Mycoplasma PCR Detection Test

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Materials

- 100 uL culture supernatant
- LookOut Mycoplasma PCR Detection Kit (Sigma Aldrich Catalog #MP0035)
- JumpStart *Taq* DNA Polymerase (Sigma Aldrich Catalog #D9307)
- Barrier pipette tips of various sizes
- DNA LoBind Eppendorf Tubes 1.5 mL (Fisher Scientific Catalog #022431021)
- Molecular Biology Grade water (UltraPure™ DNase/RNase-Free Distilled Water; Catalog #10977015))
- 1 Kb Plus DNA Ladder (Invitrogen Catalog #10787018)
- Agarose Powder (VWR; Catalog #N605)
- 1 X TAE Buffer (Corning; Catalog #46-010-CM)
- GelRed Nucleic Acid Stain (Biotium: Catalog #41003)

Equipment

Microcentrifuge, Vortex mixer, Thermal cycler, Heating block, Electrophoresis gel tank, Fluorescent imager

Procedure

Preparation of Sample Material

Supernatant samples should be collected from confluent cultures. For a sample from an older culture, DNA extraction is recommended.

1. Transfer 100 uL of supernatant from the test culture to a sterile 1.5 mL microcentrifuge tube.
2. Incubate the sample at 95°C for 5 minutes
3. Briefly centrifuge (5 seconds) the sample to pellet cellular debris before adding the supernatant to the PCR reaction. These samples are stable at 2-8°C for at least 1 week.

PCR

1. Prepare the PCR reaction tubes as detailed in the table below. Each PCR tube should contain 25 uL of reagents. The test and positive control PCR tubes in the kit come supplemented with the

proper cocktail of primers, dNTPs, internal control DNA in the test tubes, non-infectious DNA fragemtns of *Mycoplasma orale* genome in the positive control tubes, and gel loading buffer/dye. The negative control sample is prepared in a test PCR tube.

Reagent	Test Sample	Positive Control	Negative Control
DNA Polymerase	0.5 uL	0.5 uL	0.5 uL
Rehydration Buffer	22.5 uL	--	22.5 uL
DNA-free Water	--	--	2 uL
Sample	2 uL	--	--
Total	25 uL	25 uL	25 uL

2. Mix samples well and incubate at room temperature for 5 minutes before proceeding to thermal cycling.
3. Run PCR according to the cycle on the right.

Temperature	Time	Number of Cycles
94	2:00	1
94 55 72	0:30 0:30 0:40	40
4	∞	

Agarose gel

1. Make 1X TAE buffer from 10X TAE *(i.e. 100 mL 10X TAE in 900 mL dH20 or MilliQ water).*
2. Prepare a 1.2% agarose gel (*i.e. 1.2g agarose in 100mL 10X TAE)* for electrophoresis.
3. Add Nucleic Acid Stain (GelRed or SYBR Safe) to agarose, diluting 10,000-fold *(i.e. 5 uL in 50 mL).*
4. Cast gel inside the electrophoresis apparatus and fill with your 1X TAE until all lanes are submerged.
5. Create your DNA ladder by mixing the following components:
    a. 1 uL (500 ng) 1 kb + DNA ladder
    b. 1 uL 10X BlueJuice
    c. 8 uL dH20
6. Add 8 uL of PCR product to each respective well.
7. Allow to run at 130 V for 30-45 minutes.
8. Image gel on fluorescent imager (@ 350nm) and document results.

Expected Results

The negative control samples should show a distinct 481bp band. The positive control should show a band at 259bp and might also show an additional band at 481bp due to the internal control. Mycoplasma positive samples show bands in the range of 260 +/- 8bp.