Reporter system with β-galactosidase (β-gal)

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β-galactosidase activity *in vitro*

Follow protocol: *Determination of β-gal activity in vitro using X-gal and ONPG*

Detection of the time needed to get a visible color change; from colourless to blue for X-gal or yellow for ONPG. Several concentrations of β-gal were tested from a range of 0.5 to 3.0 U/ml. The time points chosen to measure the absorbance in a spectrophotometer were every 5 min for the first 10 min and then every 2.5 min for 1 hour. The concentration used for both substrates was 0.2 µl/ml. The absorbance was read at 610 nm for X-gal and 420 nm for ONPG. The values obtained were used to create standard curves for later comparison with in vivo measurements.

Genomic extraction

Follow protocol: *Genomic DNA purification*

To amplify LacZ from *E. coli* MG1655 genome the Thermo Fisher Genomic DNA Purification Kit was used.

Table 1: Phusion PCR for LacZ amplification from isolated *E. coli* MG1655 genome.

Phusion mastermix	Volume [µl]
HF buffer 5x	10
dNTP	1
LacZ_T2-FW	2.5
LacZ_T2-RV	2.5
phusion polymerase	0.5
H2O	33.5
Total	50

Cloning

Type IIS Assembly (T2S)

Follow protocol: *Type IIS cloning*

TII Assembly preparation

15 nmol/µl insert (each insert)
-> length (bp)/100 = total ng needed for 10 µl reaction

Promoter: J23103
53 bp = 0.53 ng
27.1 ng/µl - use 0.2 µl

RBS: B0034
30 bp = 0.3 ng
37.5 ng/µl - use: 0.1 µl

CDS: lacZ
075 bp = 3.075 ng
122.8 ng/µl
dilution: 1.63 µl lacZ in 8.37 µl H2O for final concentration of 20 ng/µl
use 0.15 µl

Terminator:
115 bp = 1.15 ng
we have 86 ng/ul- use 0.23 ul

backbone: pSB1K03
2195 bp = 21.95 ng

Transformation: T2S-lacZ level 1 into *E. coli* DH5α.
Follow protocol: *Transformation of chemically competent E. coli DH5α cells*

For transformation, 3 µl of theT2S mix was used. Cells were plated on LB kanamycin agar. Positive and negative controls were added.

Colonies were restreaked.

Colony PCR

Follow protocol: *Colony PCR*

This was done in order to check the presence of the gene of insert in the plasmid constructs. Colonies were picked from plates.

Reagents	Volume [µl]
ddH2O	33.7
dNTP	1
VF2	5
VR	5
10x taq buffer	5
Taq DNA polymerase	0.3
Sample	1
Total	50

Liquid cultures

Follow protocol: *Overnight culture*

Cells were cultured in LB substituted with 100 µM kanamycin.

Agarose gel electrophoresis

Follow protocol: *Gel electrophoresis*

PCR products of the colony PCR were loaded on a 1% agarose gel.

Gibson Assembly

Follow protocol: *Gibson Assembly*

Gibson Assembly preparation

LacZ:
112.6 ng/µl -use 0.25 µl
GB mastermix:
use 2.5 µl
backbone pBAD24
87,7 ng/µl -dilute 1:5 and use 0.8 µl final conc is 56 ng

Positive control: ThermoFisher gibson positive control

Gibson was run at 50°C for 60 min.

Transformation

Follow protocol: *Transformation of chemically competent E. coli DH5α cells*

For transformation, 2µl and 3 µl of the Gibson assembly products were used. Cells were plated on LB ampicillin agar.

Positive and negative controls were added.

Colonies were restreaked.

Colony PCR

Follow protocol: *Colony PCR*

This was done in order to check the presence of the gene of insert in the plasmid constructs. Colonies were picked from plates.

Reagents	Volume [µl]
ddH2O	33.7
dNTP	1
lacZ GB fw	5
LacZ GB rv	5
10x Taq buffer	5
Taq DNA polymerase	0.3
Sample	1
Total	50

Liquid cultures

Follow protocol: *Overnight culture*

Cells were cultured in LB substituted with 100 µM ampicillin.

Agarose gel electrophoresis

Follow protocol: *Gel electrophoresis*

PCR products of the colony PCR were loaded on a 1% agarose gel.

Cryo-stock

Follow protocol: *Cryo-stock*

Cryo-stock made from a colony transformed with pBAD24 lacZ.

Expression level of β-galactosidase *in vivo*

E. coli cells expressing lacZ from pBAD24 were cultured overnight in LB plus 0.2% glucose. Next morning cells were diluted 1:100.

The cells were exposed to a concentration 0.2 mg/ml of Xgal/ONPG and incubated for 3 hours. Absorbance was measured for 180 min every 30 min. As a blank, cells were grown in X-gal or ONPG free media to eliminate the measurement of the cell growth. The measurements for pBAD24-lacZ were repeated three times, the average absorbance was then plotted against time. As a negative control, pSB1C3-Caff-I DH5α cells were used. The measurements for the negative control were performed only once.

Upon expression of β-gal the samples should turn blue when X-gal was added and yellow when ONPG was added. The absorbance of the negative control should stay equal throughout the measurements. They were grown in 10 mL LB-media with 0.2% glucose.