen develops

Stepping Up A Propagation

Yeast Propagation

Repeat for 1L propagate

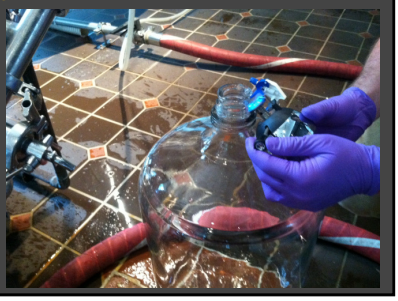
Final Yeast Propagation Outside Of Fermenter

Yeast Propagation

- Acquire a clean 5 gal carboy
- Soak with sanitizer

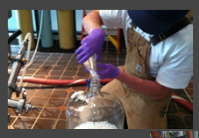
Final Yeast Propagation Outside Of Fermenter

- Drain carboy
- Flame top of carboy



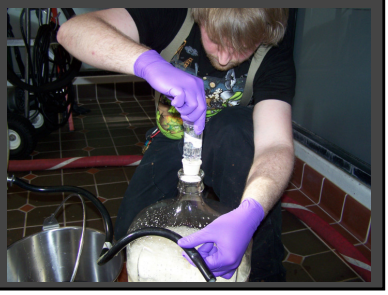
Final Yeast Propagation Outside Of Fermenter

- Fill carboy with 2.5 gallons
  - About half full
  - Again flame top of carboy
- Flame the Erlenmeyer holding the 1L propagate
- Add propagate to carboy
- Flame top of carboy



Final Yeast Propagation Outside Of Fermenter

Plug carboy with airlock and allow to ferment for 1-2 days or until krausening occurs



Sensory Analysis

Perform a sensory analysis of the propagate

- Acquire a 25mL pipet and pipet pump

Sensory Analysis

Yeast Propagation

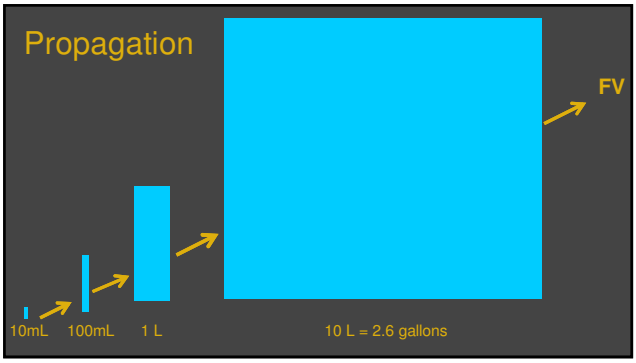
- Flame top of carboy
- Pipet 25mL from carboy and into a clean glass
- Flame carboy
- Replace airlock
- Taste

Final Yeast Propagation Outside Of Fermenter

Yeast Propagation

- Pitch beer in carboy into fermenter
- Send 1bbl of fresh wort for final propagation

Propagation



Quality Assurance

- Do regular checks for the cleanliness of your brewery
  - Checks cleaning technique, effectiveness
    - Identify problem locations, use tests to control quality of product
- Bacteria
  - *Pediococcus* and *Lactobacillus* strains
- Yeast
  - Wild yeast
    - *Brettanomyces, Kloekera, Pichia* strains etc.

Quality Assurance

- Timing
  - Same day use ONLY
  - Give 3 days from sampling for analysis
  - 1.5 active hrs over 4 total hrs

QA - Bacteria

- Be sure to clean lab before starting
- A dirty lab can lead to false positives which waste time and money
- Also keep glassware clean

QA - Bacteria

Acquire a media broth that is suitable for culturing *pediococcus* and *lactobaccilus* strains

QA - Bacteria

- To make ~35 plates at 8mL per plate begin heating 300mL of water in a 400mL beaker
- Weigh 16.5g of Lactobacilli MRS broth and add to heating water

QA - Bacteria

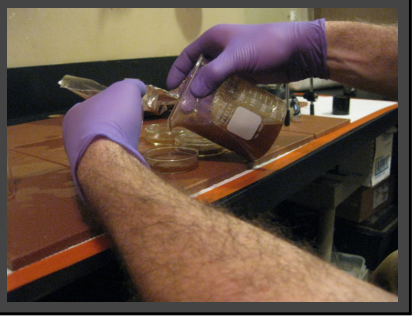
Quality Assurance

- Bring mixture to a soft boil
- Boil for 5 mins or until broth is fully mixed

QA - Bacteria

Quality Assurance

- Allow to cool for a minute
- Then begin pouring plates



QA - Bacteria

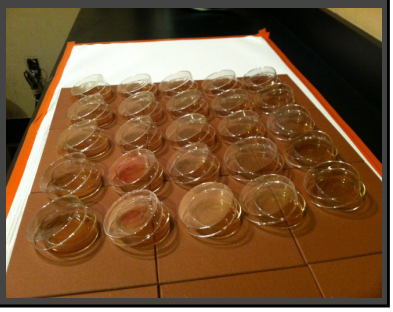
Quality Assurance

Autoclave the media plates

QA - Bacteria

Quality Assurance

Remove plates from autoclave and allow to dry on a clean countertop



QA - Bacteria

Quality Assurance

- During this time, identify the locations in the brewery that you would like to swab
  - Heat ex out
  - Filter out
  - Brewing water
  - Zwickles
  - Transfer lines
  - Yeast sample
  - Yeast propagate
  - Clean carboy

QA - Bacteria

Quality Assurance

Swabbing Technique

QA - Bacteria

Quality Assurance

- Return Plates to lab and wrap in parafilm
- Allow to sit upside down at room temp for 3 days

QA - Bacteria

Quality Assurance

Analyze plates

Quality Assurance

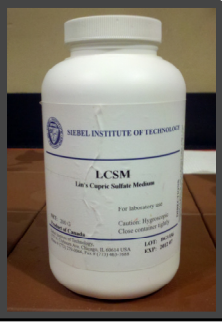
QA - Yeast

Clean Clean Clean

Quality Assurance

QA - Yeast

- Choose growth media that is appropriate for Wild Yeast
- Lyn's Cupric Sulfate Medium



Quality Assurance

QA - Yeast

- To make ~35 plates at 8mL per plate begin heating 300mL of water in a 400mL beaker
- Weigh 12.0g of Lyn's Cupric Sulfate Medium and add to heating water

Quality Assurance

QA - Yeast

Pipet 3mL of cupric sulfate and add to heating broth

QA - Yeast

Quality Assurance

- Bring mixture to a soft boil
- Boil for 5 mins or until broth is fully mixed in

QA - Yeast

Quality Assurance

- Allow to cool for a minute or two
- Then begin pouring plates

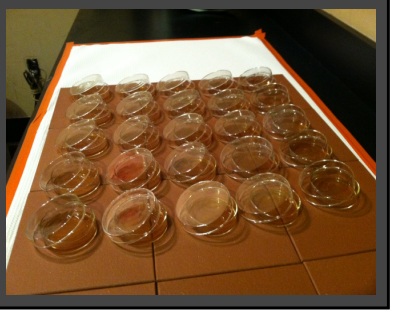
QA - Yeast

Quality Assurance

Autoclave the media plates

QA - Yeast

Remove plates from autoclave and allow to dry on a clean countertop



Quality Assurance

Yeast

- During this time, identify the locations in the brewery that you would like to swab
  - Brewing yeast
  - Heat ex out
  - Filter out
  - Clean FV/BBT
  - Zwickles
  - Transfer lines
  - Keg cleaner head
  - Yeast sample
  - Yeast propagate
  - Clean carboy

Quality Assurance

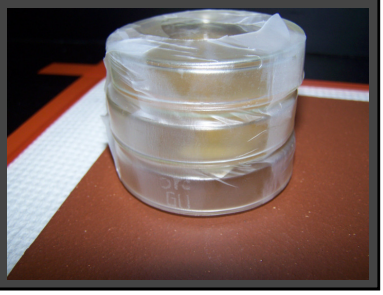
Yeast

Begin swabbing

Quality Assurance

Yeast

- Return Plates to lab and wrap in parafilm
- Allow to sit at room temp for 3 days



Quality Assurance

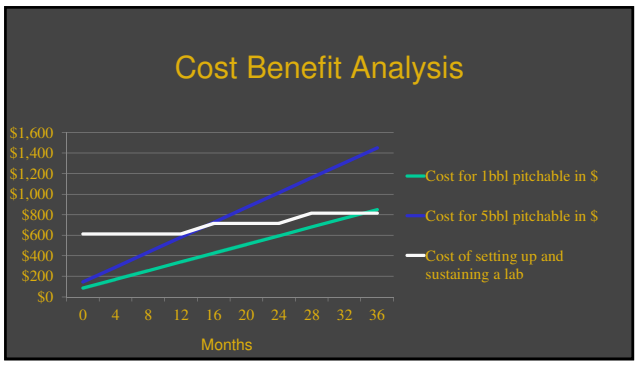
Yeast

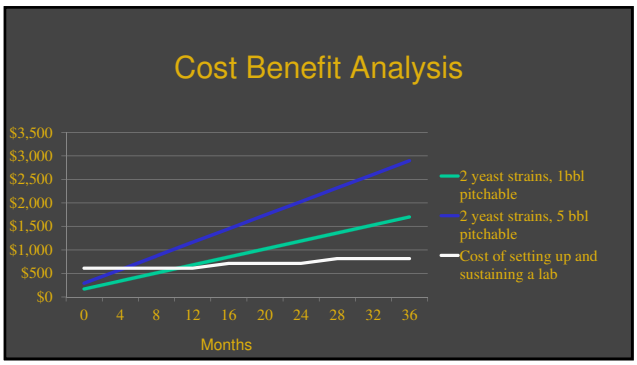
QA - Analysis

Acquire as much data as possible, utilize it to control quality.

	MRS 9/9/2011	LCSM 9/14/11	MRS 9/21
Heat exchanger	-	X	-
Steel EX Zwickle	+	-	-
Hot liquor Bottom	-	-	-
FV 1 Arm	+	-	-
FV1 Bottom	+	-	-
FV2 Arm	-	-	-
FV2 Bottom	-	-	-
FV3 Arm	-	-	-
FV3 Bottom	-	+	X
FV4 Arm	+	-	+
FV4 Bottom	+	+	+
FV5 Arm	-	-	-
FV5 Bottom	-	+	+
FV6 Arm	-	-	-
FV6 Bottom	-	-	-
FV7 Arm	-	+	-
FV7 Bottom	-	+	-
FV8 Arm	+	+	+
BBT Bottom	+	+	-
Keg Cleaner	+	-	-
Keg Line Downstairs		-	-
Keg Line Upstairs		-	+
Bottom SV5		-	-
Faucet SV5		+	+
Right transfer line Downstairs			-
Inside Clean tank			-
zwickle of clean tan			-
bottom of clean tank			+
rack arm of clean FV			-
Filter in			
Filter out			

	MRS 9/9/2011	LCSM 9/14/11	MRS 9/21
FV8 Arm	+	+	-
BBT Bottom	+	+	-
Keg Cleaner	+	X	-
	Keg Line Downstairs	-	-
	Keg Line Upstairs	-	+
	Bottom SV5	-	-
	Faucet SV5	+	+
	Right transfer line Downstairs		-
	Inside Clean tank		-
	zwickle of clean tank		-
	bottom of clean tank		+
	rack arm of clean FV		-
	Filter in		
	Filter out		
Recommedations			Noters
1. CMC Butt Valves on Racking arms and bottoms of FVs regularly and definitely before filtering/transferring			Diff sample #s
2. Cap butterfly valves on BBT and Upstairs kegging line			18-FV8 Zwickle
3. Scrubb keg cleaner keg engager after each use			23-SV3 Zwickle
+%	40.00%	34.78%	25.00%



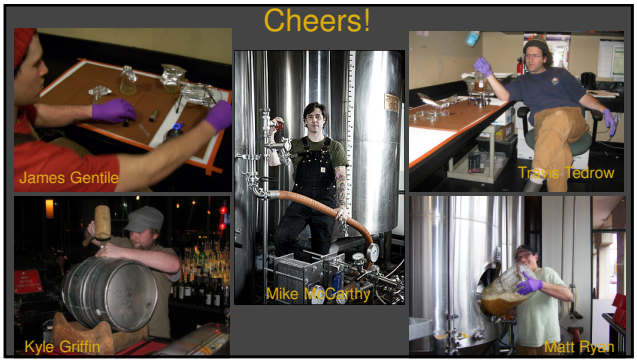


Conclusion

- These skills will make you more marketable as a brewer, and potentially can make you a more valuable employee
- Once this process is initiated, its on the schedule so its a normal task that needs to be completed
- Breweries in far off lands

- Further reading
  - The Fungus Among Us
    - Chris White Ph.D and Yussef Cherney, Fermentap, 2008
  - *Yeast Performance and Management*
    - Graham G. Stewart – Guild Review Paper
  - Yeast: The Practical Guide to Beer Fermentation
    - Chris White and Jamil Zainasheff., Brewers Publications, 2011

Cheers!



Micah Krichinsky
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@brewinggold

- Lab manuals
  - bit.ly/JQpYMg

- Further reading
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  - *Yeast Performance and Management*
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