

## Mycoplasma PCR test

### Sample preparation

- Take 1 ml supernatant of the culture to be tested (if the cells are in suspension, then get rid of the cells via 10 min 300 g centrifugation).
- The 1 ml supernatant is to be pelleted for 5 min 20.000 g.
- Suspend the pellet into 100 ul H<sub>2</sub>O, vortex it thoroughly.
- Boil for 3 min 95 °C, than vortex again, let it cool (preferably in ice), then roll down the condensation to the bottom of the tube, and use it as template for the PCR reaction (can be stored in -20 °C)

### PCR protocol

Primer1F, Primer2R (if needed, ask! It's not sensitive at all, I'm just too lazy to copy it here ☺)

RB	1,5 ul
dNTP (2mM)	1,5 ul
Primer1F	0,3 ul
Primer2R	0,3 ul
TaqMan polimerase	0,5 U
Template	1 ul
H <sub>2</sub> O	to 15 ul (practically 10,3 ul)

### PCR settings:

95 °C     3:00  
95 °C     0:30  
60,5 °C   1:20 } 40 cycle  
72,0 °C   3:00  
4 °C     inf