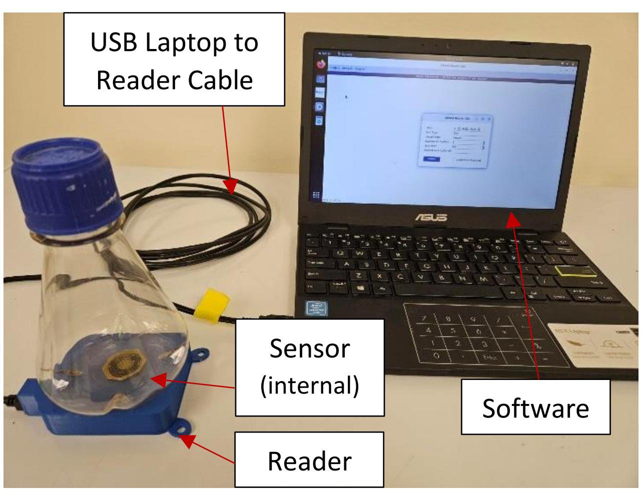
Note #001 / 2024.02.20

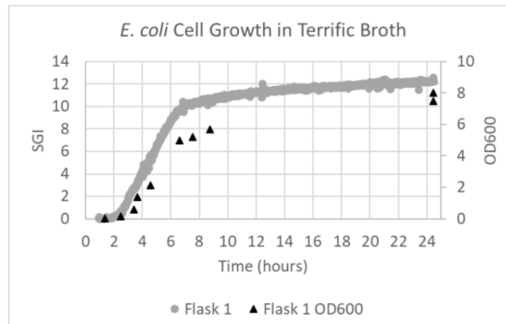
Skroot SMART Platform: Monitoring *E.coli* Growth Profiles in Shake Flasks

Motivation: Bacteria cells, such as *E. coli*, are a champion chassis for biomanufacturing due to their fast-doubling time. However, the growth patterns of these microbes are highly dependent on strain type and growth conditions. To capture the growth profile (lag, log, and stationary phase) manual sampling of the media is typically conducted over the full growth period. Absorbance of light is used to measure the amount of cell mass, typically at 600 nm (OD600), however this method is only accurate up to ~1 OD and thus careful dilutions must also be performed at higher cell densities. To capture a full growth profile, an operator must be present for a taxing 16+ hours.

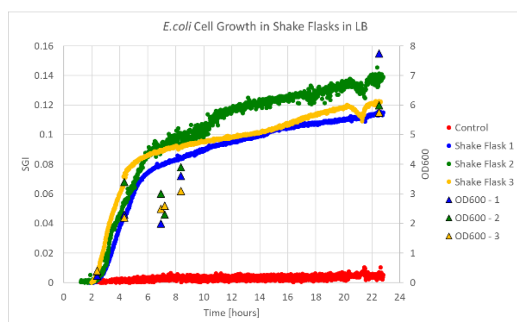
Solution: The Skroot Single-use, Metabolite Absorbing, Resonant Transducer (SMART) sensor can be used to continuously monitor the growth of cell cultures. By placing the sterile Skroot SMART sensor inside of a shake flask, the sensor can provide a minute-to-minute response to how a bacterial cell culture is growing, without the need for sampling the culture. With the application of a Skroot SMART sensor, accompanied by a reader and appropriate software, the platform reports real-time responses to bacterial cell growth. The Skroot SMART sensor absorbs naturally secreted secondary metabolites via a cell transduction layer which in turn changes the sensor's radiofrequency (RF) resonant characteristics. This is done wirelessly and passively (no onboard power needed for the sensor sticker). Additionally, due to the non-optical based sensing mechanism, Skroot SMART sensors can be used in a variety of different media, including turbid media at high cell densities.



Demonstration: To first demonstrate the use of Skroot SMART sensors in tracking bacterial cell growth, sensors were placed in 250mL shake flasks with 80mL of Terrific broth. Flasks were shaken at 200 RPM and incubated at 37°C. In conjunction with the Skroot SMART sensor output, Skroot Growth Index (SGI), 1mL media samples were evaluated with a spectrophotometer (OD600). Below is the cell growth plot as indicated by the data from the spectrophotometer and the Skroot SMART sensor system, showing direct correlation of all growth phases.



Second Demonstration: Skroot SMART sensors were used to monitor the growth of *E. coli* 5α in Luria-Bertani (LB) broth. Growth conditions were identical to the previous demonstration, aside from the difference in media. Three replicate shake flasks were seeded 1 vol-% with the same overnight culture whereas the control was supplemented with Pen-Strep to ensure no microbial growth. Note the noise that can be present in manual OD600 plots.



Intellectual Property:
1.Wireless Sensor (US Patent #11105761)
2.Transduction Cell Membrane (Application at USPTO)
3.Resonant Sensor Reader (WO Patent #236534)

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