gDNA extraction from *S. cerevisiae* cells

reagents:

- glass beads
- phenol:chloroform, pH8.0
- TSENT (2% TritonX-100, 10% SDS, 1mM EDTA, 100mM NaCl, 10mM tris Hcl, pH8)
  Add 1 ml 100x TE (Tris-EDTA, pH8) to 88ml H2O, 2ml Triton-X 100, 10ml 10% SDS, 0.58g NaCl.
- 5M Ammonium Acetate (38.54g NH3Ac in 100ml H2O)
- RNAse 10mg/ml in 10mM Sodium acetate pH7
- Tris buffer (emM pH 7.5): 250ul 2M Trizma-Hcl pH 7.5 = 100ml H2O

Protocol:

- grow cells o.n. in YPD (1% Yeast Extract, 2% Peptone, 2% D-Glucose; sterile)
- Pellet cells by centrifuging 5’ at 5000rpm, RT
- Resuspend in 1ml H2O, centrifuge as before
- add :
  - 800ul TSENT
  - 800ul Phenol:Chloroform
  - Glass beads up to 30ml
- vortex 3’
- Add 800ul TE and vortex 1’
- Centrifuge 2’
- Transfer acqueous phase in a new tube and add 1600ul Phenol chloroform
- vortex
- centrifuge 5000rpm 2’, RT.
- Transfer acqueous phase in a new tube
- Add 4ml EtOH 100% and mix by inversion
- Leave at least 20’ at -20°C
- centrifuge 5’ at 12000rpm
- Remove EtOH and dry well (air or speed vacuum)
- Resuspend in 800ul Tris Buffer.
- Add 6ul Rnase
- Incubate 5’ at 37°C
- Add 32ul Ammonium acetate + 4ml EtOH 100% and mix by inversion
- Leave p.n. at -20°C
- Centrifuge 12000rpm 15’.
- remove EtOH and dry well
- Resuspend in H2O, volume variable according to the size of the pellet, but at least 30ul (to clean the tube walls)