

**Isolation of total DNA (Goyon and Lichten (1993) Mol. Cell. Biol. 13, 373-382.**

- take 40 ODU cells (e.g. 40 ml of  $OD_{600}=1$ ), optional: fix in 50 % ethanol at  $-20^{\circ}\text{C}$
- wash 1x in TE
- resuspend in 0.5 ml 1 M sorbitol, 10 mM  $\text{NaPO}_4$  pH 7.2 (or  $\text{KPO}_4$ ), 10 mM EDTA, 1 %  $\beta$ -mercaptoethanol, 1 mg/ml zymolyase T20
- 30 min  $37^{\circ}\text{C}$
- 5 sec. microfuge, resuspend in 0.5 ml 50 mM EDTA, 0.5 % SDS, 200  $\mu\text{g/ml}$  Prot.K and incubate 30 min at  $65^{\circ}\text{C}$
- cool on ice and add 200  $\mu\text{l}$  5 M KOAc and keep 20 min on ice
- 30 min microfuge at  $4^{\circ}\text{C}$
- decant SN into new tube and add 600  $\mu\text{l}$  isopropanol
- mix by inversion and microfuge for 90 sec.
- resuspend pellet in 300  $\mu\text{l}$  TE add 5  $\mu\text{l}$  RNase, incubate 30 min  $37^{\circ}\text{C}$
- add 300  $\mu\text{l}$  5 M  $\text{NH}_4\text{Ac}$  and 600  $\mu\text{l}$  isopropanol
- mix by inversion and microfuge for 5 min
- wash pellet with 70 % ethanol and let pellet air dry
- resuspend pellet in 35  $\mu\text{l}$  TE, 10 min microfuge at  $4^{\circ}\text{C}$  to pellet debris, save SN.
- use 5  $\mu\text{l}$  for restriction digest in Southern analysis