iGEM2017 – Microbiology – BMB – SDU

Project type: iGEM2017	**Creation date:**
Project title: Cellulose degradation	**Written by:** MA
Sub project: SDS-page	**Performed by:** MA

1. SOPs in use

iGEM2017_SOP02_v02_FN_ONC_E.coli

iGEM2017_SOP27_v01_MA_SDS-PAGE

2. Purpose

To determine the presence of the different proteins encoded in the plasmids.

3. Overview

Day	SOPs	Persons	Experiments
2017.10.17	SOP02	MA	Preparation of the 7 different ONC made on strains needed to be run on a SDS-page.
2017.10.18 - 2017.10.19	SOP27	MA	Preperation for first SDS-page, and running the first one.
2017.10.20	SOP27	MA	SDS-page was run again.

4. Other comments

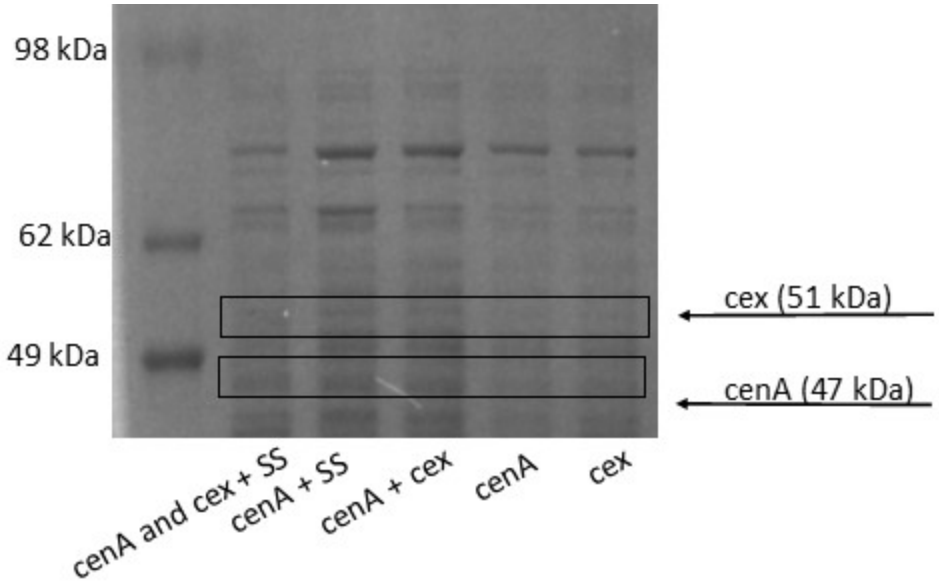
5. Experiment history

Date (YY.MM. DD)	SOPs	Alterations to SOPs and remarks to experiments	
2017.10.17	SOP02	MA	Preparation of the 7 different ONC made on strains needed to be run on a SDS-page.
2017.10.18 - 2017.10.19	SOP27	MA	75 microliter cells was spun down to harvest cells, and separate them from the supernatant, the pellet was dissolved in 75 microliters LB-media, and both the supernatant and pellet had 25 microliters 4x SDS-buffer with DTT added, and was boiled at 95 degrees celsius for 10 min, hereafter 10 microliters was added. 10 microliters of sample was loaded into each well. 4 microliters of SeeBlue™ Plus2 Pre-stained Protein Standard was loaded. The SDS-page was run for 120 min at 120 volts to get a nice spread of the proteins. The

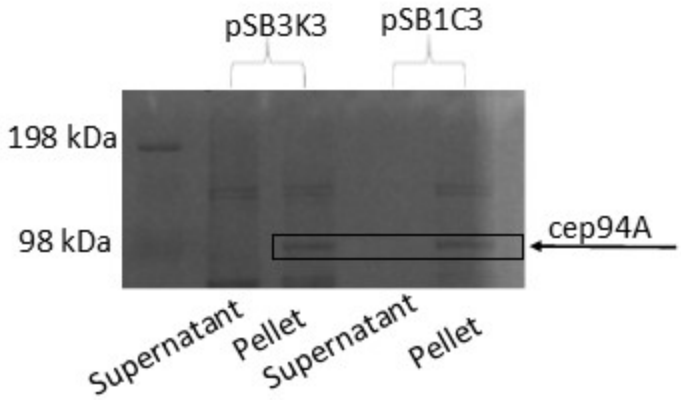
			lowest bands were run out of the gel. The gel was fixed with fixation buffer overnight. The gel was stained with Coomassie Brilliant Blue for 10 hour and detained with a mix of ethanol and acetic acid. Here after it was left in demineralised water
2017.10.20	SOP27	MA	After looking at the result, the 90 microliters left over for the supernatants of the strains containing the cellulases and secretion system, were acid precipitated with 50% TCA, and the protein dissolved in LB and SDS-buffer resulting in a 10x concentration of the proteins in the supernatants. 10 microliters of sample was loaded into each well. 4 microliters of SeeBlue™ Plus2 Pre-stained Protein Standard was loaded. The SDS-page was run for 120 min at 120 volts to get a nice spread of the proteins. The lowest bands were run out of the gel. The gel was fixed with fixation buffer overnight. The gel was stained with Coomassie Brilliant Blue for 10 hour and detained with a mix of ethanol and acetic acid. Here after it was left in demineralised water

6. Results and conclusions

The SDS-pages confirmed the presence of cep94A, but did not prove the existence of either cellulases nor the secretion system.



SDS-page of supernatants.



SDS-page with prove of cep94A.

10. Appendices