

2P03 Final Report

Individual: Pesara Amarasekera

Group Members: Pesara Amarasekera, William Baker, Felicity Rugard (only WL5), Daniel Wong (only WL4), Kevin Zhang

Individual Lab Section: L04

Individual Student Number: 400195491

Course : IBEHS 2P03 Health Solutions Design Projects II: Introduction to Genetic Engineering

Instructor name: Dr. Vincent Leung

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Introduction

From WL4-WL6 we used the BioBrick assembly cloning technique to attempt and create a genetically engineered organism to detect heavy metal contamination. It is important to detect heavy metals in the environment as it causes harm to biological lifeforms, copper is one of these metals. High concentrations of copper can affect marine life depending on their sensitivity to exposure, as well as inhibit photosynthesis in algae (Leung, p. 192). Synthetic biology provides the detection of heavy metals via the construction of biosensors, which was the desired outcome of this series of experiments (Leung, p. 192). To construct said organism we utilized the following genetic materials: amilCP chromoprotein (in the pAmilCP-promoterless plasmid) acts as the indicator of copper ions, and the pCusC promoter sequence acts as the detector of copper ions. The amilCP chromoprotein is used to turn the cells expressing the gene blue, this will act as the main indicator of the presence of copper (Leung, p. 171). The pCusC sequence is sensitive to copper ion expression; it works by a two-stage activation of the CusS/CusR system (Leung, pp. 192-193). CusS is a transmembrane protein which is used to detect the existence of extracellular copper ions, CusR is phosphorylated by CusS which activates the pCusC promoter. The BioBrick assembly technique was used as Biobrick parts make restriction ligation much more effective, by limiting the number of restriction enzymes used, it allows a potentially infinite number of constructs to be made by reusing the same enzymes and restriction sites (Leung, pp. 185-190). The collection of materials used in the experiments are available in Appendix I.

Methods

WL4

PCR amplification was used to amplify the gene of interest. PCR consists of 3 steps, denaturation of “template” DNA, the annealing of primers, then utilizing DNA polymerase to replicate the gene of interest (Leung, pp. 136-137). The gene of interest (GOI) for this lab was the pCusC promoter; it was amplified using standard BioBrick primers. Alkaline Lysis with the detergent SDS (sodium dodecyl sulfate), was used to create the Minipreps (MPs) used in our labs. Exposure of bacterial suspensions to the strongly anionic detergent at high pH opens the cell wall and denatures chromosomal DNA and proteins while releasing the plasmid to the supernatant, this helps isolate the plasmid we wish to use (Leung, p. 134). The Miniprep backbone was pAmilCP-promoterless. It was usually the case that gel electrophoresis was used in each lab. We used agarose gel electrophoresis- with a PCR control, PCR reaction, and miniprep- to estimate the number of base pairs (bps) present in the samples to determine if the procedure was successful.

WL5

A Restriction Digest was used in WL5 to cut the backbone plasmid and the PCR amplicon (pCusC promoter). The backbone was cut using XbaI and EcoRI-HF endonucleases, and the GOI with SpeI and EcoRI-HF endonucleases to expose overhangs (Leung, pp. 213-214). This was then purified using the DNA purification kit [2]. Ligation was used to combine backbone and insert. Each fragment has different overhangs, one with EcoRI/XbaI overhangs and the other with EcoRI/SpeI overhangs on either end because of the use of two different restriction enzymes. Because both were digested with EcoRI, the XbaI site on the plasmid can hybridize with the SpeI site on the insert (Leung, pp. 210). T4 DNA Ligase was used to ligate the overhangs together. A control was created without ligase present to determine the effectiveness of ligation using electrophoresis. Transformation was used to put the ligated product into the E. Coli vector

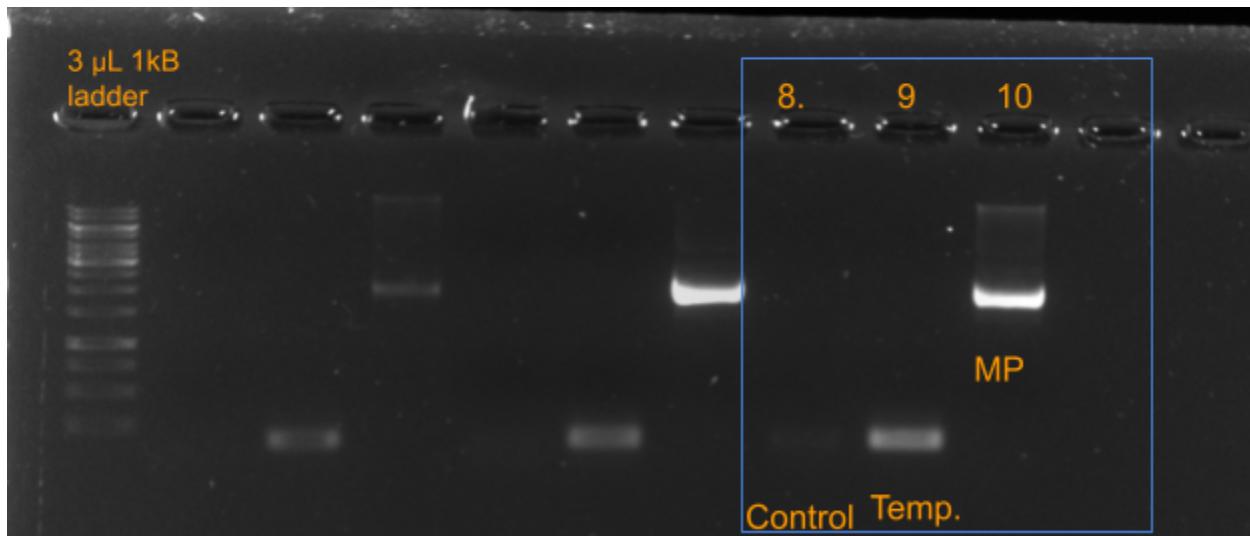
(DH5a). The plasmid must contain a resistance gene that can be constitutively expressed in the cell. This makes a selective pressure for the bacteria to obtain the plasmid and this can be used for screening transformants in a differential media. These LB-agar plates contained an antibiotic (chloramphenicol) for selection of cells that were successfully transformed with a viable plasmid, and 1mM CuSO₄ (copper (II) sulfate) to differentiate cells with a working device from those without.

WL6

A MP and a Digest of the transformed product from WL5 was created. Spectrophotometry was used to measure the efficacy of the circuit by varying concentrations of copper and measuring absorbance at a maximally absorbed wavelength. The Diagnostic Digest with pCusAmil-IBM digested with EcoRI and SpeI was compared to a control plasmid with no insert digested the same way.

Results

WL4



8. The Control is approx. 250 bp
9. The Template (pCusC BioBrick PCR Ampicillin) is approx 250 bp as well
10. The MP is approx 2000bp

Figure 1: The MP (Miniprep) was expected to appear bright and around 2kbp (as in the coursepack) and as expected from the plasmid map [3], the MP contains a linear plasmid taken for the backbone that was digested. The template appears fairly concentrated enough and appears fairly nearly where it is expected (being 157 bp long) (Leung, pp. 193). The control should have no bands but it is slightly visible.

WL5

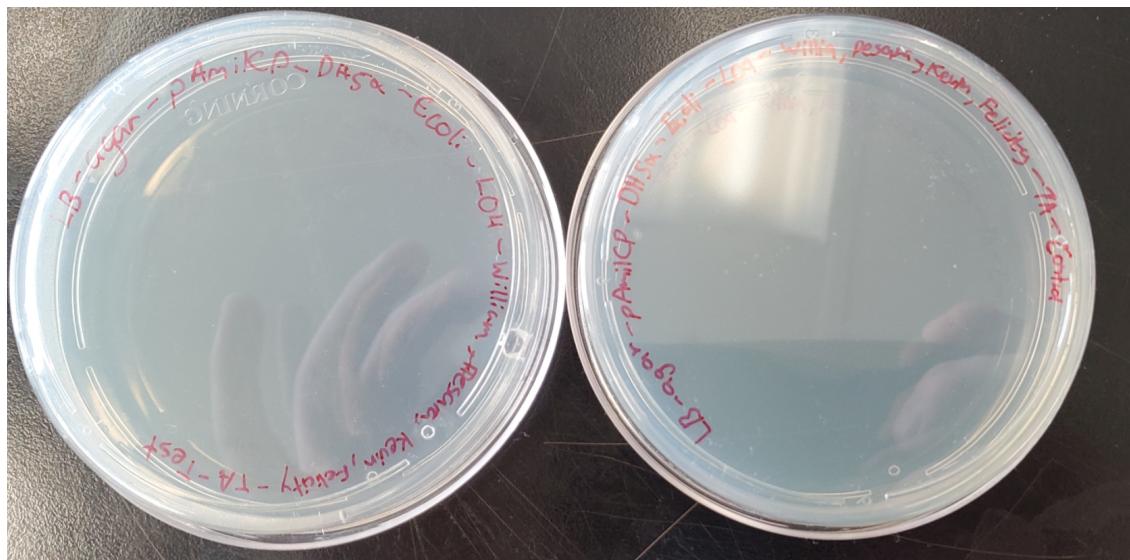


Figure 2: The test plate (left) had no cultures growing on it and the control plate had very few cultures growing on it that are white. Since no culture is visibly growing in the test plate this indicates that transformation was not successful as there is neither the desired mechanism (which is blue) nor any white colonies indicative of successful transformation of the plasmid into the vector. The control plate had the desired effect with an extremely low amount of growth being exhibited which can be attributed to the antibiotic and/or CuSO₄ not being concentrated enough in the areas of growth. Since the antibiotic chloramphenicol should inhibit the growth of non-transformed Bacteria, and CuSO₄ should inhibit growth due to being a heavy metal.

WL6

Absorbance Assay

Table 1: The absorbance values for a wavelength of 589 nm, for the triplicate experiments. The 1st column contains the variation of CuSO₄ concentration (in μM). As can be seen from the calculated average value (5th column) and standard deviation (6th column) of the replicated measurements the values do not have a high variance and are close to the average measurement, which means that the measurements are close enough to be considered similar. The trend indicates that the absorbance increases proportional to the amount of copper ions present.

	WELL 1	WELL 2	WELL 3	Average Wells	STD wells
BLANK (water)	0.023	0.041	0.009	0.02433333333	0.01604161255
0	0.108	0.119	0.094	0.107	0.01252996409
0.5	0.289	0.278	0.273	0.28	0.008185352772
0.8	0.459	0.481	0.473	0.471	0.01113552873
1.1	0.612	0.578	0.756	0.6486666667	0.09449514979
1.4	0.792	0.834	0.757	0.7943333333	0.03855299383
1.7	0.921	0.878	0.933	0.9106666667	0.0289194283
1.9	1.132	1.039	1.16	1.1103333333	0.06334298172

Gel Image

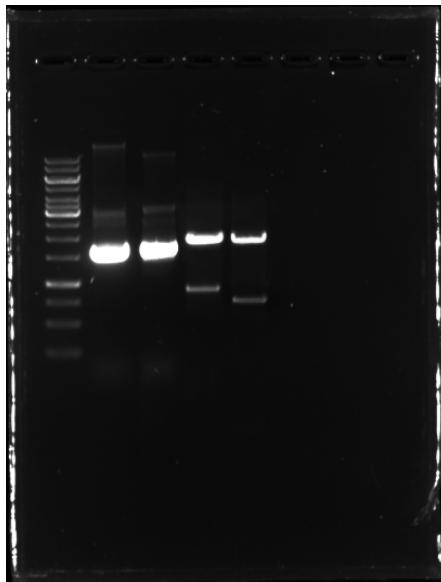


Figure 4: A 1% gel. Well (from the left) contains a GenRuler 1kB ladder (3 μ L). Well 2 contains the pAmilCP-promoterless (10 μ L). Well 3 contains the pCusAmil-IBM (10 μ L). Well 5 contains pAmilCP promoterless plasmid digested with EcoRI-HF and SpeI (10 μ L). Well 4 contains pCusAmil-IBM plasmid digested with EcoRI-HF and SpeI (10 μ L).

Conclusion

To create the desired mechanism we first created the PCR product with the desired gene of interest and isolated the backbone plasmid for insertion, while making sure to adhere to the BioBrick assembly specifications. Next, we digested the backbone and insert, purified these products, ligated them, and transformed them into our vector (DH5a). Finally, we (through WL6) measured the efficacy of our products by subjecting them to tests of absorbance for different concentrations of copper ions. The methods used from WL4-WL6 theoretical should suffice in creating a genetically engineered organism that can be used in detecting copper ions, as indicated by the results in WL6 (which were performed by more experienced experimentalists), our own construction of the organism was not successful as indicated by our results in WL5. It should be noted that our results in WL4 indicated that our MP and template were close to the expected amount of bps. Our methods for measuring the efficacy and screening transformants seem reliable, as the controls used indicated a clearly observable change when a reactant was unused. Thus our controls can be used to fine tune our own procedure and be used by others to repeat our experiments and obtain the desirable construct.

References

Book

[1] Leung, V. (2022). *Ibehs 2P03 Student Course Pack 2022 V9*. McMaster University.

Websites, Articles, and Manuals

[2] *PureLink PCR purification kit - thermo fisher scientific*. (n.d.). Retrieved March 16, 2022, from https://assets.thermofisher.com/TFS-Assets/LSG/manuals/purelink_pcr_man.pdf

[3] *Sequence analyzer: AMILCP chromoprotein promoter-less sequencing result*. Addgene. (n.d.). Retrieved March 15, 2022, from <https://www.addgene.org/browse/sequence/252164/>

Appendix I : Materials

WL-4 Materials

PRODUCT	COMPANY	CAT. #	LINK
2X PCR Master Mix	New England Biolabs (NEB)	M0270L	https://international.neb.com/products/m0270-taq-2x-master-mix#Product%20Information
FWD Primer	Integrated DNA Technologies (IDT)	N/A	N/A
REV Primer	IDT	N/A	N/A
Template	IDT	N/A	N/A
Quick Plasmid Miniprep Kit	Invitrogen	K210011	https://www.thermofisher.com/order/catalog/product/K210010
6x Gel Loading Dye	Invitrogen	R1161	https://www.thermofisher.com/order/catalog/product/R1161?SID=srch-srp-R1161
	Invitrogen	SM0314	https://www.thermofisher.com/order/catalog/product/SM0314?SID=srch-srp-SM0314

GeneRuler 1KB DNA ladder			
SYBR Safe Gel Stain (Gel Red)	Invitrogen	S33102	https://www.thermofisher.com/order/catalog/product/S33102?SID=srch-srp-S33102
Agarose	BioShop	AGA001.500	https://www.bioshopcanada.com/Products/Details/AGA001
Nuclease-free water	Invitrogen	10977023	https://www.thermofisher.com/order/catalog/product/10977023?SID=srch-srp-10977023
50x TAE Buffer	Bioshop	TAE222.1	https://bioshopcanada.com/Products/Details/TAE222
Chlorampheni col	Sigma	C0378-5G	https://www.sigmaldrich.com/CA/en/product/sigma/c0378

WL-5 Materials

PRODUCT	COMPANY	CAT. #	LINK
10x RE Buffer 2.1	NEB	B7202	https://international.neb.com/products/b7202-nebuffer-2-1#Product%20Information
XbaI	NEB	R0145L	https://international.neb.com/products/r0145-xbai#Product%20Information
EcoRI-HF	NEB	R3101L	https://international.neb.com/products/r3101-ecori-hf#Product%20Information

SpeI	NEB	R3133S	https://international.neb.com/products/r0133-spei#Product%20Information
PCR Purification Kit	Invitrogen	K310002	https://www.thermofisher.com/order/catalog/product/K310001
LB Broth Powder (Miller)	Bioshop	LBL407	https://bioshopcanada.com/Products/Details/LBL407
Nuclease-free water	Invitrogen	10977023	https://www.thermofisher.com/order/catalog/product/10977023?SID=src_h-srp-10977023
T4 DNA Ligase	NEB	M0202S	https://international.neb.com/products/m0202-t4-dna-ligase#Product%20Information
10x Ligase Buffer	Bioshop	B0202S	https://international.neb.com/products/b0202-t4-dna-ligase-reaction-buffer#Product%20Information
DH5α Competent Cells	Invitrogen	18265017	https://www.thermofisher.com/order/catalog/product/18265017

WL-6 Materials

PRODUCT	COMPANY	CAT. #	LINK

Copper (II) Sulfate	Sigma	451657-10G	https://www.sigmaaldrich.com/CA/en/ substance/copperii sulfate1596177589 87
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