

# iGEM2017 – Microbiology – BMB – SDU

**Project type:** iGEM2017

**Creation date:** 22/10-2017

**Project title:** Biobrick test

**Written by:** MA & JB

**Sub project:** Growth experiment

**Performed by:** MA, FN & JB

## 1. SOPs in use

iGEM2017\_SOP02\_v02\_FN\_ONC\_E.coli

iGEM2017\_SOP08\_v03\_MA\_M9\_minimal\_medium

iGEM2017\_SOP14\_v02\_EG\_Table\_Autoclave

## 2. Purpose

To test the ability of *E. coli mg1655* with different genes live on cellobiose and cellulose.

### 3. Overview

Day	SOPs	Persons	Experiments
17.10.23	SOP02 SOP08	MA	Prepared M9 medium and ONCs
17.10.24		MA	Set up of growth experiments
17.10.24 - 17.10.27		MA, JB, FN	Measurements

### 4. Experiment history

Date (YY.MM.DD)	SOPs	Alterations to SOPs and remarks to experiments	
17.10.23	SOP02 SOP08	MA	<p>Prepared M9 minimal media 3*100 mL with CML, 1x100 mL with KAN and 1x100mL with both CML and KAN.</p> <p>Five <i>E.coli mg1655</i> ONCs were prepared from freeze stock Lac+RBS control, cep94A on pSB1C3 backbone, bglx, cep94A pSB3K3 backbone and <i>E. coli</i> MG1655 which both got cep94A on pSB3K3 and the full cellulase secretion system from BBa_K2449026</p>
17.10.24		MA	<p>Each ONC were diluted to an OD600 of 0,005 in 10 mL M9 media with the right antibiotic in six erlenmeyer flasks.</p> <p>Casaminoacids was added to all flasks, 3/6 flasks from each ONC had added cellobiose, while the other 3 was left without a carbon source. The bacteria containing genes for the secretion system got cellulose instead of cellobiose. The start OD600 was measured to make sure all cultures was at OD600 0,005. All flasks was placed in a waterbath with a constant temperture of</p>

			37°C, and at rotation at 40RPM (note that 40RPM is a slow rotation speed, but we experienced that slow speed worked best for cep94A)
17.10.24 - 17.10.27		MA, JB, FN	After 8, 16, 24, 32, 40, 48, 56, 64 and 72 hours 1mL sample was taken from each erlenmayer flask and the OD600 measure and plotted into our graphs with a triple determination. After measuring OD600 the 1mL sample was poured back in the flask

## 6. Results and conclusions

The experiment showed that the cep94A on pSB1C3 makes *E.coli* able to live on cellobiose. While without cep94A it wasn't able to live on cellobiose.

There can't be said anything about if the cellulase secretion system is degrading cellulose to cellobiose from this experiment, because of the cellobiose experiment showed that even if the cellulose is degraded to cellobiose the low copy plasmid pSB3K3 isn't enough for the *E. coli* to live on cellobiose. For more information check out our wiki section with demonstrations and results.