SOP: Mycoplasma Testing

Approvals:

Preparer: Dr. Maggie Bryans — Date: 17FEB14
Reviewer: Jason McMillan — Date: 19FEB14

1. **Purpose:**
   1.1. Testing of samples for presence of mycoplasma.
2. **Scope:**
   2.1. Applies to testing of solutions including media and cultures for presence of mycoplasma.
3. **Responsibilities:**
   3.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.
   3.2. It is the responsibility of the students/technicians to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
4. **References:**
   4.1. Takara mycoplasm testing protocol
   4.2. Amersham Bioscience puReTaq Ready to go PCR beads protocol
   4.3. thermocycler SOP
   4.4. gel documentation System SOP
5. **Definitions:** N/A
6. **Precautions:**
   6.1. Ethidium Bromide is a mutagen. Use care and wear double gloves.
   6.2. UV light can damage eyes, wear UV shields when using the UV light box
7. **Materials:**
   7.1. Takara Mycoplasma Detection PCR Kit (Catalog number: 6601)
      7.1.1. Takara forward primer diluted 1:5 with PCR grade water
      7.1.2. Takara reverse primer diluted 1:5 with PCR grade water
      7.1.3. Takara control template diluted 1:5 with PCR grade water
   7.2. Amersham Bioscience pure Taq Ready to Go PCR beads (Catalog number: 27-9557-01)
   7.3. PCR grade water
   7.4. mineral oil
   7.5. agarose
   7.6. 10x TBE buffer
   7.7. 1% ethidium bromide solution
   7.8. DNA sample buffer
   7.9. 1kb DNA ladder (New England Biolabs, Catalog number: 3232L)
   7.10. sterile filter pipet tips (0-30µL and up to 200µL)
   7.11. thermal cycler
   7.12. horizontal electrophoresis box
   7.13. power Supply
   7.14. gel documentation system with UV light
8. **Procedure:**
   8.1. **PCR amplification**
      8.1.1. **Preparation of samples**

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8.1.1.1. Verify that a bead is visible at the bottom of each tube. If necessary tap the tube against a hard surface to force the bead to the bottom of the tube.

8.1.1.2. Label tubes as: sample, +C1, +C2, -C
Write the date and your initials on all tubes.

8.1.1.3. Add the following to each tube containing a PCR bead:

	Sample	+ Control **1**	+ Control **2**	- Control
Sterile distilled water	18µL	22µL	17µL	23µL
Forward primer	1µL	1µL	1µL	1µL
Reverse primer	1µL	1µL	1µL	1µL
Control DNA	n/a	1µL	1µL	n/a
Sample DNA	5µL	n/a	5µL	n/a

8.1.1.4. Mix the contents of the tubes by gently pipetting up and down.

8.1.1.5. Overlay each reaction mix with 50µL mineral oil.

8.1.2. **Amplification of samples**

8.1.2.1. Turn on instrument.

8.1.2.2. Verify wells in the thermocycler have mineral oil and add mineral oil if needed.

8.1.2.3. Verify that PCR program number 47 has not been changed.
Program should be:

94C	5min	1 cycle
94C	1min	\
55C	2min	35 cycles
72C	1min	/
4C	10min	

If necessary, edit the program.

8.1.2.4. Place all tubes in the thermal cycler.

8.1.2.5. Start program number 47.

8.1.2.6. When program is complete, shut off instrument and remove tubes.

8.1.2.7. Store tubes at -20°C.

8.2. **Analysis by Electrophoresis**

8.2.1. **Preparation of 1x TBE Running Buffer**

8.2.1.1. Dilute the 10x TBE to 1x TBE by combining 100mL 10x TBE with 900mL DI water. Mix well.

8.2.2. **Preparation of 2% Agarose Gel**

8.2.2.1. Assemble gel box WITHOUT the comb.

8.2.2.2. Weigh out 2 ±0.1 grams of agarose and place into a 250mL Erlenmeyer flask.

8.2.2.3. Add 100mL 1xTBE buffer to the agarose.

8.2.2.4. Microwave the agarose mixture until it begins to boil (~3 minutes).

8.2.2.5. Carefully remove from microwave and swirl to mix.

8.2.2.6. Pour agarose mix into the gel box tray until the height of the agarose mix is approximately 0.5cm.

8.2.2.7. Add 10µL ethidium bromide and mix with the pipet tip.

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Reminder: Ethidium bromide is a mutagen, use care when handling and wear double gloves.

8.2.2.8. Place the comb into the tray.
8.2.2.9. Allow the gel to set (approximately 30 minutes).
8.2.2.10. When the gel is set, remove the comb, reverse the tray and add enough 1X TBE buffer to just cover the gel.

8.2.3. **Running the Gel**

8.2.3.1. Remove PCR tubes from the -20°C freezer and IMMEDIATELY remove as much mineral oil from the top as soon as possible before the samples thaw.
8.2.3.2. Add 10µL sample buffer to each tube and mix well.
8.2.3.3. Load 15µL of each sample into individual wells. Record position of samples.
8.2.3.4. Load 10µL of DNA ladder into an adjacent well. Record position of ladder.
8.2.3.5. Connect the lid to the electrophoresis apparatus.
8.2.3.6. Connect the leads to the power supply making sure to use the color codes.
8.2.3.7. Turn on the power supply and run at 100Vfor approximately 1-2 hours.
8.2.3.8. Turn off the power supply.

8.2.4. **Analysis**

8.2.4.1. Wearing double gloves, carefully remove gel and tray from the apparatus and place into a storage container designated for ethidium bromide.
8.2.4.2. Observe under the UV light box.

Reminder: Use the UV shield.

8.2.4.3. Take a photograph of the gel.
8.2.4.4. Molecular weight of the + Control PCR band should be 810bp.

9. Attachments:

9.1. Data Table
9.2. Picture of 1kb DNA Ladder

10. History:

Name	Date	Amendment
Deb Audino	02Feb05	Initial Release
Deb Audino	10Oct05	Added catalog number for the Takara kit. Added concentration of ethidium bromide stock solution. Reduced volume of ethidium bromide solution for gel. Added attachments.
Deb Audino	04Apr08	College name change
Jason McMillan	19Feb14	College name change

Lane	ID of PCR	Presence (+) or

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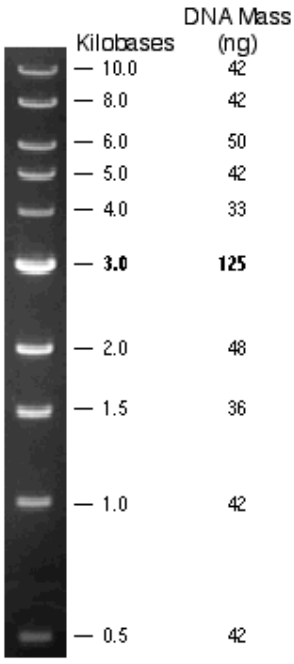
Position	reaction loaded	Absence (-) of 810bp band
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		
11		
12		
13		
14		



1kb DNA Ladder (New England Biolabs)