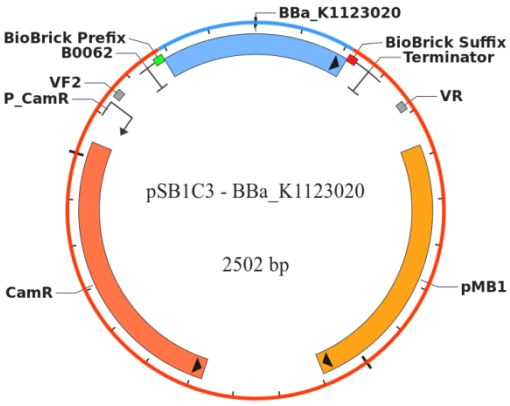
BBa_K1123020

Basic Information

Basic Function: The expression of POLY(THREONINE--LYSINE)
Description: This part holds the DNA information for the POLY(THREONINE--LYSINE) protein, so that it can be placed Behind a promoter and expressed.

Related Parts: BBa_K1123008
Authors: Ardjan van der Linden
Data Collection: Ardjan van der Linden
Affiliation: TU-Eindhoven
Team: TU-Eindhoven 2013
Student Contact: igem2013@tue.nl
Faculty Contact: m.merkx@tue.nl
Date Submitted: 31-08-2013
Date Updated: 31-08-2013
Biosafety: Risk Group 1
Availability: Unavailable
Sequence: Confirmed



Additional Comments: This Brick has been tested in the pET28a vector and not the pSB3C1. All experiments were performed in this vector. The part has been tested using this vector to check the protein expression, the protein itself and if expressed properly will then be used functionally in the brick BBa_K1123009

Reference:

Device Information

Device Name: BBa_K1123020
Device Type: Coding
Description: Protein sequence
Components: none

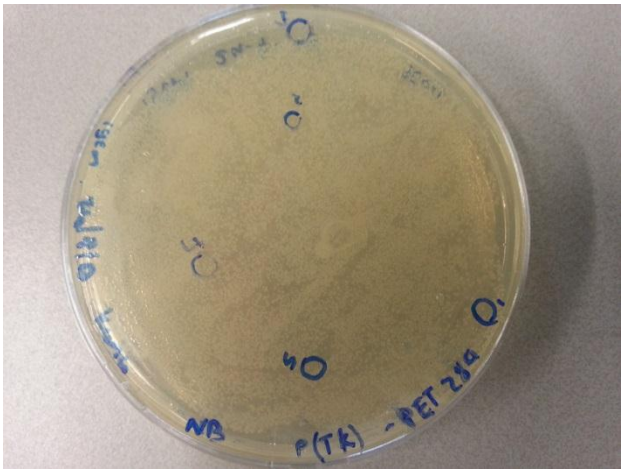
Assembly: BioBrick
Protocol: -
Scars: No
Insertion: Plasmid
Vector: pET28a

Additional Comments: The DNA of this part was not submitted to the registry. It was therefore not transferred to the pSB1C3 vector. The biobrick was therefore only tested in the pET28a vector.

Reference:

Plate Imaging

BASIC INFORMATION
Purpose: Control of ligation into pET28a vector
Chassis: E.coli
Strain: NB (NovaBlue)
Protocols: Standard plating procedure
Date: 24/07/13



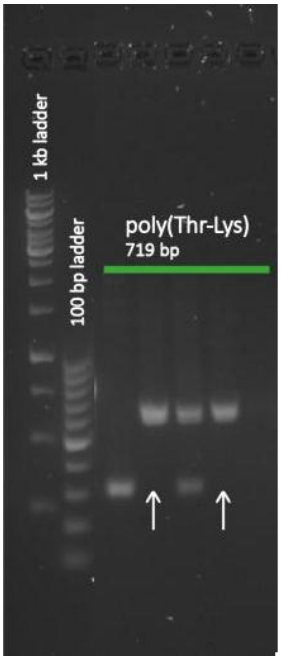
GROWTH CONDITIONS
Media Type: Agar + Kanamycin
Antibiotic: Kanamycin (30µL/mL)
Vessel: Petri dish
Incubation: 37°C
Growth Time: 15 hours

MEASUREMENT INFORMATION
Data Type: Picture
Location: TU-Eindhoven BioLab
Machine Name: Digital Camera
Reporter Used: none

Additional Comments: The growth of colonies was seen simply by photographing the growth plates after incubation.
Reference: None

Restriction Mapping

BASIC INFORMATION
Purpose: Check ligation and PCR results, also used to check protein sequence length.
Chassis: E.coli
Strain: NB (NovaBlue)
Protocols: Standard gel electroforesis
Date:19/08/2013



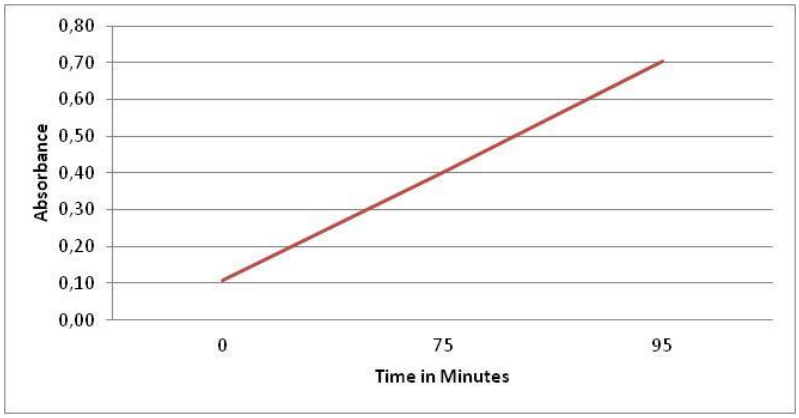
MEASUREMENT INFORMATION
Data Type: Gel electrophoresis
Location: TU-Eindhoven BioLab
Machine Name: N/A
Enzymes Used: PCR primers
Total Time: 60 minutes
Voltage Used: 100 V

Ladder Used: New England Biolans 100 bp ladder

Additional Comments: We performed colony PCR on the plates shown above using T7 FW and RW primers These samples were then run on gel giving an indication of correct ligation into pET28a.
Reference: none

Growth Curve

BASIC INFORMATION
Purpose: To prepare for protein expression.
Chassis: E.coli
Strain: BL21
Protocols: Standard Expression protocol
Date:20/08/13



GROWTH CONDITIONS
Media Type: LB + Kanamycin
Vessel: culture flask
Volume: 400mL
Incubation: 37°C, 250rpm

MEASUREMENT INFORMATION
Data Type: Growth Curve (OD vs Time)
Location: TU-Eindhoven Biolab
Machine Name:N/A
Time Interval: N/A
Total Time: N/A

Additional Comments: The optical densities were measured upto an optical density of 0.600 as we would then be inducing protein expression.
Reference: None

Future Work

This brick will later be used in a composite part, BBa_K1123008, once in that part, the same protein will be expressed anaerobically. We will need to then perform the same controls on the composite part to show that the expression remains equal, aerobically as well as anaerobically.