**STANDARD OPERATING PROCEDURES – Jessica Hill and Andrew Moore**

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**Title: Methanol kill fluorescent reporter bacteria to produce a negative control for colonization experiments in *C. elegans***

**Version:** **1.0** (Unique identification number/letter code) **Effective Date:** **20231117**

**Approval:** NAME **Date: YYYYMMDD**

**Biosafety Approval:** NAME **Date: YYYYMMDD**

**Final Approval:** NAME **Date: YYYYMMDD**

**Applicable To** (List of all people the SOP is applicable to)

If you are assessing bacterial colonization within the *C. elegans* intestine via live imaging or bactoFISH (smFISH for bacteria), this protocol should be used to generate negative controls for each fluorescent reporter bacteria being imaged.

The idea being, that these negative controls will still fluoresce (thus be detectable) but won’t colonize the intestine. To grow worms on these negative controls, we need to mix them with a small portion of their live, unlabeled counterparts.

**Purpose** (State the objective of a procedure including additional background information and limitations)

To kill bacteria with methanol treatment yet maintain their internal fluorescence reporter. These dead but fluorescent bacteria can then serve as a negative control for imaging experiments.

**Safety Considerations (Risks)** (Identify unique risks and mitigation steps specific to the procedure)

Be careful when handling the fixatives.

Always verify that the fixation worked, by streaking a bit of the methanol treated bacteria onto an LB plate and monitoring for growth.

**Equipment** (List equipment to be used in the procedure, include any special instructions about its use)

Shaking incubator

Centrifuge

**Materials** (List materials to be used in the procedure)

Room temperature 100% methanol

Sterile ddH20

Culture tubes

Bacteria of interest

**Procedure** (Describe the procedure in a step-by-step, chronological manner)

Grow bacteria of interest (i.e., strain A and fluorescent reporter version of strain A) overnight in liquid culture.

Next day, pull overnight cultures out of the incubator. Place strain A off to the side, will use later.

Pellet fluorescent reporter version of strain A (3,000 g for 10 min at RT), and aspirate off the supernatant leaving 5 mL.

Now add 5 mL of 100% methanol and incubate at RT while shaking for a total of 30 min.

Post-incubation, pellet sample (3,000 g for 10 min at RT) and aspirate supernatant.

Resuspend sample in 10 mL of sterile ddH20, vortexing gently.

Pellet sample (3,000 g for 10 min at RT) and aspirate supernatant.

Resuspend sample in 1 mL of sterile ddH20, vortexing gently.

Now plate 200 uL of methanol killed fluorescent reporter bacteria onto an NGM plate.

Then pipette 100 uL of strain A onto the spot of methanol killed fluorescent reporter version of strain A. That way they are somewhat mixed together.

Let plates dry (usually takes a few hours), then store at 4C.

**Definitions** (As needed)

none

**References** (Traditional references as needed. SOPs necessary to fulfill the requirements for this SOP.)

none

**Appendix** (As needed)

none

**Revisions** (Newly added material or material that has been edited in the SOP)

none