

Testing cultured cells for Mycoplasma contamination

To prepare the DNA for the test:

METHOD: Instagene DNA purification (Biorad)

Grow cells for **at least 3 days in antibiotic free medium** before preparation of DNA. If you have more than one sample make sure you USE FILTER TIPS to avoid cross-contaminating samples. Use sterile PBS and milliQ H₂O. Instagene matrix is available from Fiona Houghton, Rm 109, ext 30661.

1. Count cells and resuspend at 30,000 cells / 1 ml PBS. Centrifuge 1ml at 10,000 rpm 30sec, R.T. to pellet cells.
2. Remove supernatant and resuspend cells in 1 ml of sterile water
3. Add 20µl cell suspension to 200 µl of Bio Rad Instagene matrix 6% (make sure the beads are well mixed)
4. Incubate for 30 min at 56 °C
5. Vortex, heat to 100 °C for 8 min
6. Vortex for 10 sec
7. Centrifuge at full speed in microfuge (12,000rpm) for 2-3 min
8. Aliquot into 4 new sterile PCR tubes (0.6ml tubes) 20ul each (use 20 µl /PCR reaction ~ 50 cells/reaction). Add the remaining DNA solution to 1 new sterile 1.5ml tube.
9. Store at -20 °C until ready to carry out PCR