Polymerase Chain Reaction Protocol for Mycoplasma Detection

Materials and reagents:

- 0.2 ml PCR tubes + rack
- pipettes
- ice / cooler
- autoclaved ddH_2O
- reagents:
  - reaction buffer (10 x Optimized DyNAzyme React.Buffer (Thermo Fisher))
  - DNA polymerase (DyNAzyme (Thermo Fisher))
  - dNTPs (10 mM (Sigma))
  - primers (12.5 pM, Mycoplasma forward and reverse (IDT))
  - template DNA sample (DNA from Gl261, SW480 cells)

Procedure

1. prepare reaction mixture without DNA for 3 samples:

   samples: a. SW 480 (Mycoplasma +)
   b. Gl261 (Mycoplasma –)
   c. blank (= sample without DNA)

   mixture: [40 µl final volume / sample]

- water (ddH_2O):	27 µl
- 10 x reaction buffer:	5 µl
- 10 mM dNTP:	1 µl
- 12.5 pM Primers:	3-3 µl
- DNA polymerase:	1 µl
for 1 sample:	40 µl

2. add 40 µl reaction mixture into the PCR tubes
3. add DNA into the PCR tubes: 50 ng DNA + ddH_2O (final volume: 10 µl)
4. carefully flick the tubes
5. place them into PCR machine and set the program:

   cycle 1 95 °C 3 minutes
   cycle 2 step 1. 95 °C 30 sec
   step 2. 50 – 70 °C 30 sec } (40x)
   step 3. 72 °C 1 min
   cycle 3 72 °C 1 min

   hold 4 °C or take the tubes immediately and keep them at 4°C.

Primer sequences: reverse: 5'– TTC TTT TCA CCT TTC CCT CAC GGT AC - 3'

forward: 5'– GGT GAA TAC GTT CTC GGG TCT TGT ACA CAC – 3'