The RNAi Consortium

TRC Library Protocols
Protocol Title: Mycoplasma Detection
Current Revision Date: 4/23/2014
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<u>Culturing Cells for Mycoplasma Detection</u>

1. Thaw cells according to your specific labs protocols (usually in a T25), if using our BL2+ space, please use the quarantine incubator located in 2100A
2. When confluent (or close) seed new T25 to at least 50% density.
3. Allow cells to grow to confluency.
4. Lets cells sit in media (unchanged) for at least 3-5 days after reaching 100% density
5. Remove 100ul – 1mL of media from flask and place in clean microcentrifuge tube or cryo vial.
6. Place sample in box labeled "Jess's Mycoplasma Box" on top shelf of freezer -20 F.D in room 2073 (box has red arrow on top of it).

<u>Using Ciprofloxin to decontaminate Myco positive cultures.</u>

Ciprofloxin is used at a 10 ug/mL final concentration. It has been noted that this process will cure ~70% of cell lines, kill ~10% of cell lines, and have no effect on ~20% of cell lines. Plan accordingly. (Sigma Aldritch, 17850-5G-F)

1. Cells are maintained in standard growth media supplemented with 10 ug/mL ciprofloxin for 2 weeks, with the standard passaging for the given cell line. If passages are >4 days apart, media should be replaced after 3 days with fresh growth media + ciprofloxin.

2. You should look at the cells during this period for morphological changes, and general health of cells. It is not uncommon for the cells to proliferate a little slower than usual, and look "unhealthy"

3. After two weeks of treatment, cells should be passaged for 2 weeks in growth media alone (same passaging regimen).

4. After the 4 weeks of treatment, media should be re-tested for contamination as above.