Control of S-Linalool Production with Two Inducers

Group 4

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Construction of synthetic *Escherichia coli* producing s-linalool

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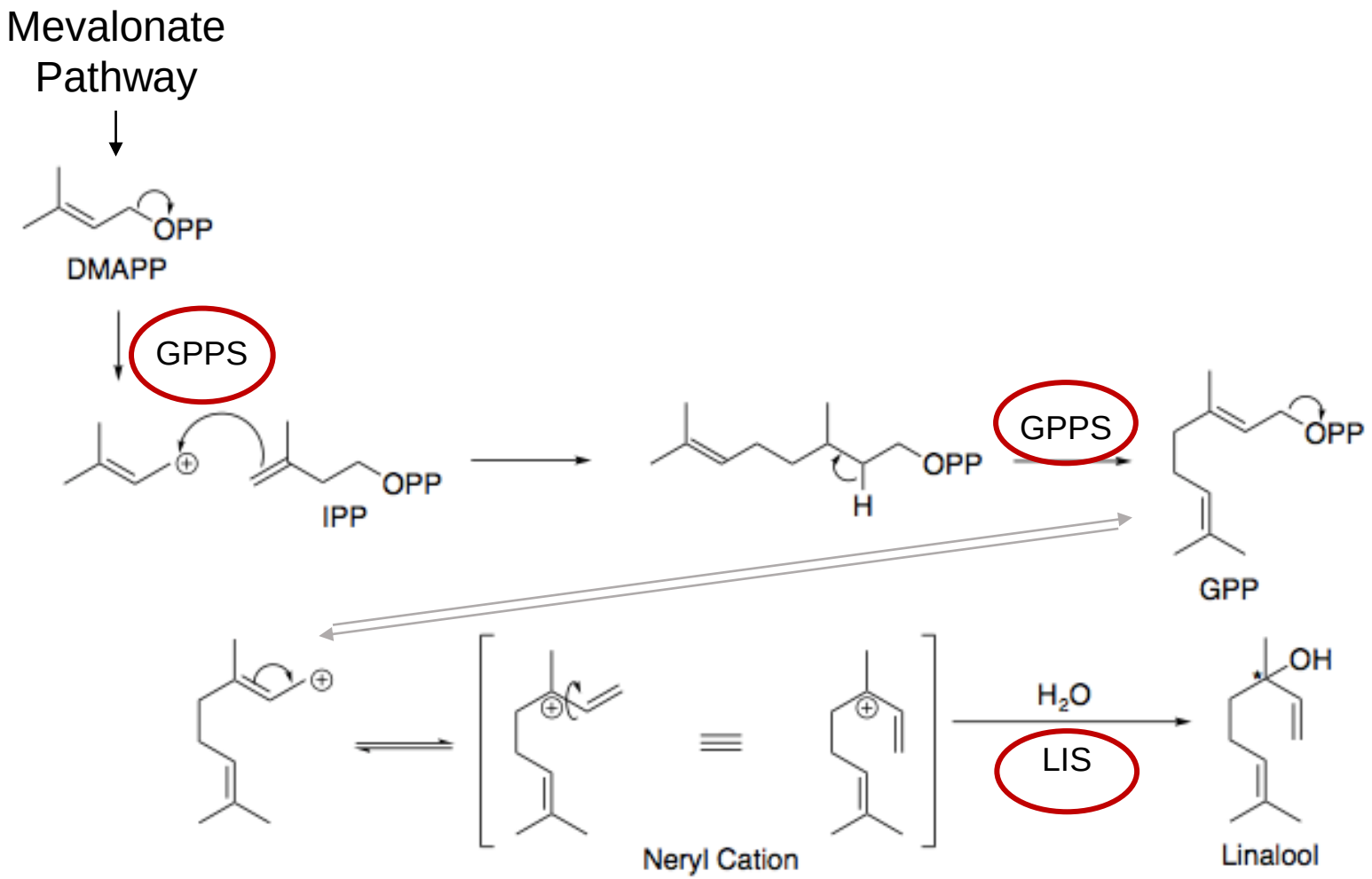
- https://core.ac.uk/download/pdf/82201273.pdf

Introduction

- A fragrance compound found in essential oils, including petitgrain, coriander and lavender.
- Used in aromatherapy together with massage for relaxing and wakefulness as well as for improving scent.
- Artificial scents made using chemical methods are high-cost and the production yield of linalool from plants is low.
- Microbial production systems offer the possibility for production of target compounds in a clean and simple metabolic environment that minimizes the risk of formation of unwanted side products.

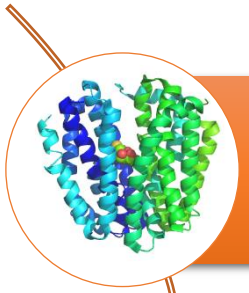
Linalool Biosynthesis

- Geranyl pyrophosphate synthase (GPPS)
- S-linalool synthase (LIS)



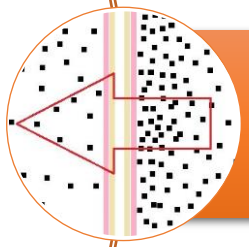
Adapted from https://commons.wikimedia.org/wiki/File:Linalool_Biosynthesis.png (CC BY-SA 4.0)

Inducers



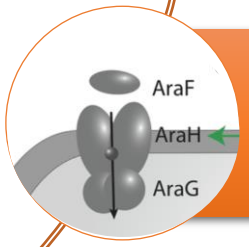
IPTG

- Lactose permease - *LacY*



aTC

- Passive diffusion

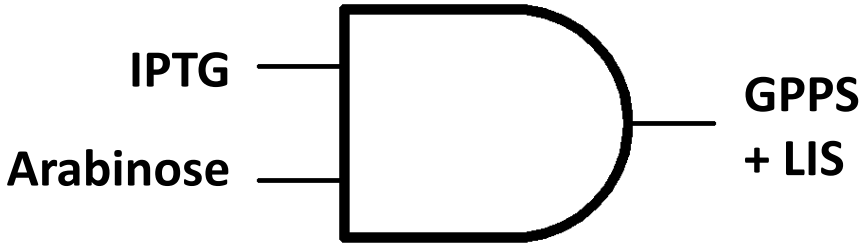


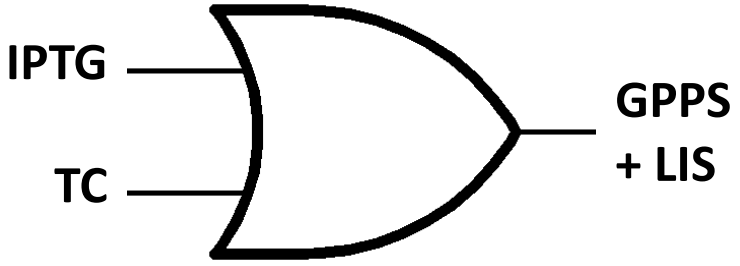
Arabinose

- Arabinose transporters – *AraA-F*

Sensors

- Expressing two enzymes for the synthesis of s-linalool, which is used for floral scent.
- Two methods for control with two inducers:
  - AND gate and OR gate

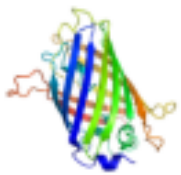




Promoter, controlling expression of a gene

Repressible promoter, active if repressor absent or inactive, binding sites for different repressors can be present

Gene, can encode signal protein or repressor



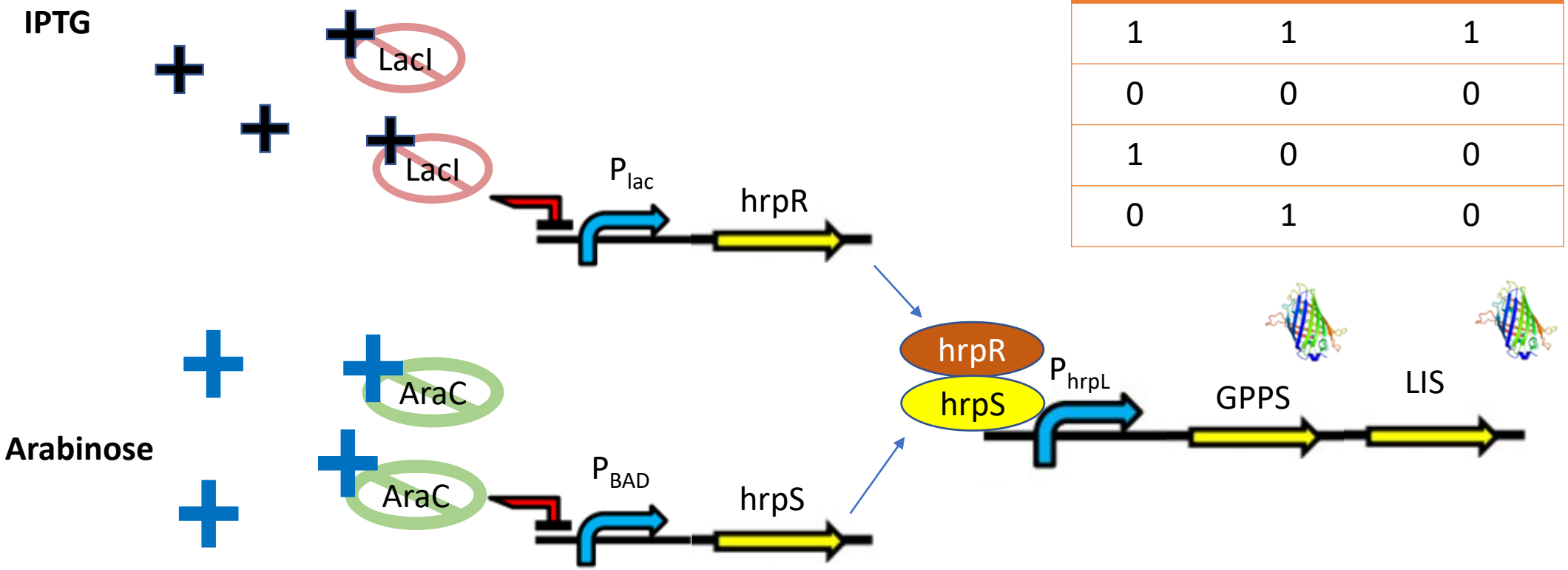
Protein, output signal

Repressor, is a protein that a has binding side within promoter region

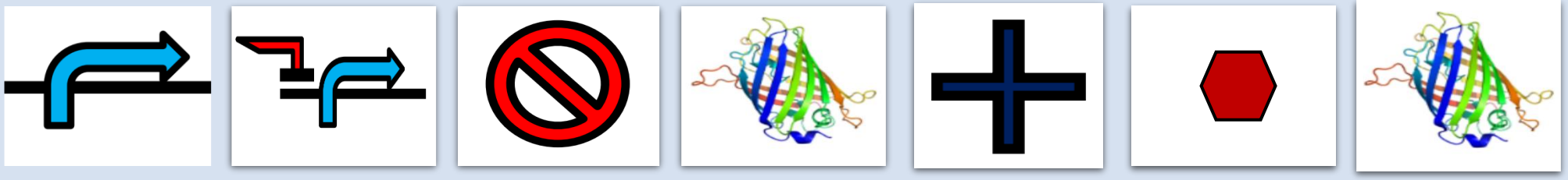
Chemical inducer, inactivating repressor

AND Gate Circuit

IPTG	Arabinose	S-Linalool
1	1	1
0	0	0
1	0	0
0	1	0



AND Gate Parts



Promoters

- HrpR & HrpS regulated promoter P_{hrpL} - BBa_K2967011

Repressible Promoters

- lacI regulated promoter P_{lac} - BBa_R0010
- AraC regulated promoter P_{BAD} - BBa_K113009

Repressors

- LacI repressor - BBa_C0012
- AraC repressor - BBa_K2442103

Signal proteins

- HrpR - BBa_K2967008
- HrpS - BBa_K2967009

Inducers

- IPTG
- Arabinose

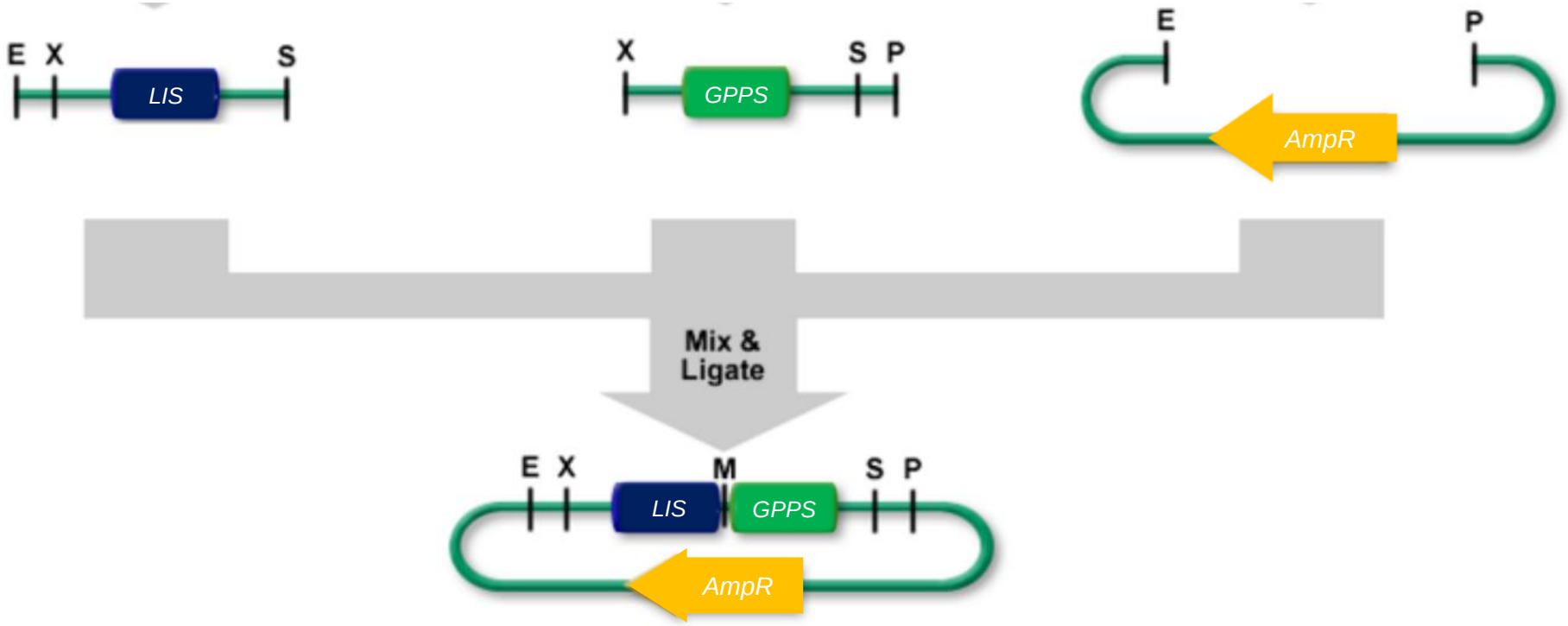
Other parts

- RBS.1 (Strong) - BBa_B0030
- Double terminator - BBa_B0015

Output

- GPPS
- LIS

Example of Assembly Standard RFC[10]



EcoRI

5′...G▼AATTC...3′
3′...CTTAA▲G...5′

SpeI

5′...A▼CTAGT...3′
3′...TGATC▲A...5′

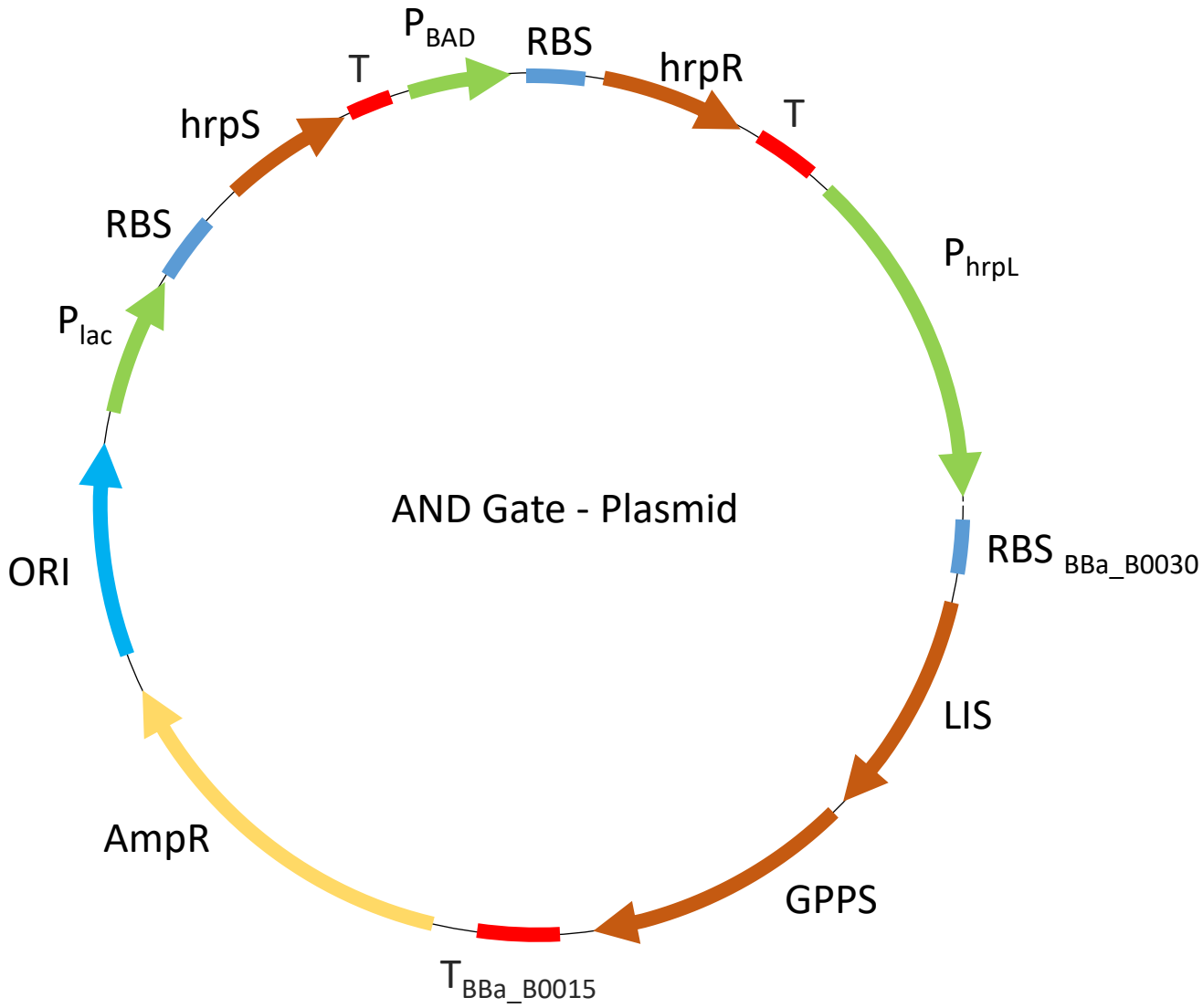
XbaI

5′...T▼CTAGA...3′
3′...AGATC▲T...5′

PstI

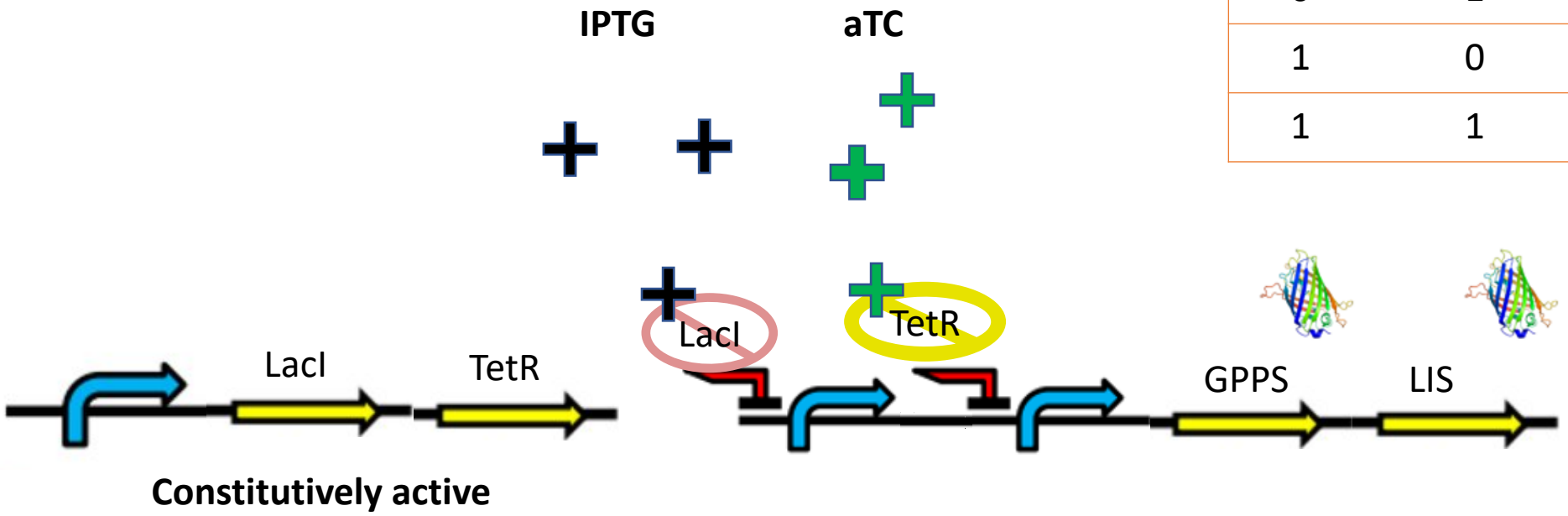
5′...CTGCA▼G...3′
3′...G▲ACGTC...5′

AND plasmid

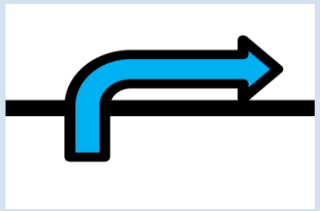

P BAD
RBS
hrpR
T
P hrpL
RBS BBa_B0030
LIS
GPPS
T BBa_B0015
AmpR
ORI
P lac
RBS
hrpS
T
AND Gate - Plasmid

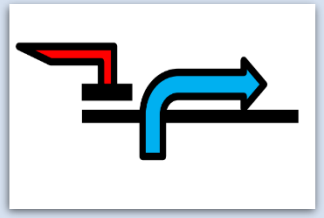
OR Gate Circuit

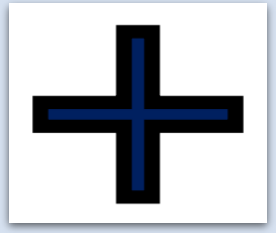
IPTG	TC	S-Linalool
0	0	0
0	1	1
1	0	1
1	1	1

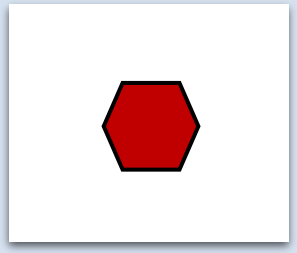


OR Gate Parts









Promoters

- Constitutive promoter (e.g BBa_J54200)

Repressible Promoters

- lacI regulated promoter - BBa_R0010
- TetR regulated promoter - BBa_R0040

Repressors

- LacI repressor - BBa_C0012
- Tetracycline repressor - BBa_C0040

Inducers

- IPTG
- Tetracycline

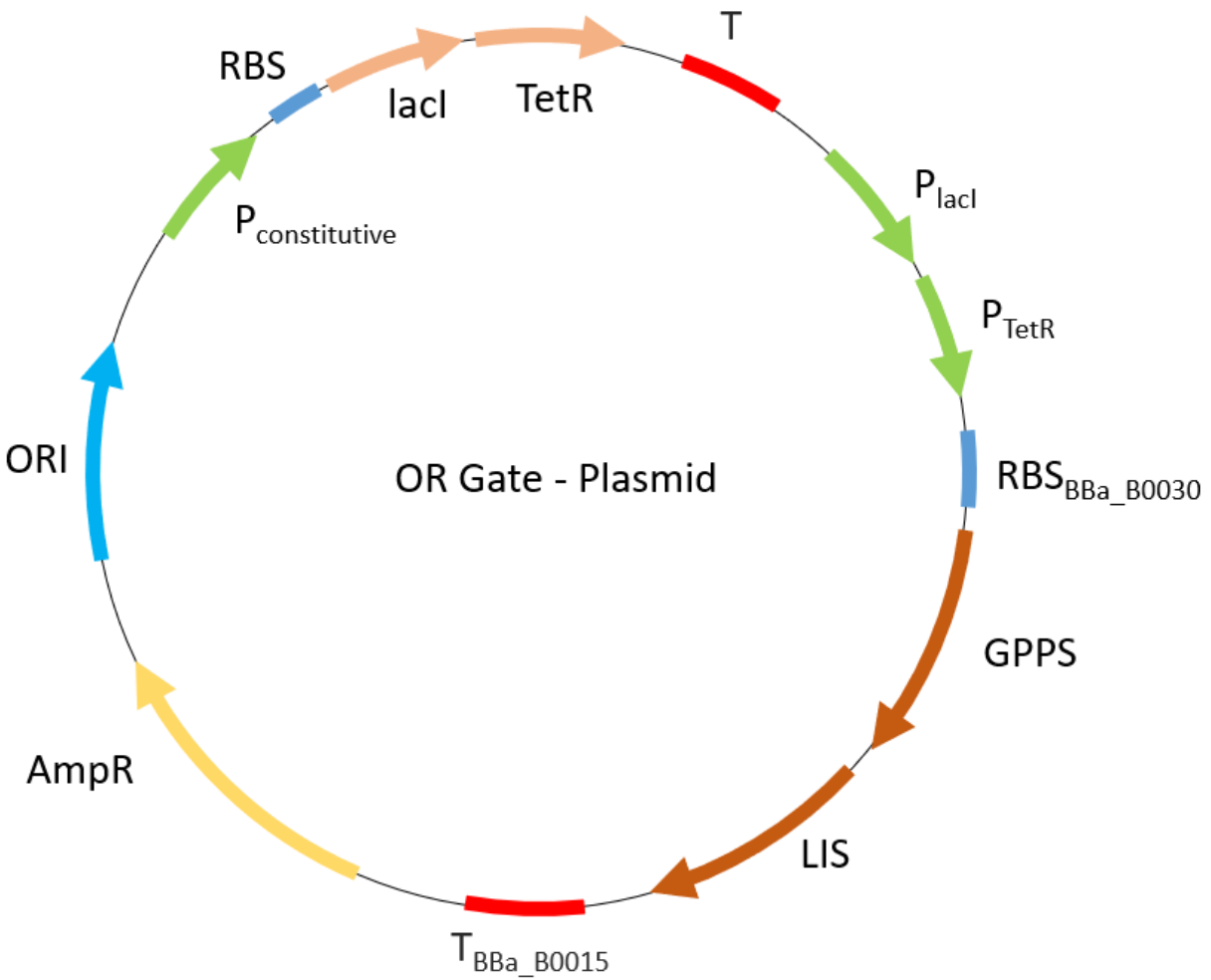
Other parts

- RBS.1 (Strong) - BBa_B0030
- Double terminator - BBa_B0015

Output

- GPPS
- LIS

OR plasmid


RBS
lacI
TetR
T
P lacI
P TetR
RBS BBa_B0030
GPPS
LIS
T BBa_B0015
AmpR
ORI
P constitutive
OR Gate - Plasmid

Thank you for listening!
Any questions?

References

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