Growing yeast in big volume and use alpha factor to induce enzyme

Purpose:

Grow yeast in big volume to have enough yeast to secret out an adequate amount of enzyme. Use alpha factor to induce W303 to secret out xylanase, glucanase and lipase.

Material:

- Liquid Selection medium (-LEU)
- YPD
- Alpha factor

Procedure:

1. Liquid culture 4 tubes of 181-W303 strain in 10 ml –LEU medium+ 10 μl Adenine at 30^{o}C overnight (over 14 hours).
2. Calculate OD_{600} for the sample in next day. The OD600 should be over 1.
3. Prepare two Erlenmeyer flask and add in 500ml of –LEU medium in each of the flask. (operating in the hood)
4. Add two tubes of liquid yeast into each of the flask.
5. Incubate in 30^{o}C and 200 rpm for 5-7 hours, OD_{600} should between 0.3-0.5.
6. Centrifuge at 4^{o}C, 2000 g, RT for 30 mins.
7. Discard supernatant and resuspand pellet with 50 ml YPD.
8. Prepare two Erlenmeyer flask and add in 550 ml YPD in each flask.
9. Add resuspand yeast in each of the flask, OD_{600} should be at 0.1.
10. Incubate yeast in 30^{o}C and 200 rpm for 30 minutes-1 hour, OD_{600} should between 0.1-0.3.
11. Add 600 μl alpha factor in one flask.
12. Incubate both flask in 30^{o}C and 200 rpm for 5 hours, test OD_{600} and write it down.
13. Take 10 μl of yeast, both induced and none induced. Look under microscope.
14. Centrifuge at 4^{o}C, 11000 g, RT for 10 mins.
15. Collect the supernatant and centrifuge at 4^{o}C, 11000 g, RT for 10 mins.
16. Collect 200 ml supernatant and filtered rest of it in 0.22 μm filter.

Small reminder:

- everything work with yeast need to be auto claved.
- Any liquid medium need to operate in the hood
- OD need to be recorded.

Making synthetic complete media (SCM) plate

Purpose:

Building selection plate to select out yeast that has been successfully transforms correct plasmid.

Material:

Table 1.

0.2% Adenine	10ml	4ml
Bacto-yeast nitrogen base	6.7g	2.68g
Dextrose (2%)	20g	8g
A.A mix	depend on preparation	
Agar	20g	8g
	1L	400ml

Table 2.

〈a.a〉

Arginine-HCl	0.02g
Tryosine	0.03g
Isoleucine	0.03g
Phenylalanine	0.05g
Glutamic acid	0.1g
Aspartic acid	0.1g
Valine	0.15g
Threonine	0.2g
Serine	0.4g
	1.08g/L
	(0.432g/400ml)

Table 3.

〈selection〉

Adenine	0.02g
Uracil	0.02g
Tryp	0.02g
His	0.02g
Met	0.02g
Leu	0.06g
Lys	0.03g
0.19g/L (0.076g/400ml)	

Procedure:

400ml can make 20 plates

1. Put 8 gram Agar into Erlenmeyer flask and add 200ml of water
2. Put agar mix to autoclave
3. Make A.A mix with Table 2 and Table 3, delete your selection amino acid in Table 3
4. Put everything beside agar in Table 1 into Beaker
5. Add 200 ml of ddH2O into beaker and stir for 20 minutes.
6. Put 200 ml fluid from step 5 into .22 μ m filter, add 4 ml adenine.
7. Take out autoclaved agar and add the A.A buffer into agar
8. Pour the fluid onto the plate
   (This step need to be fast or agar will condense.)

Tips:

§ When adding A.A mix with agar, you want to make sure your fluid is flow through the side of flask so it won't have huge amount of bubbles.

Making yeast extract, peptone, dextrose (YPD) plate

Purpose:

YPD is a rich medium that yeast like to grow on, so we make YPD plate to take out yeast from perm and also plating new yeast cell.

Material:

Table 1.

Yeast extract 1%	4g
Peptone 2%	8g
Dextrose 2%	8g
agar 2%	8g
	400ml

Adenine

Process:

1. Put everything in table 1. Into Erlenmeyer flask
2. Add 400 ml ddH_2O
3. Pour the fluid onto plate

Transformation protocol

Purpose:

put composite plasmid into yeast strain W303 to express xylanase, glucanas and lipase.

Material:

- YPD (yeast extract, Peptone, Dextrose)
- Adenine
- Lithium Acetate
- 40% PEG8000 in 0.1M LiAc
- ssDNA
- ddH_2O

Procedure:

17. Liquid culture SCSY 79 strain in 3 ml YPD+30 μl Adenine at 30° C overnight.
18. Calculate OD_{600} for the sample in next day. The OD600 should be over 1.
19. Dilute the OD_{600} to 0.05 and put refresh sample at 30° C for 3.5-4 hours. The new OD600 should be between 0.3-0.5.
20. Take 1.5 ml in 15 ml centrifuge tube, and centrifuge at 1000g ,RT for 15 mins. Turn on the dry bath machine, and set the temperature at 95° C.
21. Discard the supernatant.
22. Suspend with 200 μl 0.1M LiAc
23. Centrifuge at 1000g ,RT for 15 mins. Heat the ssDNA at 95° C for 10 mins.
24. Discard the supernatant.
25. Suspend with 200 μl 0.1M LiAc
26. Mix the following component in a new 15ml centrifuge tube:
    - ✓ 40% PEG8000 in 0.1M LiAc: 700 μl
    - ✓ LiAc treated yeast: 100 μl
    - ✓ ssDNA: 5 μl
    - ✓ composite Plasmid: 500 ng
27. Rotate the tube at 30° C for 2 hours. Turn on the dry bath and set the temperature at 42° C. Take selection plate in RT.
28. Add 100 μl DMSO.
29. Heat shock: 42° C, 20mins.
30. Add 5 ml YPD, flip and put in RT for 5mins.
31. Centrifuge at 1000g ,RT for 15 mins.
32. Discard the supernatant, and suspend with1 ml ddH_2O.
33. Centrifuge at 1000g ,RT for 15 mins.
34. Discard the supernatant, and suspend with1 ml ddH_2O.
35. Centrifuge at 1000g ,RT for 15 mins.

36. Discard the supernatant, and suspend with200 μ l ddH_2O.
37. Spread 20 μ l on_____ plate with glass beads.
38. Incubate in 30° C for 2 days.