Background info

Mycoplasmas (and acholeplasmas) are wall-less eubacteria that exist as parasites within eukaryotic cells. As the smallest, simplest, and most primitive forms of self-replicating prokaryotes, they consist of only three major components—a plasma membrane, ribosomes, and a double-stranded genome of approximately 580 kb. Because of their limited synthetic capacity, these organisms depend on host cells as a source of nutrients.

Mycoplasmas pose a recurring problem for investigators who use cells grown in culture. When mycoplasmas infect cell cultures, they dramatically effect virtually every aspect of cellular metabolism, without any apparent influence on cellular morphology. Often, only an increased cell doubling time indicates culture contamination. To avoid these artifactual effects, cell lines should be periodically evaluated.

Most agree that it is vital to test your cultures for mycoplasma regularly, although testing frequency in some labs may vary from weeks to months. How often you test may depend on how many people come into contact with the cells, as well as how often your lab acquires new cultures. However frequently you decide to test, you have a number of choices for the testing method, whether PCR, biochemical assays, ELISA, or imunofluorescence. Methods based on RT-PCR can often detect non-obvious mycoplasma contamination with clean controls.

Protocol

1. Grow cells in the absence of antibiotics for at least 48h.

2. Take supernatant (0.1-1mL) from cell culture and boil it for 10min.

3. Spin down for 2min @ high speed and dilute it 1/10th with PCR grade H_2O.

4. Take 10uL of boiled and diluted supernatant and transfer it to a clean tube.

5. Add PCR mix (15uL):

    2.5uL 10xBuffer*

    0.5uL 10mM dNTPs

    0.5uL 10uM Myco-1 primer (5' `GGCGAATGGGTGAGTAACACG` 3'; Tm**=59.7C)

    0.5uL 10uM Myco-2 primer (5' `CGGATAACGCTTGCGACCTATG` 3'; Tm**=59.9C)

    0.5uL Taq polymerase*

    H_2O up to 15uL

*Check $MgCl_2$ concentration for optimal PCR conditions. This parameter varies based on the polymerase employed.

** Tm = 81.5 + 16.6(log10([Na+])) + .41*(%GC) - 600/length. Na+ is set at 0.1M.

5. Run PCR:

1. 5' @95C
2. 30"@95C
3. 30"@59C
4. 30"@72C**
5. Go to step 2 35 more times
6. 5' @72C
7. Store @ 4C

**Check enzyme processivity (commonly 1Kb/min).

6. Load samples onto a 1-1.2% agarose gel.

7. Analyse gel. Positive samples contain a 400bp DNA fragment.

Reagents

-10xMRB (see restriction buffer recipes).
-DNA polymerase (we routinely employ Taq polymerase from Biotools; the 10x buffer for this enzyme contains $MgCl_2$).
-Agarose.
-DNA running buffer (we routinely employ TAE).
-H_2O (PCR grade)