Identification of secreted Proteins by LC-MS/MS

Solutions

2xSDS loading buffer		
Ingredients	Volume	Final concentration
1 M Tris ph 6.8	600 µl	60 mM
Glycerol	5 ml	50%
20% SDS	1 ml	2%
Bromphenolblue	a really small tip of a spatula	-
1 M DTT	1 ml	100 mM
H_2O	2.4 ml	-
Final	10 ml	-

Sample preparation for LC-MS/MS

1. Inoculate transgenic culterses in 10 ml TAP
2. Dilute pre cultures to 2*10^5 in 50 ml TAP, let them grow for 7 days
3. Harvest 2 ml in reaction tubes at 5000 g for 5 min
4. Repeat centrifugation to remove residual cells from medium
5. Freeze the supernatant in falcons at -80°C for at least 2 hours
6. Put the samples in the lyophille over night
7. Resuspend the lyophilized samples in 2x SDS loading buffer
8. Load the samples onto a 8% SDS gel
9. Wash the gel 2 times with dH_2O for 5 min
10. Dye the gel with colloidal Coomassie over night (40 ml Coomassie + 10 ml Methanol)
11. Destain the gel with 1% acetic acid
12. Cut the desired bands out of the gel and incubate them in 850 µl destainer over night at 4°C
13. Remove destainer
14. Incubate the samples in 300 µl 50 mM ammonium bicarbonate (pH 7.8-8.0) for 5-10 min
15. Remove ammonium bicarbonate and incubate samples for 5-10 min in 300 µl 70% acetonitrile
16. Remove acetonitrile
17. Repeat steps 14.–16. 3 times
18. Incubate samples in 300 µl 100% acitonitrile for 10 min
19. Remove acetonitrile
20. Dry the gel pieces in the speedvac for 5-10 min
21. Incubate the dried gel pieces in 30 µl Trypsin (0,01 µg Trypsin in ammonium bicarbonate) for 20 min
22. Add 30 µl – 40 µl ammonium bicarbonate, incubate samples over night at 37°C
23. Add 2% FoAc and incubate for 20 min
24. Transfer the supernatant in fresh reaction tubes and put them in the speedvac
25. Add 100 µl elution buffer 1 to the samples with gel pieces and incubate them for 1 h
26. Transfer the supernatant again into the reaction tubes in the speedvac
27. Add 200 µl elution buffer 2 to the samples with gel pieces and incubate them for 30 min
28. Transfer the supernatant again into the reaction tubes in the speedvac
29. Prepare stage tips

30. Take the samples out of the speedvac
31. Dissolve the samples in 50 µl buffer A and centrifuge at full speed for 3 min
32. Activate the stage tips like followed:
    a. Add 50 µl 100% acetonitrile, centrifuge at 300 g for 2 min
    b. Add 50 µl buffer B, centrifuge at 300 g for 2 min
    c. Add 50 µl buffer A, centrifuge at 300 g for 2 min
33. Add the samples onto the stage tips, centrifuge at 300 g for 2 min
34. Wash the samples 3 times with 50 µl buffer A and cetrifuge at 300 g for 2 min
35. Eluate the samples with 70 µl buffer B into reaction tubes
36. Evaporate the eluated samples and resuspend them in 20 µl buffer A
37. Vortex the samples and put them into ultrasonic bath
38. Repeat step 37. and centrifuge the samples at 30.000 rpm for 3 min
39. Transfer samples into MS-tubes