Engineering the Bacteriochlorophyll Biosynthesis Pathway for the Development of New Energy Sources

UC Berkeley - Lawrence Berkeley Laboratory Team

Team Members: Konniam Chan, Joyce Chan, Mimi Ho, Laina Magaya

Advisors: Connie Kang, James Carothers, Jay D. Keasling

Motivation

- Purpose: Engineer *E.Coli* to produce biofuel
- Utilize the vastly untapped solar energy

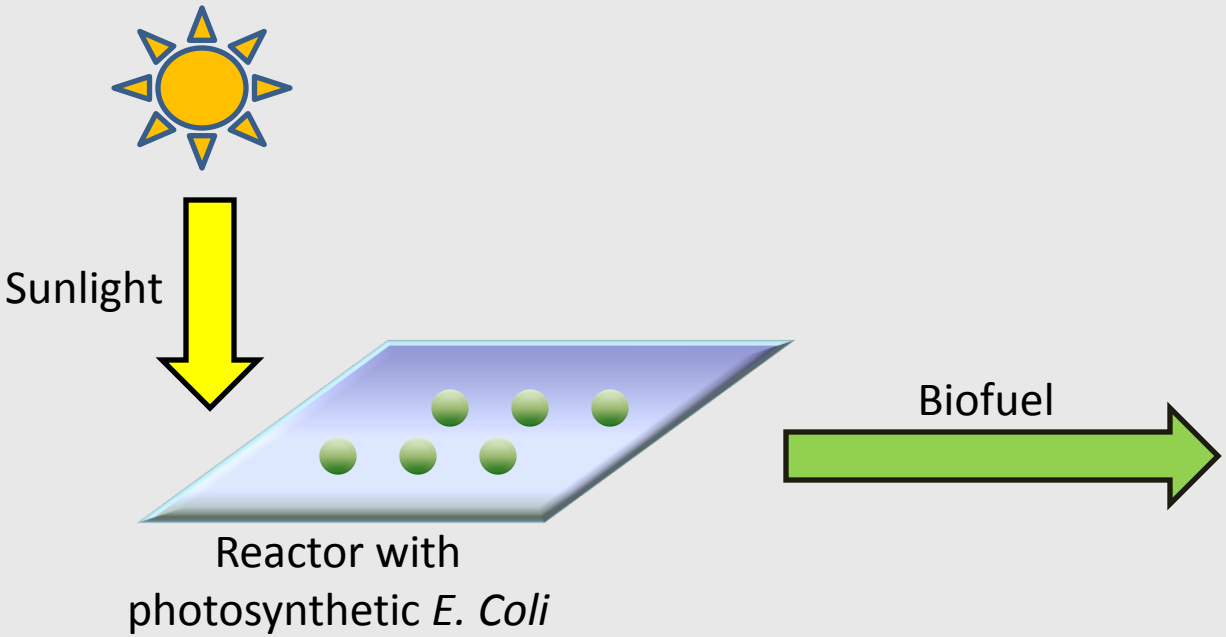
Solar Energy Available	3850 x 10^{21} Joules per year
World Consumption of Energy	0.471 x 10^{21} Joules per year

- Complimentary approach to photovoltaic cells

	Photovoltaic cells	**Biofuel Reactor (photosynthetic *E.Coli*)**
Process	Light Energy → Electrical Energy	Light Energy → Chemical Energy
Materials	Amorphous Si, CdTe	*E. Coli*
Use	Electricity Generation	Fuel production

Photosynthesis

- Process in plants and bacteria by which light energy is converted to chemical energy
- Incredibly efficient process (95% of solar energy incident on molecular reaction center is transferred to chemical energy)
- Minimal energy input costs



Phase 1 Approach

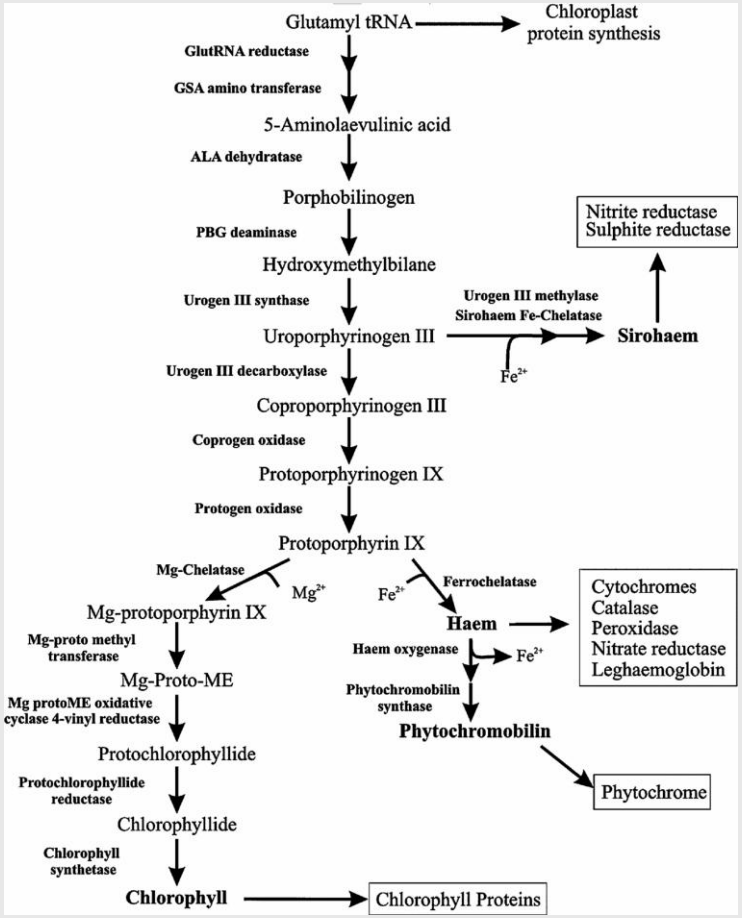


Figure 1. Overview of the metabolic pathway for the synthesis of chlorophyll in plants

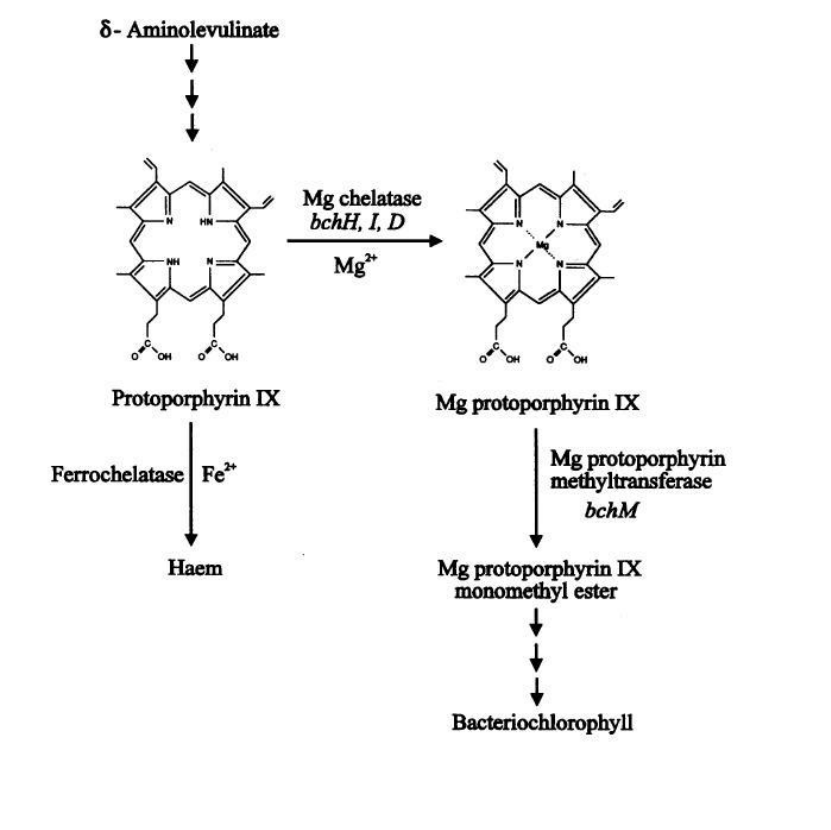
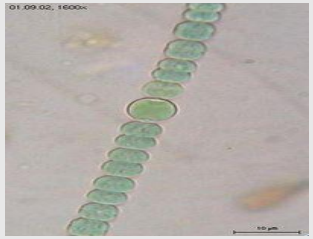


Figure 2. The conversion of protoporphyrin IX to Mg-protoporphyrin IX via the enzyme Mg-chelatase as the first unique step in the bacteriochlorophyll pathway.

Two Main Photosynthetic Organisms

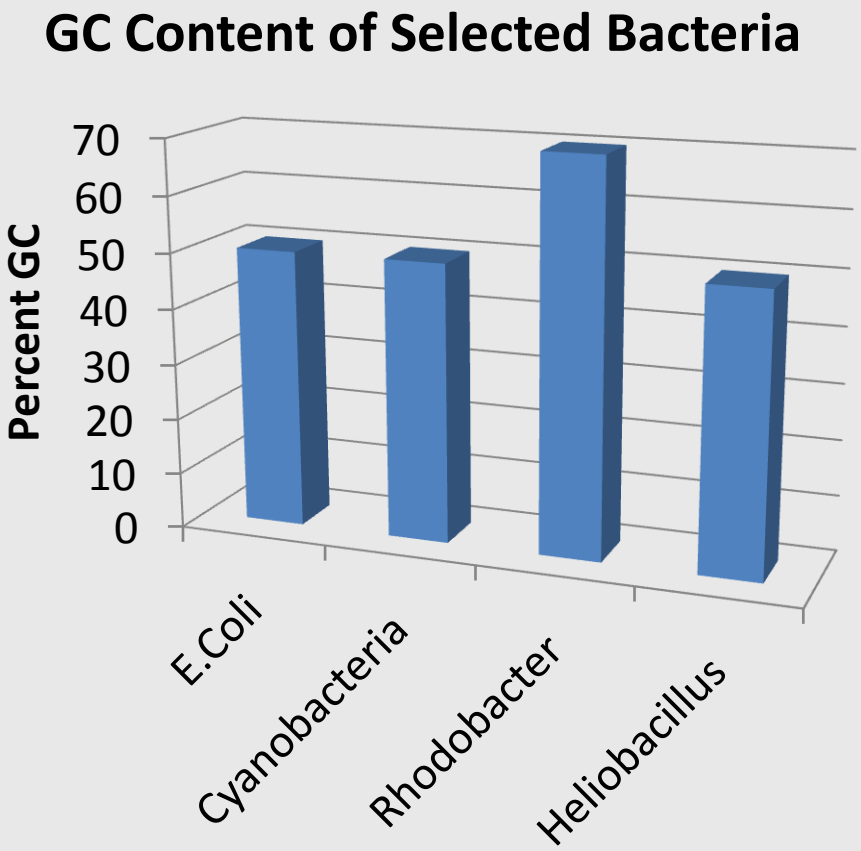
- Subclone the genes for Mg-chelatase, for two photosynthetic bacteria:
  - ***Rhodobacter sphaeroides*** (purple bacteria)

  - ***Synechocystis sp.*** (cyanobacteria)



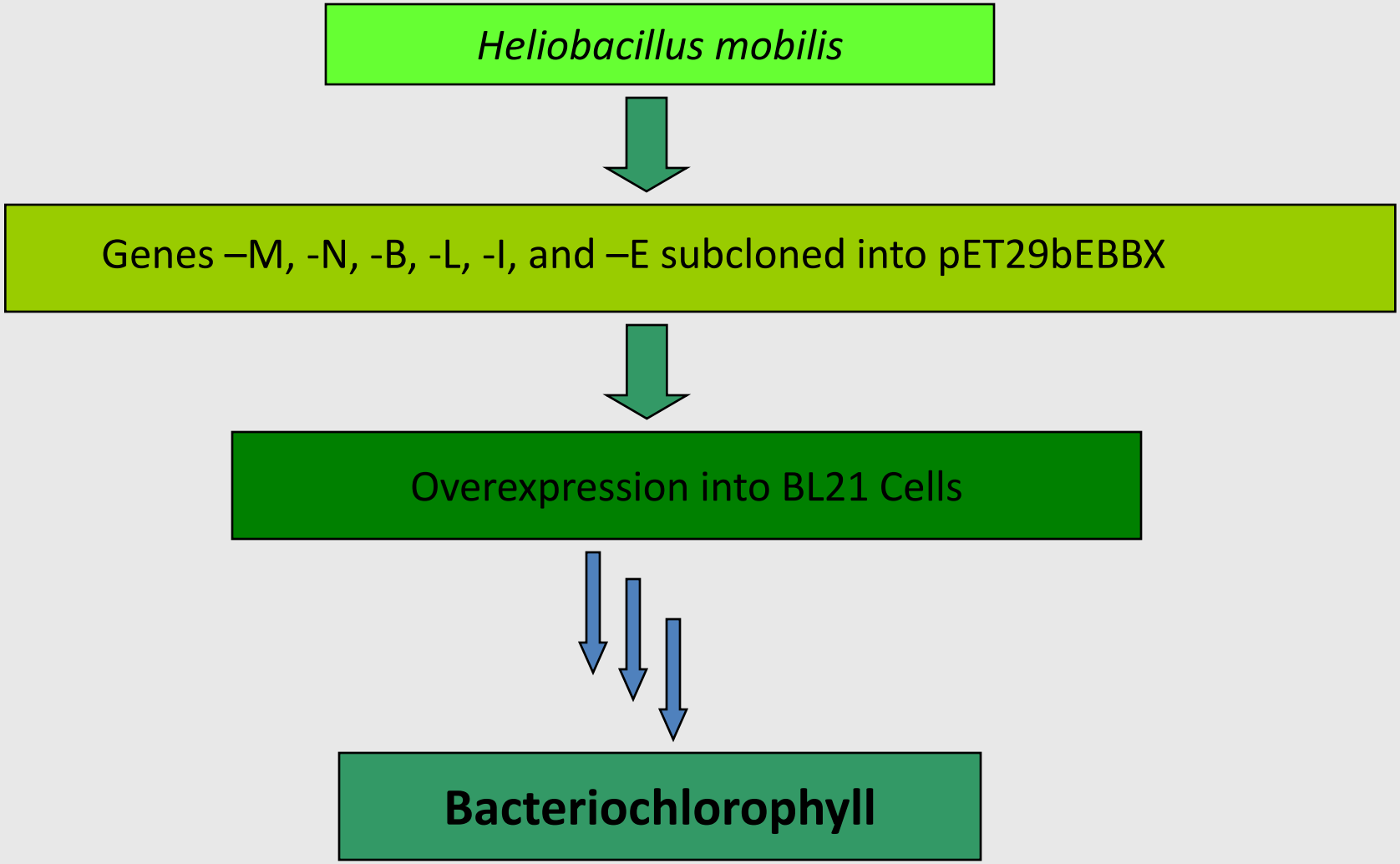
- Enzyme Activity
  - Protein Expression
  - Spectroscopy

GC Content

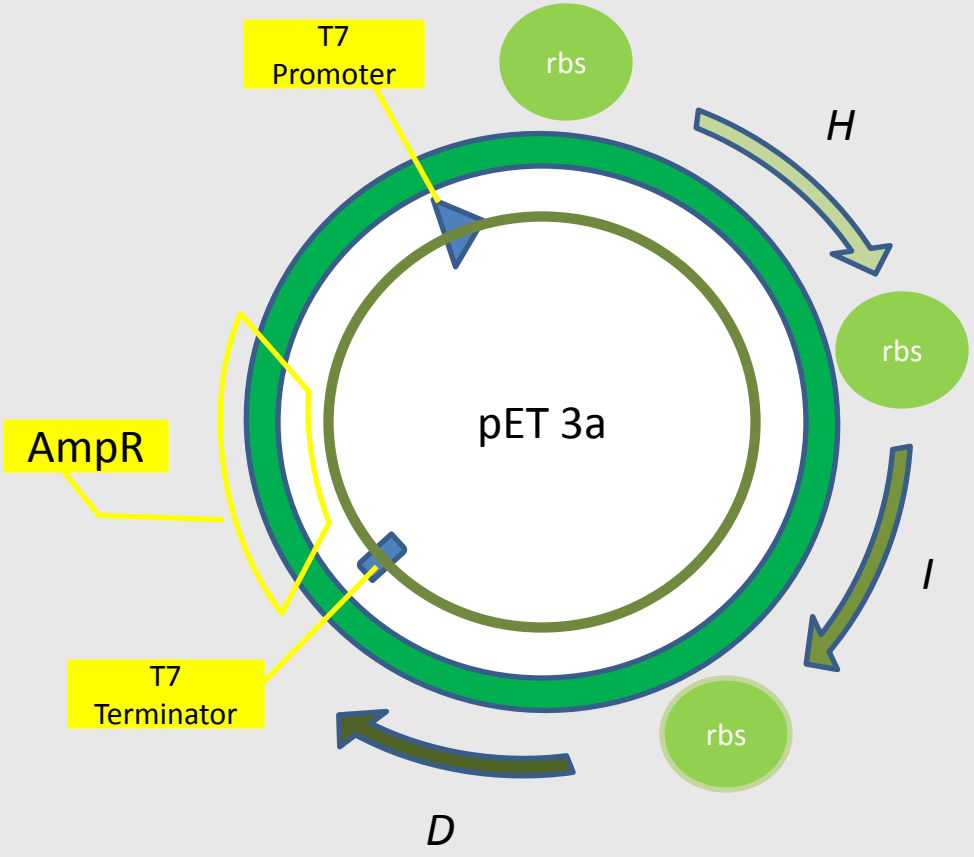


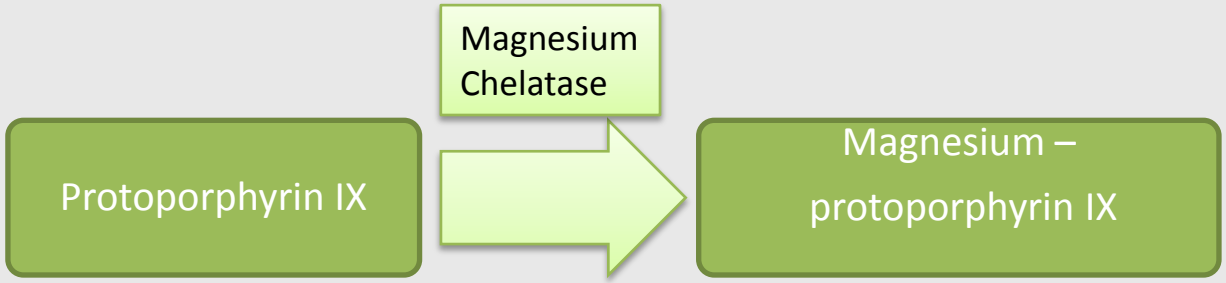
- Expected Cyanobacteria to exhibit the most enhanced activity
- Expected Rhodobacter to exhibit the least activity
- Difficulties in amplifying Rhodobacter genes due to high GC content

Phase 2 Approach

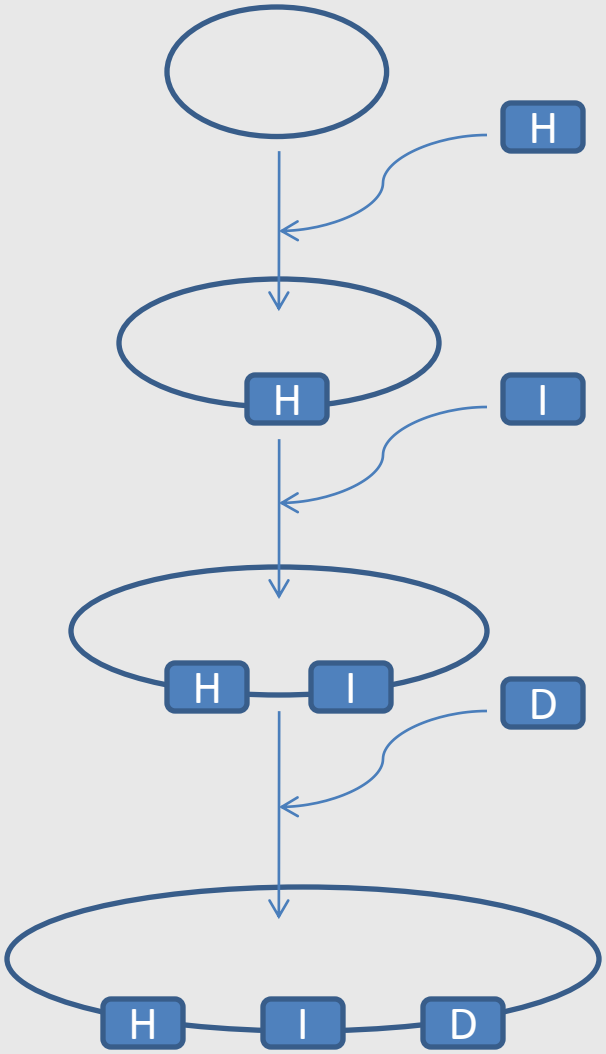


Biological Parts Construction

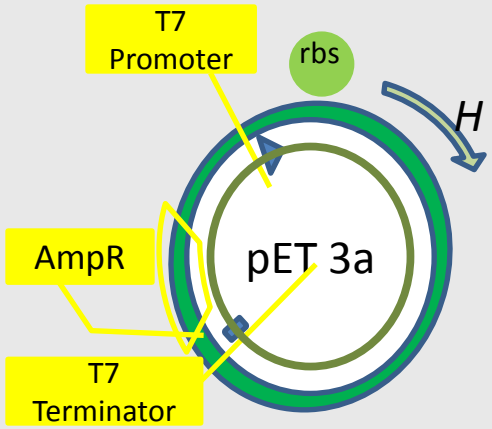


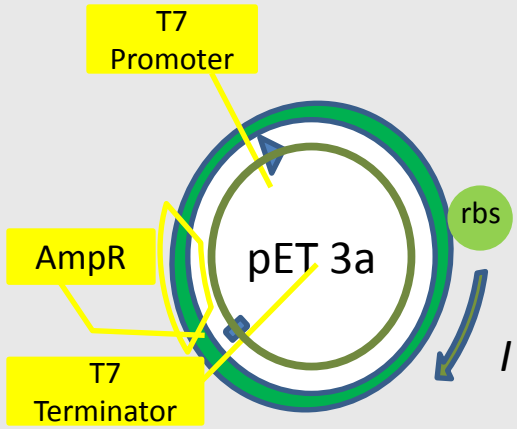


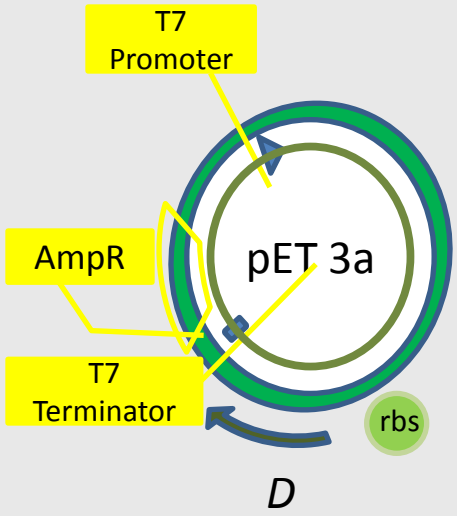
Sequential System Building Method



Biological Parts Construction





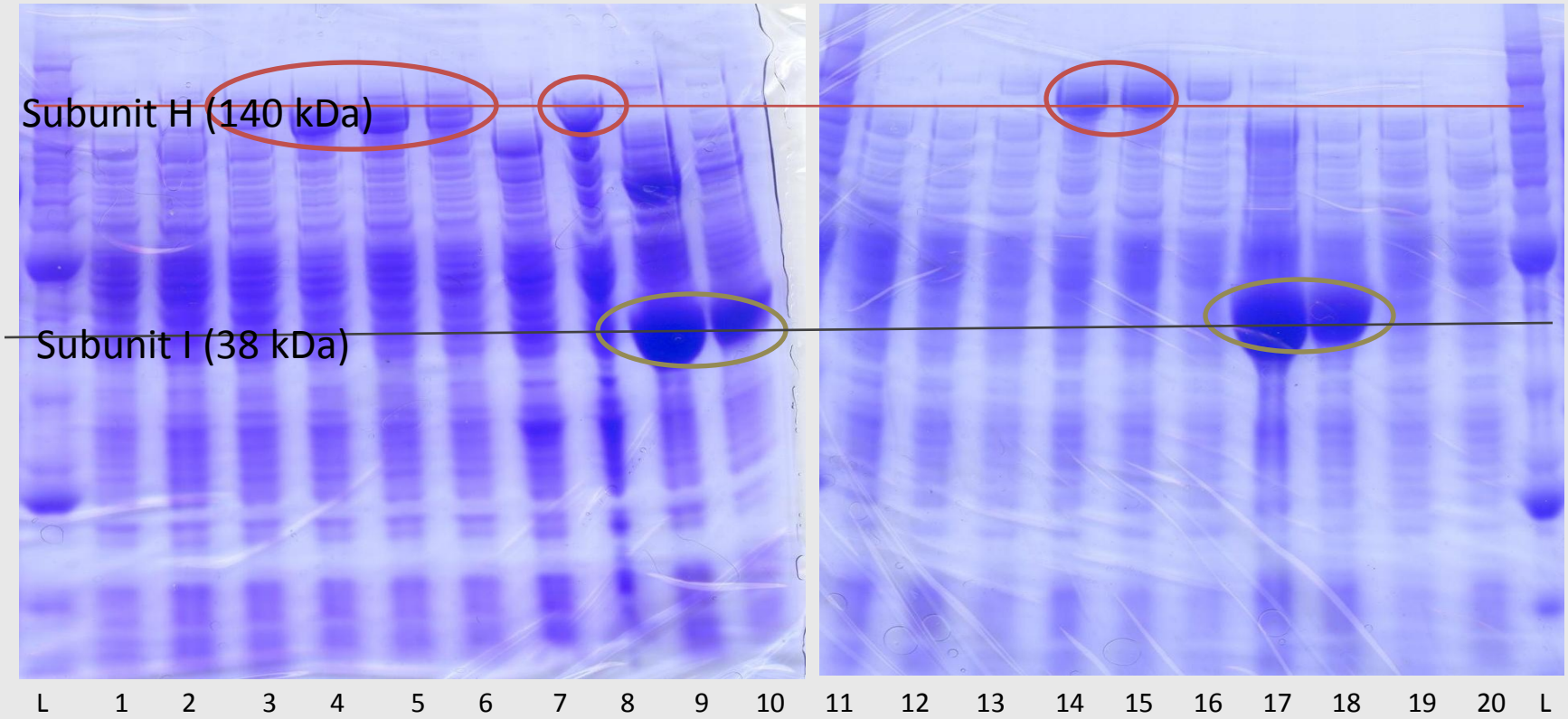


Construct with
-H gene only

Construct with
-I gene only

Construct with
-D gene only

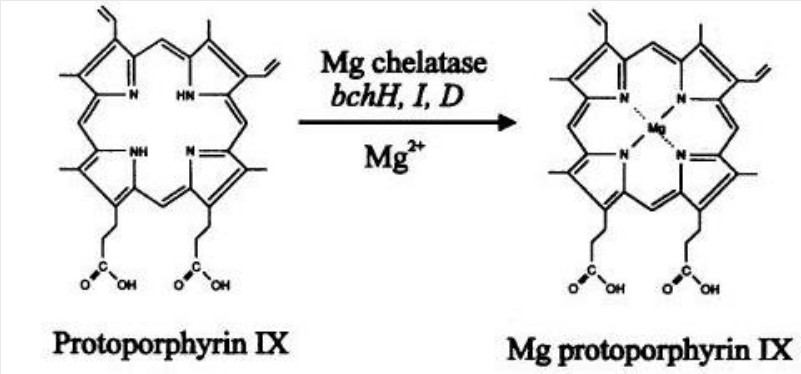
Investigation of Protein Expression



Expected Distinctive Protein Bands	Expected Molecular Weight
Subunit H	140 kDa
Subunit I	38 kDa

Spectroscopy

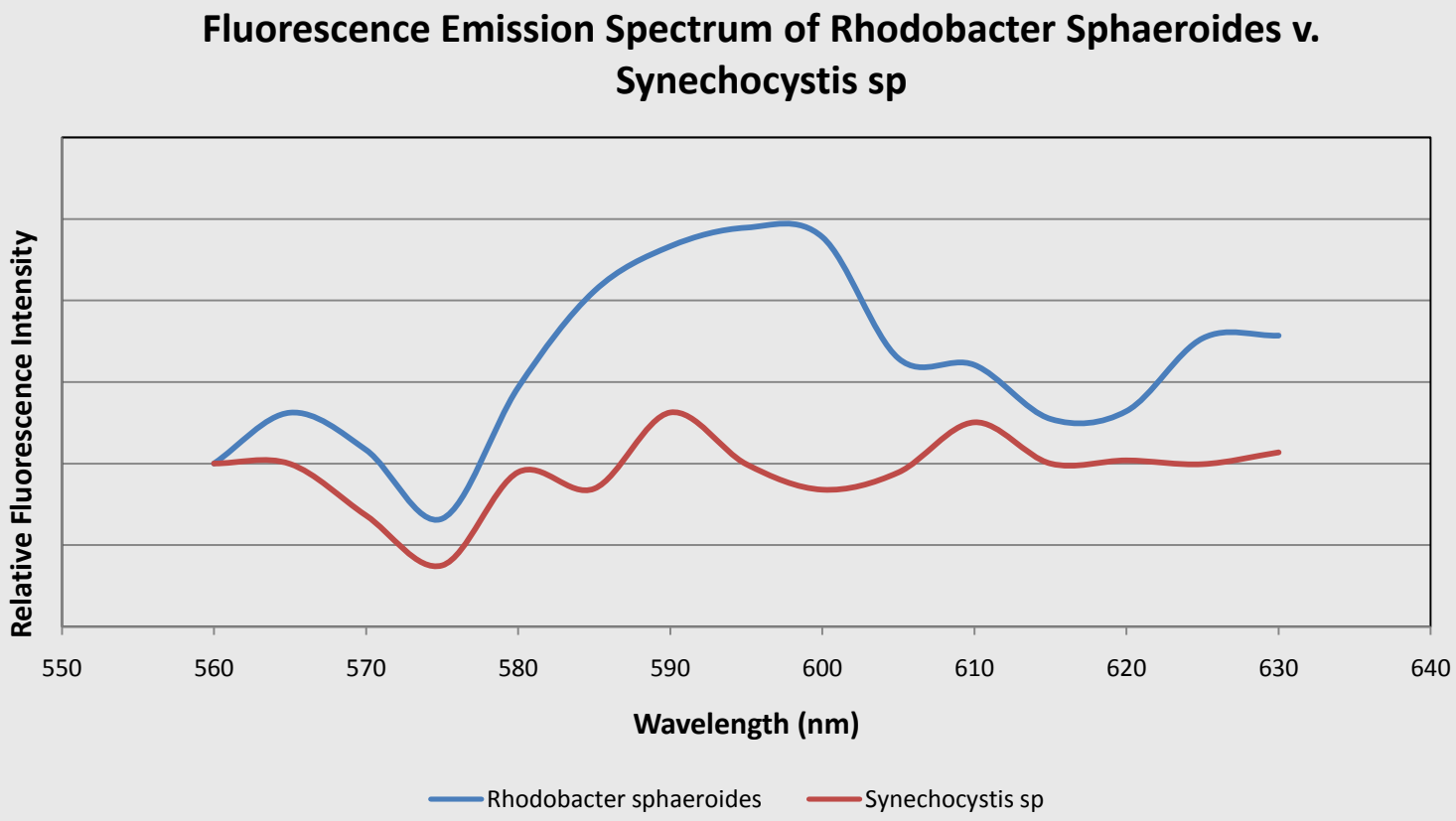
- Because thermodynamics of reactions are equal for all Mg-chelatases, the difference in activities lies in kinetics
- Assume: relative concentration of products correlated to relative reaction rates



Absorbance, Fluorescence measurements → Concentration of Mg-protoporphyrin → Reaction rates of Mg-chelatases → Enzyme Activities

Spectroscopy Results

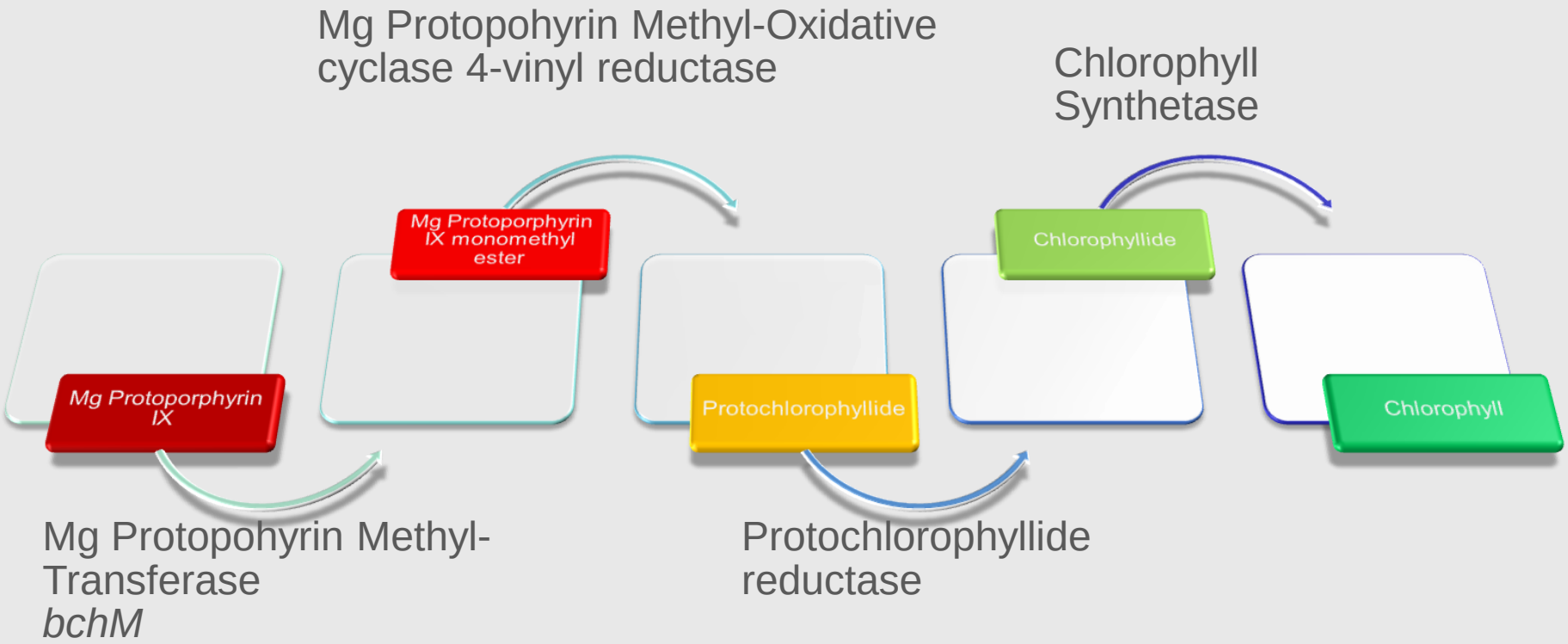
- Absorbance inconclusive (possibly due to interference)
- Fluorescence Emission Spectrum
  - *Rhodobacter* has more efficient Mg-chelatase



Bacteriochlorophyll Phase II: Chlorophyllic Device for Expression Optimization

In studies done by Xion et al 1998, complete sequence analysis of cosmid pHM6, which completed mutations in the protochlorophyllide reductase genes bchL, bchL and bchB, indicated that it contained an 8355bp region that codes for several genes involved in bacteriochlorophyll biosynthesis (bchM, bchE, bchL, bchN,bchB,bchI and bchD). Included in this region is the reaction center (RC) structural polypeptide coded by pshA gene.

Pathway - Phase II



Goal

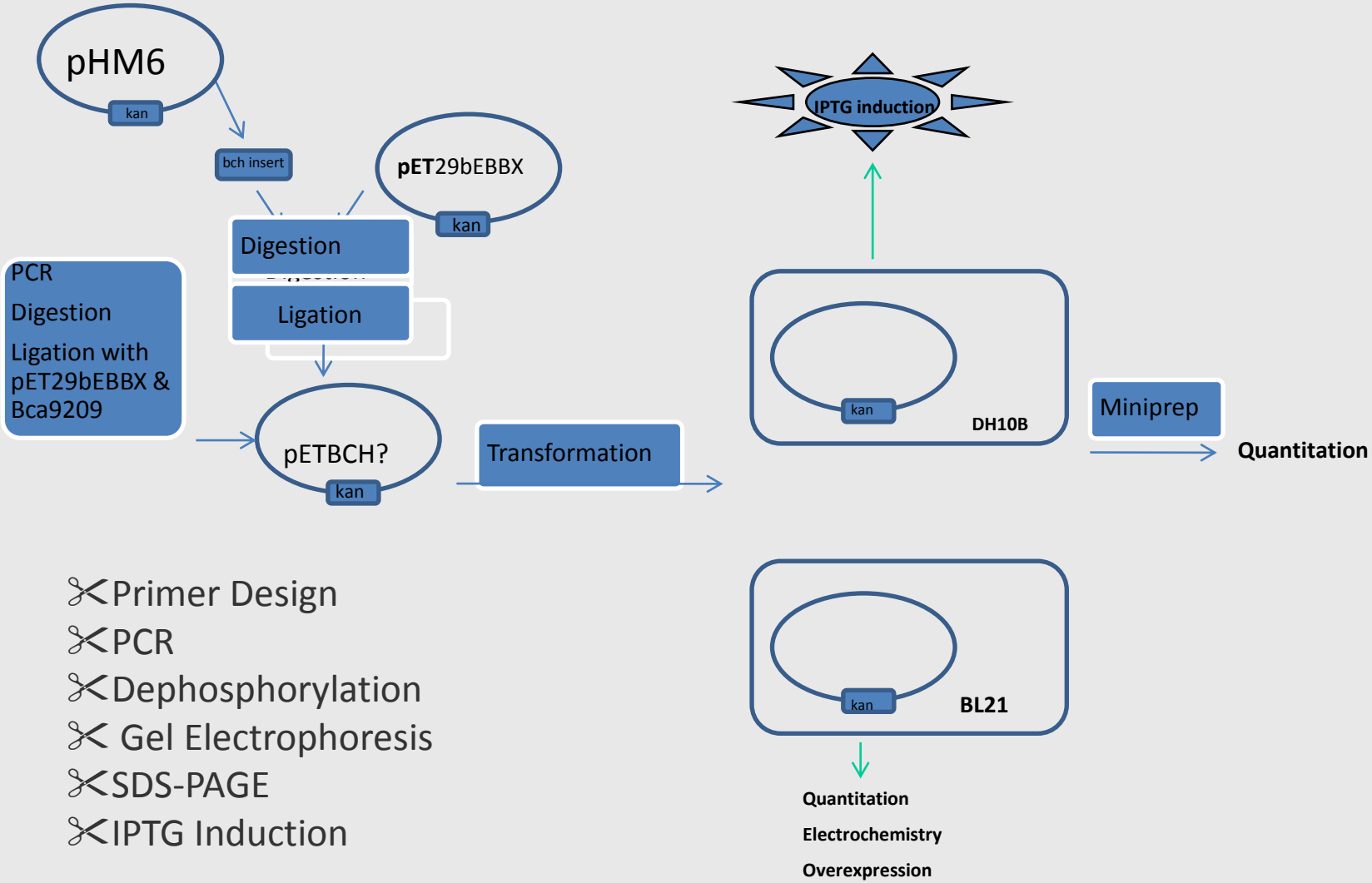
- Design a set of reconstitutive devices for chlorophyll synthesis
- To demonstrate that the 3 proteins BCHM, BCHE and BCHLNB are overexpressed in a heterotrophic prokaryotic system and are are sufficient for cyclase, reductase and transferase activity.
- Need to determine, that the clustering of genes would lead to sequestering of protoporphyrin IX.

Mode of Action

- Primer pairs were designed to amplify 6 fragments of the pHM6 bch gene cluster and construction of a chlorophyllic device that would be used for **overexpression and redirection.**

- **Restriction Mapping using Biobrick 2.0 on the MCS of the pET29bEBBX**
- **Subclonning and partial sequencing**.
- A genomic library was constructed by partial digestion with EcoRI/BamHI and BgII/XhoI followed by ligation of the DNA fragments into EcoRI/XhoI and BgII/XhoI site of the cosmid vector pET29bEBBX.

Methods



- ✂Primer Design
- ✂PCR
- ✂Dephosphorylation
- ✂ Gel Electrophoresis
- ✂SDS-PAGE
- ✂IPTG Induction

Conditions

- Cells cultures @ 37° C in LB + kan
- Ligation cultures 37° C in LB agar
- Transformants @ 30° C degress in LB + kan + shaking
- DH10B and BL21 cells

Constructs

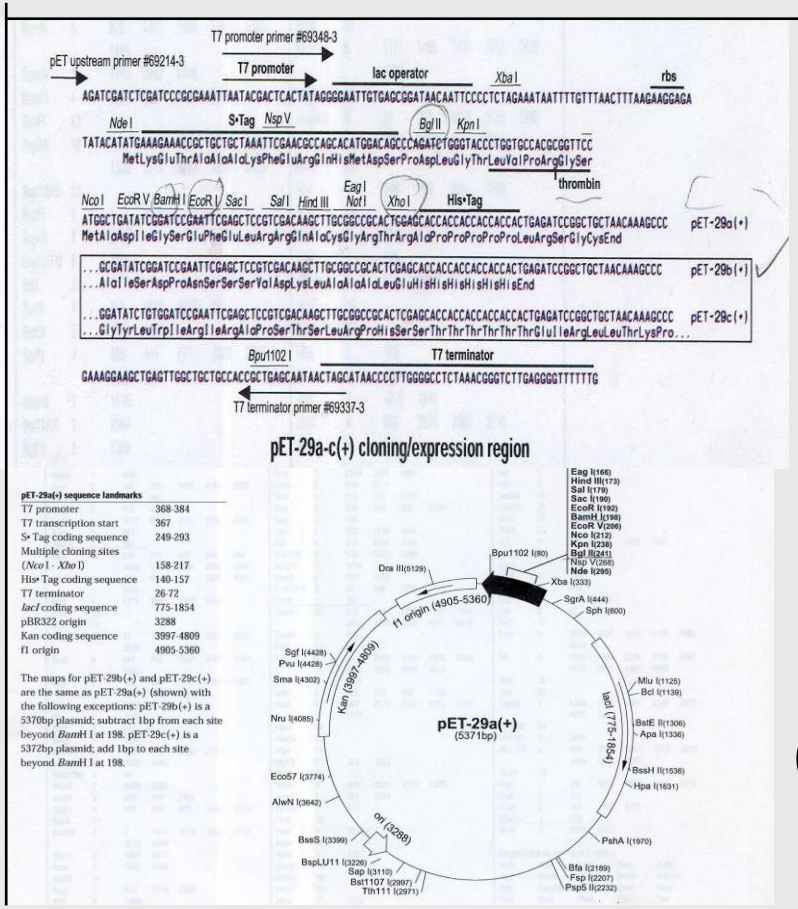
bchB	bchE	bchL	bchM	bchN

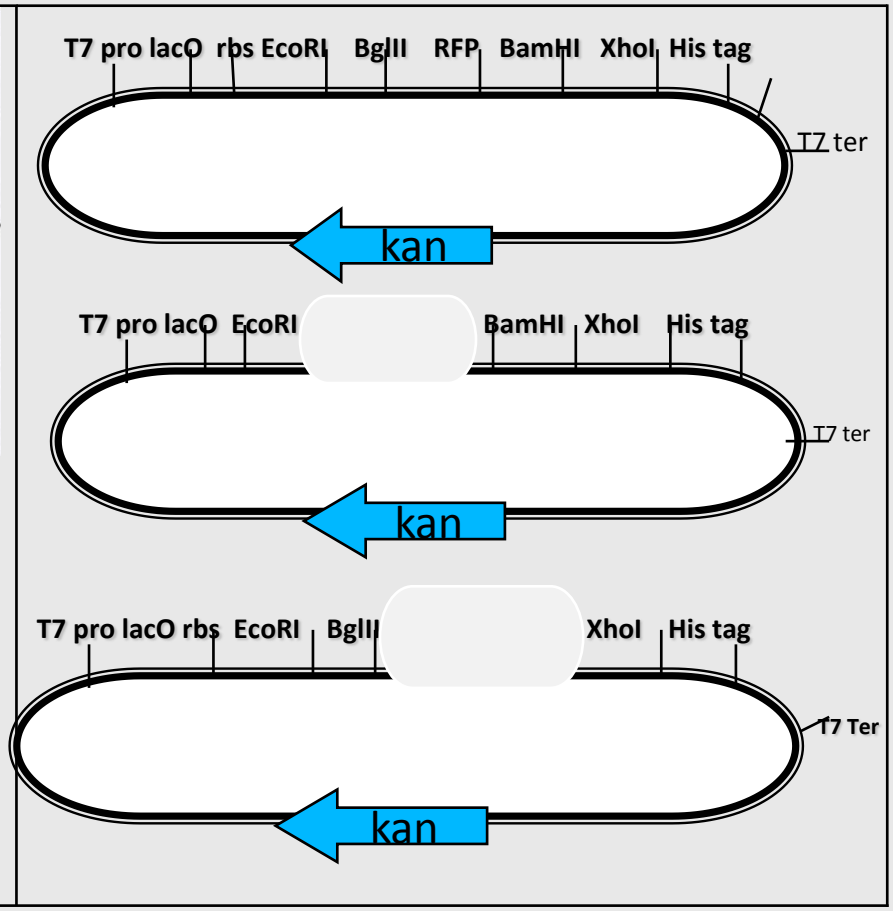
Experimental Setup

bch inserts + pET29bEBBX no RFP		controls Bca9209+GFP Bca9209(L) pET29bEBBX +RFP

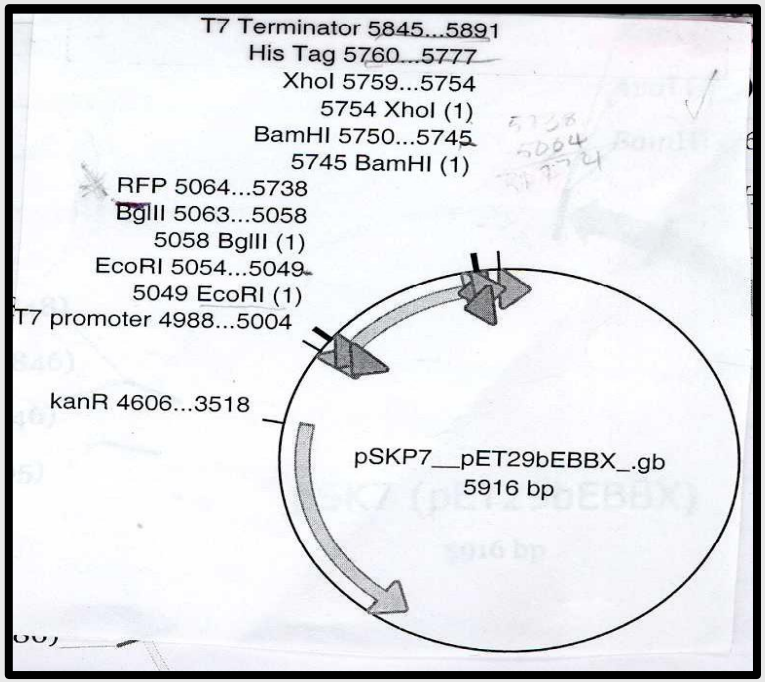
lac induced T7-promoter system

- High expression levels of
- Lac operator to reduce leaky expression
- Cytoplasmic expression
- Kanamycin gene for greater selection in E.coli.

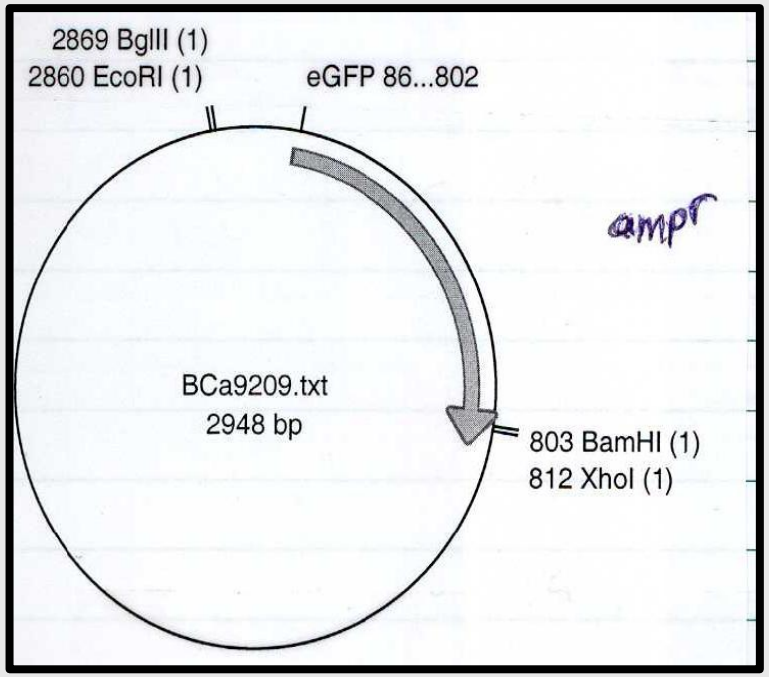




Plasmid Maps



pET29bEBBX



Bca9209 Positive Controls

The Device

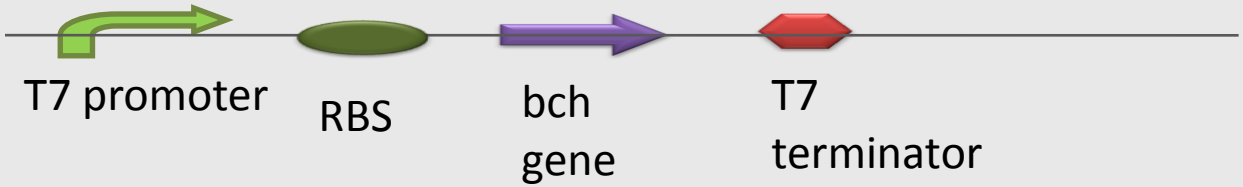
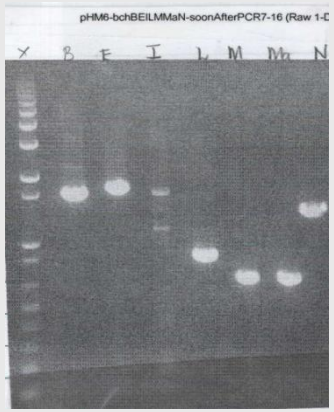


	Table 1. Function of ORF's of Heliobacillus mobilis	
gene	function	size (basepairs)
bchL	**""""""**	864
bchN	**subunit of light-independent protochlorophyllide reductase**	1323
bchB	**""""""**	1587
bchE	Mg protoporphyrin IX monomethyl ester oxidative cyclase	1701
bchM	Mg protoporphyrin Methyl Transferase	684
bchI	subunit of the Mg-chelaste	1092

- Enhance transcription-T7
- Increase gene product
- Transformation efficiency

Gene Isolation and digests

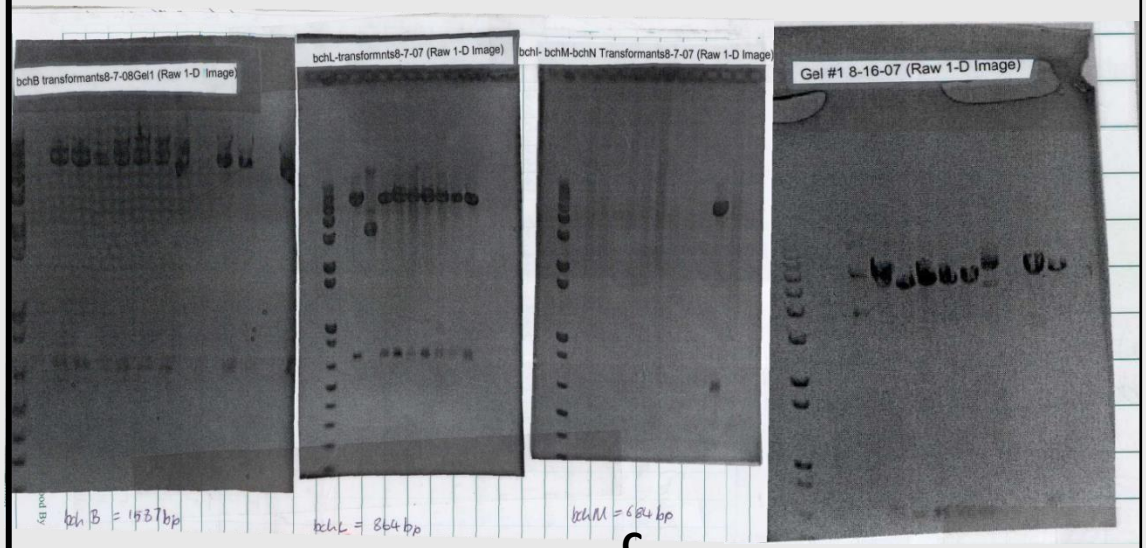


A

B

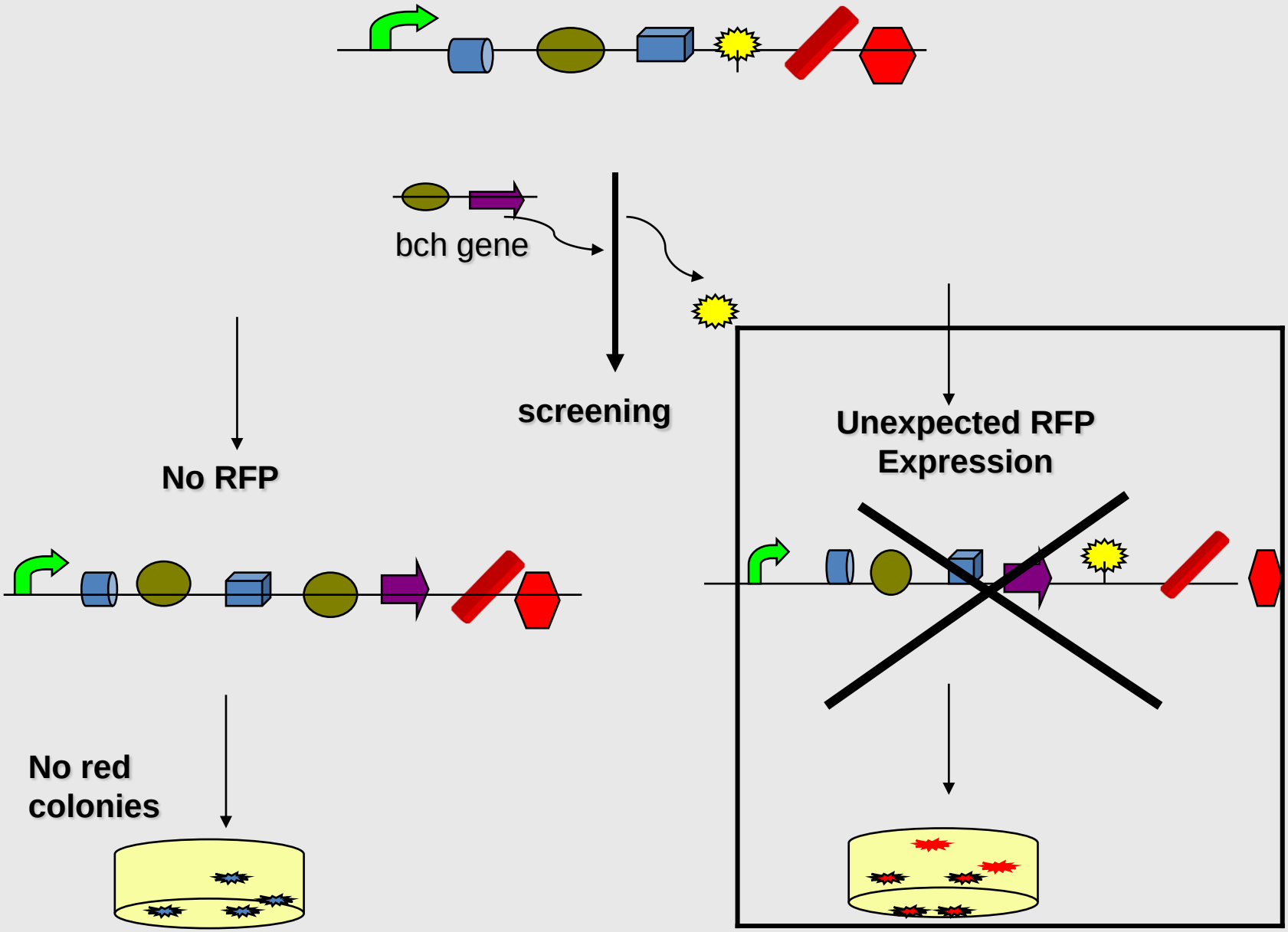
A-bch inserts

B-plasmid digest



C

C-subcloning products


bch gene
screening
No RFP
Unexpected RFP
Expression
No red
colonies

Phase II Results

bch gene cluster Insert Values

Insert	CFU's		RFP	DNA Ng/ul -digests	Transformation efficeincy %
bchB	13		X	126.4	11.5
bchE	2		X	96.6	2
bchI	10		X	86.6	11.6
bchL	10		X	80.5	12.4
bchN	5		X	98.2	5
bchM	4		X	97.2	4.11

Transformation Efficiency= CFU/DNA conc (ng/ul)

- Reconstitution of bch B,E,I,L,M,N from *Heliobacillus mobillis*
- Successful subcloning although with low transformations efficiencies
- Subcloning worked and confident that the device encoded necessitates transcription effciency/optimization.

Conclusion

- Biological parts are able to perform their intended functions
- *Rhodobacter sphaeroides* has the better enzyme
  - GC Content has no effect
- Subcloning M, N, B, L, I, E successful
- Future Research:
  - Quantitative rate expressions
  - Explore the rest of bacteriochlorophyll biosynthesis pathway
- Applications:
  - Industrial-scale production of biofuels using bacteria
  - Production of chlorophyll for research purposes
  - Use of novel enzymes to insert specific metal ions into compounds such as protoporphyrin IX.

Acknowledgements

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- Connie Kang
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- Kevin Costa
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- SynBERC
- Lawrence Berkeley National Laboratory

References

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