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Protocol status: Working
We use this protocol and it's working

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GFP-sacB Characterization

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ABSTRACT

2023 NUS-Singapore iGEM team followed this protocol to characterise their New Composite Part "GFP-sacB" with IPTG and sucrose solution at various concentrations.

MATERIALS

- LB media
- M9 Media
- Correct Antibiotics
- IPTG Solution
- Sucrose Solution
- DI Water

SAFETY WARNINGS

- Proper lab PPE must be worn at all times.
- Since cells are used in this protocol, a Biosafety Cabinet (BSC) is required to ensure safety.

PROTOCOL integer ID:
88978

Keywords: sacB, GFP, Negative Selection, Negative Selection Marker, Characterization

Cell Inoculation and Incubation (Day Before Characterization)

1 Inoculate cells with GFP-sacB gene from the cell stock.

2 Add 5 mL of LB media and 5 µL of the appropriate antibiotic to a Falcon tube.

3 Incubate the Falcon tube at 37 °C in an incubator.

Sample Preparation (96-well Plate)

4 Decide the concentrations of IPTG and sucrose solutions for characterization.

5 Prepare new Falcon tube(s).

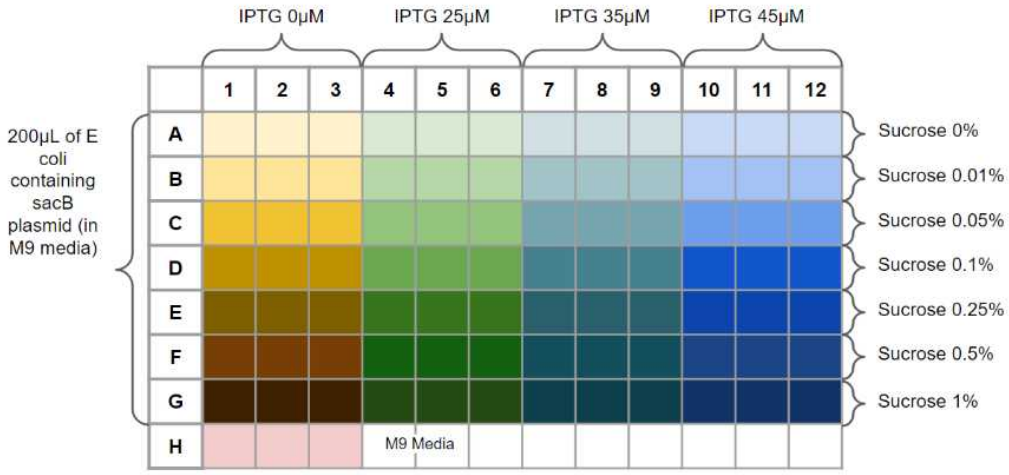
6 Add 5 mL of M9 media, 5 µL of the appropriate antibiotic, and 100 µL of cells cultured the previous day.

7 Add the required volume of IPTG to reach the desired concentration.

8 Incubate the cells for 02:00:00 at 37 °C. 2h

9 Prepare a sterile 96-well plate.

10 Design an appropriate plate map, each sample (with a particular IPTG and sucrose concentration) must be repeated 3 times. Example of plate map:



One of the actual plate map used by the NUS-Singapore iGEM 2023 team when characterizing the GFP-sacB gene.

11 Adjust sucrose concentration by adding DI water and sucrose solution to each well (final volume of 20 μL) according to the plate map.

12 Add 200 μL of cultured cells to each well already containing DI water and sucrose solution (final well volume of 220 μL).

13 Add at least 3 wells of 220 µL of M9 media as the blank (negative control).

Characterization and Plate Reader Reading

14 Place the 96-well plate into the plate reader.

15 Create a protocol in the plate reader's software according to the following setting:

	A	B
	Plate Type	96 WELL PLATE
	Set Temperature	Setpoint 37°C
		Preheat before moving to next step
	Start Kinetic	Runtime 6:10:00 (HH:MM:SS), Interval 0:30:00, 13 Reads
	Shake	Orbital: Continuous
		Frequency: 282 cpm (3 mm)
	Read 1	Absorbance Endpoint
		Full Plate
		Wavelengths: 600
		Read Speed: Normal, Delay: 100 msec, Measurements/Data Point: 8
	Read 2	Fluorescence Endpoint
		Full Plate
		Excitation: 485, Emission: 528
		Optics: Top, Gain: 100
		Light Source: Xenon Flash, Lamp Energy: High
		Read Speed: Normal, Delay: 100 msec, Measurements/Data Point: 10
		Read Height: 7 mm
	End Kinetic	

16 Start the continuous reading.

17 After the reading is complete, remove the 96-well plate from the plate reader.

18 Save the data as a CSV file for future analysis.

19 Discard the used 96-well plate in a biohazard bin.