Sugden Lab.

Classic Hirt (from Hearing)

Buffers:

Resuspension: 10mM Tris-HCl, pH8.0, 10mM EDTA

Lysis: 10mM Tris-HCl, pH8.0, 10mM EDTA, 1.2% SDS

5M NaCl

Procedure:

1. Resuspend PBS washed cells in resuspension buffer at 10^7/2ml

2. Add equal v. of lysis buffer, mix gently and incubate for 20' at RT.

3. Bring to 1M NaCl .

4. Stand on ice for >16 hours

5. Spin the lysates in 50Ti at 17000 rpm for 2hours at 4o. (25000g)

6. Treat the supernantant with 300ug/ml proteinase K at 37o for ½ hour.

7. PC,C extraction. Add equal v. of isopropanol plus 20ug per ml of glycogen at-80o for 30' or -20 o/n.

8. Spin at 3000 rpm in table top centrifuge for 20' at 4o.

9. Dissolve the pellet in TE plus 300mM NaOAC and ethanol precipitate it again.

10. Wash with 70% ethanol, and dissolve in TE containing 20ug/ml RNase A. or in hH2O plus 1xproper NEB buffer (400ul/10^8) and digest with restriction enzymes plus 100ug/ml RNase A for 2hours. (For MluI and BssHII, buffer 3 and , 1unit/ug DNA, in 500ul total Vol. for 2hours; then add 300mM NaOAC and 2 vol. of isopropanol)

Last Modified 06/05/05