

iGEM 2018: NUS Singapore-A



Sterilisation Workflow

Our sterilisation workflow for our dyes consists of 2 parts, a decontamination protocol and an experimental design to demonstrate the effectiveness of our decontamination protocol.

1. Decontamination Protocol

1. Spin down bacterial culture at 5000 rpm for 5 minutes.
2. Transfer the supernatant to a 50ml falcon tube.
3. Filter the supernatant with a 0.22 μm filter.

The filtered supernatant contains the decontaminated dye.

2. Experiment Demonstrating the Absence of Our Modified Microorganisms

1. Plate 100 μL of filtered supernatant on an LB agar plate **without** the antibiotics our microorganisms are resistant to, for example, kanamycin and chloramphenicol. **Label this Plate A.**
2. Plate 100 μL of filtered supernatant on an LB agar plate **with** the antibiotics our microorganisms are resistant to, for example, kanamycin and chloramphenicol. **Label this Plate B.**
3. Incubate plates A and B overnight at 37°C.
4. Inspect plates A and B for colonies.

Experimental Design Rationale

The absence of colonies on a LB agar plate plated with filtered supernatant after overnight incubation is sufficient to demonstrate that the decontaminated dye is free from any microbes.

However, if there are colonies on this plate, this indicates either the failure of our decontamination protocol, or contamination by bacteria present in the environment. We would be unable to determine if our engineered microbes are present or absent from such a result. Thus, we also need to plate our filtered supernatant on an LB agar plate with antibiotics. These antibiotics should be the antibiotics the engineered microorganisms are resistant to.

Interpretation of Results

Colonies absent on Plate A and B: Successful decontamination.

Colonies present on Plate A only: It is highly probably that there are microorganisms in the filtered supernatant. While these microorganisms are not our engineered microorganisms, this indicates contamination of the filtered supernatant due to improper microbiological practices. Another possibility is that only Plate A was contaminated. Repeat the experiment.

Colonies present on Plate B only: Plate B was contaminated with microbes carrying antibiotic resistant genes. Repeat the experiment.

Colonies present on Plate A and B: The supernatant contains the engineered microbes. Decontamination was unsuccessful. Repeat the workflow. If colonies are still present on both plates, this would point to a flaw in the design of your decontamination protocol.

Appendix - A guide on safely bringing biomanufactured products out of the lab in the context of the iGEM competition

What the iGEM Safety Committee expects:

- A well-designed decontamination protocol
- Experimental results to prove the absence of your team's engineered organisms in your biomanufactured product
- Wiki documentation of the steps your team went through to get permission to take your product out of the lab, for transparency
- A mention of how your team obtained this permission when interacting with other teams and the general public, to raise awareness of safety issues and good safety practices

How to be granted permission to bring your biomanufactured products out of the lab and/or to the Jamboree:

1. Write in to iGEM's Safety Committee about the nature of your product and your intention to bring it out of the lab.
2. Design a safety workflow, which includes:
 - a. Lab protocol to decontaminate the product
 - b. Experiment to demonstrate that no modified organisms are present in the decontaminated product
3. Send this to the iGEM Safety Committee for approval
4. Test the approved workflow using your own biomanufactured product
5. Send experiment results to iGEM Safety and Security Committee for approval
6. Bring your product to the Jamboree!