DNA EXTRACTION USING CTAB

1. A saturated (1.6 x 10e9 cells in 8 ml) overnight culture was pelleted at 3000g for 2 min, washed in ice cold spheroplasting buffer, and pelleted again.

2. Cells were resuspended in 500 µl spheroplasting buffer plus 1% (v/v) β-mercaptoethanol, 0.5 mg/ml Zymolyase 100T (ICN), and incubated at 37 C for 5 minutes.

3. Sheroplasts were pelleted and the supernatant removed completely. The pellet was resuspended gently in 500 µl of extraction buffer, followed by the stepwise addition of β-mercaptoethanol to 0.16% (v/v) proteinase K to 0.5 mg/ml, and Rnase to 20µg/ml.

4. The homogenate was incubated at 37 C for 15 minutes with occasional mixing, extracted with 200 µl chloroform:isoamyl alcohol (24:1 v/v) by vigorous vortexing, and centrifuged at 16,000 g for 3 minutes.

5. ALL SUBSEQUENT PRECIPITATIONS AND WASHES WERE PERFORMED WITHOUT CENTRIFUGATION.

6. The aqueous phase was transferred to a 5 ml round-bottomed tube, and 1.5 ml of dilution buffer was added. Invert tube gently 5x until a diffuse precipitate forms, incubate for 10 minutes at TR, and then invert a further 20x until a discrete precipitate appears. (reduces RNA ppt.)

7. Remove supernatant and wash ppt. twice with 2ml ice cold was buffer. Add an aliquot of 0.5 ml ice cold NaCoHEx solution and gently agitate tube until the DNA precipitate becomes translucent.

8. Transfer the susupension (with DNA) to a 1.5 ml tube, add 1 ml of ethanol (RT) and invert the tube until a discrete ppt. forms. Remove the supernatent and wash the ppt. with 1 ml 70% ethanol + 30% (v/v) 1 mM $Co^{3+}(NH_3)_6Cl^-_3$.

9. Resuspend the DNA on ice in NaCoHex solution and precipitated with ethanol as above. The DNA is then resuspended on ice in 100 µl of ice cold TMNa solution and precipitated with 200 µl of ethanol (RT).

10. The DNA was washed with 200 µl 70% (v/v) ethanol + 30% (v/v) 10 mM $MgCl_2$ and resuspended on ice in 100 µl of ice cold TMSpe buffer.