Genomic DNA purification

Introduction

For yeast in general, not specific for Y. lipolytica.

Materials

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Procedure

Isolation of chromosomal DNA from yeast (genomic DNA)

1. Inoculate cells in 2 ml YPD media and incubate overnight at 30°C.
2. Harvest cells by centrifugation (e.g. 2 minutes in the table centrifuge).
3. Wash pellet in 1 ml milli-q water and centrifuge again.
4. Remove supernatant and resuspend in 200µl breaking buffer.
5. Transfer suspension to a “fast prep” tube.
6. Add 200µl of the small glass beads.
7. Go to the fumehood, put on glasses and gloves and add 200µl phenol.

   *** After the phenol has been added, gloves and glasses should be worn and everything should be carried out under the fumehood until step 16.
8. Mix samples in the Fastprep machine 3 x 20 seconds at 4 rpm.
9. Centrifuge samples 5 minutes at 10.000G. The tube should now contain a phenol phase at the buttom and a waterphase at the top. Usually the two phases are separated by some white cell debris.
10. Transfer supernatant (waterphase) to a new eppendorf tube. The waterphase with the DNA is often small. It can be made bigger by adding 200 µl TE buffer before centrifugation.
11. Precipitate DNA by adding 1 ml ice-cold 96% ethanol[1] to the eppendorph tube.
12. Centrifuge samples 5 minutes at 10.000G
13. Wash sample with 96% ethanol.
14. Centrifuge again for 5 minutes at 10.000G
15. Wash sample with 70% ethanol.
16. Discard supernatant and evaporate remaining ethanol in the fumehood.
17. Resuspend DNA in an appropriate volume of milli-q water or TE buffer (e.g. 50-100µl).

Notes

18. [1] Both the 96% and the 70% ethanol solution are stored in the freezer to make sure it is ice-cold.

19. Breaking buffer:

Table1

	A	B	C
1	Compound	Final conc.	Prep of 100 ml buffer from stock solutions
2	Triton	2%	2ml
3	SDS	1%	10ml of a 10% solution
4	NaCl	100mM	10ml of 1M
5	Tris-HCL, pH 8	10mM	0.2ml of 0.5M
6	EDTA	1mM	78ml H2O