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E. coli growth curve assay in BG-11 + sucrose

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To obtain growth curves of KJK01 and pCSCX-KJK01 in BG-11 + sucrose and to characterize their growth. This also serves to estimate sucrose consumption and butanol production over time.

This can be adapted for any other bacteria as well by giveing proper growth conditions, and using appropriate wavelength to measure the OD and giving proper time intervals based on whether the bacteria is slow growing or fast growing.

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Primary culture

1 Inoculate 1 colony of *E. coli* KJK01 in **5 mL** of 1X LB

2 Inoculate 1 colony of *E. coli* pCSCX-KJK01 in **5 mL** of 1X LB with **5 µL** Kan^{50} and **5 µL** Amp^{100}

3 Leave both cultures in a shaker incubator **Overnight** at **37 °C** and **180 rpm**

Secondary culture

4 The next morning, inoculate **900 µL** of 1° culture of KJK01 in **90 mL** BG-11 and **10 mL** of sucrose. This serves as a control for sucrose consumption.

This culture is expected to show negligible/no growth and no butanol production.

5 Inoculate **900 µL** of 1° culture of pCSCX-KJK01 in **90 mL** BG-11 and **10 mL** of sucrose with **90 µL** Kan^{50} and **90 µL** Amp^{100}. This serves as a control for butanol production.

This culture is expected to show fastest growth and low butanol production.

6 Inoculate **900 µL** of 1° culture of pCSCX-KJK01 in **90 mL** BG-11 and **10 mL** of sucrose with **90 µL** Kan^{50}, **90 µL** Amp^{100} and **10 µL** IPTG (**0.1 Milimolar (mM)**)

This culture is expected to show slow growth and maximum butanol production.

7 Place these three cultures in a shaker incubator at **37 °C** and **180 rpm**

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Preparing samples 6h 15m

8 At **06:00:00** intervals, draw 2 **1 mL** samples from each culture 6h

9 Measure the OD of 1 sample from each culture at 600 nm and note it down for the growth curve assay

10 Centrifuge all 6 samples at **5000 rpm, 7°C** for **00:15:00** 15m

11 Decant the solution and store the supernatants at **-20 °C**

11.1 Submit 1 sample of each culture for the sucrose assay. This helps determine consumption of sucrose over time.

11.2 Submit 1 sample of each culture for NMR analysis. This helps determine butanol production over time.

12 Continue this procedure for **96:00:00** 4d

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