



GENE CIRCUITS DESIGN

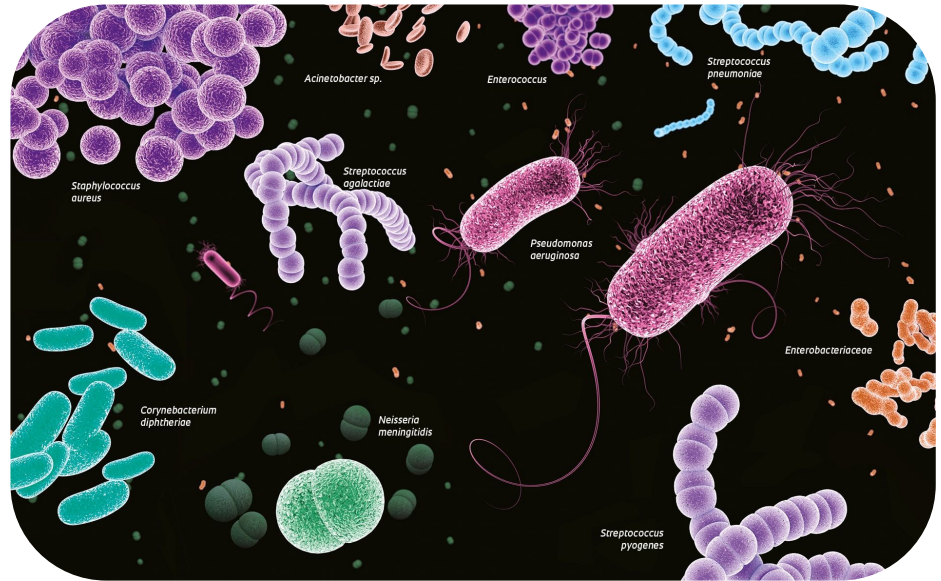
iGEM FCB-UANL

PRIMEIROS PASOS

O QUE QUERO FAZER?

Qual bacteria vou escolher?

- Condições de uso
(ecologia, saúde, etc)
- Antecedentes
- Propósito final

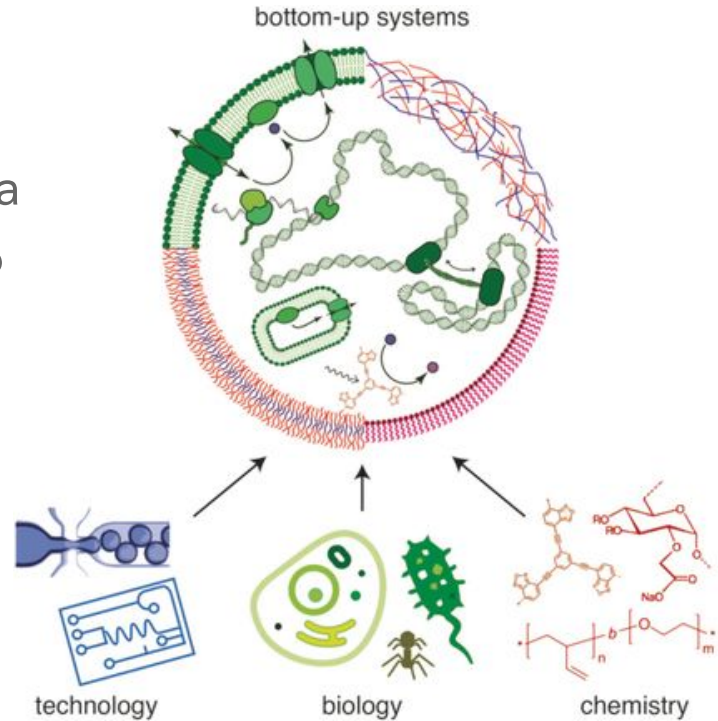


PRIMEIROS PASOS

O QUE QUERO FAZER?

É importante conhecer o problema para poder definir as generalidades do novo circuito

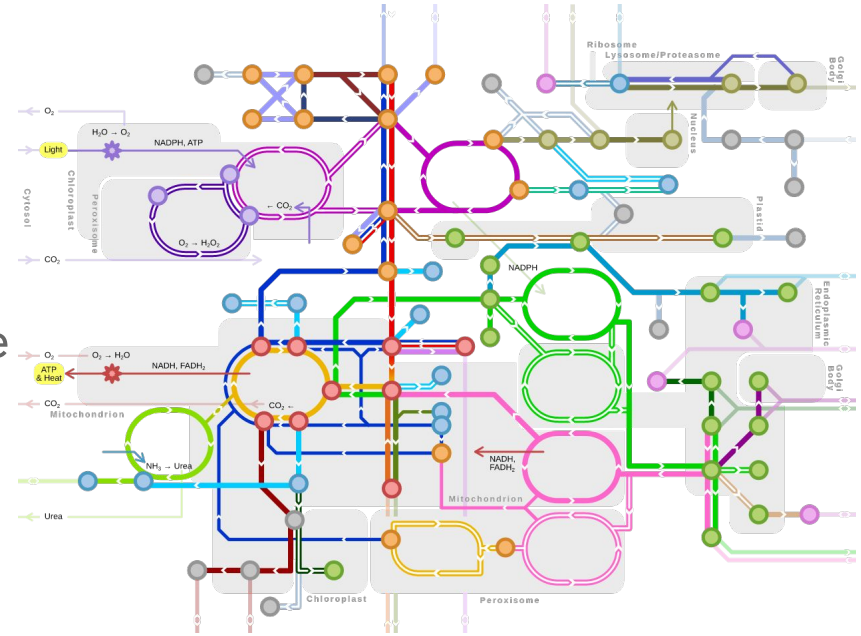
- Sensibilidade
- Tipo de resposta
- Tasa de expressao
- Eficiência
- Leaky expression



PRIMEIROS PASOS

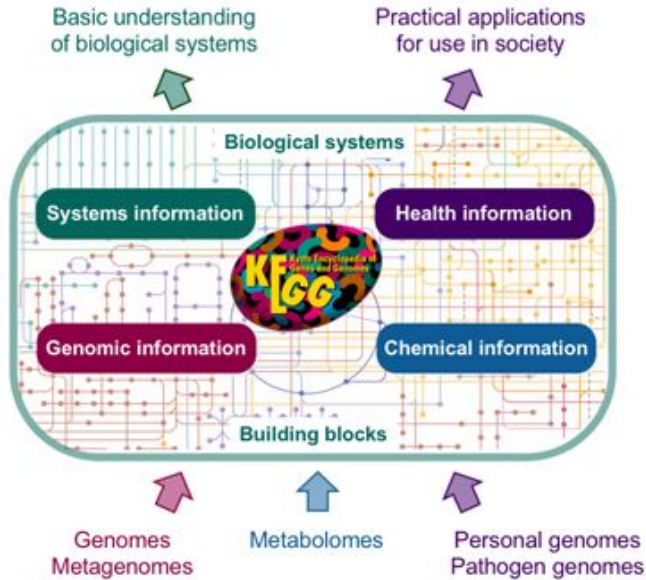
CONHECENDO O SISTEMA

- Expressão recombinante: quais são os antecedentes?
- Metabolismo natural: o que poderia acontecer se eu editasse a rede metabólica?



PRIMEIROS PASOS


CONHECENDO O SISTEMA



The screenshot shows the SubtiWiki website interface. At the top, the "SubtiWiki" logo is displayed in blue. Below the logo is a search bar with the text "Genes or proteins" and a dropdown arrow. To the right of the search bar are "Go" and "Search" buttons. Below the search bar, there is a section titled "Subti-Apps" with five buttons: "Pathways", "Expression", "Interactions", "Regulations", and "Genome". Below this is a section titled "Quick links" with three buttons: "Labs", "Plasmids", and "Methods". At the bottom, there is a "Citation" section with the following text: "Zhu B, Stülke J. SubtiWiki in 2018: from genes and proteins to functional network annotation of the model organism *Bacillus subtilis*. Nucleic acids research. 2017 Oct 09; 46(D1):D743-748. doi:10.1093/nar/gkx908."

PRIMEIROS PASOS

CONHECENDO O SISTEMA

**BioCyc**
Database Collection

LOGIN | Why Login? | Create New Account

Q Enter a gene, protein, metabolite or pathway... Quick Search Gene Search

Searching *Bacillus subtilis* SRM103576 change organism database

Sites Search Genome Metabolism Analysis SmartTables Help

gene
spo0A
Bacillus subtilis SRM103576

polypeptide
sporulation transcription factor Spo0A

Add to SmartTable

Provide Feedback

Twitter Facebook

Accession IDs	ES969_RS12710	Length	804 bp / 267 aa
	ES969_12710	Map Position	[2,342,100 <- 2,342,903] (58.37 centisomes, 210°)
		Location	cytosol

View in Genome Browser

SummaryGO Terms (1)Protein FeaturesOperonsReferencesShow All

Summary

Derived by automated computational analysis using gene prediction method: Protein Homology.

Molecular Weight of Polypeptide 29.691 kD (from nucleotide sequence)

Unification Links

NCBI-Protein	WP_003226427.1
UniProt-via-RefSeq	WP_003226427.1

Relationship Links

Pfam In-Family	PF00072, PF06769
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OPERATIONS
Sequences

- Get Protein Sequence
- Get Nucleotide Sequence
- BLAST the Nucleotide Sequence
- BLAST the Protein Sequence
- Save Nucleotide Sequence to File
- Save Protein Sequence to File


Comparison Operations

- Change Organisms/Databases for Comparison Operations
- Show This Gene in Another Database
- Search for This Gene in Multiple Databases
- Show Orthologs (with Operon Diagrams) in Multiple Databases
- Align in Multi-Genome Browser
- Align Gene Nucleotide Sequence with Orthologs
- Align Gene Product Amino Acid Sequence with Orthologs

PRIMEIROS PASOS

CONHECENDO O SISTEMA

HOME
Classic view


The Comprehensive Enzyme Information System

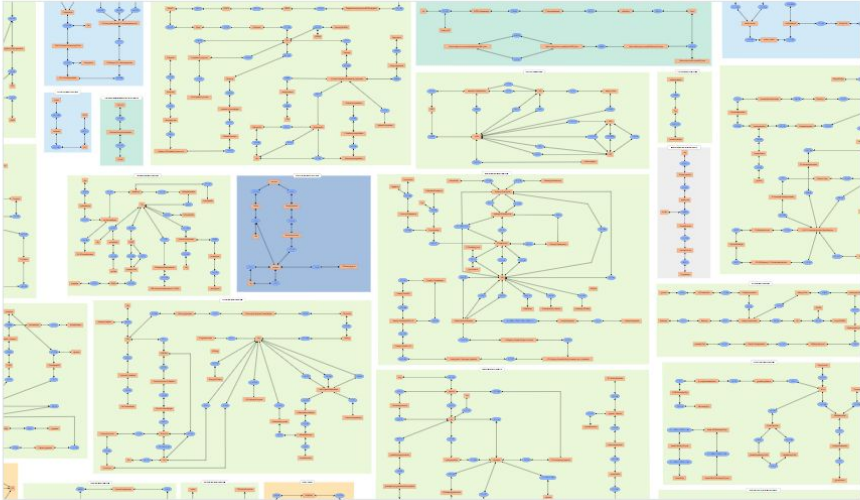
Contact
login history all enzymes

> Search & Highlight

> Plot Your Dataset

> Download map as svg

Help Center view



Pathway Categories

- Central and energy metabolism
- Lipid metabolism
- Amino acid metabolism
- Nucleotide and cofactor metabolism
- Carbohydrate metabolism
- Fermentation and other catabolism
- Xenobiotics and secondary metabolism
- Other pathways

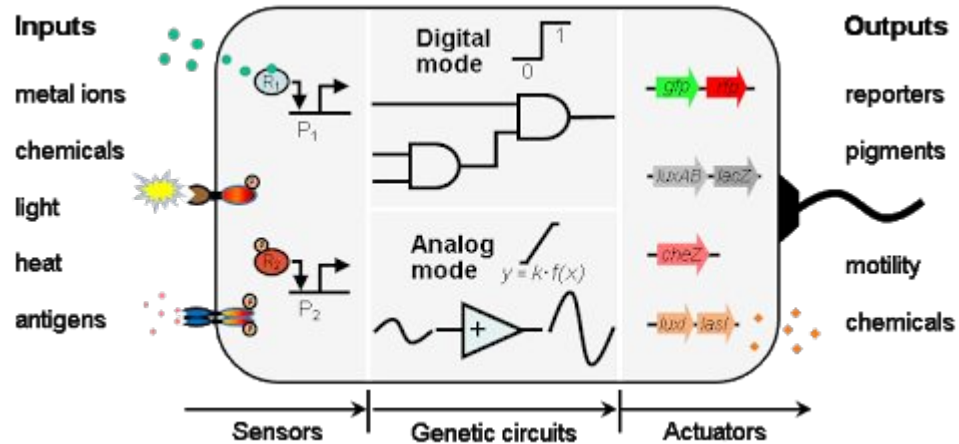
Show interpathway links

Show or hide cofactors on the map:

Toggle on zoom

PRIMEIROS PASOS

FERRAMENTAS

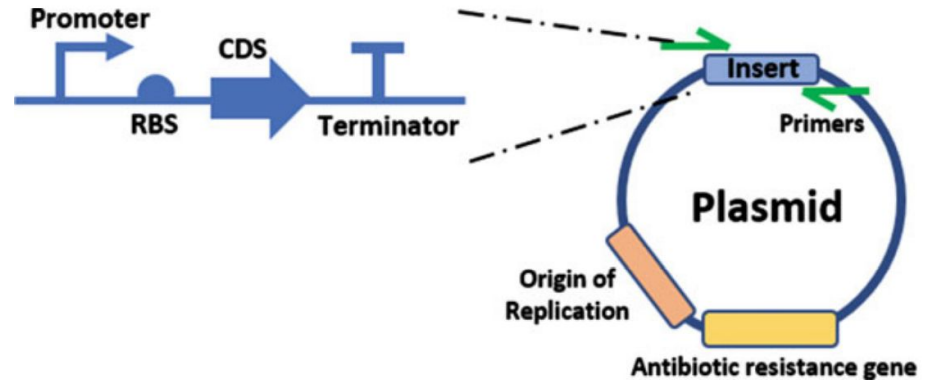


- Vias metabólicas sintéticas
- Novos circuitos
- Modelos *in silico*
- Engenharia de proteínas

PARTES DO CIRCUITO

CIRCUITO GENÉTICO BÁSICO

El componente básico de un **circuito genético** (izquierda) y cuando se inserta en un **plásmido** (derecha) (Yeoh et al., 2019).

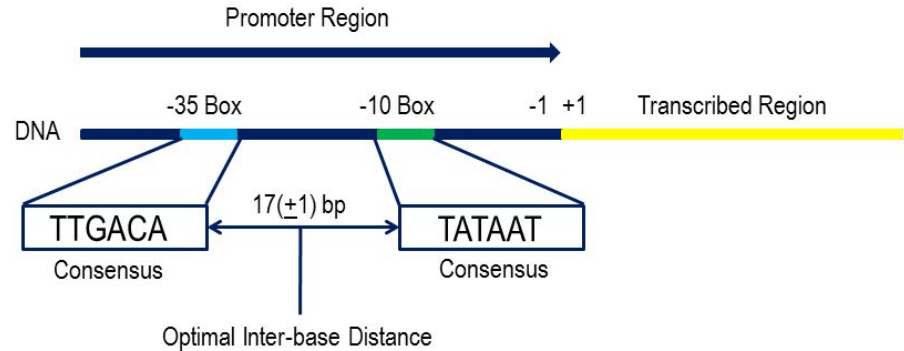


PARTES DO CIRCUITO

PROMOTORES

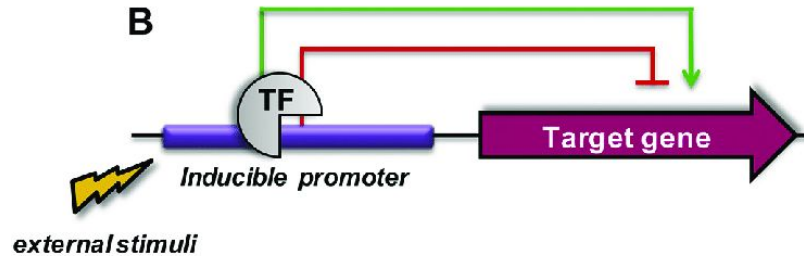
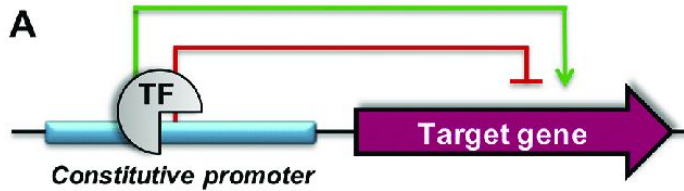
Controlan la transcripción de la **proteína** de interés y la convierten en ARN, la **unión de la ARN polimerasa** y los **factores de transcripción** y, en última instancia, afecta a la **expresión del gen** (Yeoh et al., 2019).

1. Constitutivos
2. Inducibles
3. Reprimibles



PARTES DO CIRCUITO

PROMOTORES



(PDF) Engineered microbial host selection for value-added bioproducts from lignocellulose," 2019)

PARTES DO CIRCUITO

PROMOTORES - BASES DE DATOS

Encontrados en artículos científicos, otras bases de datos o wikis de equipos iGEM:

parts.igem.org › Promoters › Catalog ▼ [Traducir esta página](#)

[Promoters/Catalog - parts.igem.org](https://parts.igem.org)

These **promoters** were developed by the 2007 USTC **iGEM** team. Browse by regulation and RNA polymerase. Most **promoters** are designed for a particular RNA ...

molbiol-tools.ca › Promoters ▼ [Traducir esta página](#)

[Promoters - Online Analysis Tools](https://molbiol-tools.ca)

PromoterHunter - is part of phiSITE **database** which is a collection of phage gene regulatory elements, genes, genomes and other related information, plus tools. (...

epd.epfl.ch ▼ [Traducir esta página](#)

[EPD The Eukaryotic Promoter Database](https://epd.epfl.ch)

EPD is a collection of eukaryotic **promoters** derived from published articles. Instead, the EPDnew **databases** (HT-**EPD**) are the result of merging **EPD promoters** ...

[Access EPDnew](#) · [H. sapiens](#) · [Access EPD](#) · [M. musculus](#)

- Constitutive
- Cell Signalling
- Metal sensitive
- Phage
- Madras
- USTC

PARTES DO CIRCUITO

PROMOTOR - CONSIDERACIONES

Es importante tener varias cosas en **consideración** a la hora de buscar un promotor, tales como:

- La **caracterización** (¿Qué tan caracterizado está?)
- El **plásmido** en el que se está usando
- El **organismo** en el que se usa (promotores de *E. coli* pueden funcionar diferente en *B. subtilis*)
- **Fuga** del promotor ("Que tan preciso")



PARTES DO CIRCUITO

RBS - RIBOSOME BINDING SITE

Reclutamiento del ribosoma para iniciar la traducción. Entre más fuerte, mayor tasa de traducción (Yeoh et al., 2019).

La colección de bibliotecas **Anderson** ofrecen RBS de distintas intensidades.

RBS Calculator: **predice la fuerza** con el objetivo de **diseñarlos con una fuerza específica**.

[parts.igem.org](#) > Anderson ▾ [Traducir esta página](#)

[Ribosome Binding Sites/Prokaryotic/Constitutive/Anderson ...](#)

Description. The **Anderson RBS** family are suitable for general protein expression in E. coli or other prokaryotes. The family is known to cover a range of ...

[salislab.net](#) > software ▾ [Traducir esta página](#)

[De Novo DNA: The Future of Genetic Systems ... - Salis Lab](#)

RBS Calculator. Predict Translation Rates · **RBS Calculator**. Control Expression Levels · RBS Library Calculator ... Synth Success Calculator. Optimize DNA ...

[RBS Calculator Predict...](#) · [RBS Calculator](#) · [RBS Library Calculator](#)

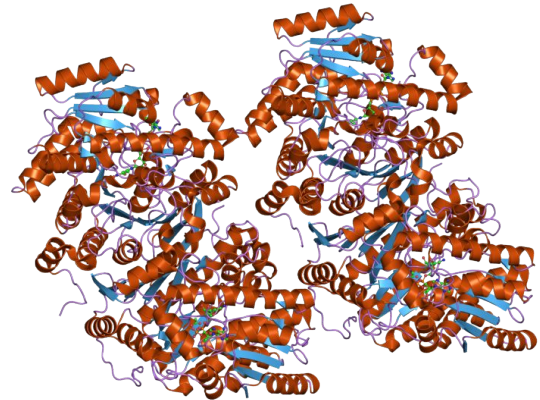
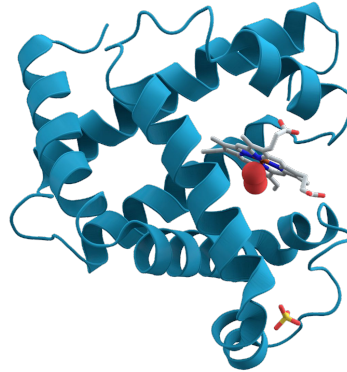
PARTES DO CIRCUITO

CDS - CODING DOMAIN SEQUENCE



Es la **proteína de interés** que se expresará en el **circuito** genético diseñado con funciones como:

1. Digestivas
2. Transporte
3. Estructurales
4. Hormonas
5. Defensa
6. Contracción
7. Almacenamiento
8. ¡Y muchísimas más!

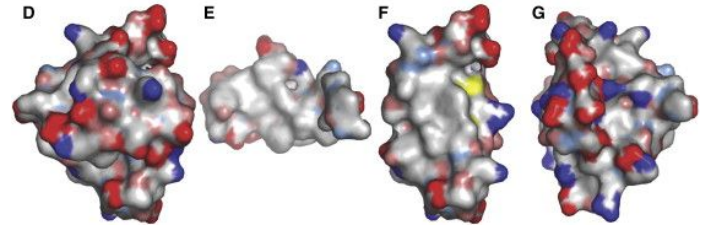


PARTES DO CIRCUITO

CDS - PRECAUCIONES



- Debe comenzar **siempre con un codón de inicio** y **terminar con un codón de parada**.
- Asegúrese siempre de que **no haya un codón de parada prematuro** en en medio de la CDS.
- **Si** el CDS **se deriva** de otras especies o huéspedes, la **optimización de los codones debe realizarse** con las herramientas adecuadas para equilibrar el uso de los codones y mejorar la expresión de la proteína.



PARTES DO CIRCUITO

CDS - CODING DOMAIN SEQUENCE

Existen muchas **bases de datos, artículos y reportes** de los cuales se pueden obtener **secuencias de CDS** de proteínas, entre ellos están:

National Center for Biotechnology Information

Welcome to **NCBI**. The **National Center for Biotechnology Information** advances science and health by providing access to biomedical and genomic information.

UniProt

The mission of **UniProt** is to provide the scientific community with a comprehensive, high-quality and freely accessible resource of protein sequence and functional ...

Protein coding sequences - parts.igem.org

Protein coding sequences are DNA **sequences** that are transcribed into mRNA and in which the corresponding mRNA molecules are translated into a polypeptide ...

PARTES DO CIRCUITO

CDS - CODING DOMAIN SEQUENCE

Como se mencionó, **las funciones** de proteínas **son muy variadas** y pueden dar lugar a **todo tipo de proyectos y circuitos genéticos**.

- Reporteros
- Reguladores transcripcionales
- Marcadores de selección
- Enzimas
- Biosíntesis
- Modificación del ADN
- Proteasas
- Enzimas de modificación postraducciona
- Proteínas de membrana
- Receptores y ligandos
- Proteínas de lisis
- Y más...

PARTES DO CIRCUITO

TERMINADORES



Los terminadores son secuencias que **terminan la transcripción**, se recomienda **usar un doble terminador** para evitar que la RNA polimerasa continúe.

[Terminators/Catalog - parts.igem.org](https://parts.igem.org/Terminators/Catalog)

E. coli **terminators**. There are several E. coli transcriptional **terminators** available via the Registry. The most commonly used type of **terminator** is a forward ...

[WebGeSTer DB—a transcription terminator database](#)

por A Mitra · 2011 · Mencionado por 93 — The **database** comprises of a million **terminators** identified in 1060 bacterial genome sequences and 798 plasmids. Users can obtain both graphic and tabular ...

PARTES DO CIRCUITO

HERRAMIENTAS BIOINFORMÁTICAS

Existe una gran variedad de **aplicaciones y programas** que pueden permitirnos **diseñar circuitos**, entre ellos:



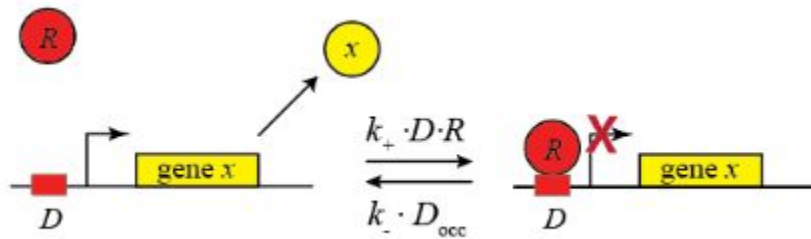
Benchling



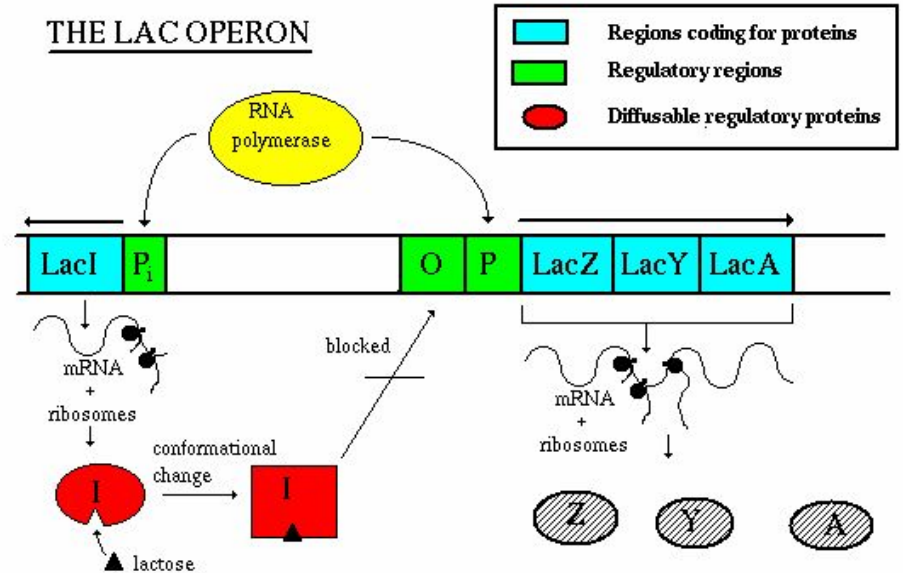
SnapGene®

REGULAÇÃO DE EXPRESSÃO

Generalidades

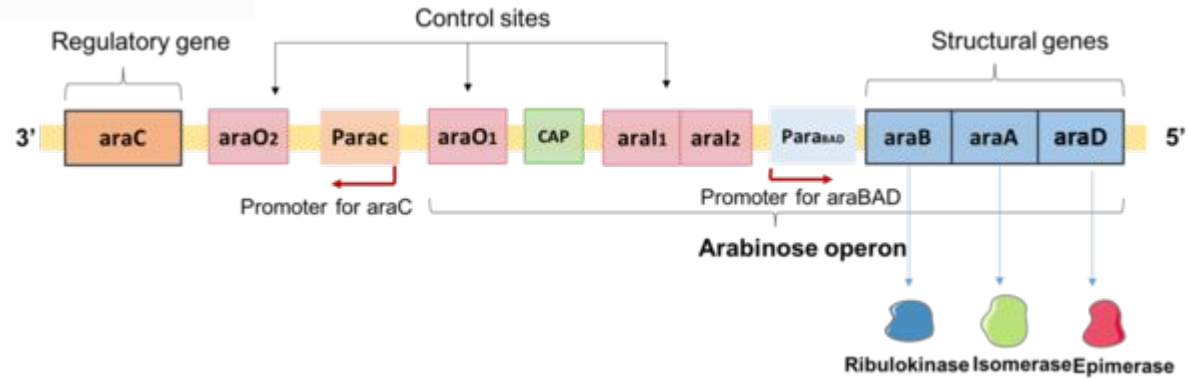
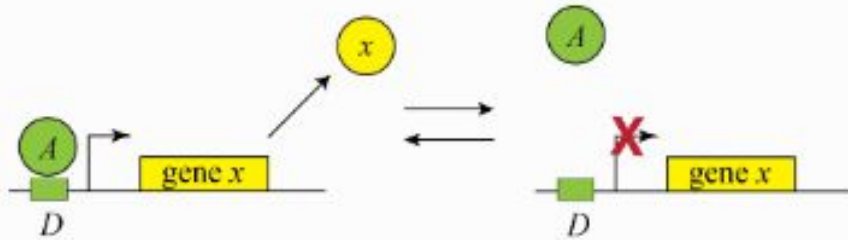


THE LAC OPERON



REGULAÇÃO DE EXPRESSÃO

Generalidades



REGULAÇÃO DE EXPRESSÃO

Exemplos

- Killswitch

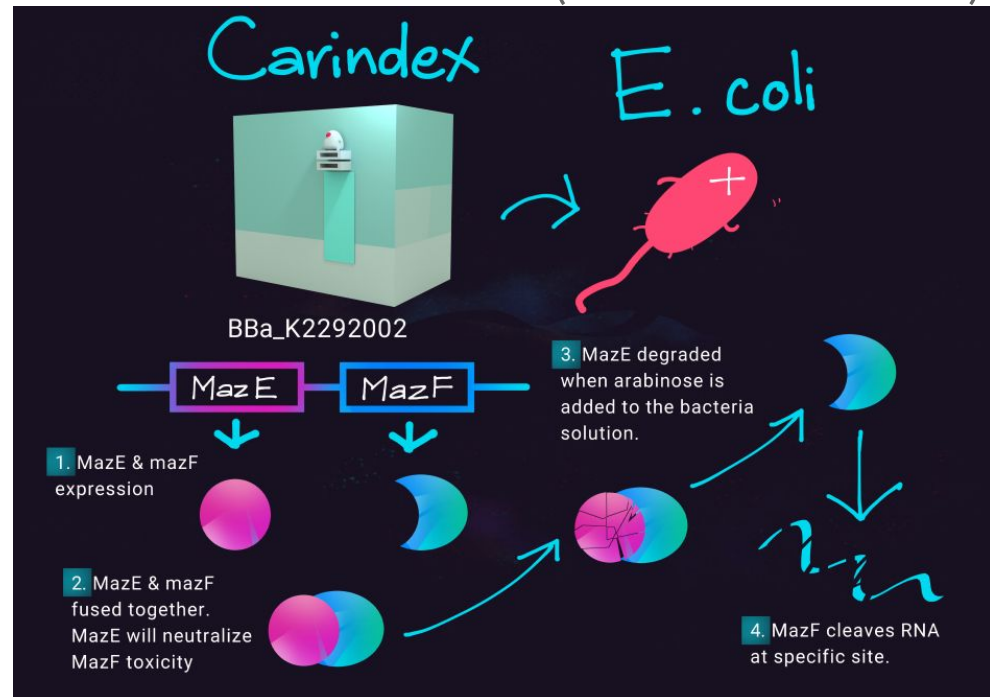


Kill switch

Here are parts that could trigger cell death through disruption at the level of the protein and the cell.

Name	Type	Description	Length
BBa_K1074011	Composite	PsdRf1+RBS+SDP	2207
BBa_K117000	Coding	Lysis gene (promotes lysis in colicin-producing bacteria strain)	144
BBa_K1172901	Coding	Alanine racemase from "E. coli"	1080
BBa_K1172902	Device	alanine racemase ("alr") under the control of the P _{lac} promoter	1134
BBa_K1172908	Device	Part 1 of the Biosafety-System Lac of Growth (pRha lacI air)	2509
BBa_K1172909	Device	Biosafety-System araCtive (AraC)	3281
BBa_K1172911	Device	Biosafety-System Lac of Growth (lacI)	3600
BBa_K1172912	Device	Part 1 of the Biosystem TetOR alive (pRha TetR air)	2042
BBa_K1184000	Coding	KillerRed	723
BBa_K1197007	Composite	IPTG dependent B subtilis Kill Switch construct	2006
BBa_K1223001	Project	P.A.S.E. 1 cassette	2148
BBa_K1223002	Project	P.A.S.E. 2 cassette	1210
BBa_K1405008	Composite	A Kill Switch with "memory" time repressed by IPTG	2926
BBa_K1616005	Coding	Holin/endolysin reversed - RBS and double T7 terminator	1379
BBa_K1616013	Coding	ccdB reversed - RBS and double T7 terminator	575
BBa_K1639022	Composite	pCons-TetR-pTet-TevProtease Suicide Switch	1546

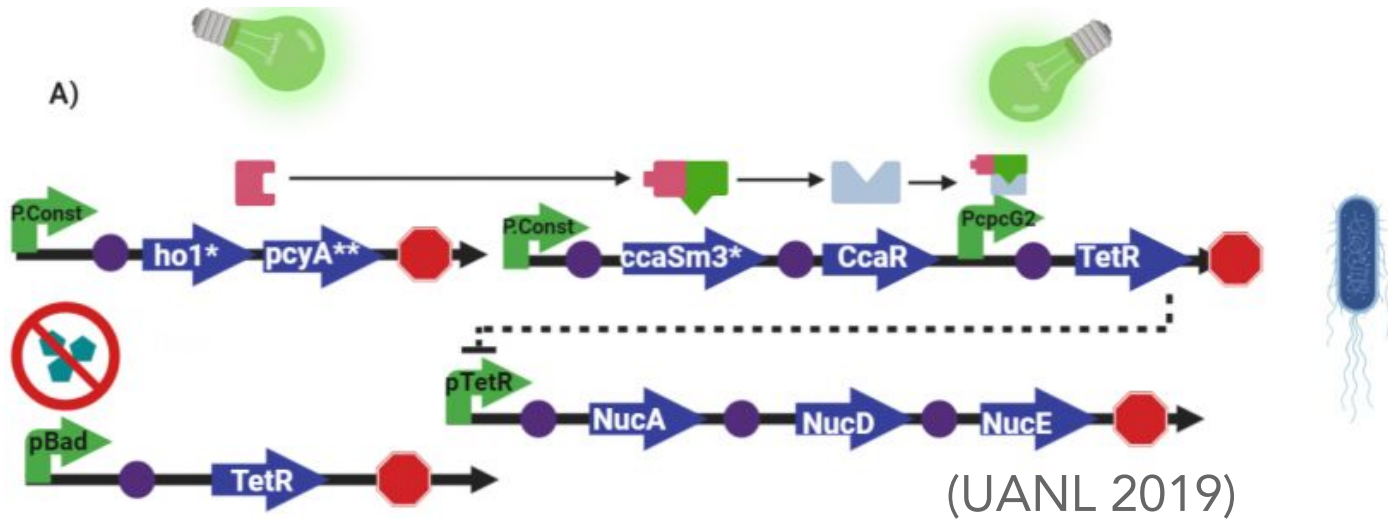
(CCU Taiwan 2017)



REGULAÇÃO DE EXPRESSÃO

Exemplos

- Killswitch con activación y represión



REGULAÇÃO DE EXPRESSÃO

Exemplos

- Biosensor

Biosensor

