Introduction

Maintaining and manipulating your own yeast bank can be a very rewarding hobby. Anyway, be prepared to invest a conspicuous amount of time and resources to such an activity.

Although the subject is quite advanced, you don't need to be a veteran brewer to achieve good results, but some technical background and an understanding of the underlying principles obviously helps. Thus before setting up your own yeast ranch, I strongly suggest you read some of the very fine books available on brewing science and techniques.

Don't be scared by the seemingly complex steps involved: the description might be wordy, but most manipulations are actually performed in a matter of seconds, and after some practice they will become second nature to you.

You will notice the stress on **sterility** throughout these pages. While a homebrewer can easily be satisfied with sanitizing his equipment, we will be manipulating very small amounts of yeast cells in a bacteria friendly environment.
Though very simple in essence, these procedures are performed by professional labs in sterile rooms or under laminar flow hoods.
Since we poor homebrewers generally don't have access to such facilities, we must settle for manipulations that can guarantee nothing more that a high *probability* of sterility.
Nevertheless, we really should concentrate our efforts, and strive for sterility.

The concepts and procedures I describe are the ones I use daily (well, almost. I do have a life, you know...), and are derived mostly from the works of G. Fix, P. Rajotte and information gathered on the internet (HBD and The Brewery), adapted to my own conditions and possibilities, and reviewed by professional biologists.
Special thanks to Joseph Kish for bringing to my attention the distilled water storage method.
Homebrewers are known to be an opinionated lot, and I'm sure each one of you will find ways to improve and adapt to his own taste.

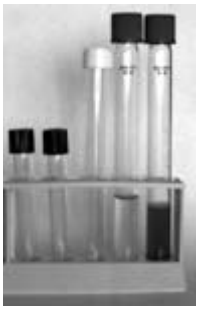
This is in no way meant to be a reference, just a report of what I do. Apparently with a good degree of success, but as always, YMMV.

Summary

- Acquiring a new strain
- Storage

Materials to Have On Hand

- A pressure cooker or canner, to heat sterilize your tools
- A good supply of sterile disposable plastic Petri dishes (10 cm diameter), poured with agar/malt solid media. As an alternative you might want to use glass Petri dishes, which can be reused as many times as you want, but need to be sterilized in an autoclave



- Screw cap culture tubes (best size is 18x160mm), filled with wort and sterilized (autoclaved)
- Screw cap culture tubes (best size is 16x100mm), filled with 6 ml of distilled water and sterilized (autoclaved)
- A good supply of sterile canned wort, for use as starter media, preferably in 50, 250, 700 ml sizes

- Inoculation loop. An aluminum rod handle carrying a nickel/chrome steel wire about 7 cm long, terminated in the form of a 3-4 mm loop. You can make one yourself with a length of Ø 0,5 mm steel wire and an X-acto handle.

- 1 ml glass pipettes, sterilized. They should have a cotton plug on the mouth end, to keep your mouth bacteria out

- sterile 100 ml Erlenmayer flask, or small bottle, with screw cap, rubber closure, or glass cap (best)
- sterile 500 ml Erlenmayer flask, or bottle, with screw cap, rubber closure, or glass cap
- sterile 1000 ml Erlenmayer flask

- sterile airlock with rubber bung, fitting the above 1000 ml flask
- Sterile disposable 5cc syringes

- A good source of flame, either a Bunsen burner or a handheld propane torch

The Flame Zone

We will be using a flame to sterilize the inoculation loop and the openings of various containers.
As you can imagine, direct contact with a flame destroys bacteria, molds and spores.
But there is a second advantage to the use of a flame: it heats the air immediately surrounding it, creating an ascending air flow.
At a distance of less than 5 cm from the flame, we are inside this protected area called *the flame zone*, in which it is very unlikely that any airborne dust particle may fall.

Alcohol

Even though alcohol is an inadequate sanitizer, it is always good practice to use it to wipe the outside of the glassware you'll be using, to remove dust particles that may have accumulated.
CAUTION: alcohol is extremely flammable, wait for complete evaporation before lighting any flame!

Speed

All procedures should be performed as quickly as possible to minimize the chance of contamination.
Practice, practice and then practice some more.
Also, remember to work in flame zone, if at all possible.

Multi Strain Yeast

There is no easy way for a non professional to maintain a mixed-strain yeast: if you use plating to select colonies, you will probably end up with only one of the strains of which the mix is composed.
So I advise you to stay away from such yeasts. After all, a brand new Wyeast pack costs only a few bucks...

Cleaning Glassware

Since you will be using several pieces of glassware, cleaning them after use can became an issue.

Even though specific detergents are available, I think regular automatic dishwasher detergent dissolved in hot water is more than adequate.

These detergents usually contain sodium metasilicate, and are designed to be very effective at high temperatures. They also do not require excessive rinsing.

At times it might be necessary to scrub away stubborn organic residues with a bottle cleaning brush.

Heat Sterilization

Microbiological manipulations require **sterility**.
This means that every form of life, even spores, should be removed from objects that come in contact with wort or yeast.
Sterilize your tools and glassware in advance, so you will have a supply of sterile containers ready when you need them.

We can achieve sterility either with dry or wet heat.

Dry heat

To sterilize an object with dry heat, you need to place it in an oven at a certain temperature for a certain time.
Authors of textbooks on microbiology do not agree on the temperature and time required, but I find the recommendations of the UK Department of Health quite reasonable: **45 minutes at 170 °C**.

Obviously this procedure can be applied only to objects that can stand such conditions.
Glass pipettes are good candidates, as they are generally too long to fit into a pressure cooker.

To sterilize a pipette, first you must substitute the cotton plug on the mouth side. Then wrap the pipette in aluminum foil, to preserve sterility until you need to use it.
Place in the oven and slowly rise temperature to 170°, then keep for 45 minutes.
Turn off the oven and let cool very slowly to avoid breakage due to thermal stress.

Wet heat

Sterilizing with wet heat (water vapor) is the most common method to obtain absolute sterility. It is also the most effective, since spores can resist dry heat, but **20 minutes of exposure to steam at 121°C** will kill anything.

Such temperature can be reached by pure water vapor under the pressure of 15 psi or 1,05 Kg/cm^2 in an autoclave or pressure cooker.
Notice I said pure vapor: if the atmosphere inside the pressure cooker is a mix of air and water vapor, the temperature would be lower.
This means that you should let the steam escape from your cooker for a few minutes before closing the valve, in order to expel the air.

CAUTION Remember that a pressure cooker or canner, if not handled properly, can become a dangerous object: read and follow the instructions that came with your cooker.

As a general guideline:

1. Pour hot water in the cooker up to a level of 3 cm from the bottom.
2. Place the objects to sterilize inside the cooker. I suggest you wrap the objects you want to autoclave in aluminum foil. Thus when you need them you won't have to worry about dust or external contamination: just discard the foil and use them.
3. Close cooker and leave valve open. Apply heat.

4. When steam begins to vent, wait 5-10 minutes then close valve
5. Wait until required pressure (15 psi or 1,05 Kg/cm2) is reached, then start timing.
6. After 20 minutes remove from heat and let cool naturally down to room temperature. Do not force cool.
7. Open cooker, remove objects and store in a clean place until needed.

Canning Wort

If you consider that for each starter you need at least 1 or 2 liters of sterile wort, I'm sure you will agree that having a good supply of sterile wort ready for use is of paramount importance.

Of course, you could always prepare it on the fly, but I think that canning offers the most convenient solution.

Canned wort can be kept at ambient temperature almost indefinitely

CAUTION Boiling is not enough, you really need to pressure can.
Wort pH is not acidic enough, and the chance of developing a *Clostridium Botulinum* infection (botulism) is non-trivial.
Remember, your life is at stake on this.
You should read a good book on home canning, or at least refer to the site of the National Food Safety Database.
And remember also that a pressure canner, if not handled properly, can become a dangerous object: read and follow the instructions that came with your canner.
I know, I know... But beeing paranoid does not mean they're not out to get you... :-)

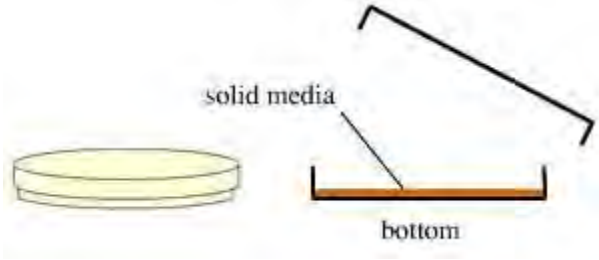
- Prepare a 1,030 OG wort using light dry malt extract for consistency. If you want you can add about 15 IBUs of hops.
- Boil for at least 30 minutes to achieve a good hot break, then chill to room temperature.
- Filter the resulting wort into jars of various sizes, as you see fit. Remember to fill some screw cap culture tubes with 10 ml of wort, for use in the first steps of propagation
- Close jars with their lids, tighten caps on tubes, put in pressure cooker and autoclave for 20 minutes at 15 psi.
- When done, remove from heat and let cool. When safe, open cooker and extract jars and tubes.
- Observe the lids: if everything went well, they should be depressed in the middle, indicating a good vacuum seal. If not, use the wort immediately (it is sterile after all, it just won't keep that way for long).
- Store in a dark, clean place.

You will notice an incredible amount of protein break on the bottom, due to the high temperature boil, but this is normal and should not be a problem. When you need the wort, just decant it carefully and leave the trub behind.

Also note that when boiled at high temperatures the wort becomes considerably darker.

Pouring Plates

For selection, we need to grow yeast on a solid media in a Petri dish.
This solid media is obtained by dissolving agar into wort (which provides the nutrients).



The basic property of agar is that it melts above 95°C, and remains liquid until cooled to about 45°C. Thus you can pour the liquefied media into the plates and let if solidify.

Instead of pouring many plates at once, I store the solid sterile media in small 100 ml bottles, sealed with a rubber closure.
When the need arises, I simply heat a bottle until media liquifies, then pour it in 5-6 plates.

The quantity of agar needed varies greatly, depending on the source, grade and form of the agar you use, so you need to experiment a little.
Too much agar and the surface becomes too hard, too little agar and the surface remains too soft and you risk cutting it with the loop when you streak.
I use flaked agar from health food stores, and 15 grams dissolved into 1 liter of wort works nicely for me.

Prepare the media

- Take a jar of canned wort and pour it in a clean pot, leaving the trub behind.
- Add the agar and mix
- Allow the agar to soak for 10 minutes
- Apply heat and bring to boil, stirring until agar is completely dissolved. Beware, the mix has an astonishing boil-over potential
- Pour the hot liquid into 100 ml bottles and place rubber stoppers. During sterilization, pressure develops inside the bottle and the stopper will pop out. To avoid this, insert a piece of cotton string between the the rubber stopper and the bottle mouth. This creates an airway to release pressure during sterilization
- Cover the bottle closure with aluminum foil
- Place bottles in pressure cooker and autoclave for 20 minutes at 15 psi (1,05 kg/cm2)
- When done, remove from heat and let the pressure cooker cool until you can open it safely
- Remove the bottles, pull out the pressure relief strings. The strings are now wet, and should come away with no effort
- Allow to cool then store in refrigerator

Pour the plates

The usual precautions apply: scrub the table surface and your hands with alcohol. Wear a mask, keep your work area as dust-free as you can.
Since you need to work as quickly as possible, you should practice with water and empty Petri dishes ahead of time, to gain the necessary dexterity.

1. Put a bottle of solid media in a water bath in a pot and apply heat until it melts
2. Allow to cool to about 50-55°C (monitor water bath temperature)
3. In the meantime, take 6 sterile Petri dishes and place them on the table, cover on top, smaller media half on bottom
4. When the temperature of the bottle reaches 50°C, quickly proceed as follows
5. Hold the bottle in your right hand, agitating continuosly to keep the temperature uniform and avoid partial solidification
6. Remove the rubber stopper and discard it
7. Flame the bottle mouth
8. Partially lift the cover of the first dish
9. Pour about 15-20 ml of liquid to cover at least 2/3 of the plate surface
10. Gently swirl the plate to spread the liquid media on all the surface. There should be a layer of about 3-4 mm of liquid media
11. Close the plate cover
12. Repeat steps 8 through 11 with the other Petri dishes
13. When done, wait until media solidifies without moving the plates. Having poured the media at a relatively low temperature (50°C), very little condensation will form on the inner side of the cover
14. Turn the dishes upside down (media on top, cover on bottom). From this moment on, plates should always be kept upside down
15. Incubate at 30°C for 24 hours in a dry, dark, clean, dust-free. Condensation will disappear
16. Check media surface for mold or bacteria growth, and discard contaminated plates
17. Seal with electrical tape and store in refrigerator or use immediately

Petri dishes with solid media keep for several weeks if refrigerated, but even if sealed with tape after a few weeks the agar releases water and becomes unsuitable for healthy yeast growth.
At times I have observed mold contamination, possibly due to spores from the sealing tape.
It might thus be advisable not to seal the plates, and simply stack them and store them in sealed sterile plastic bags.
Of course, the best solution is the one adopted by professionals, that is to seal the plates with Parafilm™ and store in sterile bags.

Plating a suspension

The purpose of this procedure is to obtain well separated yeast colonies on a surface of solid media, starting from a yeast suspension in a test tube, for later selection.

The suspension could be a master storage in distilled water, slurry from a previous batch, dregs from a commercial bottle, etc.

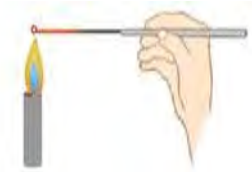
Since each colony on the solid media in most cases originate from a single yeast cell, to get well separated colonies we need to spread and dilute the suspension as much as possible. Our goal is to inoculate the solid media with less that 50 yeast cells.
To obtain such a low number, we can dilute the original suspension (possibly 1:100) or we can streak 2 plates in a row. Results are similar, but I prefer the 2 plates method.

As an alternative, you can perform a single streak with a sterile cotton swab. With the loop you deposit a drop of suspension on the media, then you spread it with the cotton swab.

The usual precautions apply: scrub the table surface, the test tube and your hands with alcohol. Wear a mask, keep your work area as dust-free as you can, work in flame zone.
Since you need to work as quickly as possible, you should practice with empty test tubes and Petri dishes ahead of time, to gain the necessary dexterity.

Place on the table 2 Petri dishes upside down (media on top, cover on bottom), test tube with suspension (standing in its rack), inoculation loop.
When ready, proceed as follows:

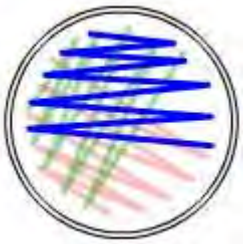
- Hold inoculation loop with right hand
- Flame loop from tip to handle until it's red hot, then keep it in the flame zone



- While the loop is cooling near the flame, take test tube with left hand
- Grab tube cap with right hand little finger, unscrew by turning tube with left hand. Hold cap between the little finger and the palm of your right hand

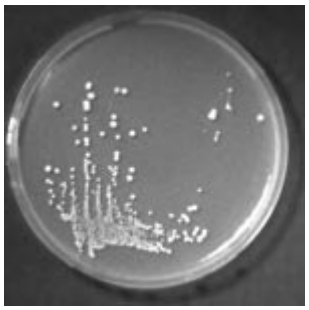
- Flame tube mouth
- Working in the flame zone, dip loop in the solution trying not to touch the glass walls
- Remove the loop and keep in flame zone. Be extra careful not to heat the yeast on the tip
- Flame the tube mouth again and place cap back on, again using left hand to screw it
- Put away tube on rack

- With your left hand, lift the media half of the first Petri dish and hold it vertical (to minimize the chance of dust falling on the agar), leaving the cover on the table
- Streak the loop on 1/2 of the media surface, in a zig-zag pattern, being careful not to scar the surface
- Turn the plate 60° clockwise and repeat streaking. Do not flame the loop between streaks
- Turn the plate 60° clockwise once more for final streaking

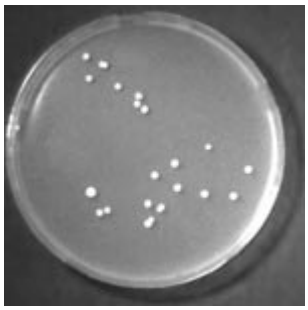


- Put plate back on its cover. From this moment on, Petri dishes must be kept upside down (media on top, cover on bottom) at all times
- Without reinoculating, repeat the above steps with the second Petri dish
- Flame inoculation loop and put it away
- Incubate plates in a warm (>27 °C) clean and dry place

Depending on the concentration of yeast cells in the original suspension, yeast colonies on the first dish will probably grow too close to each other. However on the second dish the number of cells carried over on the loop should be minimal, and colonies should grow well separated and healthy.



First plate



Second plate

The rule is that if you can see yeast on the inoculation loop, then there is too much of it: after all, a yeast cell size is less than 10 µm.
It may seem that nothing remains on the loop after streaking the first plate, but there will be a few cells anyway, and this is exactly what we want.

After a few days (depending on the viability of original cells) you will have large, well separated colonies on the second plate, suitable for further selection or storage.

Should mold or bacteria colonies be present, I suggest you discard the plate and repeat the procedure.

Solid to Liquid Transfer

The purpose of this procedure is to transfer one or more yeast colonies grown on a solid media to a test tube containing a liquid media, maintaining sterile conditions.
The liquid media could be sterile distilled water for long term storage, or sterile wort for fermentation and propagation.

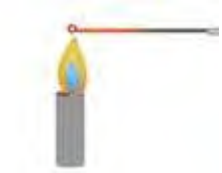
The usual precautions apply: scrub the table surface, the test tube and your hands with alcohol. Wear a mask, keep your work area as dust-free as you can, work in flame zone.
Since you need to work as quickly as possible, you should practice with empty test tubes ahead of time,

to gain the necessary dexterity.

Examine the colonies on the plate and select a few round, healthy, well isolated ones. You can mark them with a felt tip pen on the bottom of the dish.
Place on the table the Petri dish upside down (media on top, cover on bottom), test tube with liquid media (standing in its rack), inoculation loop.

When ready, proceed as follows:

- Hold inoculation loop with right hand
- Flame loop from tip to handle until it's red hot, then keep it in the flame zone



- With your left hand, lift the media half of the Petri dish and hold it vertical (to minimize the chance of dust falling on the agar), leaving the cover on the table
- Touch the media surface with the loop, to cool it
- With the loop, scrap one or more of the selected colonies
- Place the loop near the flame (not too near, of course...) and put plate back on its cover

- Take test tube with left hand
- Grab tube cap with right hand little finger, unscrew by turning tube with left hand. Hold cap between the little finger and the palm of your right hand

- Flame tube mouth
- Working in the flame zone, dip loop in the liquid without touching the glass walls, swirl it until yeast comes off the loop.
- Remove loop and put away
- Flame the tube mouth again and place cap back on, again using left hand to screw it

The plate is about useless now, and you can discard it.

If you are preparing a **master** for storage, you can wrap the tube in aluminum foil, label it and store in the refrigerator.

If you inoculated a tube of sterile wort, you should incubate it in a warm (>27 °C) dry clean place. Expect signs of fermentation within 12 hours. Crack the cap open to release CO2, then proceed with starter propagation or plate again for further selection.

Liquid to Liquid Transfer

Use this procedure to step up a starter.
That is, to transfer fermenting wort from one flask to a larger one containing more sterile wort.

The usual precautions apply: scrub the table surface, the flasks and your hands with alcohol. Wear a mask, keep your work area as dust-free as you can, work in flame zone.

Proceed as follows:

- Take smaller flask with left hand, swirl to bring yeast in suspension
- Grab flask cap with right hand little finger, remove and discard it
- Hold the larger flask with your right hand
- With left hand, remove cap from larger flask, hold cap between the little finger and the palm of your left hand. Pay attention not to spill the contents of the smaller flask
- Flame mouth of both flasks
- Working in the flame zone, pour the suspension from the smaller into the larger flask. The two containers should never touch
- Flame again the mouth of the larger flask, then place cap back on

Wort to Flask Transfer

Use this procedure to prepare a sterile flask with canned sterile wort for use as a starter.
That is, pour the content of a previously canned wort jar into a sterile flask.

The usual precautions apply: scrub the table surface, flask, jar and your hands with alcohol. Wear a mask, keep your work area as dust-free as you can, work in flame zone.
Place on the table a sealed jar of sterile canned wort and a sterile flask of the appropriate size.

When ready, proceed as follows:

- Open the wort jar but do not remove cap
- Hold empty flask in left hand
- Remove flask cap with right hand little finger, hold cap between the little finger and the palm of your right hand
- With right hand, remove cap from the wort jar
- Flame the mouth of both the jar and flask. A portable propane torch is best suited for this task
- Carefully pour the wort from the jar into the flask. The two containers should never touch
- Put away the jar
- Flame the mouth of the flask, put cap back in place (or fit airlock)

When the flask is a large one, say 1000 ml, a considerable amount of CO2 is produced during fermentation, so you might want to use an airlock.
The flask would probably have been autoclaved capped with aluminum foil, and the airlock and rubber bung would have been soaked in a disinfectant solution.

Preparing a Starter

To pitch an adequate amount of yeast in your wort, you should always prepare a starter.

In professional brewing, a count of 3 million cells per ml for every 4 °Plato is a standard for an ale yeast, while a bottom fermenting yeast would require twice as much.
In my experience, pitching 1 liter of actively fermenting and highly viable starter provides a count of about 5 million cells per ml, which is considered acceptable for homebrewing purposes, leading to a lag time of less than 6 hours. For a high gravity ale or a lager, you need 2 liters of starter.
If you own a microscope and a hemacytometer, you can perform an accurate cell count, but this is not strictly necessary.

When you acquire a new yeast strain in a Wyeast pack, you will pitch about 50 ml of active culture in a flask with 250 ml of sterile wort.
In other situations however, a few more steps are required.

From master culture or slant

If you are starting from a master stored in distilled water, I suggest you first plate it, then use solid to liquid sterile transfer. Or you can skip the plating and use liquid to liquid sterile transfer.

If you are starting directly from a plate or slant, use solid to liquid sterile transfer.

In either case you will end up inoculating a culture tube containing 10 ml of sterile wort.
Incubate this tube in a warm (>27 °C) dry clean place. Expect signs of fermentation within 12 hours.
Periodically crack the cap open and swirl to release CO_2.

First step
As soon as fermentation begins, you should transfer the content of the tube to a small bottle or Erlenmayer flask containing 50 ml of sterile wort. This larger container should have a screw cap, rubber closure, or glass cap.

Prepare the flask using the wort to flask sterile transfer technique.
The flask now contains about 50 ml of sterile wort.
Now you can transfer the content of the culture tube into the flask, using liquid to liquid sterile transfer.

Incubate the flask in a warm (>27 °C)dry clean place. Expect signs of fermentation in a few hours.
Periodically crack the cap open to release CO_2.

Second step
As soon as fermentation begins, you should transfer to a larger flask (500 ml) containing 250 ml of sterile wort.
To prepare this larger flask, again use the wort to flask sterile transfer technique.
Then transfer the contents of the 50 ml flask to the 500 ml flask using liquid to liquid sterile transfer.

Having now a flask with approximately 300 ml of fermenting starter, you proceed with the final step as explained later.

From a Wyeast pack

Smack the pack as usual and incubate in a warm environment. When the pack swells to about 3 cm thickness, but before it becomes a balloon, you should draw a sample for selection and storage, and pitch the rest in your starter.

The usual precautions apply: scrub the table surface, test tube, Wyeast pack, scissors and your hands with alcohol. Wear a mask, keep your work area as dust-free as you can, work in flame zone.

Following the wort to flask sterile transfer technique, prepare a 500 ml flask containing 250 ml of sterile wort.

Next, have everything ready on the table: test tube with liquid media (sterile wort) standing in its rack, syringe, scissors, a sterile 500 ml flask with cap, containing 250 ml sterile wort.

When ready, proceed as follows:

1. Since you can't flame the pack (try it :-), thoroughly scrub it with alcohol
2. Take the syringe out of its package, hold it in right hand and briefly flame tip of needle
3. Perforate the pack and draw about 1 ml of yeast suspension
4. Keep the needle in the flame zone
5. Take test tube with left hand
6. Grab tube cap with right hand little finger, unscrew by turning tube with left hand. Hold cap between the little finger and the palm of your right hand
7. Flame tube mouth
8. Inoculate content of syringe into tube
9. Flame the tube mouth again and place cap back on, again using left hand to screw it
10. Discard syringe
11. Hold scissors in right hand and flame blades
12. Hold pack in left hand
13. Cut a corner of the pack, and keep opening in flame zone. Put scissors away
14. Hold flask in right hand
15. Remove flask cap with left hand little finger, hold cap between the little finger and the palm of your left hand. Pay attention not to spill the contents of the pack
16. Flame mouth of the flask
17. Carefully pour the wort from the pack into the flask. The two should never touch
18. Flame the mouth of the flask, put cap back in place

Now you have a culture tube inoculated with fresh yeast. Incubate it in a warm (>27 °C) dry clean place. Expect signs of fermentation within 12 hours. Crack the cap open to release CO_2, then proceed with plating for selection and storage.

You also have a flask with 300 ml of starter. This also should show signs of fermentation within a few hours. As soon as it's fermenting, step it up to a larger flask containing 700 ml of sterile wort.

Final step
First you have to prepare a 1000 ml flask containing 600 ml of sterile wort.
To do this, follow the now familiar wort to flask sterile transfer technique.
Then transfer the contents of the 500 ml flask to the 1000 ml flask using liquid to liquid sterile transfer.

When this last step is actively fermenting, you are ready to pitch into your wort.

If you are worried about introducing a large quantity of starter wort in your batch, you can allow the last step to ferment almost out, discard most of the liquid portion, and add fresh wort after the boil and

chilling. By the time you have racked to primary, removed trub, aerated, etc. you should be ready to pitch.

For a lager or a very high gravity beer, the common procedure is to double the last step: after you have 300 ml of starter, transfer it in 2 flasks with 700 ml of sterile wort each. This won't require any extra time, only more wort and another flask, but you will end up with twice as much yeast to pitch.

Acquiring a New Strain

In most cases you acquire a new yeast strain either by buying a new Wyeast pack or by reculturing from a commercial bottle.

In the first case, I suggest you keep the package inactive until you actually intend to make a batch from it. You can then proceed as detailed in the preparing a starter section.

If you want to **reculture yeast from a commercial bottle**, first of all you should gather information on the yeast characteristics: is it a bottling strain or the primary fermentation yeast? Is it a pure strain or a multi-strain? Bottom or top fermenting?

If it is a pure strain, you can successfully try to reculture it, provided it has not been sitting in the bottle for too long. There is obviously no point in trying to reanimate dead yeast :-)

The usual precautions apply: scrub the table surface, culture tube and your hands with alcohol. Wear a mask, keep your work area as dust-free as you can, work in flame zone.

Have ready a culture tube with 10 ml of sterile wort and a sterile 1 ml pipette.
Proceed as follows:

- Open the bottle and decant in a pitcher (you can enjoy it later...) leaving behind some beer and the yeast dregs
- Flame bottle mouth
- Swirl bottle to rise the sediment
- Take the sterile pipette and hold with right hand, discard the aluminum foil
- Flame the bottle mouth again
- Plunge the pipette in the bottle and suck up 1 ml of suspension. The cotton plug in the pipette must not come into contact with the liquid
- Take out the pipette, keeping it sealed with your right index finger
- While keeping the pipette tip in the flame zone, take the test tube in your left hand
- Grab tube cap with right hand little finger, unscrew by turning tube with left hand. Hold cap between the little finger and the palm of your right hand
- Flame tube mouth
- Working in the flame zone, release the content of the pipette into the test tube. Pipette and tube should never touch
- Flame the tube mouth again and place cap back on, again using left hand to screw it
- Put test tube in its rack and incubate in a warm (>27 °C) clean and dry place

Since the viability of the yeast cells you inoculated is probably very low, you might have to wait for several days before you see any sign of fermentation.
As soon as the wort starts fermenting, plate it for selection and storage.

Long Term Storage in Distilled Water

After having plated and selected yeast colonies on solid media, you need to preserve them in time.

You might store yeast on Petri dishes or slants in the refrigerator, but the shelf life of these media is limited, and after 3-4 month you need to reculture the yeast.
I was looking for a more permanent storage method, when Joseph Kish on the AHA Forum brought to my attention an article by M. D. Graham on the sourly missed Brewing Techniques magazine.

The article was about an underestimated method of preservation of yeasts and other fungi: **distilled water**.

Though skeptical at first (the words *reverse osmosis shock* do come to mind) I decided to give it a try. Since then I have adopted it as my standard long term storage method.

It is extremely simple. First you fill a culture tube with distilled water and autoclave it to ensure absolute sterility.
Then you transfer one or more colonies of yeast from a plate to the tube, using solid to liquid sterile transfer technique.

The amount of yeast carried over must be minimal: the suspension inside the tube should become only slightly blurred. If it gets milky white, then there is way too much yeast in suspension.

When you need to use the yeast, plate a sample on Petri dishes, select then proceed to make a starter.

Authors suggest that you can store this ***master culture*** at room temperature, but I prefer to keep it refrigerated. After 6 months, I counted a percentage of viable cells around 70% on all my strains, with no apparent sign of mutation.

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