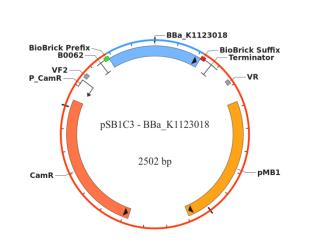
Basic Information

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

Related Parts: BBa_K1123006 <u>Authors</u>: Ardjan van der Linden <u>Data Collection</u>: Ardjan van der Linden <u>Affiliation</u>: TU-Eindhoven <u>Team</u>: TU-Eindhoven 2013 <u>Student Contact</u>: igem2013@tue.nl <u>Faculty Contact</u>: m.merkx@tue.nl <u>Date Submitted</u>: 31-08-2013 <u>Date Updated</u>: 31-08-2013 <u>Biosafety</u>: Risk Group 1 <u>Availability</u>: Unavailable Sequence: Confirmed



BBa_K1123018

<u>Additional Comments</u>: 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_K1123007.

Reference:

Device Information

<u>Device Name</u>: BBa_K1123018 <u>Device Type</u>: Coding <u>Description</u>: Protein sequence <u>Components</u>: none <u>Assembly</u>: BioBrick <u>Protocol</u>: -<u>Scars</u>: No <u>Insertion</u>: Plasmid <u>Vector</u>: pET28a

<u>Additional Comments</u>: 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

<u>Purpose</u>: Control of ligation into pET28a vector <u>Chassis</u>: E.coli <u>Strain</u>: NB (NovaBlue) <u>Protocols</u>: Standard plating procedure <u>Date</u>: 24/7/13

GROWTH CONDITIONS

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

MEASUREMENT INFORMATION Data Type: Picture

<u>Location</u>: TU-Eindhoven BioLab <u>Machine Name</u>: Digital Camera <u>Reporter Used</u>: none



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

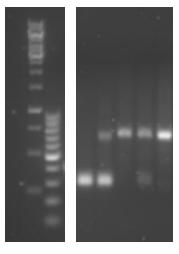
Restriction Mapping

BASIC INFORMATION

<u>Purpose</u>: Check ligation and PCR results, also used to check protein sequence length. <u>Chassis</u>: E.coli <u>Strain</u>: NB (NovaBlue) <u>Protocols</u>: Standard gel electroforesis <u>Date</u>: 08/0813

MEASUREMENT INFORMATION

<u>Data Type</u>: Gel electrophoresis <u>Location</u>: TU-Eindhoven BioLab <u>Machine Name</u>: N/A <u>Enzymes Used</u>: PCR primers <u>Total Time</u>: 60 minutes <u>Voltage Used</u>: 100 V <u>Ladder Used</u>: New England Biolans 100 bp ladder Ladders Kbp |100bp | Digested samples



<u>Additional Comments</u>: 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. <u>Reference</u>: none

Growth Curve

BASIC INFORMATION

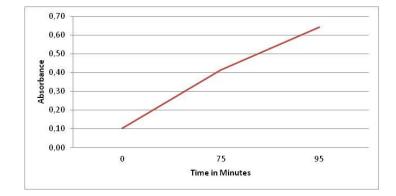
<u>Purpose</u>: To prepare for protein expression. <u>Chassis</u>: E.coli <u>Strain</u>: BL21 <u>Protocols</u>: Standard Expression protocol <u>Date</u>:20/08/13

GROWTH CONDITIONS

<u>Media Type</u>: LB + Kanamycin <u>Vessel</u>: culture flask <u>Volume</u>: 400mL <u>Incubation</u>: 37°C, 250rpm

MEASUREMENT INFORMATION

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



<u>Additional Comments</u>: 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_K1123006, 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.