Project overview

Bio capsule
Aggregation module  Bio-plastic producing module

Biobrick Review
Project overview

Bio capsule

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Biobrick Review
Aggregation using Ag43

Aggregation module

EASY

DIFFICULT
Project overview

Bio capsule

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Biobrick Review
The best way to produce Bio-plastic

Bio-plastic synthesis

R. eutropha → Poly(3HB) → E. coli
Aggregation Module

Plastic Producing Module

Bio capsule

Hard Cluster

Valuable substance

Bio capsule

Valuable Hard Cluster

Harvesting by Filtration & Decantation
Project overview

Bio capsule
Aggregation module
Bio-plastic producing module

Biobrick Review
• A bio-communication tool for iGEMers.

• You can learn more biobrick parts and communicate with other iGEMers all over the world.
Centrifugation takes much time

Centrifuge

Remove Supernatant

Add Medium

Culture Medium
Filtration can harvest continuously.

Continuous supply of medium

Filtration

Filtrate

Culture Medium
Antigen 43 (Ag43) is...

- An adhesion molecule
- A cell surface protein
- Assists aggregation
Ag43 expression image
Induction of aggregation module

L-arabinose

AraC

P_{BAD}

RBS

Ag43

dT

Translation

Aggregation
Aggregation was confirmed

- 5ml LB medium
- Rotary shaking at 180 rpm, 37°C
- Medium is clear in the presence of arabinose

24 hrs later
Aggregation result in reduction of optical density

Graph showing the change in OD600 of medium over time for different conditions:
- Arabinose (-)
- Arabinose (+)

Insert image showing medium samples with and without arabinose.
Harvesting method

Mesh

Stroking

Filtrate

Culture Medium
Example of filtration

36 hrs cultivation (5 ml LB)  
mesh size: 250 µm
Summary

• Characterization our aggregation module

• Establishment of an efficient method for aggregating cells to a cluster
Submitted parts

Aggregation module (BBa_K759001)

- pBAD + AraC
- RBS (BBa_I0050)
- Ag43 (BBa_K346007)
- dT

Characterization module (BBa_K759012)

- pBAD + AraC
- RBS (BBa_I0050)
- eCFP (BBa_E0020)
- RBS
- Ag43 (BBa_K346007)
- dT

BBa_B0034
BBa_B0015
What is Poly(3HB) ?

Poly-3-hydroxy-butyrate, Poly(3HB) is **bio-plastic**.

It is made by bacteria as an energy-storage molecule.
→ **bio-degradable**

It is made from **glucose or soybean oil**.
It is a part of carbon cycle.

It is made from glucose or soybean oil.
P(3HB) is made from acetyl-CoA in 3 steps

Three enzymes encoded in phaCAB operon works to produce bio-plastic.
Product identified as Poly(3HB) with GC/MS.

▲ direct standard

Peak comparison

Products by pGEM ▼
We quantitatively analyzed Poly(3HB) production among various conditions. TB is the best medium to produce Poly(3HB)!

Transformed plasmid: pGEM(PhaCAB)
Best condition for Poly(3HB) production

- **TB** helps bacteria growth and Poly(3HB) production.

- **Pantothenic acid** and **Glucose** are substrates of the pathway.

- **JM109** is more closely related to wild-type than DH5α.
We successfully identified and quantified Poly(3HB)!

✓ We found a substitute for common plastic and produce it in *E.coli*.

✓ We **identified** the product as bio-plastic, Poly(3HB).

✓ We **established the best condition** to produce bio-plastic.
Bio capsule system

Plastic producing module

Aggregation module
Experiments

• We transformed a plasmid of “plastic producing module” into the cell of “Aggregation module”

• The “Aggregation module” is induced by the addition of 1% Arabinose.

• The “Plastic producing module” is constitutively expressed and supplemented with addition of glucose.
Stronger aggregation produced clearer supernatant

Ara: (+)  (+)  (-)
Glu: (-)  (+)  (+)

Conditions 37°C, 180 rpm, 1.5 ml

24 hrs

48 hrs
Clusters of cells

Bio capsule!
Fast precipitation was observed after incubation of 48 hrs, 180 rpm, 50 ml. With both modules precipitation was even faster!
The production of P3HB was confirmed

(+) Aggregation module
(−) Plastic producing module

Nile red staining

Bright field
The production of P3HB was confirmed by HPLC test. The results for Arabinose induction showed (+) for both assays, while Glucose induction had (-) for the first assay and (+) for the second assay.
We can collect cells by filtration or by decantation in large scales!

The expression plastic increases the strength of aggregation!
We submitted eight parts

We are now testing the production of plastics with our parts!
Contribution to manufacturing

- Bio capsule system largely contributes to the method of harvesting bacterial cells!
- The model system for producing P3HB can be extended to other kinds of plastic production!

Easy and low costing!
Human Practice

A Brand New Bio-communication Tool
Our Issues

Too detailed information in PartsRegistry

Unexpected behaviors of BioBrick Parts
Solution

We want to share OUR opinions.
“Biobrick Review”
“Biobrick Review”

Discuss issues of BioBricks!

Learn characters of BioBricks!

Enjoy Ranking Events as GAMEs!
How to Use BB Review

Visit http://www.bbreview.net
How to Use BB Review

Sign Up!

username: hokkaidou
email address: hokkaidou@example.com
Select your team: HokkaidoU_Japan
The list ordered by alphabetically. If you don’t belong to a team, select “unknown”.
password: ********
password_confirmation: ********

Get your account
How to Use BB Review

Find a BioBrick
How to Use BB Review

Post your review!!
For Development

WE NEED YOUR PARTICIPATION
Enjoy
www.bbreview.net
RIGHT NOW!
Achievements

✓ Submitted and characterized 11 biobricks
  2 biobricks for aggregation module
  9 biobricks for bio-plastic producing module
✓ Succeeded in making “Bio capsule” by combining aggregation protein expression and bio-plastic synthesis
✓ released biobrick social site “biobrick review” to evaluate each biobrick through enjoying ranking like a game
✓ Participated in all Japan pre-jamboree convention
✓ Had a boot camp for beginners education
✓ Found a few faulty registered parts and improved them
✓ Succeeded in synthesizing bio-plastic and confirming the product by GC/MS
✓ Had lots of fun Threw a barbeque, go skiing, and having some parties!!!!!
References

Aggregation module


• http://2010.igem.org/Team:Peking/Project/Bioabsorbent/InductiveAggregation

• http://openwetware.org/wiki/Arking:JCAOligoTutorial8

• http://www.jcat.de/
References

Bio-plastic synthesis


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Sponsors
Thanks for listening!