Towards the construction of a chitin-binding domain BioBrick: PCR Amplification of the *Pseudomonas aeruginosa chiC* chitin-binding domain

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SUPPLEMENTAL

FIGURES

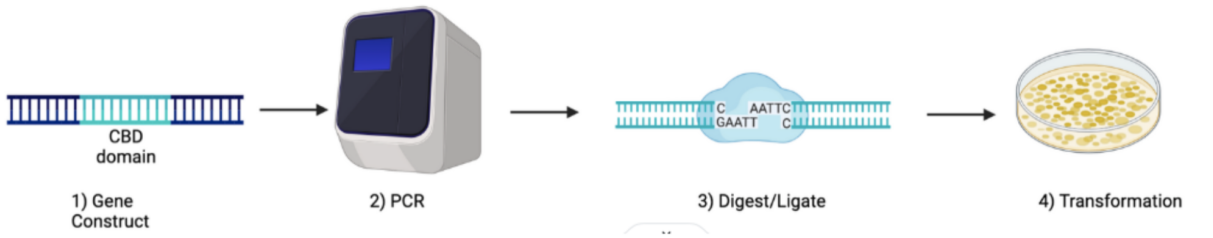

FIG S1 Overview of experimental steps of the study design explored within this paper.
A gene block containing the CBD was constructed using SnapGene ® (v6.2.1), PCR was carried out to amplify the gene blocks, the gene blocks underwent restriction/ligation at HindIII and EcoRI sites for insertion into the pET-28a(+) plasmid, and finally transformation into DH5α *E. coli* cells. The gene construct was successfully amplified. These last steps of digestion, ligation and transformation failed. Figure created using https://www.biorender.com/

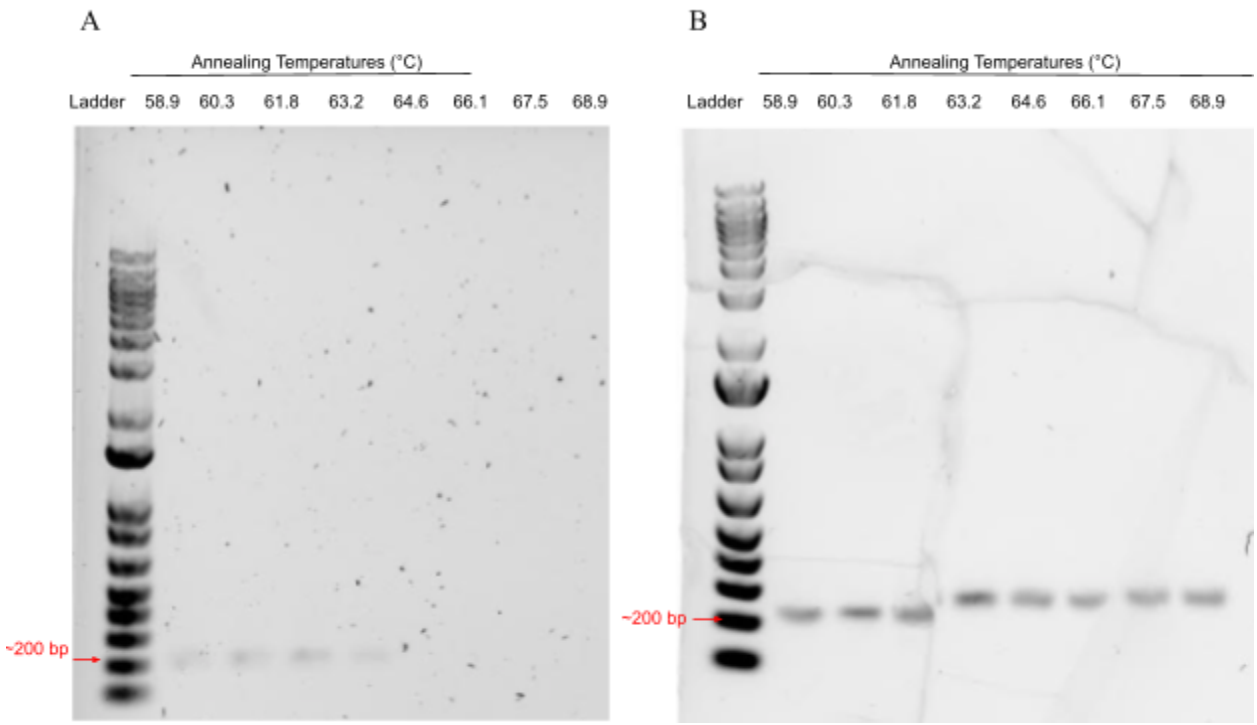

FIG S2. Greater and more stable amplification of WT pCbd was achieved with 21 base primers compared to 15 base primers. 0.001 ng (1/1000 dilution) of WT pCbd template was amplified using 15 base primers and exhibited a weak band at ~200 bp for 58.9-63.2°C annealing temperatures (A). Meanwhile, the WT pCbd gene block amplified using 21 base primers exhibited strong bands at ~200 bp for all annealing temperatures tested (B). Primer sequences were designed with the only difference being the addition of six bases in the longer primers (C). Negative control that included nuclease free water as the template showed no bands, and pUC19 positive control exhibited band at ~2686 bp as expected (not shown).

TABLES

TABLE S1 Relevant PAO1 ChiC Chitin binding DNA and expected amino acid translations. These sequences were used in the design of the gene blocks, and associated primers.

Sequence name	Sequence type	Sequence (5'-3')
PAO1 ChiC Chitin binding domain (Lim et al., 2022)	DNA	GCGGTGGATCCGCAATTCCCGCAATGGCGGGAGA ACCAGGCCTATCGGGTCGACGATGGGGTGACCTA CGAGGGGCTGCGCTATCTCTGCCTGCAGGCGCAC ACCTCCAACAGCGGCTGGACGCCGCCGGTAGCC TTCACCCTCTGGCGGCCGCTGCGCTGA
	Amino Acid	AVDPQFPQWRENQAYRVDDGVTYEGLRYLCLQAH TSNSGWTPPVAFTLWRPLR*
WT pCbd gene block construct	DNA	gcaGAATTCGCGGCCGCTTCTAGAGCAGTTGATCC ACAATTCCCACAATGGAGAGAGAACCAGGCATAT AGAGTTGACGATGGGGTGACCTACGAGGGGTTA AGATATTTATGCTTACAGGCACACACCTCCAACA GCGGCTGGACGCCGCCGGTAGCATTCACCTTATG GAGACCGTTAAGATAATACTAGTAGCGGCCGCTG CAGAAGCTTgct
	Amino Acid	AEFAAASRAVDPQFPQWRENQAYRVDDGVTYEGL RYLCLQAHTSNSGWTPPVAFTLWRPLR*
C30A pCbd gene block construct	DNA	gcaGAATTCGCGGCCGCTTCTAGAGCAGTTGATCC ACAATTCCCACAATGGAGAGAGAACCAGGCATAT AGAGTTGACGATGGGGTGACCTACGAGGGGTTA AGATATTTAGCATTACAGGCACACACCTCCAACA GCGGCTGGACGCCGCCGGTAGCATTCACCTTATG GAGACCGTTAAGATGATACTAGTAGCGGCCGCTG CAGAAGCTTgct
	Amino Acid	AEFAAASRAVDPQFPQWRENQAYRVDDGVTYEGL RYLALQAHTSNSGWTPPVAFTLWRPLR*

TABLE S2 Information relevant to primers used in all PCR conducted

Primer Type	Primer name	Primer Sequence
Forward Primer Set #1	fw_pCbd	gcaGAATTCGCGGCC
Reverse Primer Set #1	rv_pCbd	agcAAGCTTCTGCAGC
Forward Primer Set #2	fw_pCbd_21bp	gcaGAATTCGCGGCCGCTTCT
Reverse Primer Set #2	rv_pCbd_21bp	agcAAGCTTCTGCAGCGGCCG

TABLE S3 Phusion Green PCR amplified gene block sequencing results. Inaccurate or undefined (N) basecalls were observed in the first *ca.* 30 and the last *ca.* 10 basecalls (5' to 3') likely due to primer binding at the start and regions of high GC content at the end.

Primer	Gene block	Sequence results
Forward Primer Set #1	WT pCbd	NNNNNNNNNNNNCNNCATGGAGAGAGAACCANNNATATA GAGTTGACGATGNGGGTGACCTACGAGGNGGTTAAGATAT TTATGCTTACAGGCACACACCTCCAACAGCGGCTGGACGC CGCCGGTAGCATTCACCTTATGGAGACCGTTAAGATAATAC TAGTAGCGGCCGCTGNNAAGCTTGNTNGN
Reverse Primer Set #1	WT pCbd	NNNNNNNNNNGTCTCNNAAGGTNNNGCTACCGGCGGCGT CCAGCCGCTGTTGGAGGTGTGTGCCTGTAAGCATAAATATC TTAACCCCTCGTAGGTCACCCCATCGTCAACTCTATATGCCT GGTTCTCTCTCCATTGTGGGAATTGTGGATCAACTGCTCTA GAAGCGGCCGCGAATTCTGNANN
Forward Primer Set #1	C30A pCbd	GGNNNNNNNNTNCNNNNGGAGAGAGANNAGGCATATAGA GTTGACGATGGGGTGACCTACGAGGGGTTAAGATATTTAGC ATTACAGGCACACACCTCCAACAGCGGCTGGACGCCGCCG GTAGCATTCACCTTATGGAGACCGTTAAGATAATACTAGTA GCGGCCGCTGCAGAAGCTTGCTN
Reverse Primer Set #1	C30A pCbd	NNNNNNNTACGGTCTNNTNAGGTGNNGCTACCGGCGGCG TCCAGCCGCTGTTGGAGGTGTGTGCCTGTAATGCTAAATAT CTTAACCCCTCGTAGGTCACCCCATCGTCAACTCTATATGC CTGGTTCTCTCTCCATTGTGGGAATTGTGGATCAACTGCTC TAGAAGCGGCCGCGNATTNTNNNN

TABLE S4 Detailed Protocols for various procedures references throughout paper

Procedure Name	Detailed Protocol
Phusion green PCR	*PCR Reaction Mix (20 µl total) For Amplification of Gene Blocks* ● dNTPs (10 mM): 0.4 uL ● Forward primer (10 µM): 0.5 µl ● Reverse primer (10 µM): 0.5 µl ● DNA template (1, 0.1, 0.001 ng/µl): 1 uL ● DMSO (3% v/v): 0.6 µl ● 5x Phusion Green GC Buffer: 4 µL ● Phusion DNA Polymerase: 0.2 µl ● Nuclease-free water: 11.8 µL *Thermocycler Cycling Conditions For Amplification of Gene Blocks* ● Initial denaturation: 95°C for 3 minutes ● Denaturation: 95°C for 30 seconds ● Annealing: 58.9°C for 30 seconds ● Extension: 72°C for 30 seconds ● Repeat steps 2-4 for 30 cycles ● Final extension: 72°C for 5 minutes ● Hold the reaction at 4°C
Hot Start Taq PCR	● dNTPs (10 mM): 1 uL ● Forward primer (10 µM): 0.5 µl ● Reverse primer (10 µM): 0.5 µl ● Template DNA (1, 0.1, 0.001 ng/µl): 1 µl ● Template DNA 1 µl ● DMSO (5% v/v): 2.5 µl ● 10x Maxima Hot Start Taq Buffer 5 µl ● $MgCl_2$ (25 mM) 4 µL ● Taq DNA Polymerase 0.25 µl *Thermocycler Cycling Conditions For Amplification of Gene Blocks* ● Initial denaturation: 95°C for 3 minutes ● Denaturation: 95°C for 30 seconds ● Annealing: 58.9°C for 30 seconds ● Extension: 72°C for 30 seconds ● Repeat steps 2-4 for 30 cycles ● Final extension: 72°C for 5 minutes ● Hold the reaction at 4°C