Take 3 tubes per location. 10 Locations.

**Materials:**

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From Pat Gibney

Hi Hong,

I took a bunch of sterile 15-mL conical tubes home with me, and then put environmental samples in each (a chunk of bark, a berry, a pinch of dirt, etc.) - I collected these samples while wearing gloves. I then mailed the samples back to princeton. Once there, I added 10 mL of media to each tube (YNB + 8% glucose), sealed the tubes with the lid and parafilm, then let them grow without agitation at RT for ~2 weeks (you will see fermentation bubbles in many of them). I then plated about 100 microliters from each onto YPD and spread with beads. There would be a mix of many different things on the plate. I checked each one under the microscope to see what it was, and if it appeared to be a yeast-type fungus, I would restreak it onto YPD to isolate a single pure colony (for some, I also re-enriched in YNB+8% dextrose for a week).

When I started, I also was using a complex media described in one of the Sneigowski papers, but Justin Gerke from the Kruglyak lab spent his Ph.D. doing natural yeast isolations, and he said that YNB+8% dextrose does a really good job of enrichment for yeast.

I hope that helps. Let me know if you have any other questions.

Talk to you later,

Pat

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From: Duccio Cavalieri [dcavalieri@CGR.Harvard.edu]

Sent: Tuesday, October 26, 2004 5:31 AM

To: Qin, Hong

Subject: RE: Yeast strain M1-2, M2-8s2, and M2-8f2

Dear Quin.

I apologize.

Due to some problems between me and my former tech he never sent you the isolates and it was my fault not to check that this had been done, as this is the second time that is happened, (and this is just one of the many problems hat he caused me), the only apologies that I can make is that it has been hard to run a lab when firing the people that used to work for you, and it is a very frantic period when you are starting a new lab, closing my old one and some serious personal family problems.

Here is the isolation protocol.

The trick is to observe the environment from which you are sampling from looking for rotting fruits, insects or sugar rich plant liquids. Harvest the sample using sterile equipment and gloves avoiding to contaminate with men or researcher born yeasts. Perform the operations in a controlled clean environment, cleaning the bench with ethanol and exposing it to UV. Having a dedicated UV hood is the ideal condition).

Inoculate the sample in 50 ml of:

**Enrichment Selective medium (ESM).**

**1% yeast extract, 2% peptone, 10% dextrose, autoclave 20 minutes. When the media is at room temperature add ethanol to a final concentration of 5%.**

After 1 week 2 and 4 weeks plate part of the sample on Enrichment selective agar (ESM plus 2% agarose). Control colony morphology, sporulation and asci morphology, growth on Agar Lysine (Sc does not grow), suc, mal, gal fermentation tests, and select the S.c. type clones for PC-RFLP analysis of **ribosomal DNA.**

Also 101s is actually SC 1014, a well established wine yeast sc type strain from DBVGP, (Perugia) Prof. MArtini's collection. All the other strains come from Polsinelli's lab and have been isolated from Mario Polsinelli and Duccio Cavalieri

Best regards

Duccio Cavalieri