ON THE FOUNDATION OF

BLOC-MAGNETO

International Cooperation Initiative on *de novo* Construction of pYMB to expand new horizons for future iGemers' crazy imaginations

Proj. Primary Executive: CHUAN-YIN, HSIA
Bloc Founder: SHANG-JUI, TSAI
NYMU-Taipei Team Leader: CHUN-YU, LAI
Team Primary Advisor Prof. CHUAN-HSIUNG CHANG

Contacts: cheyennexym@gmail.com Chuan-Yin, Hsia; sam255676@yahoo.com.tw Shang-Jui, Tsai; nick0354@hotmail.com Chun-Yu, Lai; cchang@ym.edu.tw Prof. Chuan-Hsiung, Chang

SUMMARIZED WORKFLOW

REP-1 REP-3 REP-5 REP-7 REP-9 REP-11 REP-13 REP-15 REP-17 REP-19 REP-21

REP-2 REP-4 REP-6 REP-8 REP-10 REP-12 REP-14 REP-16 REP-18 REP-20 REP-22

REP Fragment 1: 464 bp

REP Fragment 2: 464 bp

Fragment 1

Fragment 2

REP-22

REP-1

990 bp REP

Bam HI

ORF 2
mob gene
1,107 bp

pMGT
3,741 bp

ORF 1
rep gene
828 bp

Sequence preserved for 5 bp

E X S P

Shine-Dalgarno sequence
Position: 1711 to 1713

rep gene
828 bp
Position:
1727 to
2554

E X S
Ori

P_{mms16}

E X S P

psBP16

Amp^R

E X S P

psBYMo

Amp^R

E X S P

Ori

pYMB

Amp^R

Motivation—*"Throughout the history of iGem, …"*

Throughout the history of iGem, we've gained notice from exhaustive online searching that a remarkable number of previous iGem teams have continually proposed projects relevant to ***magnetosome***, ***magnetospirillum***, and ***magnetotaxis*** every year around the world.

Among these respectable pioneers, Duke University had made their magnetotactic bacteria-based project to the official jamboree which is title "*Bacterial Dynamo*", focusing on the use of AMB-1 as AC voltage generator (Eric Josephs, Hattie Chung, *et al.*, 2006), but somehow limited by the lack of genetic manipulation toolkits for *Magentospirillum magneticum* AMB-1. Much to our pity was seeing

What's worth mentioning was the diversity of ideas regarding the application of magnetotactic bacteria proposed by a great variety of iGem teams (See **Table 1**). This even led to mentioning on 2009 iGem official website about "**magnetic bacteria**" as one of the ***Ideas for Bacteria*** (See 2009 Ideas for Bacteria).

But we soon noticed that we were at the foot of *Mt. Magneto*, where rare precedent iGemers left footprints

For now, dear fellow iGem teams around the world, we've heard your voice! Those dedications or whatever crazy fantasies regarding the magnetotactic bacteria have deeply touched us. They now provides us as the major source of courage leading to the proposal of this bold initiative.

Have you been dreaming long for a magneto-bloc? Have you been fantasized by the concept of a world made of magneto-sensing biological creatures? While most of the attempted previous iGem teams gave in, mostly during their brainstorming stage, to reasons spanning from those bacteria being hard to culture, to the lacking of thorough genetic studying and engineering tools, we now decides to fight the hesitation head-on in this year's project.

"*For this time, together we shall unite to make changes*"; this is how we NYMU-Taipei iGemers have kept in mind when we started typing this proposal, and hopefully this will become contagious thereafter.

Critical Relevance—*"Our Own Story Begins at …"*

Our own story begins at the aim of NYMU-Taipei this year—to develop a wireless neuro-stimulation system. We have been through a long, heated brainstorming process and finally settled down with the gram-negative bacterial strain *Magnetospirillum magneticum* AMB-1 as our candidate species, showing the probability of linkage between electromagnetic probing and optogenetic neuromodulation. To achieve our goal of this year, specific genetic manipulations to AMB-1 thus followed our detailed project design.

But we soon noticed that we were at the foot of *Mt. Magneto*, where rare precedent iGemers left footprints in the trails. We were presented with sparse guidelines needed to lead us the way in and out.

Table 1 | Magneto-related iGEM Teams in the History

iGEM Team (University)	Abstract	Reference
Duke 2006	**Project: "*Bacterial Dynamo*"**	http://parts.mit.edu/wiki/index.php/Duke_2006
Brown 2006	**Aimed to isolate magnetosomes which can be used as a cellular biotin reporter that could possibly be detected via magnetic resonance imaging.**	http://parts.mit.edu/wiki/index.php?title=Brown:2006/Magnetotactic_Bacteria_project&redirect=no&printable=yes
PennState University 2007	**Lab Book Discussion about Bacterial Magnetosomes**	http://openwetware.org/wiki/IGEM:PennState/Labbook/GalenLynch#Bacterial_Magnetosomes
TUDelft 2008	**Brainstorming and discussing for the application of Magnetotactic bacteria.**	http://2008.igem.org/Team:TUDelft/Brainstorm
Cambridge 2008	**Aimed to produce magnetic organelles in *E .coli* and yeast that resemble magnetosomes naturally formed in magnetotactic bacteria.**	http://openwetware.org/wiki/IGEM:Cambridge/2008/Notebook/Magnetic_Bacteria
	Modeling animal nervous system in prokaryotic chassis has a wealth of potential applications in synthetic biology.	http://openwetware.org/wiki/IGEM:Cambridge/2008/Concept
Cornell 2009	**Magnetotactic bacteria is used for heavy-metal decontamination of water.**	http://www.news.cornell.edu/stories/Feb09/iGEMteam.html
BCCS-Bristol 2009	**Describing the magnetotaxis of the magnetic bacteria in the direction of the magnetic field.**	http://2009.igem.org/Team:BCCS-Bristol/BSim/Features/Magnetotaxis
Northwestern 2010	**Project Voting: Magnetite synthesis by magnetic bacteria.**	http://2010.igem.org/Team:Northwestern/Brainstorm
TUDelft 2010	**Magnetic Bacteria coupled to Styrene sensing and binding**	http://2010.igem.org/Team:TU_Delft/5_May_2010_content
Uppsala-Sweden 2010	**Aimed to insert the chemotactic (thermotactic) protein into magentotactic bacteria**	http://2010.igem.org/Team:Uppsala-SwedenWeek2
Toronto 2011	**Aim to incorporate a biomineralization pathway for magnetite in *E. Coli*, and creating a method of novel gene expression using magnetic fields.**	http://2011.igem.org/Team:Toronto
Washington 2011	**Aims to put some of magnetotactic bacterial genes into *E. coli* to attempt to show production of magnetosomes, or magnetosome intermediates.**	http://2011.igem.org/Team:Washington

We then decided to pioneer our way into the forest, aiming to establish a BioBrick-compatible genetic engineering system on AMB-1 which never existed before in Parts Registry.

Background Info—*"For the upon-listed reasons…"*

For the upon-listed reasons and current hurdles in front of us, we set off searching information outside iGemer's realm. We later happily found sound theoretical supports from papers published from Matsunaga T.'s laboratory in Tokyo University of Agriculture and Technology.

In their pioneering works as far back in the 1980s to recent publications regarding ubiquitous applications of magnetotactic bacteria (mainly related to AMB-1; see **Table 2**; see also Tomoko Yoshino, *et al.*, 2010 for full review of recent progresses), they have achieved fruitful discoveries

spanning from optimized culturing methods (Chen-Dong Yang, *et al.*,2001), identification of endogenous strong promoters (Tomoko Yoshino, *et al.*, 2005) to construction of a cryptic plasmid, **pUMG**, as a high copy number shuttle vector between Escherichia coli and AMB-1 (Yoshiko Okamura, *et al.*, 2003).

We've been excited to find out such good news to us, and subsequently tried e-mailing several times before June 2 to ask for the possibilities of testing the vector pUMG. To our disappointment, access to pUMG was the declined due to strict controlling of this vector to other researchers by Matsunaga T.'s laboratory.

To our great relief, the construct of pUMG is very simple, with **pUC19** ligated on ***BamHI*** site with **pMGT**, an endogenous plasmid found in *Magnetospirillum magneticum* MGT-1. With historical records of the first identification of this strain by Matsunaga T., *et al.* (Matsunaga T., et al., 1990), and some responses from magnetotactic bacteria researchers all over the world (we e-mailed Long-Fei Wu, Radu Popa, Arash Komeili, and Grant Burgess, *et al.*) that claimed not having such strain, we inferred that it should be next to impossible for us to isolate pMGT from fresh culture of MGT-1. Therefore, *de novo* synthesis of genes playing crucial roles in **pUMG** seems to become the only way to the solution.

Our Solution—*"The construction of…"*

The construction of a new shuttle vector named **pYMB** is therefore to be the primary aim of our cooperative initiative.

Table 2 | Selected References from Tokyo University of Agriculture and Technology:

Authors	References	Comment
Chen-Dong Yang, *et al.*	**Effects of growth medium composition, iron sources and atmospheric oxygen concentrations on production of luciferase-bacterial magnetic particle complex by a recombinant Magnetospirillum magneticum AMB-1 (2001)**	Optimized Cultivation and **Growth Medium Composition**
Tomoko Yoshino, *et al.*	**Design and Application of a New Cryptic-Plasmid-Based Shuttle Vector for *Magnetospirillum magneticum* AMB-1 (2003)**	Establishment of an Efficient Expression System on AMB-1 with ***Shuttle Vector*** and ***Strong Promoters***
Tomoko Yoshino, *et al.*	**Development of efficient expression system for protein display on bacterial magnetic particles (2005)**	
Tomoko Yoshino, *et al.*	**Inducible Expression of Transmembrane Proteins on Bacterial Magnetic Particles in *Magnetospirillum magneticum* AMB-1 (2010)**	establishment of a ***tet-ON*** system on AMB-1
Tomoko Yoshino, *et al.*	**Bioengineering of Bacterial Magnetic Particles and their Applications in Biotechnology (2010)**	Review of the Current Trends and Achievements
Tadashi Matsunaga, *et al.*	**Molecular analysis of magnetotactic bacteria and development of functional bacterial magnetic particles for nano-biotechnology (2007)**	Review of Major Achievements Prior to 2007

pYMB is described to contain the ***ori*** (origin of replication), ***rep*** gene (required for replication) and/or ***mob*** gene (required for mobilization) of **pMGT**. To work on the safe side, we plan to test the necessity and sufficiency of the synthesized ***rep*** gene and ***mob*** gene for replication and maintenance of **pYMB** within AMB-1. Once the synthetic work is done, **pYMB** will be constructed by equipping the ***ori***, the appropriate promoter for AMB-1 (P_{mms16} and P_{msp3} as our candidate) and ***rep*** gene and/or ***mob*** gene on BioBrick backbone pSB1A3. The constructed vector is then anticipated to be capable of replicating within both E. coli and AMB-1, fully sufficing a competent shuttle vector for genetic engineering the magnetotactic bateria.

The achievement of **pYMB** synthesis, designed to be compatible with biobrick standard, will provide a powerful genetic engineering platform for the official introduction to the whole iGem community about *Magnetospirillum magneticum* AMB-1, the dreamed-species for iGemers every year around all corners of the world.

Furthermore, our *de novo* synthetic work will directly open a door to an immense number of new ideas associating non-contact force (i.e., magnetic force) with biochemical pathways, allowing interdisciplinary integration of physics and biochemistry in future years of iGem competitions, revolutionizing traditional thoughts of “circuit design” in which we see the majority of current Biobrick parts function only by way of pure biochemical driving forces, and most genetic circuits being slow-reacting on the time scale of minutes or even hours.

It’s time to make a difference! We badly need all of you in setting the major milestone on top of *Mt. Magento* this year. Join us in this wave of revolution!

Fig. 1 | *Mt. Magneto* (Beitou Hills); the prospect point of NYMU. (GPS coordinate: 120.323026, 22.678469)

Oligonucleotide-assembling gene synthesis:

Overall Workflow

1. Determine the graphic of whole pYMB (circularized form) *in silico*.
2. Computational design of optimized oligonucleotides for PCR-Cycling Assembly (PCA)
   - ✓ Using DNAWorks / GeMS for designing.
   - ✓ Adding BioBrick prefix and suffix to the target sequence.
3. Purchase oligonucleotides and PCR-cycling assembly:

Theoretical Basis

We base our *de novo* gene synthesis method on the paper published by Ai-Sheng Xiong, *et al.* in *Nature Protocols* (Ai-Sheng Xiong, *et al.*, PCR-based accurate synthesis of long DNA sequences, *Nat. Protocals*, **Vol. 1(2)**, pp791-97 (2006)); the following paragraphs are selected excerpts from the context:

"

a) In recent years, a number of oligonucleotide-based methods for synthesis and assembly of DNA sequences have been described. Early methods were based on enzymatic ligation or the *FokI* method. Subsequently, self-priming PCR, PCR assembly and template-directed ligation (TDL) were developed. More recently, methods for the synthesis and assembly of long DNA sequences were published. Representatives of these methods are the PCR-based thermodynamically balanced inside-out (TBIO) method, two-step total gene synthesis using both dual asymmetrical PCR (DA-PCR) and overlap-extension PCR (OE-PCR), successive PCR, PCR-based two-step DNA synthesis (PTDS), and the sequential ligation and polymerase cycling reaction method. Major drawbacks of total gene synthesis are the high cost and high error rate. Here, we describe an improved version of the PTDS method for synthesis of long, accurate DNA sequences. The PAS protocol is relatively simple (two PCR steps), rapid (**~1 wk** from the design of a target gene to the final product), accurate (≤**<1 error per 1,000 bps** synthesized) and inexpensive.

b) The PAS (PCR-based Accurate Synthesis) protocol involves the following five steps:
   (i) Design of the DNA sequence to be synthesized and of 60-bp overlapping oligonucleotides to cover the entire DNA sequence;
   (ii) Purification of the oligonucleotides by PAGE;
   (iii) First PCR, to synthesize DNA fragments of 400-500 bp in length using 10 inner (template) and two outer (primer) oligonucleotides;
   (iv) Second PCR, to assemble the products of the first PCR into the full-length DNA sequence;

(v) Cloning and verification of the synthetic DNA by sequencing and, if needed, error correction using an overlap-extension PCR technique.

This method, which takes ~1 wk, is suitable for synthesizing diverse types of long DNA molecule.

c) Based on the DNA sequence, 60-bp oligonucleotides that overlap neighboring oligonucleotides by ~21 bp (56 ° C melting temperature (*Tm*)) are designed and chemically synthesized. To reduce errors in the synthetic gene, we recommend that all oligonucleotides are purified using denaturing PAGE. Groups of 12 adjacent 60-bp oligonucleotides are then used to produce DNA fragments 400-500 bp in length using PCR

"

Through these days, we NYMU-Taipei iGemers have been designing oligonucleotides for our gene synthesis using DNAWorks (web server). The principles adopted by us are mainly based on the *Nature Protocols* paper with 60-mers as length of the majority of the designed oligonucleotides for PCR assembling. *Tm* (Annealing Temperature) of each pair of oligonucleotides is controlled within 58 to 62, with overall *Tm* range being 5℃.

The ***rep*** gene to be synthesized is designed to be flanked by its endogenous Shine-Dalgarno sequence, several reserved sequences aimed to optimize the range of pair-wise melting temperatures, and BioBrick prefix and suffix, making a total length at about 990 bps. As for ***ori***, it is currently planned to be synthesized in NYMU, with total length being ~140 bps, centered by the putative ***ori*** sequence. To add a few, the synthetic ***ori*** sequence will also be flanked by additional Biobrick prefix and suffix (already covered in the ~140 bps-long synthetic DNA).

Upon assembling the oligonucleotides, instructions from the *Nature Protocols* "PCR-based accurate synthesis of long DNA sequences" are highly recommended. Heat-resistant *pfu* polymerase, as suggested by the paper, is preferred for its high fidelity required for accurate PCR-based assembly of oligonucleotides; commercialized *pfu* buffer is also confirmed by us (*in silico*) to be optimal for fine PCR-based assembly without additional $MgSO_4$ or other minerals. In case *pfu* buffer is inaccessible, the following recipe can be referred to (David M. Hoover, *et al.*, 2002):

"

The final concentrations of components were 0.2 ng/μl for each oligonucleotide, 20 mM for Tris-HCl (pH 8.8), 10 mM for KCl, 10 mM for $(NH_4)_2SO_4$, 6 mM for $MgSO_4$, 0.1% (v/v) for Triton X-100, 0.1 mg/ml for bovine serum albumin, 0.2 mM for each dNTP and 2.5 U for *Pfu* polymerase."

"

For detailed experimental steps, please follow the Nature Protocols paper by Ai-Sheng Xiong, *et al*. We will be more than happy to discuss with all of you in later stages of our collaboration and we're open to making rational alterations to the protocol.

Provided below are a graph (**Fig. 2**) demonstrating the final construct of **pYMB** and a flowchart graph (**Fig. 3**) showing computer-aided design of oligonucleotides used for PCR-based Accurate Synthesis (PAS). In the final construct of **pYMB**, there is designed to contain an ***ori*** with an appropriate promoter for magnetotactic bacteria, followed by the ***rep*** gene. This final construct is currently planned to be finished in NYMU, with promoter- and ***ori***-contained **pSB1A3** (also named **pSBYMo**) as vector for back-insertion of the ***rep*** gene. Detailed information about the ***rep*** gene, our optimized results from DNAWorks for oligonucleotide designing, and codon usage of *Magnetospirillum magneticum* AMB-1 are listed within the **Supplementary Information**.

The total time for the construction of **pYMB** is planned to span about two weeks from the beginning of the synthetic works, which means, theoretically we will be expecting the total construction to be finished in late August.

Fig. 2 | Construction of pYMB from *de novo* synthetic oligonucleotides and BioBrick pSB1A3 backbone.

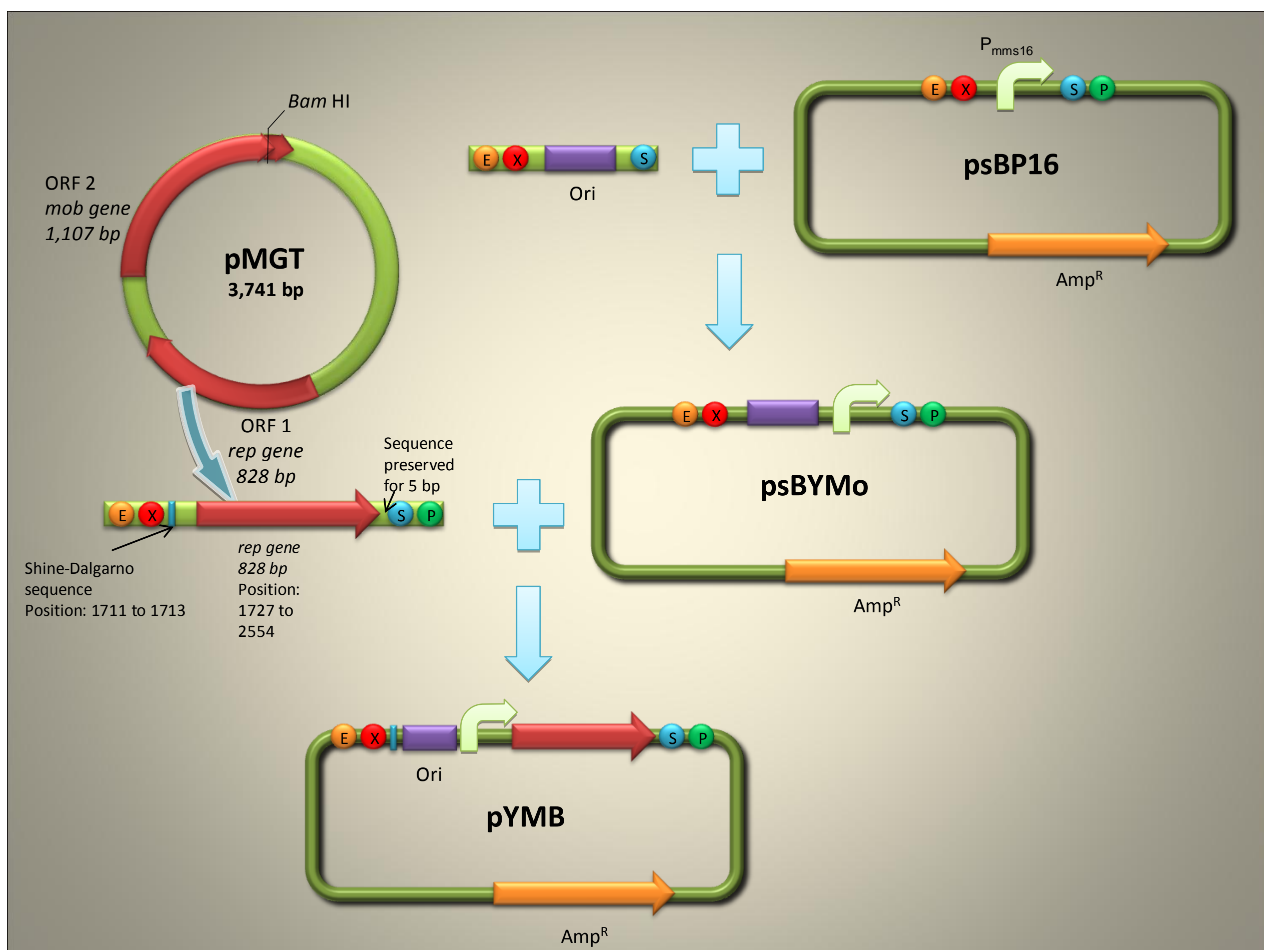
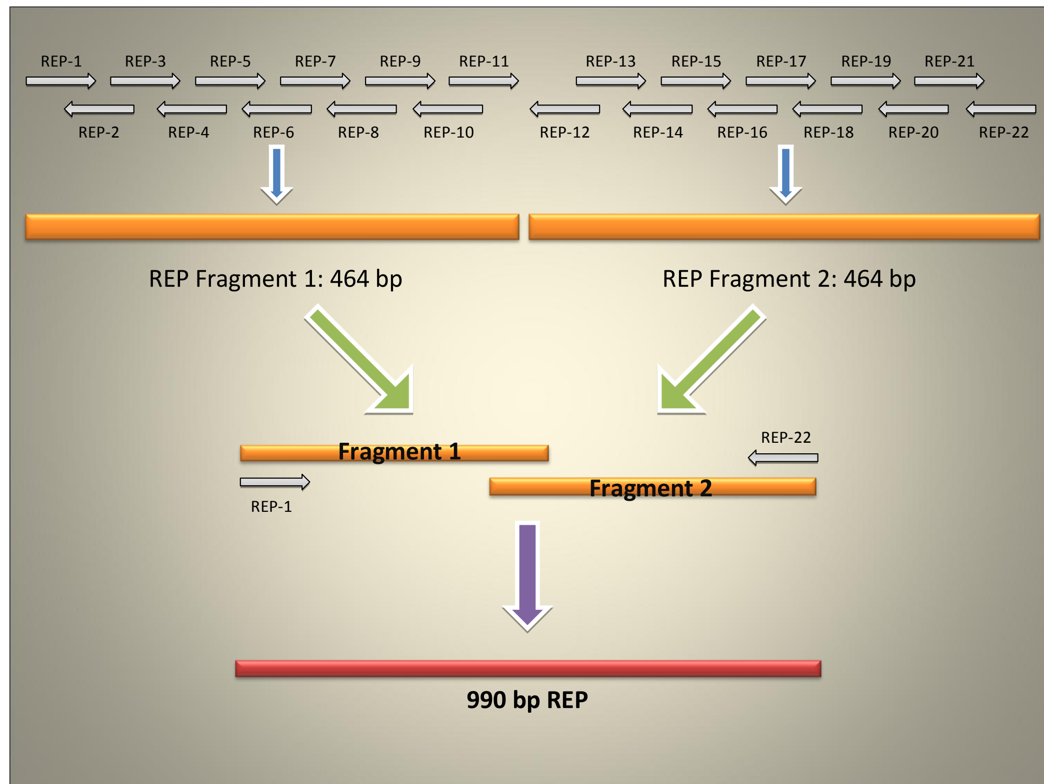
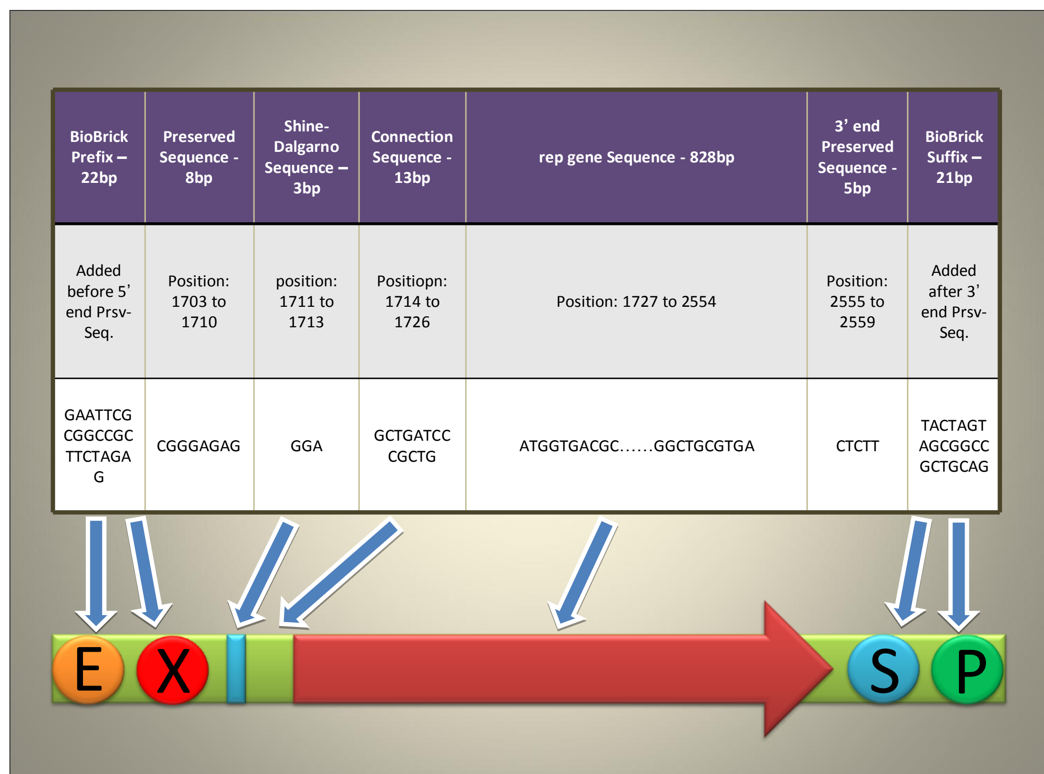


Fig. 3 |【A】Oligonucleotide synthesis of ***rep*** gene from pMGT include running PAS with 22 fragments (REP-1 to REP-22) to derive the intermediate REP Fragment 1 & 2. Later PAS between Fragment 1 and 2, using REP-1 and REP-2 as forward and reverse primers, give the final ***rep*** gene product.**【B】**The ***rep*** gene construct comprises of Biobrick prefix, a 5' reserved region, Shine-Dalgarno sequence and the open reading frame followed by another 3' reserved region and Biobrick suffix, adding up to 990 bps in length.

A.



B.



BioBrick Prefix – 22bp	Preserved Sequence - 8bp	Shine-Dalgarno Sequence – 3bp	Connection Sequence - 13bp	rep gene Sequence - 828bp	3' end Preserved Sequence - 5bp	BioBrick Suffix – 21bp
Added before 5' end Prsv-Seq.	Position: 1703 to 1710	position: 1711 to 1713	Positiopn: 1714 to 1726	Position: 1727 to 2554	Position: 2555 to 2559	Added after 3' end Prsv-Seq.
GAATTCG CGGCCGC TTCTAGA G	CGGGAGAG	GGA	GCTGATCC CGCTG	ATGGTGACGC……GGCTGCGTGA	CTCTT	TACTAGT AGCGGCC GCTGCAG

Postscript—*“Brainstorming in the Cloud …”*

“***Brainstorming in the Cloud***” has been called to realize the exchange of iGemers’ fantasies regarding magnetotactic bacteria.

Recently we’ll be adding you to the Facebook club “**BLOC-MAGNETO worldwide | 2011 iGem @NYMU**”, please feel free to post your opinions onto the wall, and we will be holding an online brainstorming session focused on the topic: “***Transformations and Improvements of Past iGem Team Projects If Magnetotactic Bacteria Were Introduced in the First iGem competition***”, (***i.e.*** imaginarily rewriting the history of iGem, given magnetotactic bacteria as a user-friendly chassis in the very beginning).

Later in August or September, we will be expecting another international online symposium on **proposed future generation of iGem team projects**, with easy-to-engineer genetic toolkits easily accessible for each team around the world.

Set free on your fantasies about magneto-biology. And make sure to be prepared for going wild in picturing the future of iGem while altering its own history at once! Got ready to show your heavy impacts?

Go!!!

About NYMU-Taipei—*“We erds…”→”We’re Magneto-es”*

NYMU-Taipei team consisting of enthusiastic undergraduates from a wide range of fields and disciplines including: Bioinformatics, Biomedical Engineering, Physical Therapy, Medical Technology, Life Sciences, and Medicine. We’ve been participating iGem since 2007, awarded with gold medal for twice, silver for once and bronze for once. We **got into the finalist in 2008** with topic “***BacToKidney***”. We are dreaming big (sometimes too big XD) this year and feel excited to be part of the iGem community. We also sincerely welcome any form of contact or opinion, just feel free to give us your feedback!