












Design of a gene expression system for *Rhodobacter sphaeroides*

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H. Scott Hinton

-  **Promoters (?)**: A promoter is a DNA of the downstream DNA sequence.
-  **Ribosome Binding Sites (?)**: A ribos can bind and initiate translation.
-  **Protein domains (?)**: Protein domain a protein coding sequence. Some prot the protein for cleavage, or enable it to
-  **Protein coding sequences (?)**: Prote Note that some protein coding sequer protein from start codon to stop codor also included here.
-  **Translational units (?)**: Translational They begin at the site of translational i codon.
-  **Terminators (?)**: A terminator is an R causes transcription to stop.
-  **DNA (?)**: DNA parts provide functional spacers, recombination sites, conjuga
-  **Plasmid backbones (?)**: A plasmid i: base pairs that replicate within the cel plasmid sequence beginning with the l and ending with the BioBrick prefix.
-  **Plasmids (?)**: A plasmid is a circular, that replicate within the cell independe propagate or assemble plasmid backt Registry that are only available as circ that these plasmids largely do not cor
-  **Primers (?)**: A primer is a short single sequencing. Although primers are not sequences here.
-  **Composite parts (?)**: Composite part

Browse parts and devices by chassis

Unless otherwise specified, most parts in the Registry work in Escherichia coli.

Catalog List

-  **Escherichia coli (?)**: Most parts in the Registry function in *E. coli*.
-   **Yeast (?)**: Yeast are simple eukaryotes.
-   **Bacteriophage T7 (?)**: Bacteriophage T7 is an obligate lytic phage of *E. coli*.
-  **Bacillus subtilis (?)**: Bacillus subtilis is a model gram-positive bacterium.

Unless otherwise specified, most parts in the Registry work in Escherichia coli

Outline

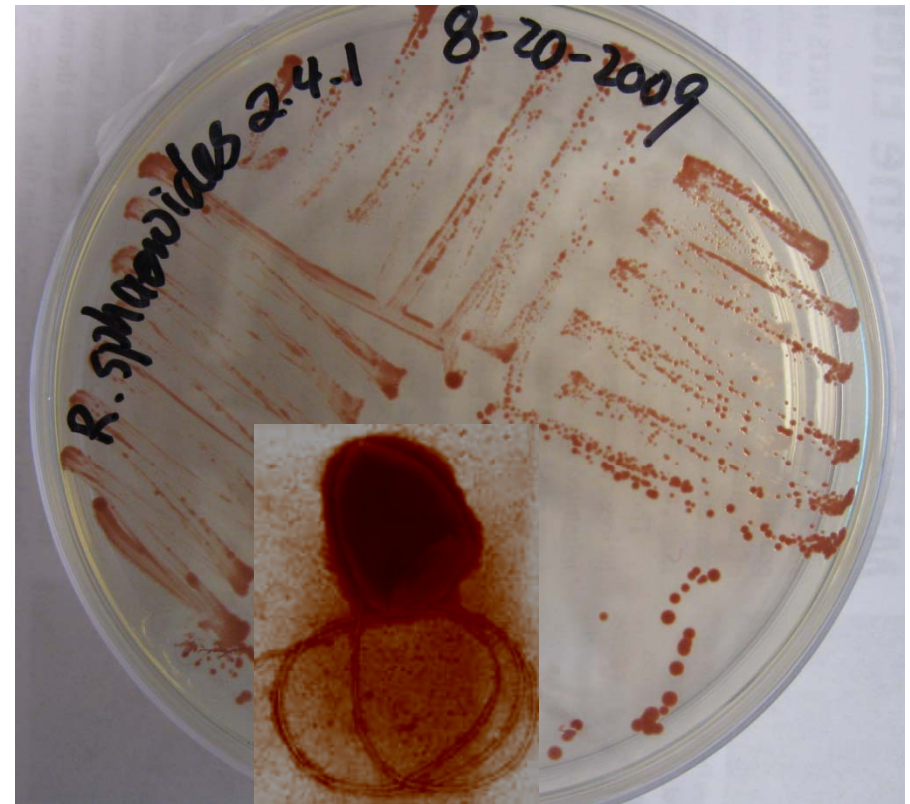


Introduction

- Design a gene expression system for *R. sphaeroides*
 - Cloning vector
 - Promoters
 - Ribosome Binding Sites
 - Terminator

Rhodobacter Sphaeroides

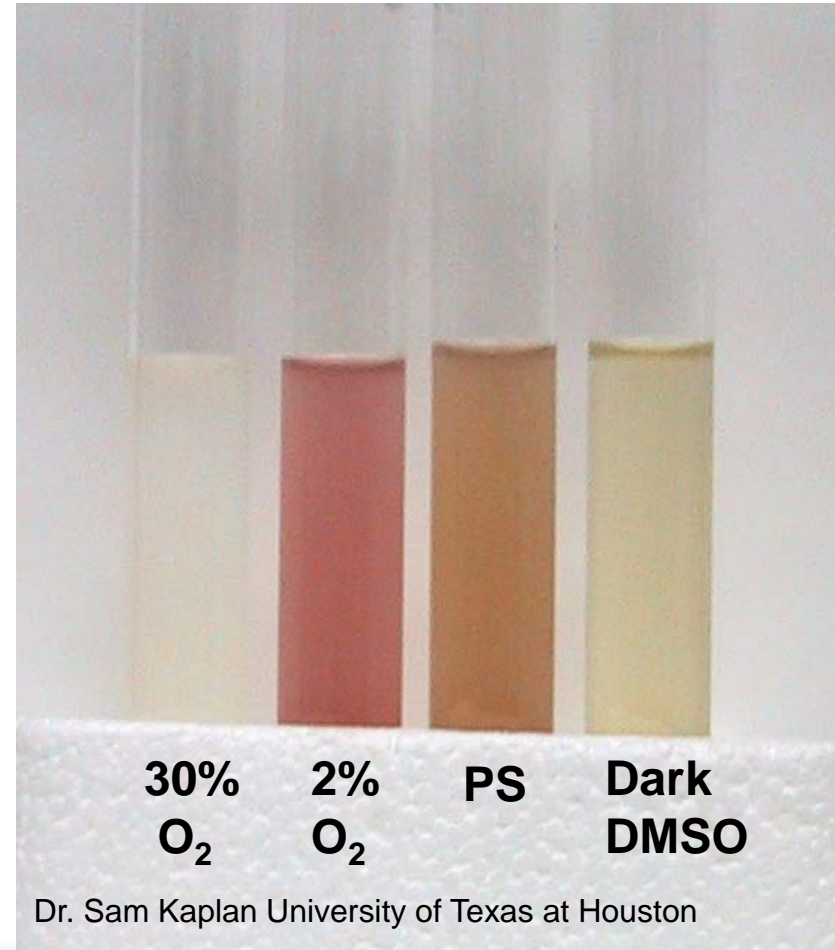
- Gram-negative: do not retain crystal violet dye in the gram staining protocol.
- Purple, non-sulfur: cannot use hydrogen sulfide as electron donor for the reduction of carbon dioxide.
- Facultative: can live with or without oxygen
- Phototrophic: light as the ultimate source of energy
- An-oxygenic: No oxygen produced
- Alpha proteobacteria.



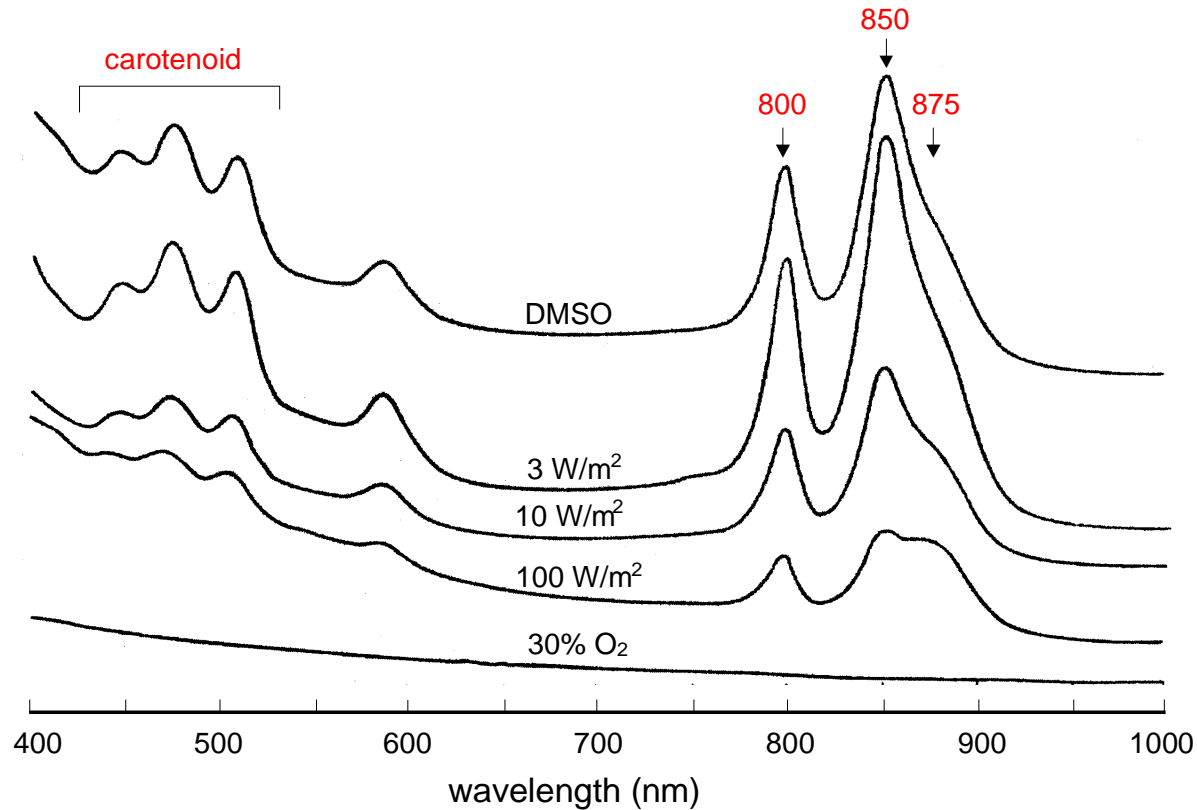
<http://www.nottingham.ac.uk/biology/contacts/sockett/research.php?S=&ID=128&pubID=NTE2NTly>

Rhodobacter sphaeroides

- Aerobic respiration
- Anaerobic respiration using Dimethyl sulfoxide (DMSO), Trimethylamine N-oxide (TMAO), NO_3
- Anoxygenic photosynthesis
- Fermentation
- Spectral complex formation occurs only under O_2 -limiting and anaerobic conditions

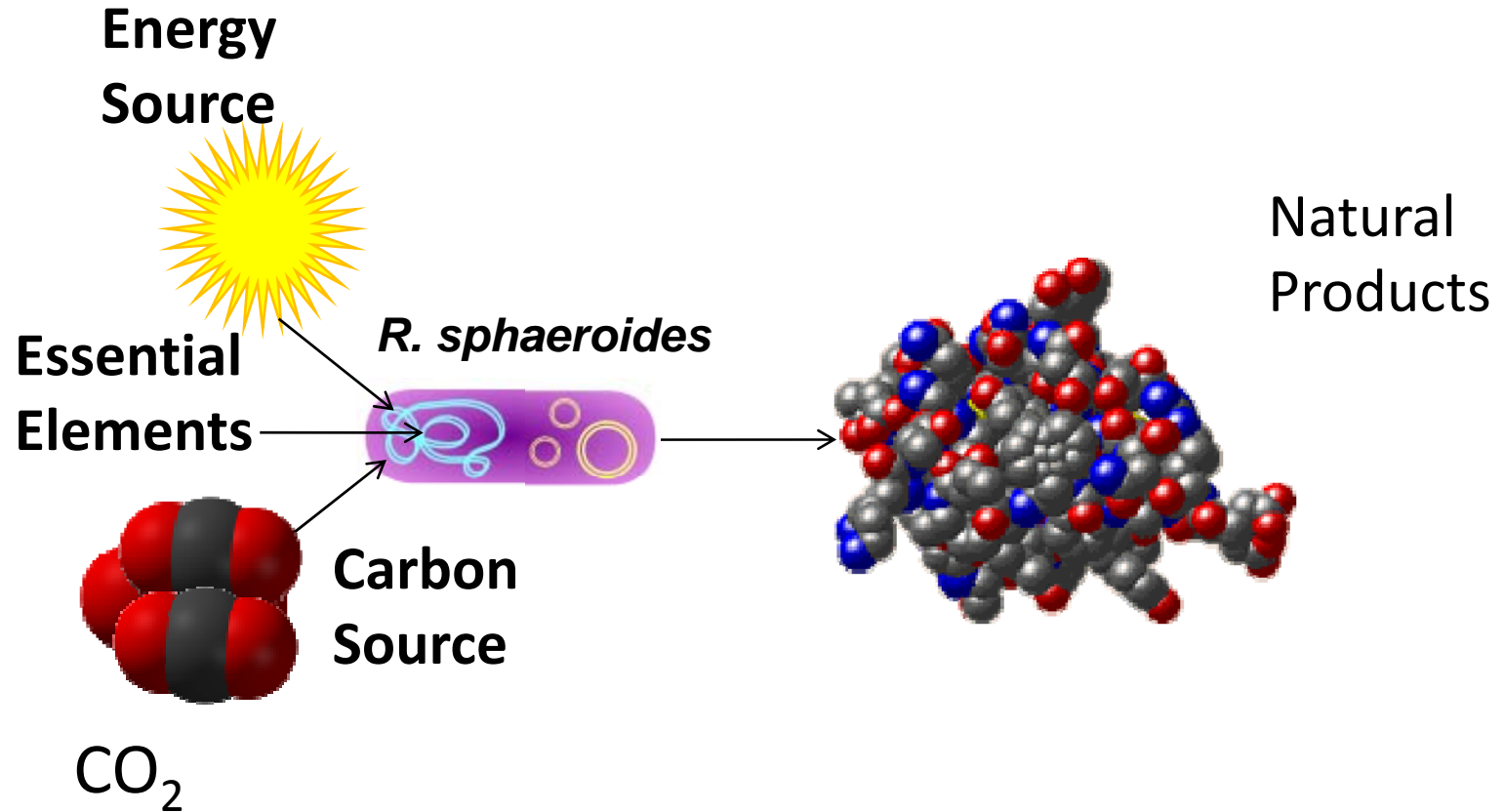


Absorption Spectrum



Dr. Sam Kaplan University of Texas at Houston

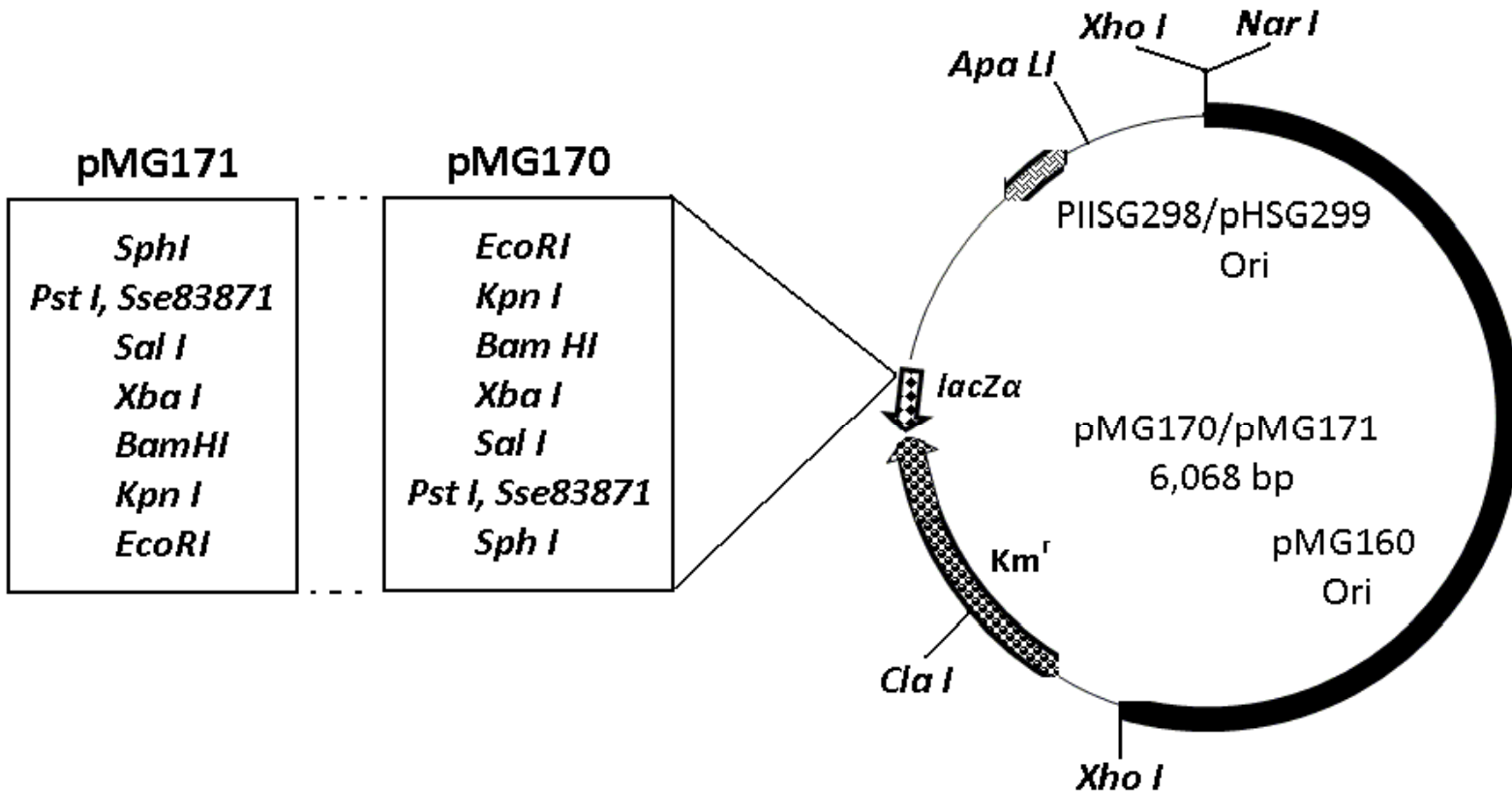
Our Dream



Outline

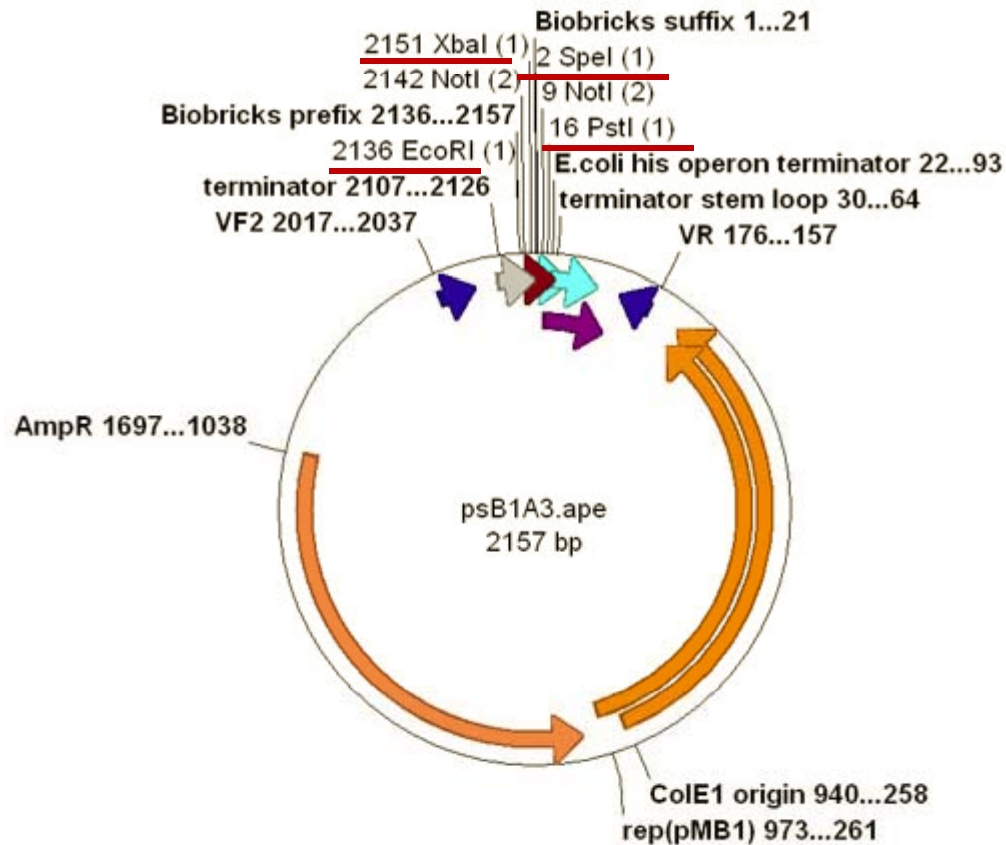
- Introduction
- ➔ Design a gene expression system for *R. sphaeroides*
 - Cloning vector
 - Promoter
 - Ribosome Binding Sites
 - Terminator

pMG170/pMG171 vector

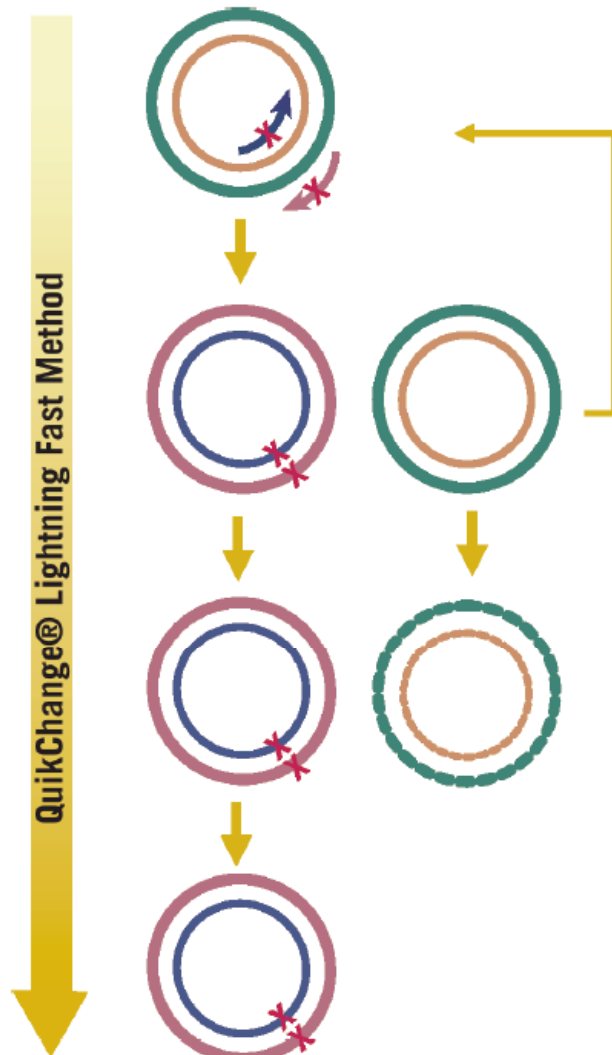


Inui, 2003. "Isolation and molecular characterization of pMG160, a mobilizable cryptic plasmid from *Rhodobcater blasticus*". Applied and environmental microbiology 69(2):725-733.

Standard BioBrick Vector



http://parts.mit.edu/igem07/index.php/Biobrick_Vector_choice



1. Mutant Strand Synthesis

Perform thermal cycling to:

- Denature DNA template
- Anneal mutagenic primers containing desired mutation
- Extend and incorporate primers with our exclusive *Pfu*-based DNA Polymerase Blend
- **Total reaction time: 1 hour***

2. Faster *Dpn* I Digestion of Template

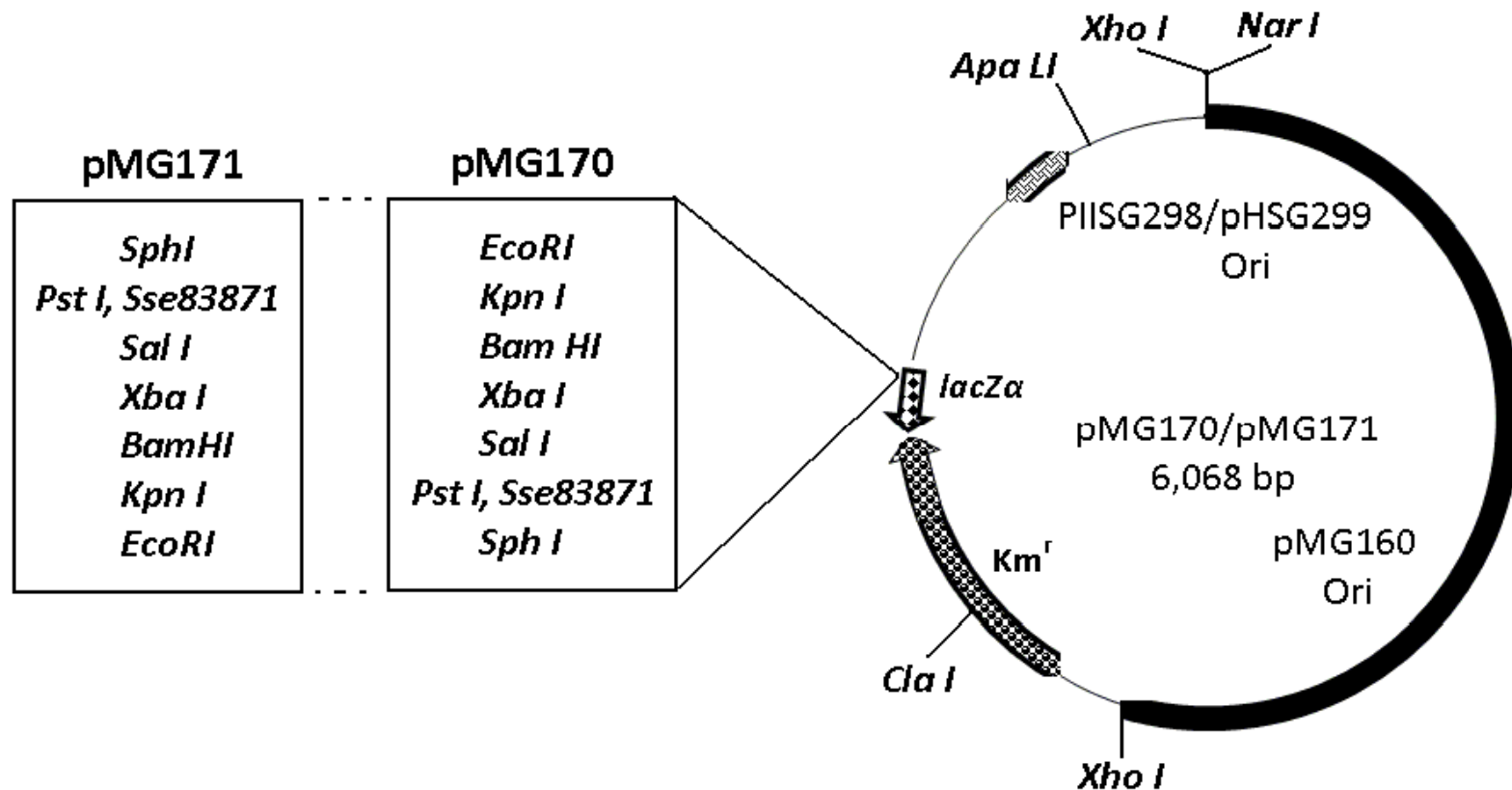
- Digest parental methylated and hemimethylated DNA with NEW *Dpn* I enzyme
- **Total reaction time: 5 minutes**

3. Transformation

- Transform mutated molecules into competent cells for nick repair
- **Total reaction time: 1.5 hours**

<http://www.qcbio.com/stratagene/210518.pdf>

pMG170/pMG171 vector



Inui, 2003. "Isolation and molecular characterization of pMG160, a mobilizable cryptic plasmid from *Rhodobcater blasticus*". Applied and environmental microbiology 69(2):725-733.

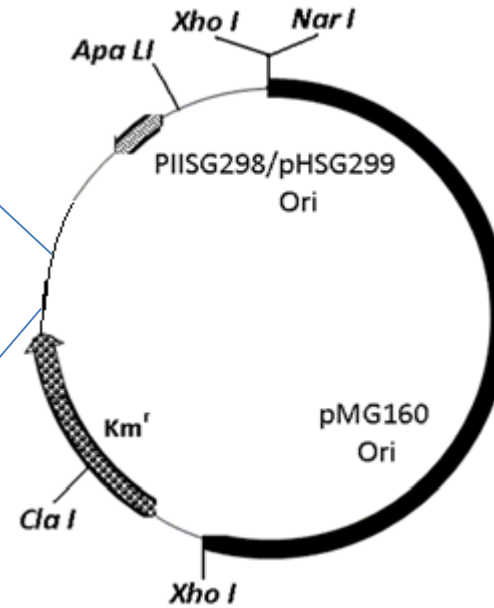
Standard Cloning Vectors

BBa_J73999

- EcoRI
- NotI
- XbaI
- SpeI
- NotI
- PstI

BBa_J73998

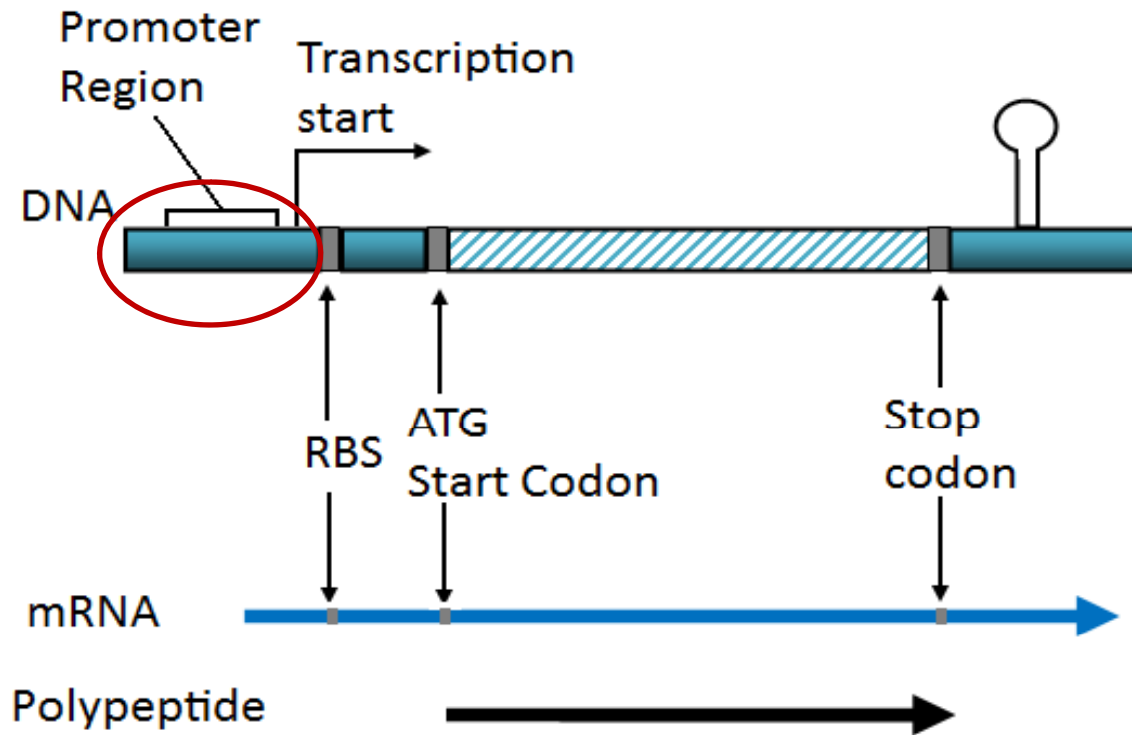
- PstI
- NotI
- SpeI
- XbaI
- NotI
- EcoRI



Outline

- Introduction
- Design a gene expression system for *R. sphaeroides*
 - BioBrick vectors
 - – BioBrick Promoters
 - BioBrick Ribosome Binding Sites
 - BioBrick Terminator

Structure of a prokaryotic gene



Lodge, J; Lund, P; Minchin, S. 2007. Gene cloning: Principles and applications.

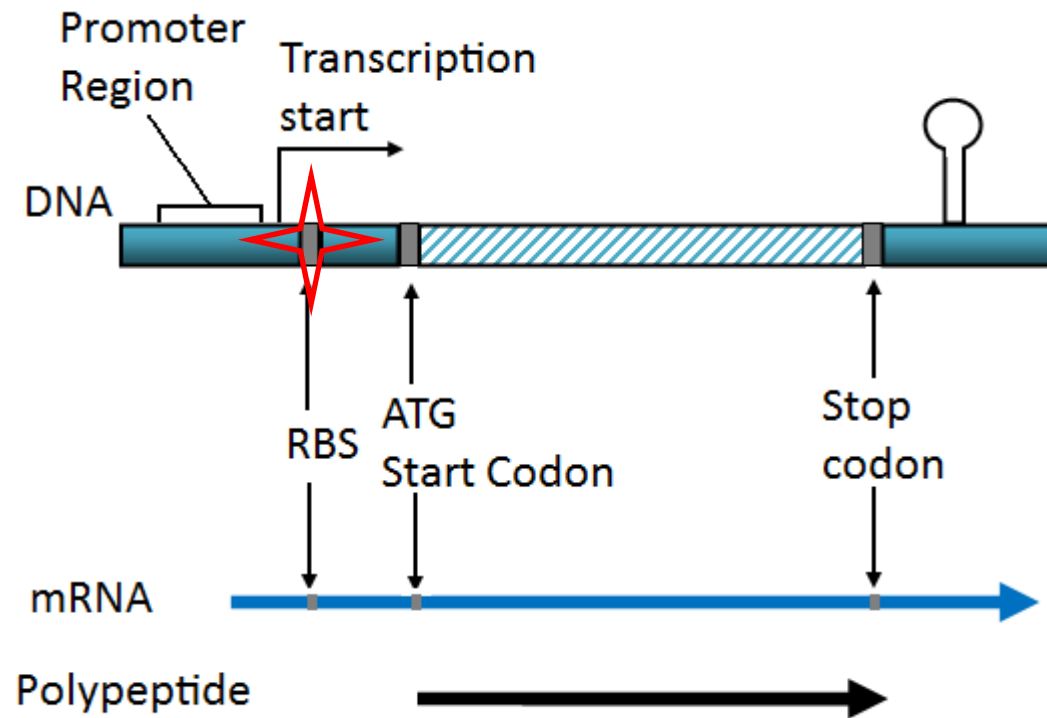
Promoter regions: *E. coli* VS *R. sphaeroides*

	$E\sigma^{70}$ consensus seq.:	TTGACA	{16-18 BP}	TATAAT	+1
<i>E. coli</i>	rrnA	CAGAAAATTATTTTAAAT	TTCTCTTGTCAGGCCGGAATAACT	CCCTATAATGCGCCACC	ACTGACACGG
	rrnB	CAGAAAATTATTTTAAAT	TTCTCTTGTCAGGCCGGAATAACT	CCCTATAATGCGCCACC	ACTGACACGG
	rrnC	CAGAAAATTATTTTAAAT	TTCTCTTGTCAGGCCGGAATAACT	CCCTATAATGCGCCACC	ACTGACACGG
	rrnD	CAGAAAAAAGATCAAAAAATA	CTTGTCAAAAAATTGGGAT	CCCTATAATGCGCCTCC	GTTGAGACGA
	rrnE	AGTCATTTTTCTGCAATTTTT	CTATTGCGGCCTGCGGAGA	ACTCCCTATAATGCGCCTCC	ATCGACACGG
	rrnG	CGATAAAGTTTTTATATTTT	TCGCTTGTCAGGCCGGAATAACT	CCCTATAATGCGCCACC	ACTGACACGG
	rrnH	TGAAAATAAAATGCATTTTT	TCGCTTGTCCTCCTGAGCCGACT	CCCTATAATGCGCCTCC	ATCGACACGG
		<hr/>			
<i>R. Sphaeroides</i>	rrnA	ATCGTCTCTTCGTCATTTTT	CCTCTTGCGGGTTTTTTT	TGCGGTTCCCTAGATAGCGCCT	CACCGAAGCGG
	rrnB	TGTTACGGAGCCCAAAAAAT	CCGCTTGCGCCCGGGGCGT	CTGCTCCTAGAAACCGCTT	CACCGAGACGA
	rrnC	TCCATGCGGCTGTCATTTTT	CCTCTTGCGGGTTTTTTT	TGCGGTTCCCTAGATAGCGCCT	CACCGAAGCGG
	$E\sigma^{32}$ consensus seq.:	TCTCNCCCTGAA	{13-17 BP}	CCCCATNTA	

Up: Sequences of the seven *E. coli* rrn P1 promoter regions (Newlands, 1993)

Bottom: Sequences of the three *R. sphaeroides* rrn promoter regions (Dryden and Kaplan, 1993)

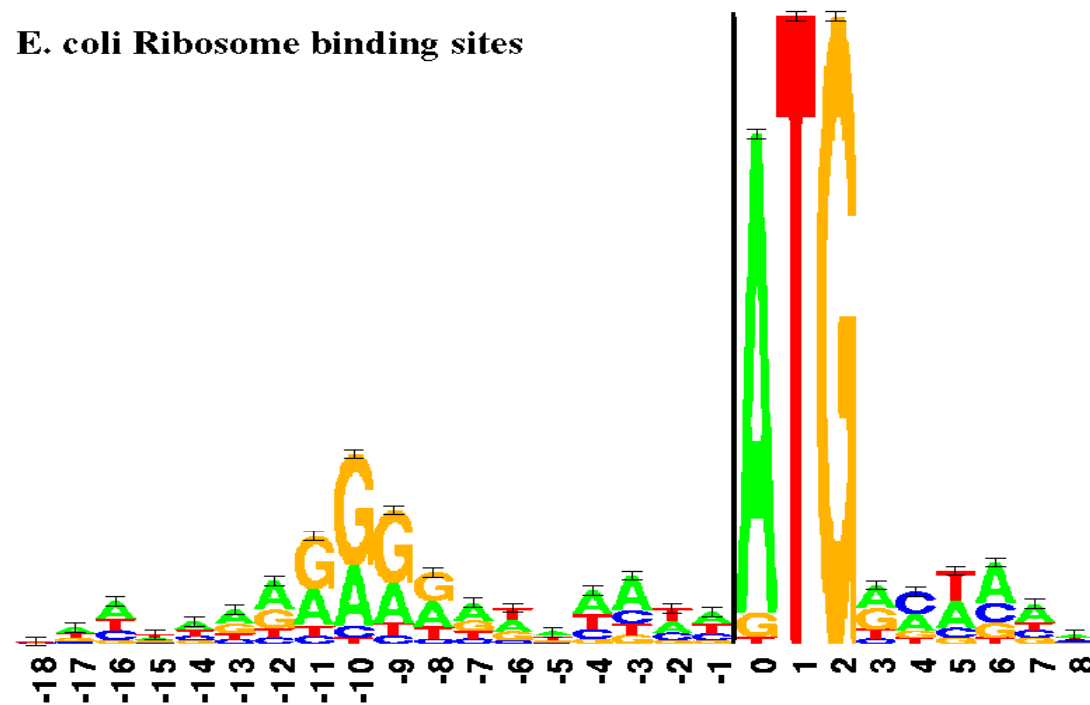
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Shine-Dalgarno Sequence

- SD-Sequence: TAAGAAGGT



Logo for E. coli Ribosome binding sites (Schneider and Stephens, 1990).

Ribosome Binding Sites (RBS)-Sequence Logo

Part of *Rhodobacter Sphaeroides* 2.4.1 Ribosome Binding Sites Training Set for creating Sequence Logo: Each line represents part of the DNA from -18 bp of a gene's upstream to +8 bp of the gene itself. **GTG** or **ATG** is the start codon of the gene.

GACATCGCCCGTGAGGCG**GTG**GACCGG

TGACGAAGGGGATGGACG**ATG**GTCTCT

CTCGATCGGATCCGTTTCATGGCCATT

GCAACGACCGAGAAGAGCATGAACCGC

CGCGCGAAAGGATGGCGGATGCCGCCC

CAAGGCGCGGGTCTACTGATGCGCGGC

CGAGGACGGTCTCGGCTGGTGC GCGAC

GAGGAGCTTCTGCAGGCCGTGCTCGCC

CGCTGGAGGTAACCCGCCATGATGCGC

AACCAGAGGAGTTGCGGAATGTATCTC

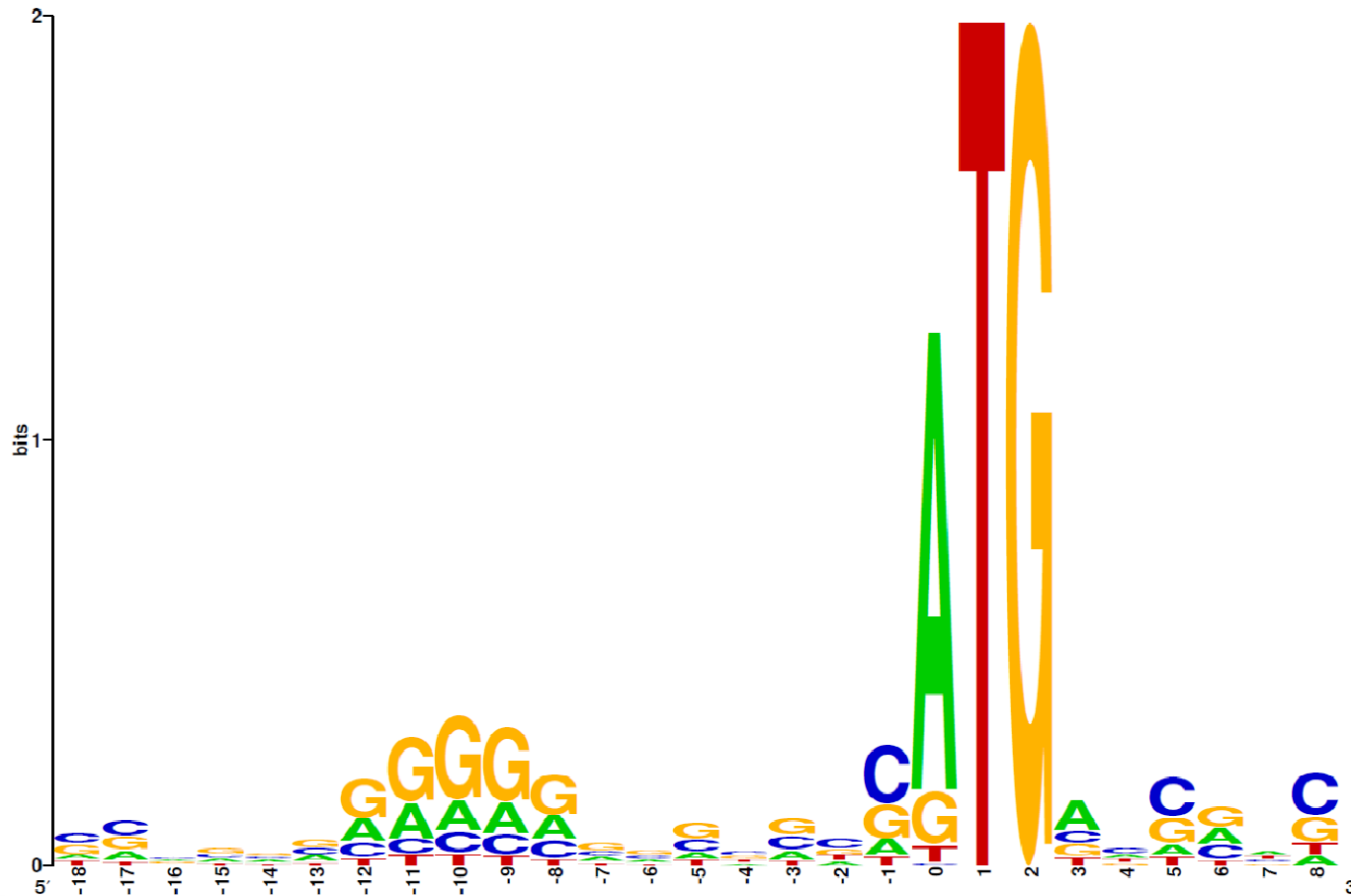
CACAACAGGAGCGGCGCGATGCCGGAA

TCGCAAGGAGTTCAGATCATGCCTCAG

CACAACAGGAGCGGCGCGATGCCGGAA

TCGGAGGAGCCTTCTGACATGTCCCCC

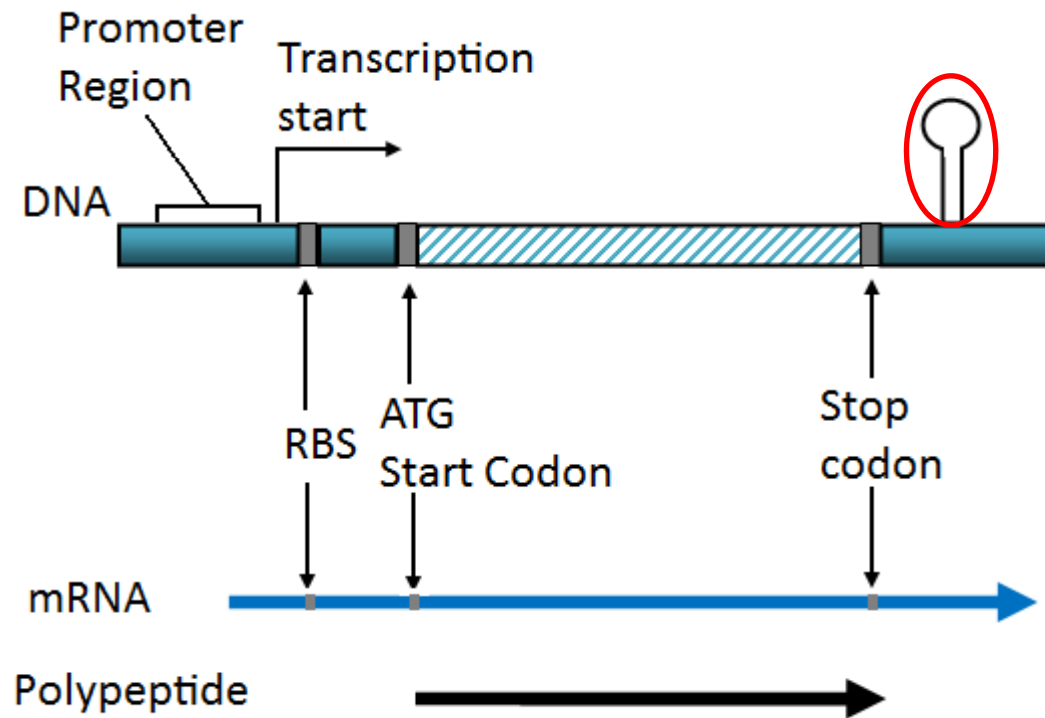
R. sphaeroides RBS sequence Logo



Potential RBS for *R. sphaeroides*

BioBrick ID	Master Sequences
BBa_K208101	CATCAACGGAGGTACTAGATG
BBa_K208102	CCTGGGGGAGGGTACTAGATG
BBa_K208103	TCAGTGGAGGGATACTAGATG
BBa_K208104	GGAGGGGAGGCATACTAGATG
BBa_K208015	GGAGGAGGGGGCTACTAGATG
BBa_K208016	TCGGAGGAGCCTTACTAGATG
BBa_K208017	GGGAGGAGGCGGTACTAGATG
BBa_K208018	GGAGGTGTTCTTTACTAGATG

Structure of a prokaryotic gene



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A typical rho-independent terminator

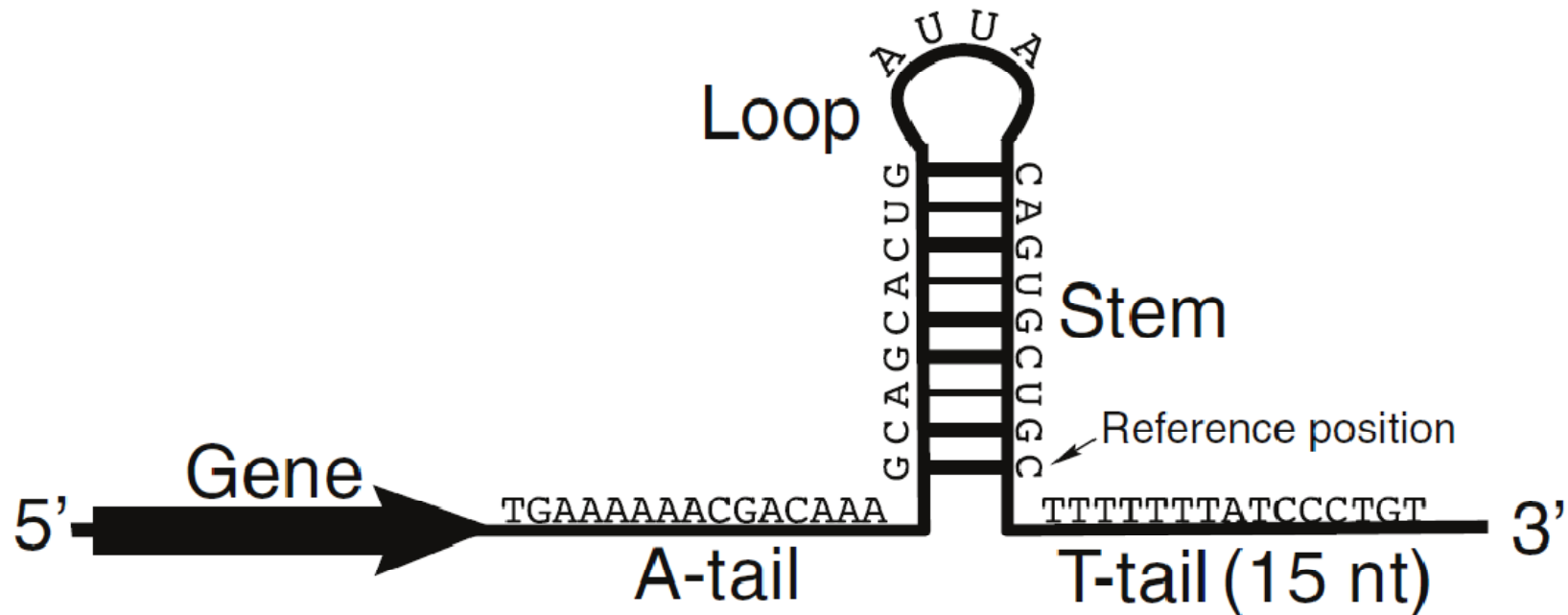
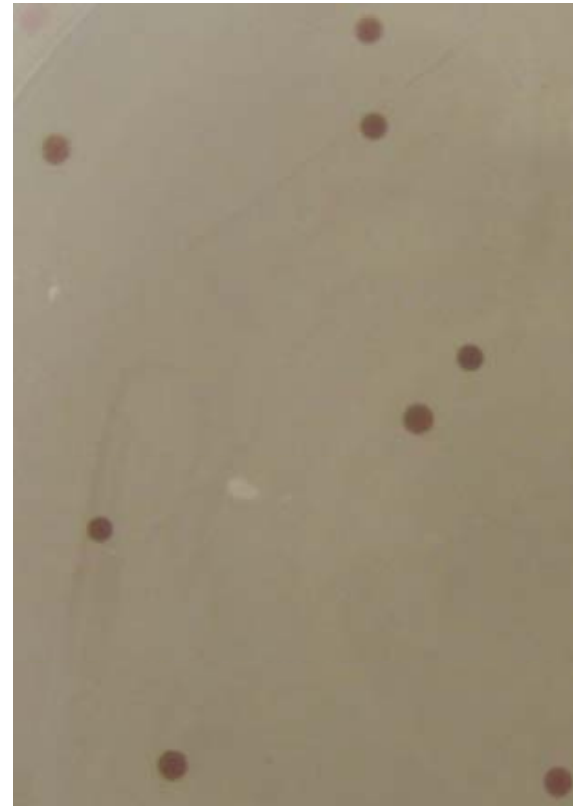
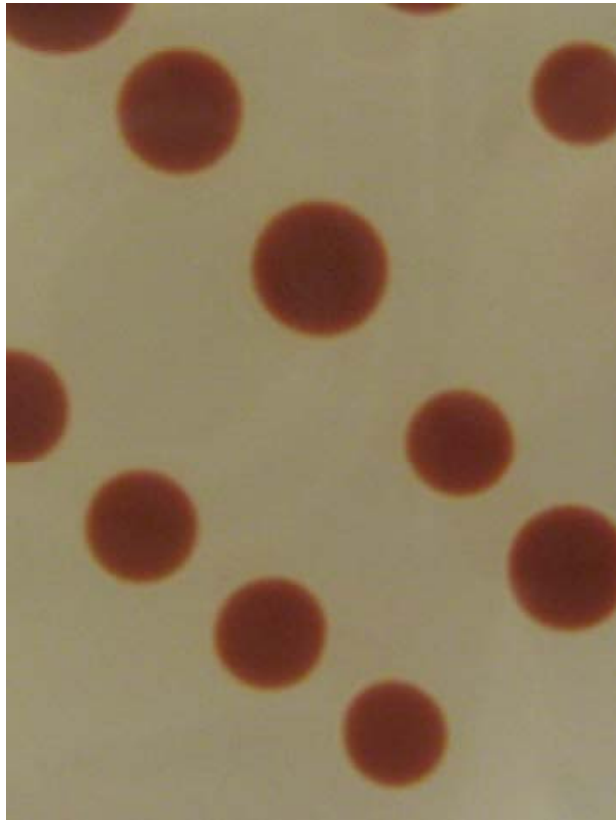


Figure 14: Structure of Omega terminator (Prentki and Krisch, 1984)

Conjugation lab



Future work

- Measure BioBrick parts activity in *R. sphaeroides*
 - Promoter strength
 - RBS activity
 - Terminator efficiency
- Demonstrate production of a protein using designed operon.

Summary

- Designed DNA sequences (BioBricks) for gene expression system in *R. sphaeroides*
 - Vectors: [BBa_J73998](#), [BBa_J73999](#)
 - Promoters: [BBa_K208109](#), [BBa_K208110](#), [BBa_K208111](#)
 - Ribosome Binding Sites : [BBa_K208101~K208108](#)
 - Terminator: [BBa_K208112](#)
- Developed a conjugation protocol
- Begin measurement and analysis of BioBricks

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Questions/Comments

Thank You!