GENE CIRCUITS DESIGN

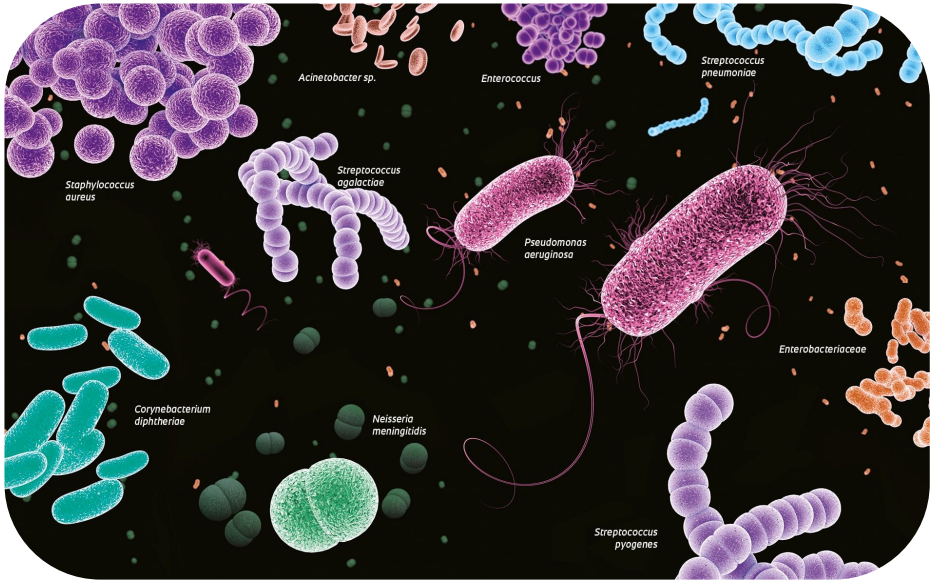
iGEM FCB-UANL

PRIMEIROS PASOS

O QUE QUERO FAZER?

Qual bacteria vou escolher?

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

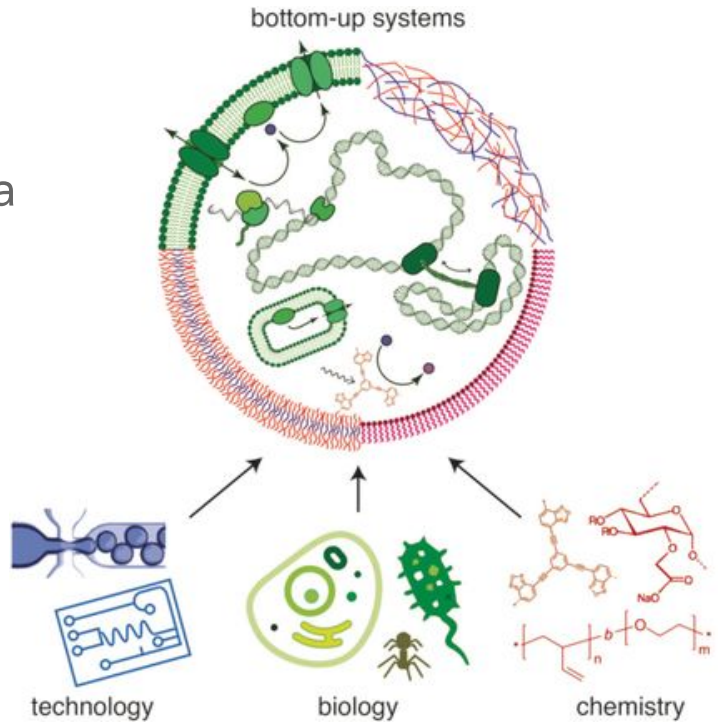


PRIMEIROS PASOS

O QUE QUERO FAZER?

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

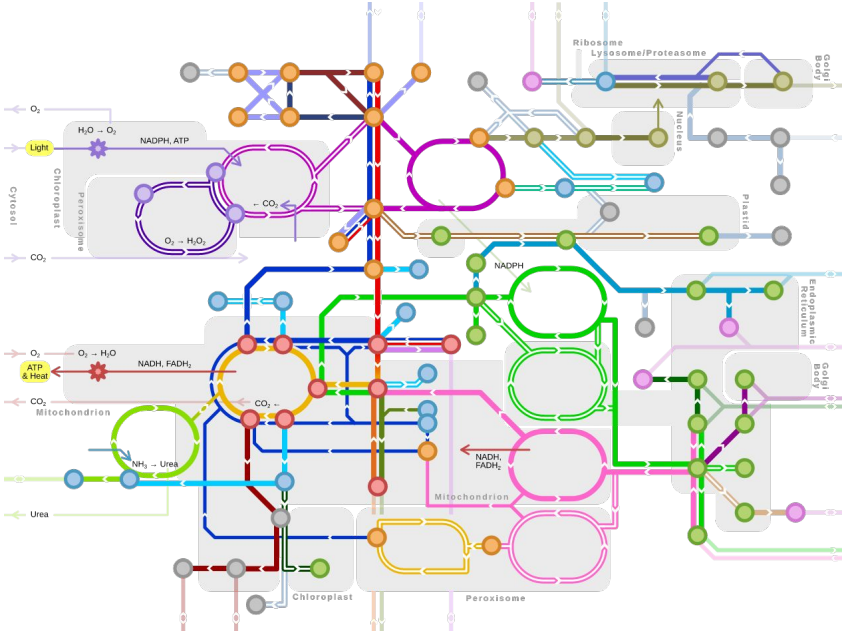
- Sensibilidade
- Tipo de resposta
- Tasa de expressao
- Eficiência
- Leaky expression



PRIMEIROS PASOS

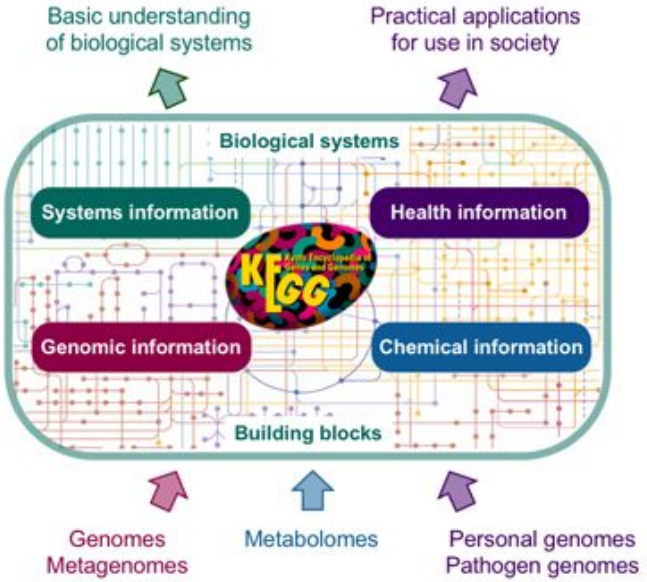
CONHECENDO O SISTEMA

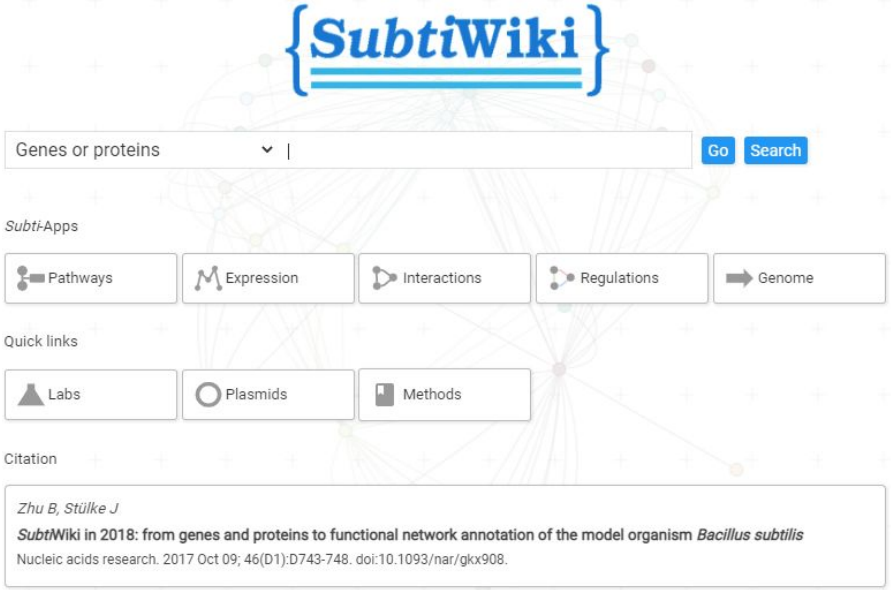
- Expressão recombinante: quais são os antecedentes?
- Metabolismo natural: o que poderia acontecer se eu editasse a rede metabólica?



PRIMEIROS PASOS

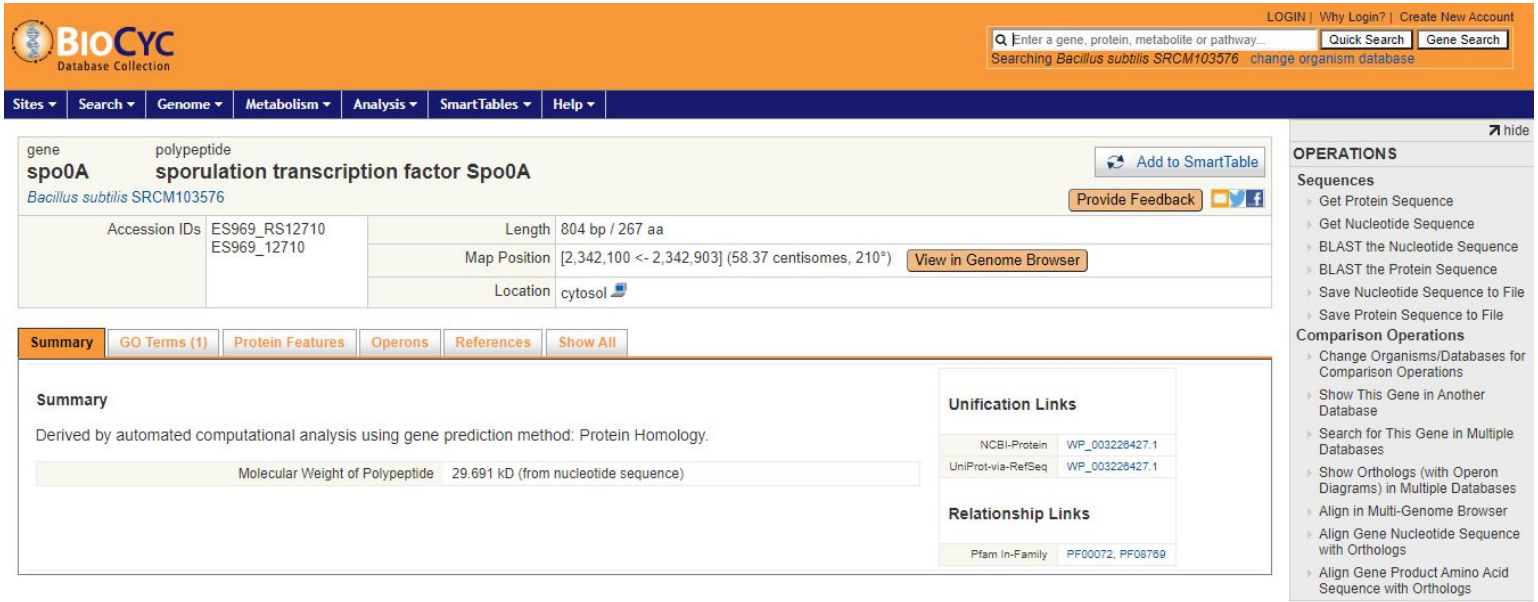
CONHECENDO O SISTEMA





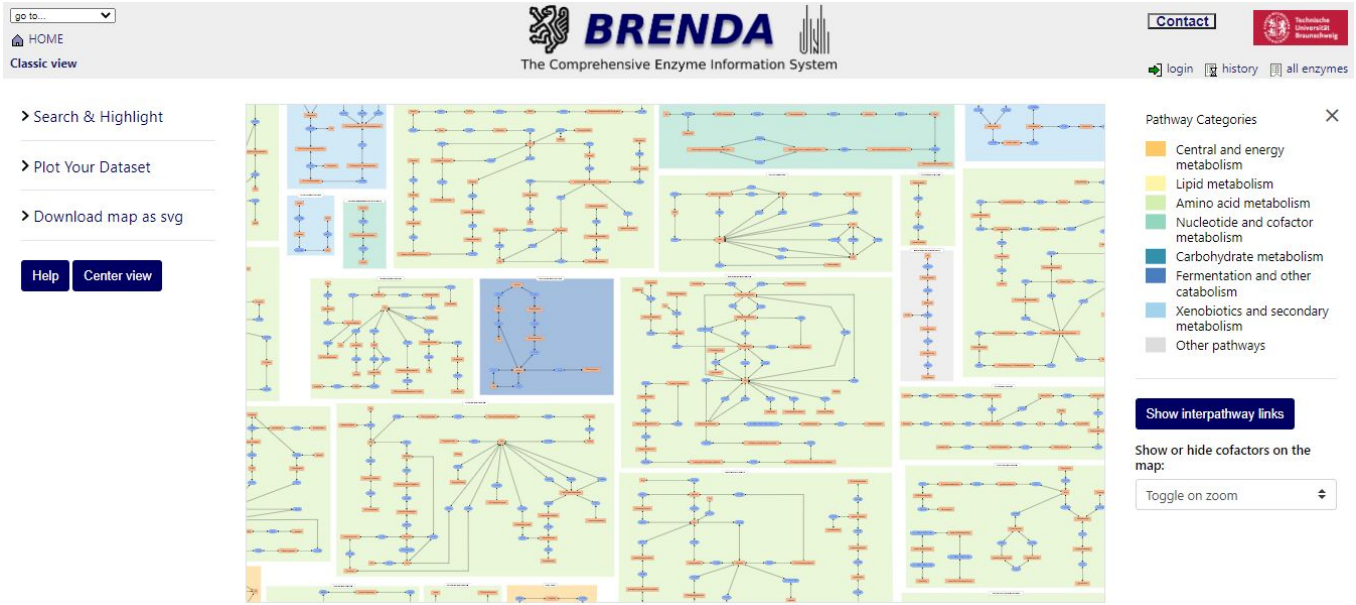
PRIMEIROS PASOS

CONHECENDO O SISTEMA



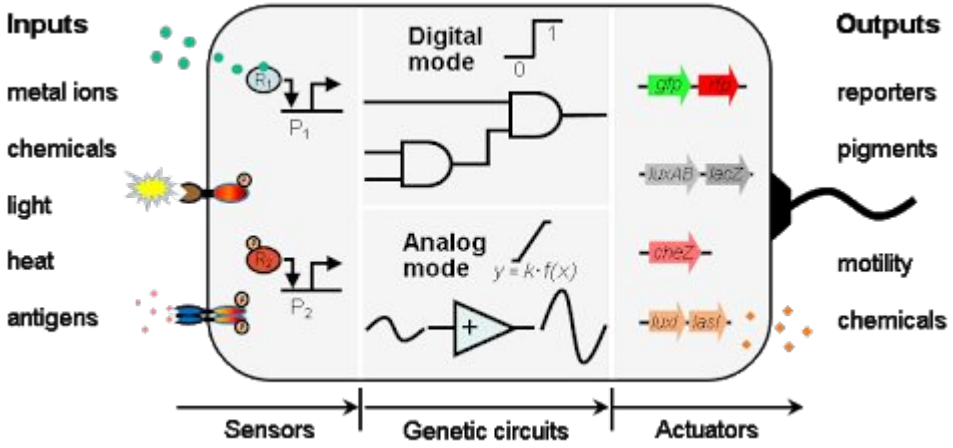
PRIMEIROS PASOS

CONHECENDO O SISTEMA



PRIMEIROS PASOS

FERRAMENTAS

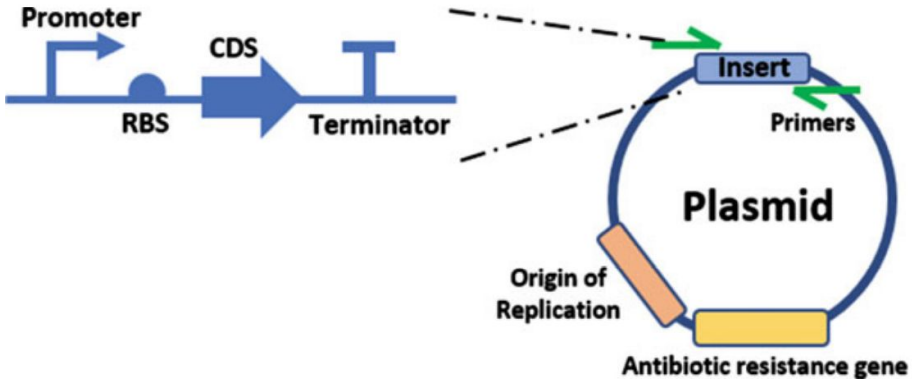


- Vias metabólicas sintéticas
- Novos circuitos
- Modelos *in silico*
- Engenharia de proteínas

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).

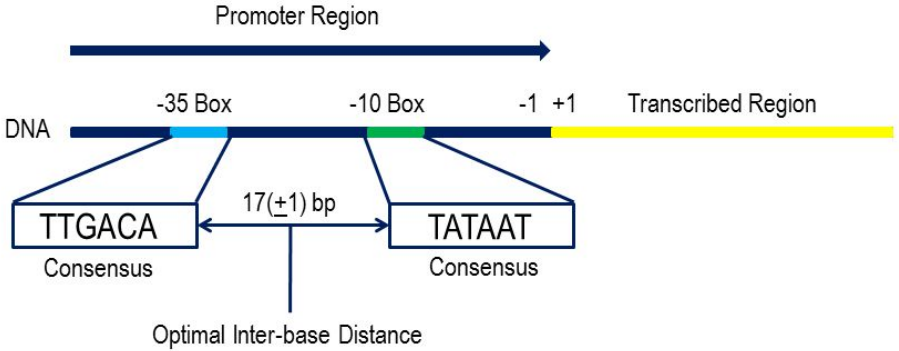


PARTES DO CIRCUITO

PROMOTORES

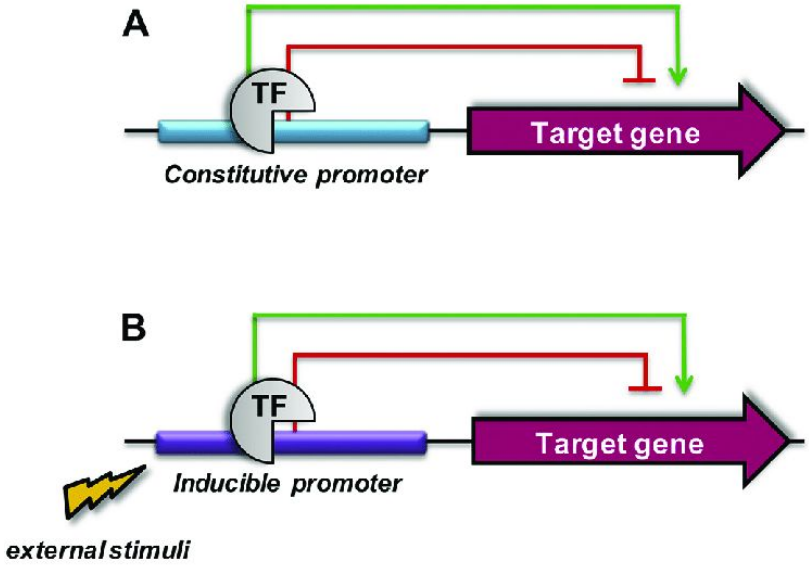
Controlan la transcripción de la proteína de interés y la convierten en ARN, la **unión de la ARN polimerasa** y los **factores de transcripción** y, en última instancia, afecta a la **expresión del gen** (Yeoh et al., 2019).

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

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

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

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 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 ...

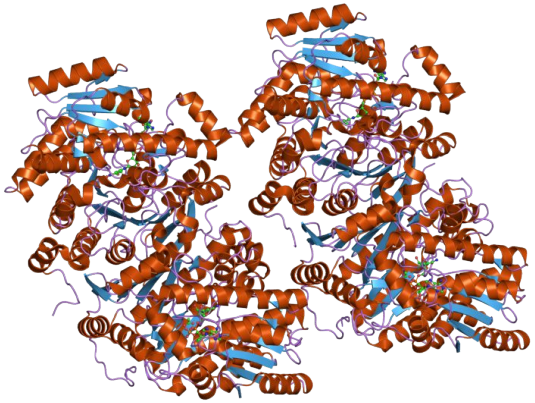
RBS Calculator Predict... · RBS Calculator · RBS Library Calculator

PARTES DO CIRCUITO

CDS - CODING DOMAIN SEQUENCE

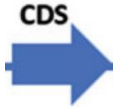
Es la **proteína de interés que se expresará en el circuito** genético diseñado con funciones como:

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

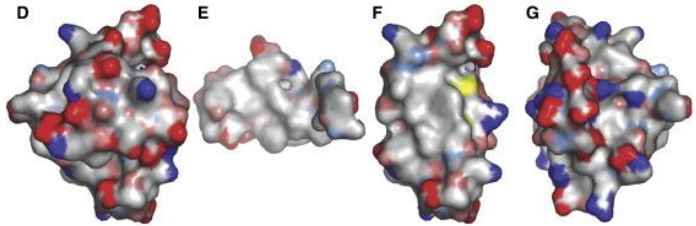


PARTES DO CIRCUITO

CDS - PRECAUCIONES

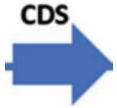


- Debe comenzar **siempre con un codón de inicio** y **terminar con un codón de parada**.
- Asegúrese siempre de que **no haya un codón de parada prematuro** en en medio de la CDS.
- **Si** el CDS **se deriva** de otras especies o huéspedes, la **optimización de los codones debe realizarse** con las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

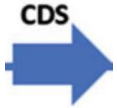
The mission of **UniProt** is to provide the scientific community with a comprehensive, high-quality and freely accessible resource of protein sequence and functional ...

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 t**odo tipo de proyectos y circuitos genéticos**.

- Reporteros
- Reguladores transcripcionales
- Marcadores de selección
- Enzimas
- Biosíntesis
- Modificación del ADN
- Proteasas
- Enzimas de modificación postraduccional
- 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 continue.

Terminators/Catalog - parts.igem.org

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:

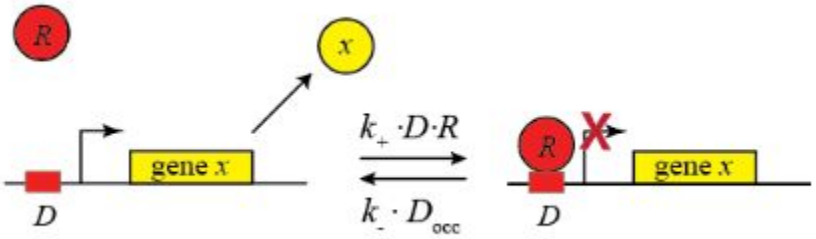
GENEius

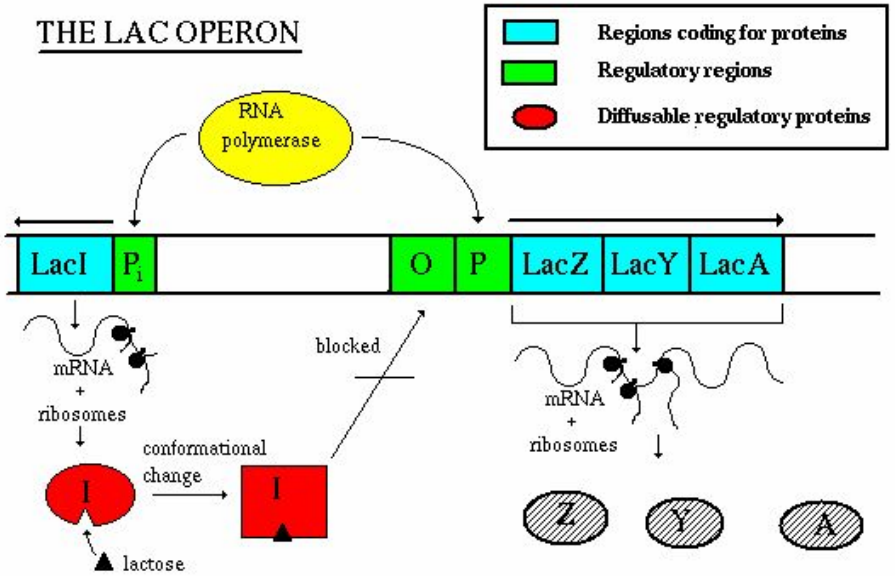
Benchling

SnapGene®

REGULAÇÃO DE EXPRESSÃO

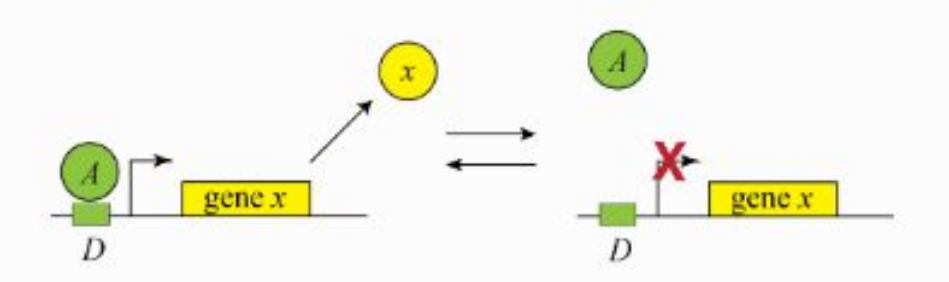
Generalidades

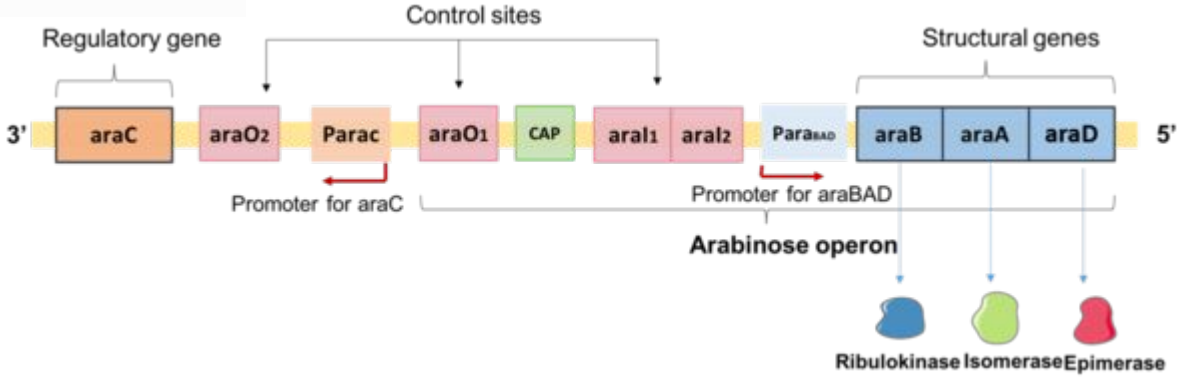




REGULAÇÃO DE EXPRESSÃO

Generalidades





REGULAÇÃO DE EXPRESSÃO

Exemplos

- Killswitch

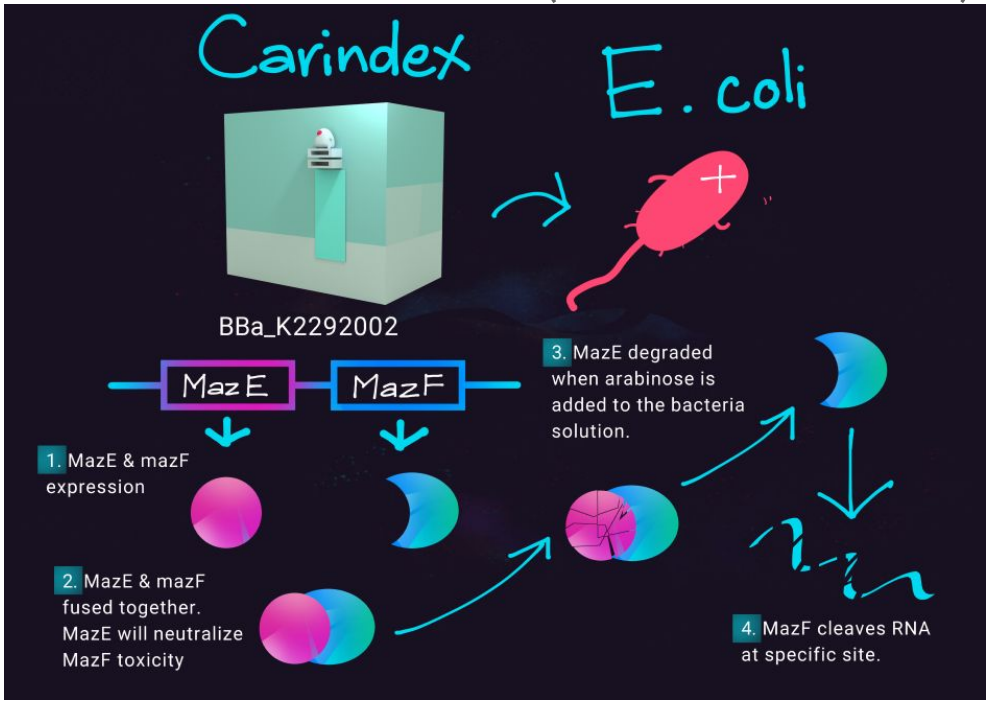
Kill switch

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

More...

Name	Type	Description	Length
BBa_K1074011	Composite	PsdpRI+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_{tac} promoter	1134
BBa_K1172908	Device	Part 1 of the Biosafety-System Lac of Growth (pRha lacI alr)	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 alr)	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

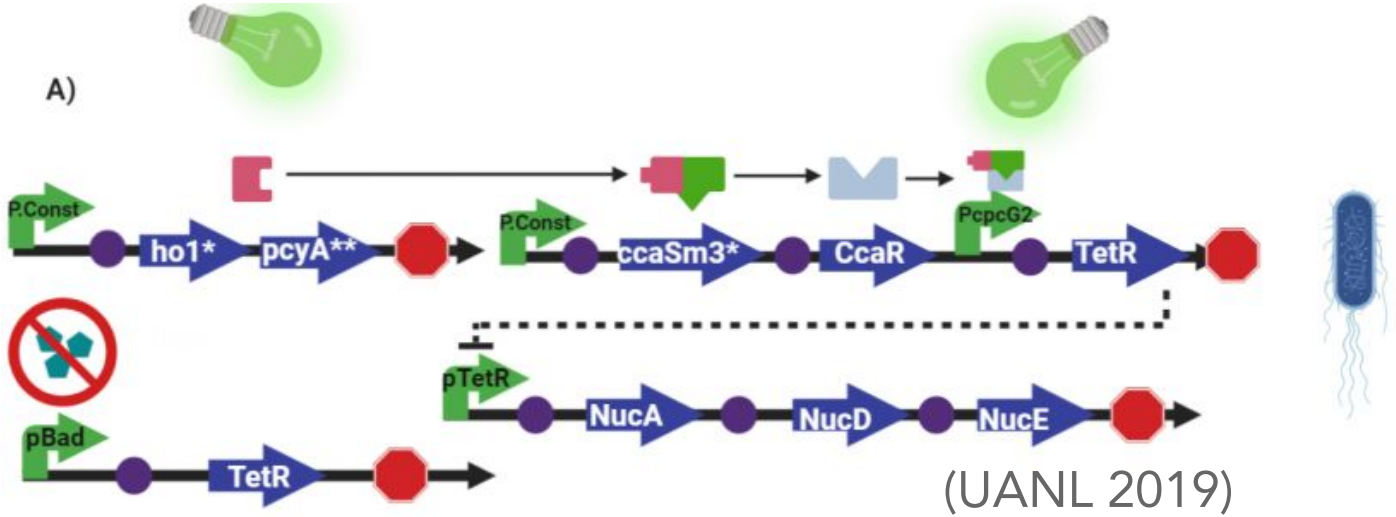
(CCU Taiwan 2017)



REGULACIÓN DE EXPRESSÃO

Exemplos

- Killswitch con activación y represión



(UANL 2019)

REGULAÇÃO DE EXPRESSÃO

Exemplos

- Biosensor

(UFAM Brazil 2013)