Yeast genomic DNA prep

AJ 21.05.19

Buffers:

Yeast Lysis Buffer

10mM Tris pH 8.0	0.5ml 1M
100mM NaCl	1.25ml 4M
1mM EDTA	100 µl
2% Triton X-100	10ml 10%
1% SDS	5ml 10% SDS
H_2O	To 50ml

Phenol:Chloroform:isoamyl alcohol
1:1:1 mix of the above

NaOAc pH 5.2
4.84g Acetic Acid
29.92g NaAcetate
100ml H_2O

Procedure:

1) Grow 10mls of yeast overnight. Grow in the presence of 1x adenine. Sometimes better if grow to low OD (DNA prep shows less red colour)
2) Pellet cells in falcon tubes; resuspend in Lysis buffer; transfer to eppendorf; spin in microfuge (top speed)
3) Resuspend in 400 µl lysis buffer. Add 400 up of Silica Beads (using a scoop made from an Eppendorf tube). Add 400 µl Phenol:Chloroform:Isoamyalcohol
4) Vortex for 5 mins in cold room
5) Spin at high speed and transfer supernatant to a new tube
6) Add 1 ml of cold 100% Ethanol. Pellet DNA. Wash pellet with 70% Ethanol. Speed vac to dryness.
7) Resuspend pellet in in 90 µl TE. Add 10 µl RNase. Incubate 37^{o}C 45 mins.
8) Add 10 µl 3M NaOAC pH 5.2 and 250 µl ice cold 100% EtOH. Pellet, wash with 70% EtOH, speed vac
9) Resuspend in 60 µl TE and boil for 5 minutes.