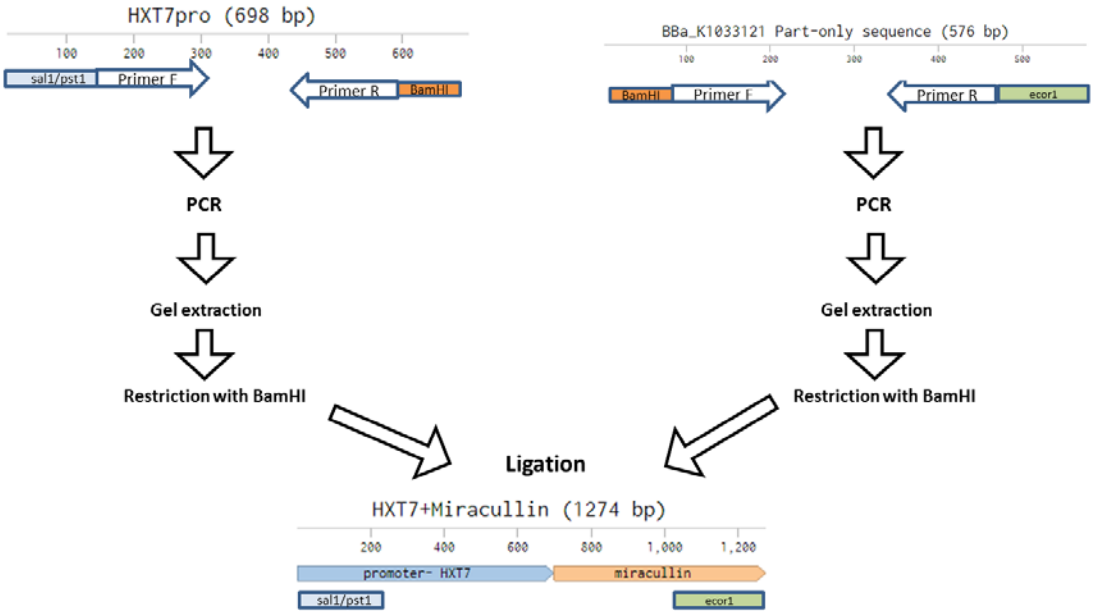
Contribution-
Improving existing BioBrick



1. **Miracullin Extraction from PSB1c3:**

DNA (PSB1c3)	13 µL
Primer R (Mir_rev)	1.5 µL
Primer F (Mir_fw)	1.5 µL
Dream-Taq	25 µL
DDW	9 µL

Mir_fw – GGATCCatggattcagcccct
Mir_rev- GAATTCgaagtaaacagtcttattaaattcaaa

2. **HXT7 :** Promoter from *Saccharomyces cerevisiae S288c*, which correlated with the extracellular glucose concentration in the culture.
   We order the promoter from IDT and we designed primers to add **PstI** and **BamHI** at the edges ;

DNA (**HXT7** promoter)	1 µL
Primer R (HXT7 rev)	1.5 µL
Primer F (HXT7 fw)	1.5 µL
Dream-Taq	25 µL
DDW	21 µL

HXT7 fw -GTCGAC **CTGCAG**CTAAAGGGAACAAA
HXT7 rev- GGATCC GGATCCACTAGTTCTA

SalI PstI BamHI EcoRI

3. After the PCR reaction, we run the products in 1% agarose gel and preformed gel extraction.
4. Digestion with **BamHI** (promoter & gene, seperatly) ;

BamH1	1 µL
DNA	1µg
Cut smart - 10X NEBuffer	3 µL (1X)
DDW	To 30 ul
Total Reaction Volume	30 µL

Incubate the restriction digest at 37C for 30min, and then 80C for 20min to heat kill the enzymes

5. Ligation of the promoter & gene:

Ligase	1 µL
Ligase buffer	3 µL
Promoter	10 µL
Miracullin	10 µL
DDW	6 µL

6. **<u>Ligate to pSB1c3 / pRS306 / pRS316</u>**

Sticky ends for DNA sequence :

DNA	250ng
CutSmart buffer	2 µL
BSA	0.5 µL
EcoR1-HF / SalI	0.5 µL
Pst1	0.5 µL
DDW	To 20 µL

Sticky ends for plasmid :

Plasmid	10ng
CutSmart buffer	2 µL
BSA	0.5 µL
EcoR1-HF / SalI	0.5 µL
Pst1	0.5 µL
DDW	To 20 µL

Incubate the restriction digest at 37C for 30min, and then 80C for 20min to heat kill the enzymes

Ligation between insert and vector:

Plasmid	2 µL
DNA	2 µL
T4 ligase	1 µL
Ligase buffer	1.5 µL
DDW	4.5 µL

O/N at 16C or 60min at 37C.

7. **Transformation to TOP10 / Yeast cells**