A MicRO*BiO*T (micro biotic robot) designed to diagnose Intestinal Candidiasis (IC)

Medford High School Honors Biology Synthetic Biology Experiment Module (Parts 2 and 3)

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Scenario: There is an Intestinal Candidiasis (IC) outbreak in the Boston-area, and you are tasked with finding which town it most likely originated from. The infection is caused by the fungus *Candida albicans*, and can be transmitted through contaminated water. A quick, noninvasive way to identify patients with IC is to test their urine for a chemical (metabolite) called arabinose. This molecule is produced by *C. albicans* in the intestines, and makes its way into the bloodstream and eventually into urine. It is rarely found in patients without IC, and is therefore a good indicator of the infection. We have collected urine samples from patients that present the symptoms of IC in various local hospitals

Overall Objective: The overall objective of this experiment is to identify the town that is home to "patient-zero" of the Intestinal Candidiasis (IC) infection using a micROBiOT.

Working Hypothesis: The working hypothesis is that the infection is being spread downstream by a local river. Therefore, the town with infected patients that is most upstream of a river may be home to patient-zero.

Approach: We will use an *E. coli*-based micROBiOT to sense the presence of arabinose, the metabolite proceed by *C. albicans*, in urine samples obtained from patients. The genetic program that confers these bacteria the ability to sense arabinose is on a plasmid. When grown in the presence of arabinose, these cell produce a fluorescent protein called GFP (green fluorescent protein). GFP confers bacterial cells a green color that is only visible under ultraviolet (UV) light illumination.

Specific Aim: Transform the plasmid into *E. coli* and spot the cells on media that has been premixed with urine from patients. Once the cells have grown on the media, use UV illumination to identify patients that have arabinose in their urine.

Safety Note: Always wear appropriate personal protective equipment (PPE) when working with chemical or biological hazards. All the biological materials in this experiment are rated Biosafety Level 1 (BSL1), which means they are no health hazards. However, to ensure the integrity of the experiment and for safety of the experimenter, it is always advisable to wear splash-resistant goggles, latex/nitrile gloves, lab coats, and closed-toed shoes.

Materials provided

You are provided 4 tubes for this experiment. Here is the key to the labels:

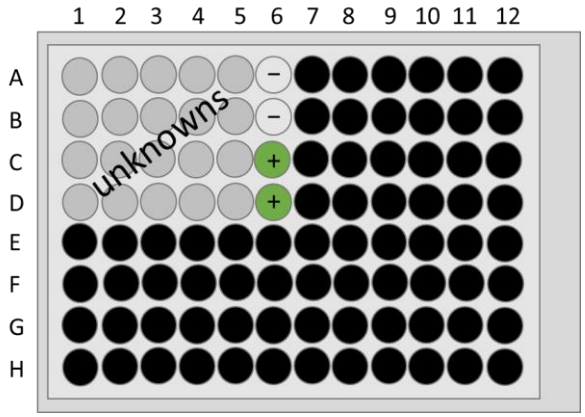
1. **C** competent cells (see **NOTE 1a** for more information)
2. **P** plasmid (see **NOTE 1b** for more information)
3. **+** positive control (see **NOTE 5b** for more information)
4. **−** negative control (see **NOTE 5b** for more information)

Experimental Protocol:

Day 1

1) Thaw the competent *E. coli* cell suspension (C) and plasmid DNA solution (P), positive (+) and negative (−) controls on ice. You may occasion check if the they thawed by gently shaking the tubes.
   NOTE 1a: Competence is ability of cells to uptake foreign DNA from the environment. Transformation is the physical process competent cells undergo when taking up DNA.
   NOTE 1b: A plasmid is a piece of DNA that can be maintained stably in a host. In this experiment, the plasmid contains the genetic program to detect arabinose.

2) Once they are fully thawed, using the P10 or P20 pipette carefully transfer 5 µL of plasmid DNA (P) into the tube containing the competent *E. coli* cells (C). Do not transfer any plasmid to controls (+, −).
NOTE 2: Do not try to mix the plasmid with cells using the pipette.
3) Seal the tube containing the mixture of competent cells and plasmid tightly. Hold the tube by the cap between your index finger and thumb and gently flick the bottom of the tube to mix the plasmid DNA with the cells.
NOTE 3: Do not invert the tube or shake vigorously. Competence is highly associated with environmental factors, and mechanical stress can significantly reduce the ability of cells to uptake DNA.
4) Incubate the tube on ice for 20 min.
NOTE 4: During this time, the cells uptake DNA.
5) Using the P20 pipette ~10 µL of transformed cells into each well of the 20 "unknown" wells containing growth media in your 96-well plate (see figure below).
Also transfer 10 µL of control cells (+, −) in each corresponding well (see figure below)
NOTE 5a: during repeated pipetting, air bubbles can get into the pipette tip leading to accidental contamination of the pipette. Be careful and pipette slowly to ensure that air bubbles are not introduced.
NOTE 5b: Most of these wells contain media mixed with urine from a different patient presenting IC symptoms. These are 20 "unknowns" (labelled A1-D5), where we don't know if the patient will test positive and negative for IC. Column 6 consists of 2 positive and 2 negative controls ("knowns"). A positive control is a sample that is known to test positive. Conversely, a negative control is known to test negative. These are to ensure a) that the sensor is working correctly in the presence or absence of arabinose, and b) the experimenter knows what a positive and negative sample looks like.



6) Leave the plate open to air dry for 5-10 min. Then close and incubate >16 h (overnight) at 37 °C.

Day 2

1) Carefully place the plates on the UV illuminator, cover with the UV-protective shield and turn on the power. Note the wells that contain green or white colonies. Make sure you compare to the controls to make your determination.
NOTE 6: It is important to ensure the UV box plastic shield is in the closed position before turning on the UV lamp to prevent UV "sunburns", which can be particularly harmful to the eyes.
2) Tally the number of positive hits from each town.
3) Use the results from the experiment and your hypothesis to guess which town is "ground zero".