LIGATION MATH

Construct: pBAD-ompT-CAM-GS-xylE-xylF

Fragment 1: pBAD-ompT-CAM-GS

Fragment 2: XylE

Fragment 3: XylF1/2

Fragment 4 : XylF1/2

FORMULA

Required mass insert (g) = desired insert/vector molar ratio x mass of vector (g) x ratio of insert to vector lengths

Vector (pSB1C3) : 25 ng/µl

pBAD-ompT-CAM-GS: 50 ng/µl

XylE: 50 ng/µl

XylF1/2: 50 ng/µl

XylF1/2: 50 ng/µl

Ratio of Insert/Vector - 6:1

Fragment	Length (bp)	Insert mass (ng)	Required volume of fragment in µl
pBAD-ompT-CAM-GS	716	51.88	1.04
XylE	963	69.78	1.40
XylF1/2	552	40.00	0.80
XylF1/2	516	37.39	0.75

CLONING PLAN

Fragments to be Digested and Ligated**,**

Fragment	Size (bp)
pSB1C3	2070
pBAD-ompT-CAM-GS	716
XylE	963
XylF1/2	552
XylF1/2	516

Reconstitution:

pBAD_RBS_ompT_CAM_GS: 500 ng (Given)
XylE: 1000 ng (Given)
XylF1/2: 500 ng (Given)
XylF1/2: 500 ng (Given)

Suspend **500 ng of DNA in 10 µl** of **TE buffer and 1000 ng of DNA in 20 µl** of **TE buffer** to get a final concentration of 50 ng/µl. Incubate the reconstituted DNA at 50°C for 20 minutes and store at 4°C.

Digestion:

pSB1C3 (Backbone)

Stock: 25 ng/µl

Molecular water	3 µl
10x Neb buffer 2.1	1 µl

DNA	5 µl
Restriction enzyme	0.5 µl EcoR1 + 0.5 µl Pst1
Total Volume	10 µl

pBAD_RBS_ompT_CAM_GS

Stock: 50 ng/µl

Molecular water	5 µl
10x Neb buffer 2.1	1 µl
DNA	3 µl
Restriction enzyme	0.5 µl EcoR1 + 0.5 µl Bsa1
Total Volume	10 µl

XylE

Stock: 50 ng/µl

Molecular water	1.5 µl
10x Neb buffer 2.1	1.0 µl
DNA	7.0 µl
Restriction enzyme	0.5 µl Bsa1
Total Volume	10 µl

XylF1/2

Stock: 50 ng/µl

Molecular water	4.5 µl
10x Neb buffer 2.1	1.0 µl
DNA	4.0 µl
Restriction enzyme	0.5 µl Bsa1
Total Volume	10 uL

XylF1/2

Stock: 50 ng/µl

Molecular water	4.25 µl
10x Neb buffer 2.1	1.0 µl
DNA	3.75 µl
Restriction enzyme	0.5 µl Bsa1 + 0.5 µl Pst1
Total Volume	10 µl

1. Prepare digestion mixture for backbone and the fragments in separate tubes.
2. Digest the mixture at 37°C celsius for 30 minute.
3. Inactivate the restriction enzymes at 80°C for 20 minute.

Ligation:

Molecular water	1.67 µl
T4 DNA ligase buffer (10X)	1.5 µl
Fragment 1	3.33 µl
Fragment 2	2 µl
Fragment 3	2 µl
Fragment 4	2 µl
pSB1C3	2 µl
T4 DNA ligase	0.5 µl
Total Volume	15 µl

1. Prepare the ligation mixture and gently mix by pipetting up and down and microfuge briefly.
2. Incubate the ligation mixture at 25°C for 10 minutes and transform 2 µl of ligated DNA into 50 µl competent cell.
3. Incubate the rest ligation mixture at 16°C overnight and transform 2 µl of ligated DNA into 50 µl competent cell next day.