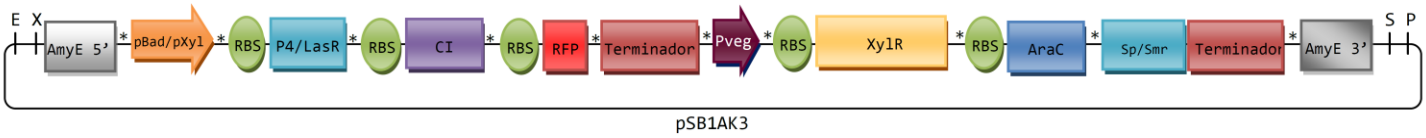
ARABINOSE-XYLOSE AND GATE

This construct was designed to function as a AND logic gate. This is due to the way the pBad/pXyl promoter was designed. We used a system that sensed l-arabinose in E.coli which was originally designed by Amelia Hardjasa and used by iGEM09_British_Columbia[1,2]. In the absence of arabinose, the repressor protein AraC (BBa_C0080) binds to the AraI1 operator site of pBAD and the upstream operator site AraO2, blocking transcription[1]. In the presence of arabinose, AraC binds to it and changes its conformation such that it interacts with the AraI1 and AraI2 operator sites, permitting transcription[1]. We also used a promoter inducible by xylose that has been designed for high expression in *B.subtilis* which was originally designed by James Chappell and used by iGEM08_Imperial_College. Xylose does not induce the promoter xylose directly, but requires the transcriptional regulator **XylR** (BBa_K143036). Our system consists in a fused promoter which includes both AraC and XylR binding sites. AraC and XylR are l-arabinose and xylose sensing, respectively, repressors. In this way, if we use these two inputs, each specific for each repressor we will have an AND gate.

To obtain our final construct we required the following Biological Parts:



Obtained from the Registry:

- BBa_K143001 (AmyE 5')
- BBa_K143002 (AmyE 3')
- BBa_E1010 (RFP)
- BBa_B0014 (Double Terminator)
- BBa_C0080 (AraC)

Synthesis Products:

- pBad/pXyl promoter
- RBS XylR

Obtained from Margarita Salas Ph.D.'s Group:

- A3 from phage phi29 from plasmid pFRC54.

Omega cassette from plasmid pHP45Ω.

We designed the following primers to add the RBS site to BBa_E1010 (RFP) and BBa_C0080 (AraC):

RFP
UPPER 5'-3'
PREFIX+RBS+SPACER+RFP
GTTTCTTCGAATTCGCGGCCGCTTCTAGAG AAAGGTGGTGAA TACTAG ATGGCTTCCTCCGAA
LOWER 5'-3'
SUFIX+RFP
GTTTCTTCCTGCAGCGGCCGCTACTAGTA TTATTAAGCACCGGT

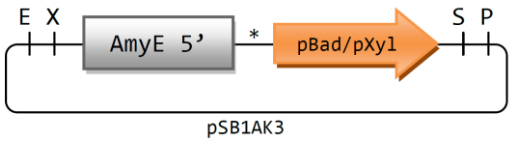
ARAC without LVA
UPPER 5'-3'
PREFIX+RBS+SPACER+ARAC
GTTTCTTCGAATTCGCGGCCGCTTCTAGAG AAAGGTGGTGAA TACTAG ATGGCTGAAGCGCAA
LOWER 5'-3'
SUFIX+ARAC
GTTTCTTCCTGCAGCGGCCGCTACTAGTATTATTA CAACTTGACGGCTAC

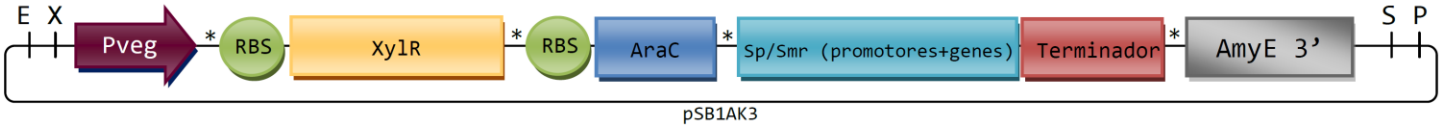
We also designed the following primers to obtain Omega cassette from plasmid pHP45Ω:

OMEGA CASSETTE
PREFIX+OMEGA CASSETTE(45 bp)
5' GTTTCTTCGAATTCGCGGCCGCTTCTAGAG CCGGGGATCCGGTGA 3'

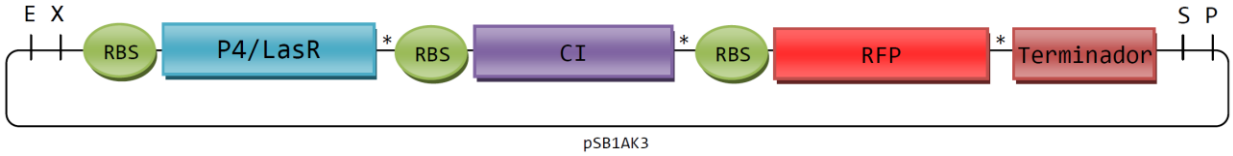
SUFIX+OMEGA CASSETTE (44 bp)
5' GTTTCTTCCTGCAGCGGCCGCTACTAGTA CCGGGGATCCGGTGA 3'

This arabinose-xylose AND team had to build the following constructs:



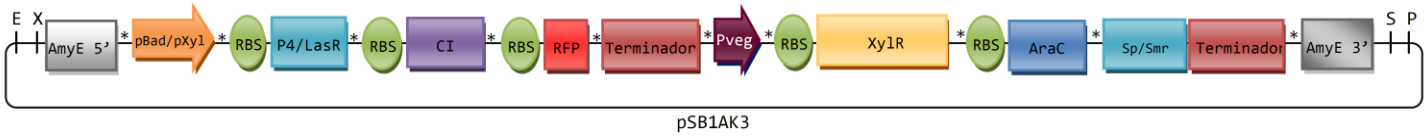


To then add the following part:



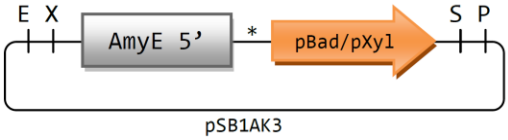
The other AND (heavy metals) team was in charge of building this part.

So we could obtain the final product:



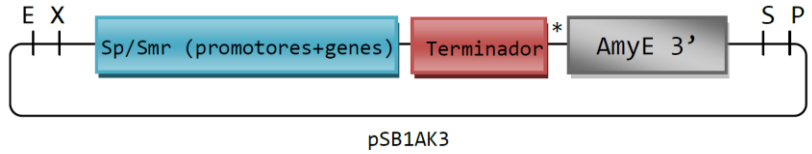
What we have up now is:

AmyE 5' – pBad/pXyl

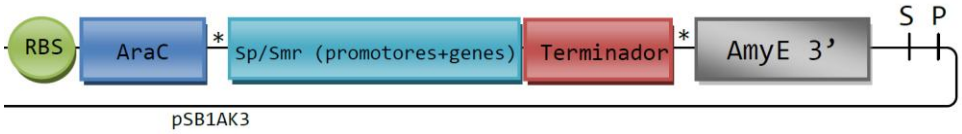


pVeg – RBS - XylR

Omega cassette – AmyE 3'

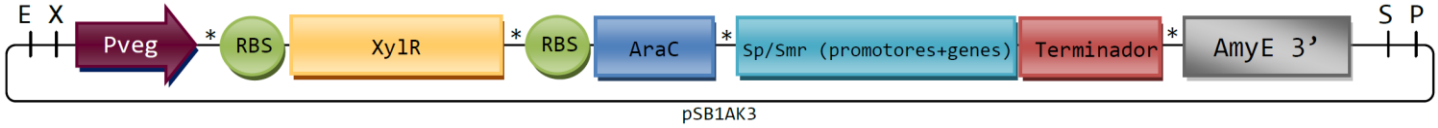


RBS – AraC – Omega cassette – AmyE 3'

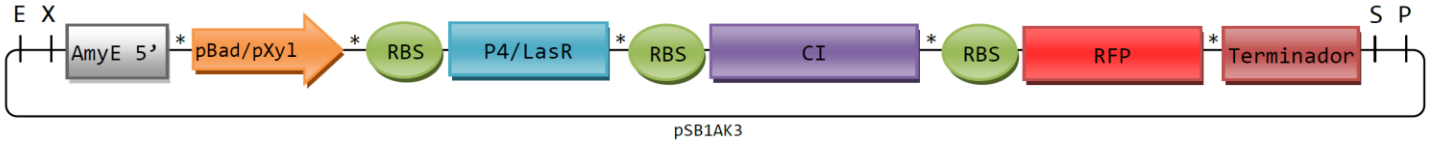


We are working hard to obtain these last two constructs in order to finish it:

pVeg – RBS – XylR – RBS – AraC – Omega cassette – AmyE 3'



AmyE 5' – pBad/pXyl – RBS – P4/LasR – RBS – CI – RBS – RFP – Double Terminador



References

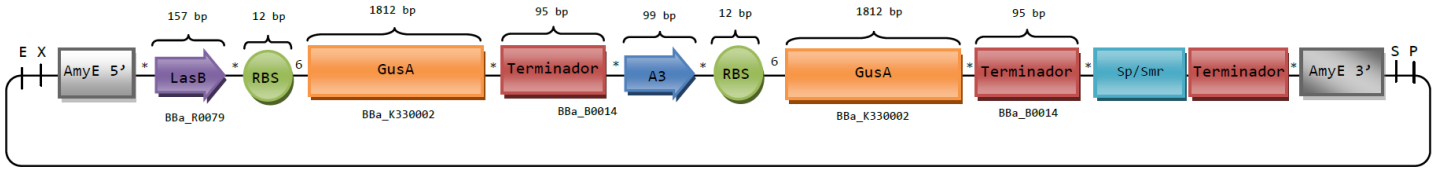
[1] Schlief, R. (2000). Regulation of the L-arabinose operon of Escherichia coli. Trends in Genetics. 16(12):559-565.

[2] Khlebnikov A, Datsenko KA, Skaug T, Wanner BL, and Keasling JD. (2001). Homogeneous expression of the PBAD promoter in Escherichia coli by constitutive expression of the low-affinity high-capacity AraE transporter. Microbiology. 147(12):3241-7.

A3- LasB (GUSA REPORTER) OR GATE

This construct was designed to function as an OR logic gate. Our system consists in two different promoters (A3 and LasB) expressing the same reporter gene (GusA). LasB (BBa_R0079) promoter (used by *NYMU-Taipei 2009, Northwestern 2011, Tokyo-tech iGEM 2011 and Tsinghua-A 2011*) requires both an activator protein, LasR, and an N-acylhomoserine lactone compound termed *Pseudomonas* autoinducer (PAI). A3 promoter is activated by P4, a regulatory protein from phage ϕ29 from *Bacillus subtilis*. In this way, if our target cell receive either LasR, P4 or both proteins, it will express GusA protein and we will have an OR gate.

To obtain our final construct we required the following Biological Parts:



Obtained from the Registry:

- BBa_K143001 (AmyE 5')
- BBa_K143002 (AmyE 3')
- BBa_R0079 (LasB)
- BBa_B0014 (Double Terminator)

Obtained from Margarita Salas Ph.D.'s Group:

- A3 from phage phi29 from plasmid pFRC54.

Omega cassette from plasmid pHP45Ω.

GusA reporter gene

We designed the following primers to add the RBS site to GusA:

GUSA
UPPER 5'-3'
PREFIX+RBS+SPACER+GUSA
GTTTCTTCGAATTCGCGGCCGCTTCTAGAG AAAGGTGGTGAA TACTAG atgttacgtcctgta
LOWER 5'-3'
SUFIX+GUSA
GTTTCTTCCTGCAGCGGCCGCTACTAGTATTATTA tcattgtttgcctcc

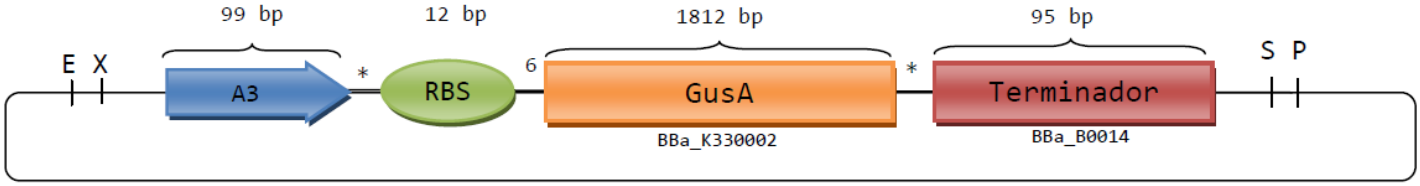
We also designed the following primers to obtain A3 from phage phi29 from plasmid pFRC54 and Omega cassette from plasmid pHP45Ω:

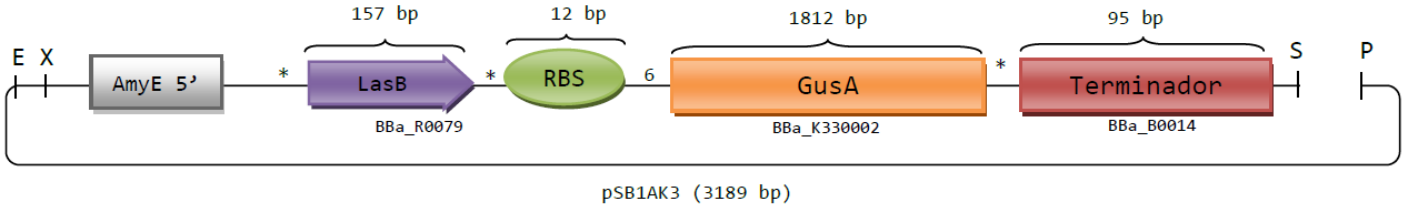
A3
UPPER 5'-3'
PREFIX+A3
5' GTTTCTTCGAATTCGCGGCCGCTTCTAGAG taacttttgcaaga 3'
LOWER 5'-3'
SUFIX+A3
5'GTTTCTTCCTGCAGCGGCCGCTACTAGTA ctacttaattatacc 3'

OMEGA CASSETTE
PREFIX+OMEGA CASSETTE(45 bp)
5' GTTTCTTCGAATTCGCGGCCGCTTCTAGAG CCGGGGATCCGGTGA 3'

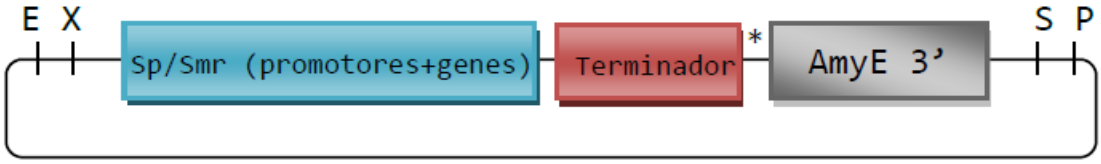
SUFIX+OMEGA CASSETTE (44 bp)
5' GTTTCTTCCTGCAGCGGCCGCTACTAGTA CCGGGGATCCGGTGA 3'

This A3-LasB OR team had to build the following constructs:



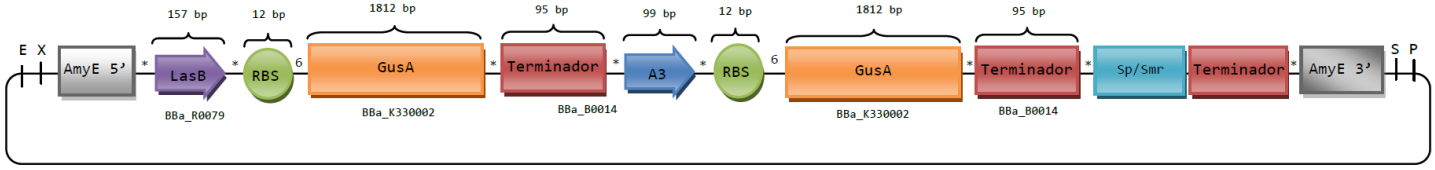


pSB1AK3 (3189 bp)



pSB1AK3 (3189 bp)

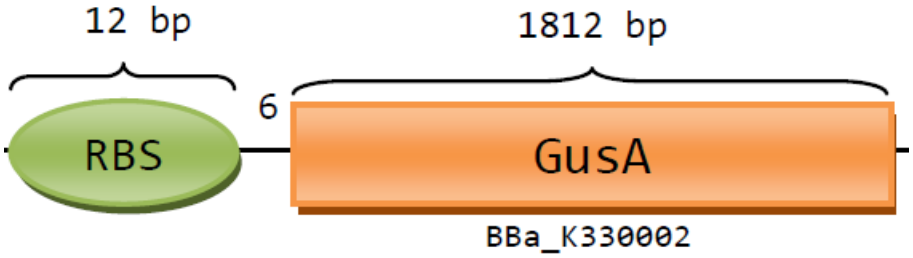
So we could obtain the final product:



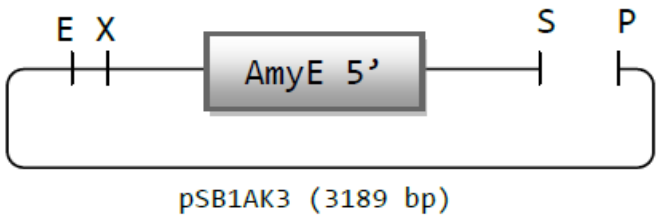
pSB1AK3 (3189 bp)

What we have up now is:

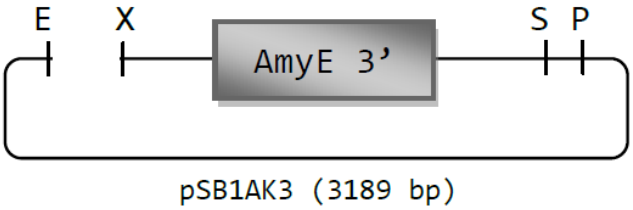
RBS - GusA



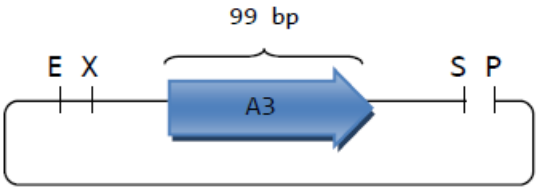
AmyE 5’ digested with S, P



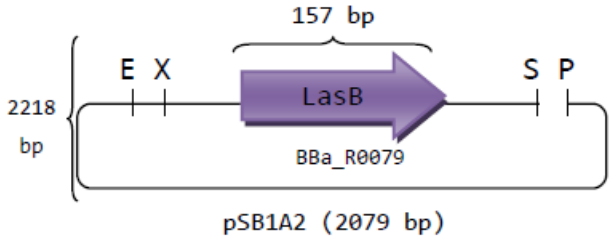
AmyE 3’ digested with E, X



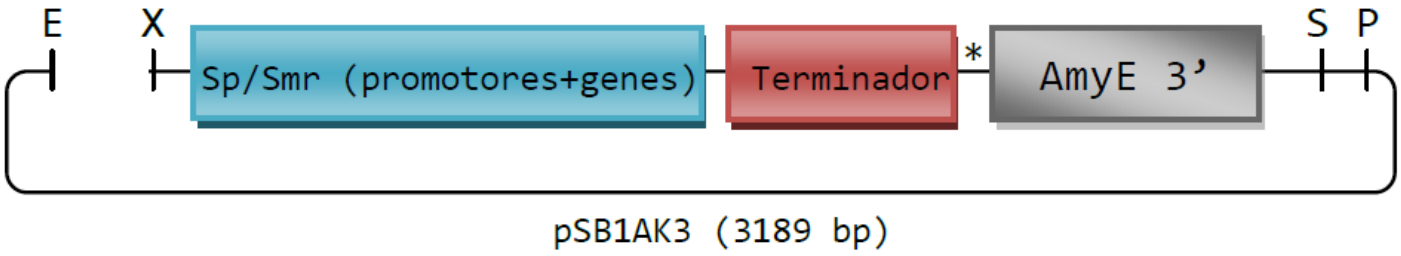
A3 promoter digested with S, P



LasB promoter digested with S, P



Omega cassette – AmyE 3’



We are working hard to obtain these last two constructs in order to finish the whole costruction.