Midland Craft Brewers Yeast Day

A workshop covering the use and storage of dried and liquid yeasts for the small scale craft brewer.

Programme: We aim to cover the basic aspects of yeast handling, short and long term storage of strains, preparation of starters and slant media. There are notes on different techniques and advice on how to manage fermentations, with practical demonstrations backed up by written notes and discussion.

We aim to cover;

- Sources of yeast including Wyeast packs, White Labs vials, Brewlab slants, dried yeast and commercial bottle conditioned beers
- Preparing a yeast starter, step-up stages and appropriate pitching rates
- Managing the fermentation
- Outline of short and medium term methods of yeast storage
- Preparing agar slants for yeast storage (*practical*)
- Preparing a starter from a home grown or Brewlab slant (*practical*)
- Preparing a starter from a bottle conditioned beer
- Transferring yeast cells from a master culture using sterile transfer techniques (*practical*)
- Streaking a slant from a white Labs vial (*practical*)
- Yeast maintenance and problems that may occur / acid washing
- Alternative techniques including a home made stir plate

General Notes

Dried Yeast

Most craft brewers have started out using dried yeast and very good results have been obtained including prizes won at national competitions. However it isn't a matter of buying any packet available and just "chucking it in," we need to take a few steps to ensure we get the best results possible. There are several well known strains available from Lallemand, Fermentis and more recently Mangrove Jack, and whilst dried yeast has a good shelf life when stored refrigerated, if stored at room temperature it can lose 20% of its viability within a year. So suggest buy from a specialist dealer and store in the fridge till needed. Dried yeast should always be rehydrated with ten times its weight in water, usually at 24 – 30^{o}C for ale strains and 21 – 25^{o}C for lager strains, however please note makers instructions. The water for rehydration should be brought to the boil first both to sterilise and remove chlorine, and then cooled to the correct temperature in a small jug. After sprinkling the dried yeast on the surface, leave for 15 minutes before stirring and then leave a further 15 minutes before pitching. If the temperature is more than 6^{o}C greater than the wort, add equal amounts of wort to the jug allowing a few minutes rest to avoid "shocking" the yeast by sudden temperature changes. On the Fermentis website www.fermentis.com there is an excellent download called *"Tips and Tricks"* which contains valuable advice about how to get the best results from dried yeast. One point to note is that if you decide *not* to rehydrate dried yeast, then it should be pitched into the wort at 20 – 24^{o}C then left to cool down to the normal fermentation range of 18 – 22^{o}C. (for ales)

Advantages of dried yeast; *Cost, convenience, no starter required, consistency.*

Disadvantages*; Limited in choice to a few commercial strains that tend in general to be more "neutral" in character than liquid yeasts.*

Liquid Yeast sources

White Labs: These yeasts were supplied in heavy duty plastic vials but more recently plastic sachets are used. Both contain approximately 100 billion cells.
Wyeast Activator: This yeast is supplied in a "smack-pack" which should be activated at least 4 hours before using by placing on a hard surface and striking to burst the inner membrane which contains a nutrient. If well within date and undamaged by heat, the pack should swell within 4 hours. However, older or heat damaged packs can take longer, even days to activate.

Both the Wyeast packs and Whitelabs vials are claimed to be sufficient for direct pitching into a 19L batch of beer, however for larger brews or incorrectly stored / handled packs a 1.5 – 2.0L starter is strongly recommended.

Brewlab slants: These can be obtained directly from Brewlab and are usually posted out within 3 – 5 working days so are guaranteed to arrive fresh and in good condition. The instructions for use recommend that a 300ml starter is prepared which is assumed to be sufficient to inoculate a 23L brew. However for larger volumes and stronger beers, a 150 – 200ml starter further stepped up to 1.5 – 2.0L would be appropriate to ensure a complete and thorough fermentation.

Bottle Conditioned Beers: Suitable examples would be Fullers Bengal Lancer and St Austell Proper Job which are available from several supermarkets with a good turnover to ensure freshness. Beer from most Micro Breweries are best avoided, the reason being few have the necessary laboratory facilities to ensure proper quality control and most use dried yeast anyway.

Advantages of liquid yeasts; *Vast number of available strains with varying characteristics to suit all styles of beer.*

Disadvantages; *Initial cost, time spent in preparing starter(s)*

Preparing a yeast starter

For both Wyeast and Whitelab packs a simple 1.8L starter can be prepared as follows. Dissolve 160 – 180g dried malt extract in 2 litres of water and boil for 10 minutes, then cool as rapidly as possible to around 20^{o}C before transferring to a suitable sterilised container such as a demijohn or 2 litre flask. After shaking the container to admit some air the vial or pack can be added, then after covering loosely with foil should be kept at room temperature for 12 – 24 hours, at which time the starter should be fermenting vigorously and be ready for pitching into a 20 – 30L batch. Ideally the starter should have an OG of 1.038 – 1.040 and a pH of around 5.0 so if your water alkalinity is high, it will be beneficial to add a few drops of A/M/S (C/R/S) or a small pinch of citric acid to help bring the starter within range. Shaking the yeast starter intermittently to disperse CO_2 build up will help to increase the total cell count, whilst a stir-plate will save you the effort! (see below) A few hops (or more conveniently hop pellets) may be added when boiling the starter, however this is not essential and note that a highly hopped starter will have a negative effect on yeast viability.

Managing the fermentation some hints and tips

- Aerate the wort well before pitching the yeast, a simple way to do this is to collect the wort in half filled gallon water containers which are capped and shaken vigorously for several seconds before adding to the FV. This should give dissolved oxygen levels of around 8 – 10 ppm and is considerably more effective than an air-stone, that is, unless the latter is delivering pure oxygen.
- Try and cool the wort to 18^{o}C before pitching the starter then insulate the FV against sudden temperature changes or better still build a fermentation chamber or cupboard. When fermentation takes hold, the heat produced will cause the temperature to rise by a few degrees to about 20 – 22^{o}C which is ideal for most ale strains.
- Some top cropping yeasts (such as St Austell for example) form a large head which usually needs gentle rousing back into the wort a couple of times a day to prevent fermentation slowing down. The very top layer of dirty yeast can be skimmed off and discarded if necessary.
- When the terminal gravity has been reached, cover the FV and rest for at least 24 hours before cooling (if possible) to assist clarification. The beer can then be bottled / kegged directly, or racked to a second enclosed tank if desired for dry-hopping or further clarification if needed. However if you do rack to a secondary vessel take great care to minimise any aeration, this can result in oxidised beer with its cardboard like taste.
- Adding a yeast nutrient such as Murphy's Yeast-Aid or Wyeast Nutrient will benefit the yeast and help prevent slow or incomplete fermentations; these can be caused by a deficiency in the wort of zinc, nitrogen or amino acids. A ¼ teaspoon added to the wort at pitching should be adequate for a 25L batch, alternatively the nutrient can be added during the last 10 minutes of the boil and the dose increased to ½ teaspoon, as some nutrient is absorbed by the trub.

Recovering yeast from a bottle conditioned beer

Noting the comments above, select a suitable beer and leave in a fridge or cool place for a few days for the yeast to settle. The yeast in St Austell Proper Job seems to adhere well to the base of the bottle and, therefore, is a good yeast to try.

Sterilise a 250ml conical flask and prepare a 150 – 200ml starter solution using 15g – 20g dried malt extract. Working in a sterile area, remove the crown cap and carefully decant all but the final 1 – 1.5cm of beer from the bottle. Then swirl the bottle vigorously several times to dislodge the yeast sediment from the bottle base. Then pour the sediment into the flask containing the starter solution, cap with kitchen foil (not too tightly) and leave at room temperature until signs of fermentation are evident (this can be as rapid as 18 hours but should ideally not take longer than 48 hours) Once the starter is fermenting vigorously the starter should be stepped up to 1 – 2L depending on the brew length.

Saving yeast for short and long term storage

For short term storage yeast can be collected from the FV and kept for up to a week in a sterilised container stored in a fridge at 1 – 4^{o}C. For collecting yeast from top-cropping strains, it's best to discard the first crop after about half way through the fermentation. Then when the head has properly reformed and before the fermentation is starting to slow down, remove some clean yeast with a sterilised spoon and place in a jug or jar which should be loosely covered with foil to allow escape of any CO_2 whilst under storage. The amount for re-pitching must be decided largely by experience, however there is a very useful calculator for this very purpose on www.mrmalty.com With bottom cropping strains the yeast may be recovered from the base of the FV after racking but

there will also be trub, dead and mutant cells present. Thus the quality will not be as high as with yeast recovered from top-cropping, however if a Conical FV is used, the first settlings (which contain most of the dead cells and trub) can be dumped and the clean yeast which settles later can be recovered.
For longer term storage some craft brewers simply omit the priming's from a particular bottle whilst packaging, then fill almost to the brim and store in the fridge for up to a couple of months. The sedimented yeast is then used to prepare a 150 - 200ml starter which should be further stepped up to 1.5 – 2.0L before pitching. Other craft brewers have experimented by storing yeast slurries under sterilised distilled water, the slurry however must first be rinsed several times to remove any beer traces.

However, the professional approach is to store the yeast on agar slants, which when grown and refrigerated will keep for up to six months. This is performed by streaking a slant with yeast using an inoculating loop, the slant is then allowed to grow at room temperature for 2 – 4 days before being refrigerated. During the growth period the cap must be vented to release any CO_2 which has built up, alternatively the caps can be loosened by a quarter turn and the slants inverted until grown. For long extended storage periods, large scale breweries and yeast banks freeze their yeast cultures under glycerol at -80^{o} C; however this is beyond the scope of most craft brewers.

Yeast strain maintenance

It makes sense to take some precautions to make sure that your yeast remains in top condition and to be aware of potential problems that may occur. A question often raised is "how many generations should I use my yeast for" and this must depend largely on your own particular technique, attention to cleaning and sterility, the strength of beers produced and the characteristics of the particular strain that is being used. Certain yeasts are more robust than others particularly in respect to mutation, autolysis and temperature extremes. Brewers propagating their own yeast for the first time would be wise to renew their strain after three generations, this could be extended to five or more generations after gaining experience and assessing beer quality. The challenge here will be maintaining a sterile culture, as cleaning and sterilisation methods which may have been adequate for dried yeast used on a "once only" basis may not be robust enough for repeated use, as any bacteria present will multiply with each successive generation to a point where beer quality becomes unacceptable. (*Brewers who ferment in plastic vessels should take extra care as these can harbour bacteria and wild yeasts, particularly if they have also been used for fermenting wine and cider)*

Some warning signs that the yeast may require renewing are briefly noted below.

- Extended fermentation times when all other factors are equal
- High terminal gravities and elevated beer pH readings (under-pitching, possible mutation, tired or stressed yeast)
- Increasingly hazy beer which may also be resistant to fining (bacterial infection or wild yeast contamination)
- Vegetal / cooked corn (common wort bacteria O. *proteus*)
- Phenolic taints and off flavours (wild yeast)
- Acetic aroma (bacterial infection)
- Autolysis – earthy smell taste, burnt rubber (stressed or tired yeast with large proportion of dead cells)
- Loss of characteristics / esters
- Poor growth when streaked on to fresh agar slant (loss of viability)

Renewing the strain at the first sign of any trouble or off flavours would be a sensible precaution, however there are a few steps that can be taken to prevent or minimise these problems.

For example, pitching a sufficient quantity of yeast and ensuring the wort pH is correct will prevent long lag times and reduce the possibility of infection with common wort bacteria. Acid washing is a relatively simple preventative measure that will kill wort and other similar gram negative bacteria, but unfortunately is not effective against gram positive (lactic) bacteria or wild yeast. Using this method, the yeast slurry for pitching is lowered to a pH of between 2.0 – 2.5 by *cautiously* adding food grade phosphoric acid whilst stirring. The slurry must be kept between 2 – 4^{o}C and held at this temperature for between one and two hours and then pitched into the wort as soon as possible. Some of the more traditional breweries acid-wash their yeast routinely after a certain number of generations, this is regarded as a sensible precaution.

Individual Techniques

A. General Information

With a strong emphasis on top-cropping strains, (for example Wyeast 1469) yeast is stored on agar slants for periods of between two weeks and six months, although for very short term use freshly cropped yeast may be stored in the fridge for up to four days before re-pitching. Home made slants are produced using 30ml polypropylene vials. When a brew has fermented more than half of the available sugars, a small amount of clean yeast is skimmed into a jug and at least four slants are streaked, and once grown stored in the coldest part of the fridge at 1 – 4^{o}C. Note that these slants should never be stored in the freezer.

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The slants are kept for up to 6 months and when needed, a 100 - 200ml starter is prepared which after 24 hours is stepped to 1.0 - 2.0L, depending on batch volume; this in turn is pitched into the brew.

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Certain strains have been kept going for many generations and more than a year using this method whilst acid washing has been used on two occasions, more as a preventive than remedial measure. Yeast-vits such as Murphy's Yeast-Aid are routinely used at the minimum recommended level; the author believes that they have been beneficial in helping to maintain yeast and subsequent beer quality. He also thinks that top-cropping yeast is advantageous for propagation purposes as the trub tends to settle as a ring on the tank sides, whilst bacteria and dead cells tend to sink to the bottom and so are not carried forward when clean yeast is skimmed from the top.

B. Preparation of agar slopes

Method can be used for either spray dried wort or product from a batch of beer in preparation.

Spray dried wort.

1. Ensure that the universals (25ml) and caps are clean.
2. Prepare wort as for the yeast seed vessel but at concentrations up to 75grams spray dried malt extract per 500ml of water.
3. Pressure cooker will take 27 universals at 10ml/universal (total 270ml). Therefore, add about 40 grams per 350 ml of water and so should end up with about 270ml. Alternatively, use about 270ml of freshly prepared wort.
3. Agar to be used at 3% (w/v) so require about 9 grams of agar for 27 slopes.
4. Add agar to hot wort, mix thoroughly and then aliquot into universals.
5. Pressure cooker for 15 minutes at 20 psi.
6. Remove carefully and allow cooling at an angle. Store cool and inspect before use.
7. Yeast stocks are restreaked every 4-5 months as they noticeably lose viability towards 6 months.

C. Yeast seed vessel-aerobic (with stirrer).

1. Bruclean a 1L duran plus lid and stirrer bar. Separately, 10ml pipette, plastic sieve, and plastic funnel.
2. Water 450ml and 0.3ml of CRS and small amount of yeast nutrients.
3. Add 45 grams of spray dried malt to cold acidified water and bring to the boil with a few hop cones (the higher the alpha acid the better). Boil 10 minutes.
4. Rinse duran and plastics thoroughly and keep lid in place on duran.
5. While hot and with swirling filter wort through sieve and funnel. Cool in cold water bath with lid on.
6. Shake thoroughly to dissolve maximum concentration of air.
7. Rinse yeast slopes (3), prepared previously with cooled wort (twice) using pipette with bulb) to transfer yeast from agar.
8. Put foil on duran and place in cupboard on stirrer plate at approximately 20^{o}C. The culture should be active in 6-18 hours, visibly opaque. Ensure stirrer provides vortex for maximum aeration.
9. To bulk up yeast, boil (10 minutes) 450 ml water with 40grams of spray dried malt, cool and aerate and add to culture after approx. 48 hours to a total of approx.800ml. I have used a 1:1 mix of sugar and malt extract at this stage without problems.
10. Add seed culture (24-36 hours later) to aerated wort in fermenter (up to 23L) and stir well.
11. It is possible to switch off stirrer a couple of hours before yeast is required so that the bulk of used spent malt extract can be decanted and this is recommended in some articles on the subject. Alternatively, the duran can be placed in the fridge overnight, decant in the morning of brewing and allowing the yeast to come to room temperature before use.

YEAST PITCHING RATES

Commercial Yeast

General Information and Assumptions

1. A vial of Whit Labs. (WLP) yeast contains 1x10^{11} yeast cells (recommended for the pitching of 19L of ale without need for seed preparation).
2. Dried yeast is usually 2 x 10^{10} cells per gram.
2. A sachet of Nottingham yeast contains 11g dry weight, 2.2 x 10^{11} yeast cells.
3. Commercial recommendation for an inoculum is 1x10^{6} cells /degree Plato/ml.
    - For ale 0.75 – 1.2 x10^{6} cells /degree Plato/ml.
    - For lager 1.50-1.80 x10^{6} cells /degree Plato/ml.

Example 1

Ale at 1040 O.G. with a volume of 23 litres requires:
1. Convert O.G. to degrees Plato (divide by 4) = 10 degrees Plato.
2. Minimum 0.75x10^{6} cells x 23,000 x 10 = 172,500 x 10^{6} cells= 1.725x10^{11} cells total.
3. Maximum 1.2x10^{6} cells x 23,000 x 10 = 276,000 x 10^{6} cells= 2.725x10^{11} cells total.
4. One sachet of Nottingham yeast contains 2.2 x 10^{11} yeast cells.

Example 2

Ale at 1048 O.G. with a volume of 23 litres requires:
1. Convert O.G. to degrees Plato (divide by 4) = 12 degrees Plato.
2. Equation 1x10^{6} cells x 23,000 x 12 = 276,000 x 10^{6} cells= 2.7x10^{11} cells total.

3. One vial of WLP insufficient and would need a 2L starter.

Recovered Yeast

Yeast can be recovered from a previous fermentation and then used to inoculate a new fermentation. One can calculate the number of cells required or this can be obtained online (e.g. Mr. Malty). However, information on the quality of the yeast is required (amount of trub present) and the slurry "thickness".

One can take these variables into consideration when one is entering this into the calculation. The percentage of trub can be assessed visually by suspending a sample of the slurry in water and allowing it to settle for 15 minutes. Alternatively, it can be removed/reduced by washing.

One can calculate the number of cells / ml in the slurry using a microscope or a spectrophotometer. In the absence of these pieces of equipment one can attempt to calculate by comparative methods.

1. WLP contains $1x10^{11}$ cells in 36ml ($3x10^{9}$ cells/ml) so slurry "thickness" can be used as a standard. The settled volume is approximately 14 ml (or $8x10^{9}$ cells/ml). If specific dilutions are done (e.g. add 72 ml of water to vial contents to $1x10^{9}$ cells/ml) then comparisons with the recovered yeast slurry can be made.
2. A similar exercise can be done for Nottingham yeast (see above).

Once an idea of slurry thickness has been estimated then this can be used for future preparations of inocula. Inoculum consistency is important and if the fermentation is not at an optimum then, in subsequent fermentations, more or less yeast can be added.

Importance of Correct Pitching Rate

Underpitching	Overpitching
Low lag time	Short lag times
Potential for contamination	Affects health of yeast over subsequent generations
Slow fermentation	Poor head retention
Reduces flavour and aroma development	Yeast autolysis produces off flavours
Low attenuation	Low attenuation
Diacetyl and acetaldehyde formation	Diacetyl and acetaldehyde formation

In general, it is better to **slightly** over, rather than under, pitch.

Post Script

There is a major difference between the production of commercial and recovered yeasts (including seeds prepared from commercial yeast). Commercial yeasts are grown aerobically and have the appropriate physiology (sterol content) to ferment. Recovered yeasts have been grown in the absence, or in very limited concentrations of, dissolved oxygen. Therefore, for repitching, **even** more attention should be given to aerate the wort prior to inoculation.