Wild Yeast Isolation Protocols and Worksheets

This is the Wild Yeast Isolation Protocol; it has everything you will need to isolate yeasts from different natural substrates. There are multiple sections to this protocol. A description of the sections is provided below. Isolating and identifying yeasts from a substrate can take up to a month to complete.

Color code for protocol

The reagents used throughout the protocol are color coded in the following manner:

- **Purple** reagents/supplies that are common use and must be put back where your got them from and should not be thrown away.
- **Orange** reagents/supplies are those that you will be used up
- **Green** reagents are those that you need to make yourself and keep at your bench or in your fridge or freezer box.
- **Blue** worksheets that can be used to record and/or calculate values, these should either be saved to your digital lab notebook or printed for your physical notebook.

Sample Collecting

Need: 1 sterile bag or tube per collected sample (bring a surplus to be safe) and a sharpie/pen, scoopula/tweezers, 100% Ethanol, Sample Collection Worksheet

1. Collect ~1 Tablespoon of substrate in sterile bag or tube (without touching the sample with your hands)
2. Record sampling information on the Sample Collection Worksheet and tube/bag

Processing

Need: One 15mL tubes/sample + 1 negative control tubes, 9mL Wild Yeast media per tube, scoopula/tweezers, First Enrichment Media Worksheet

1. Record sample information in lab notebook - Sample #, Substrate, Processed date
2. Label 15mL tubes:
    a. Sample Information
    b. Initials
    c. Date
    d. Any additional experiment information (sugar %, sugar type, isolation temperature). This is dependent upon your experiment.
3. Load samples into tubes, flame sterilize scoopula and/or tweezers between samples
4. Make the Wild Yeast media and record volumes in the First Enrichment Media Worksheet
5. Add 9mL Wild Yeast media to each tube
6. Vortex tubes and place at room temperature unless experiment dictates otherwise.
7. Check regularly for growth - bubbles and white/whitish sediment. Record this information in your lab notebook or in an excel spreadsheet to be printed for your lab notebook or to be uploaded to your online notebook.

Passaging

Need: One sterile 5mL tube per sample/control, 4mL Wild Yeast media per tube, Second Enrichment Worksheet

1. Make fresh Wild Yeast Media
2. Label 5mL tubes with information from your previous enrichment step (Step 2 above).
3. Make the Wild Yeast media and record volumes in the Secondary Enrichment Media Worksheet
4. Add 4mL of Wild Yeast Media to labeled tubes
5. Vortex passaged sample tubes
6. Add 10µL of liquid from processed sample into correspondingly labeled 5mL tube
7. Place at room temperature
8. Watch for signs of growth - bubbles and white/whitish sediment

Diluting and Plating

Need: Two 1.5mL tubes, 2X990 µL sterile H2O, and one YPD plate per sample, sterile glass beads

1. Label two 1.5mL tubes for serial dilution for each sample
2. Add 990µL sterile H_2O to each tube
3. Vortex 5mL sample tubes and add 10µL to the first dilution tube. (1:100 dilution)
4. Vortex first dilution tube and add 10µL to the second dilution tube. (1: 10,000 final dilution)
5. Label the outer edge of the bottom of YPD plates with tube information.
6. Pour ~10-15 sterile glass beads into the lid of the labeled plate.
7. Flip the plate with the lid on and pipette 100µL of vortexed 1:10,000 diluted sample (step 4) onto the glass beads. Replace lid and spread sample around the plate by shaking plate side to side.
8. Incubate plates at room temperature. Check for growth daily.

Streaking – Obtaining pure colony of each morphotype

Need: YPD plates, toothpicks

1. Split YPD plates into wedges (at least 4) for each distinct morphotype and label with sample information on plate and morphotype letter or number.
2. Streak the morphotype to its respective wedge on your plate. Use the streaking method with which you are most comfortable.
3. Store upside down at room temperature (unless drippy then store upright)
4. Watch for single colony growth.

Inoculation – Grow up the strain so we can make a freezer stock of it and extract DNA for species identification

Need: One glass test tube and 3 mL of liquid YPD per sample, toothpicks

1. Flame sterilize tube
2. Label glass test tubes with sample info and fill with 3mL of liquid YPD
3. Use toothpick to transfer single colony to test tube
4. Flame sterilize tube
5. Grow at room temperature until culture is saturated.

Freeze Down – Preserve the strain for later study

Need: 300 µL 50% Glycerol and one Cryotube per sample

1. Label cryotube with Op[*Initials*]# and document sample information in your lab notebook and in your strain database.
2. Add 300 µL of 50% glycerol
3. Add 700 µL of saturated culture to tube and mix by pipette
   Note: Some cells clump together, this is known as flocculation, ask a mentor how to deal with this before adding to glycerol
4. Put in box -80°C freezer

Stock Recipes

10X Synthetic Complete Base

Use: This is a stock to be diluted down to 1X concentrations to make synthetic complete media, which is used in yeast isolation protocols.

Liquid Recipe for **500mL** of stock adjust accordingly

Yeast Nitrogen Base (w/o AA, AS, Carb)	8.6g
Complete Dropout Mix	10g
Ammonium Sulfate	25g
Water	500 mL

Filter sterilize and store in the refrigerator

20% Sugar Stock

Use: Sugar stocks are used in growth experiments and fermentation tests and can be diluted down to any concentration that is less than 20%. These stocks should only be filter sterilized and not autoclaved because you can break down polysaccharides into their monosaccharide components.

Liquid Recipe for **100mL** of stock adjust accordingly

Sugar of interest	20g
Water	100mL

Note: Some of these will need to be heated to dissolve the sugar.
These sugars should be filter sterilized and can be stored at room temperature

1000X Chloramphenicol

Use: This is an antibiotic that is used for wild yeast isolations. This recipe makes 100 aliquots at 100µL with a concentration of 30mg/mL

Chloramphenicol	300mg
100% Ethanol	10mL

Mix well to ensure all of the powder is dissolved
Filter sterilize and store at -20°C.

1000X Ampicillin

Use: This is an antibiotic that is used for wild yeast isolations. This recipe makes 100 aliquots at 100µL with a concentration of 100mg/mL

Ampicillin (sodium salt)	1000mg
MiliQ-Water	10mL

Mix well to ensure all of the powder is dissolved
Filter sterilize and store at -20°C.

Stock Recipes (cont.)

50% Glycerol Stock
Makes 100 mL of 50% glycerol

50g (w/v)	Glycerol
50mL	miliQ H_2O

Mix well to combine water and glycerol and autoclave to sterilize.

1M NaOH Stock

2g	NaOH tablets
50mL	sterile miliQ H_2O

Mix well to ensure the tablets dissolve in the water.
NOTE: The tube will warm up as the NaOH dissolves, use a silicone grip to gently shake container.

Media/Working Reagent Recipes

Yeast Extract Peptone Dextrose (YPD)
Liquid Recipe for **1 liter** of media adjust accordingly

Yeast Extract	10g
Peptone	20g
Glucose	20g
Water	1 L

Autoclave to sterilize

Agar Plate for **1 liter** of media adjust accordingly

Yeast Extract	10g
Peptone	20g
Glucose	20g
Agar	20g
Water	1 L

Autoclave to sterilize

1X Synthetic Complete Media (Wild Yeast Media)
The recipe for this medium with a final concentration of **2% glucose**. Both the sugar and the sugar concentration can be adjusted for your specific experiment. This also makes **100mL of media** adjust the volumes accordingly.

10X Synthetic Complete Base	10mL
20% Glucose Stock	10mL
Sterile miliQ water	80mL
1000X Stock Ampicillin	100µL
1000X Stock Chloramphenicol	100µL

Leftover media can be stored in the refrigerator for up to a month to use.

Media/Working Reagent Recipes (cont.)

85% Ethanol

Makes 1mL of 85% Ethanol (scale up as needed)

850µL	200 Proof Ethanol
150µL	sterile H_2O

1. Pour or use serological pipette to transfer 850mL of Ethanol to an appropriately sized container
2. Add 150µL sterile H_2O

Worksheets

Sample Collection Worksheet
Experiment Description (if applicable):

Sample Description:
Sample Identifier:
Substrate:
Host species (if applicable):
Date:
Temperature at time of collection:
GPS coordinates:
Additional Location Information:
Replicate:

Sample Description:
Sample Identifier:
Substrate:
Host species (if applicable):
Date:
Temperature at time of collection:
GPS coordinates:
Additional Location Information:
Replicate:

Sample Description:
Sample Identifier:
Substrate:
Host species (if applicable):
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GPS coordinates:
Additional Location Information:
Replicate:

Sample Description:
Sample Identifier:
Substrate:
Host species (if applicable):
Date:
Temperature at time of collection:
GPS coordinates:
Additional Location Information:
Replicate:

First Enrichment Media Worksheet

A) # of sample: _____

B) Controls: _____

Total (add A + B): _____

Total Volume (9mL x Total): _____

Wild Yeast Media (multiply volumes by **Total Volume**)		**Total Volume**
0.9mL	10X Synthetic Complete Base	_____
0.9mL	20% Glucose Stock	_____
7.2mL	Sterile miliQ water	_____
9µL	1000X Stock Ampicillin	_____
9µL	1000X Stock Chloramphenicol)	_____

Date: _____
Notes (can include gel images here):

Secondary Enrichment Media Worksheet

A) # of sample: _____

B) Controls: _____

Total (add A + B): _____

Total Volume (4mL x Total): _____

Wild Yeast Media (multiply volumes by **Total Volume**)		**Total Volume**
0.4mL	10X Synthetic Complete Base	_____
0.4mL	20% Glucose Stock	_____
3.2mL	Sterile miliQ water	_____
4µL	1000X Stock Ampicillin	_____
4µL	1000X Stock Chloramphenicol)	_____

Date: _____
Notes (can include gel images here):