SUPPLEMENTAL MATERIAL

Existence of separate domains in lysin plyG for recognizing *Bacillus anthracis* spores and vegetative cells

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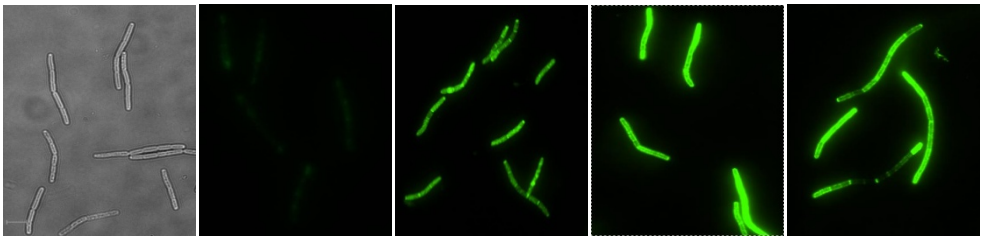
The **Supplemental material** contains the following content as shown in the below.

Figure S1. The fluorescence images of time gradient stained *B. anthracis* cells.



t=0 min t=10 min t=20 min t=30 min t=120 min

Fig. S1. The fluorescence images of time gradient stained *B. anthracis* cells. The *B. anthracis* cells were stained with EP0 at 37°C for different times ranging from 0 min to 120 min, and then the supernatant was analyzed after centrifuging at 12,000 g for 3 min. The concentration of *B. anthracis* cells was 1.4×10^{6} CFU/ml; The concentrations of EP9, EP3 and EP0 were 0.12 μM; Bar size, 5 μm.

Figure S2. Confocal analysis of *B. anthracis* vegetative cells stained with EP0.

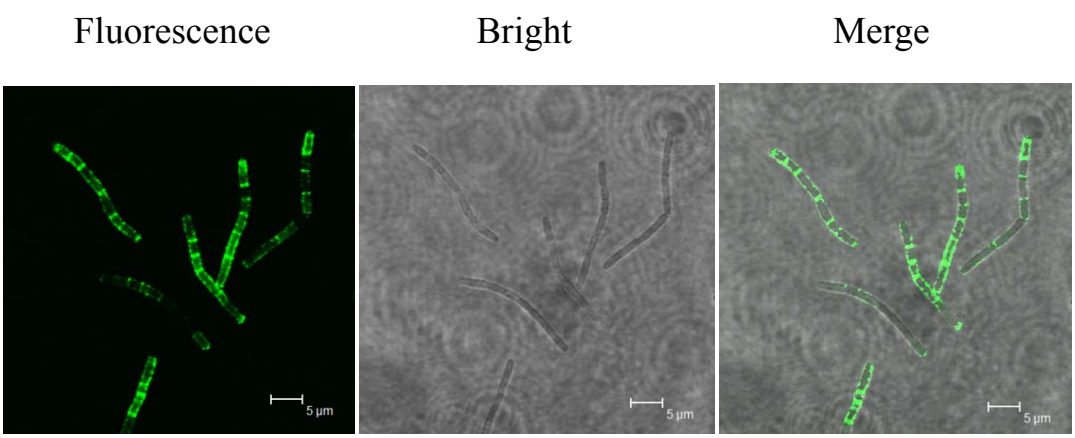
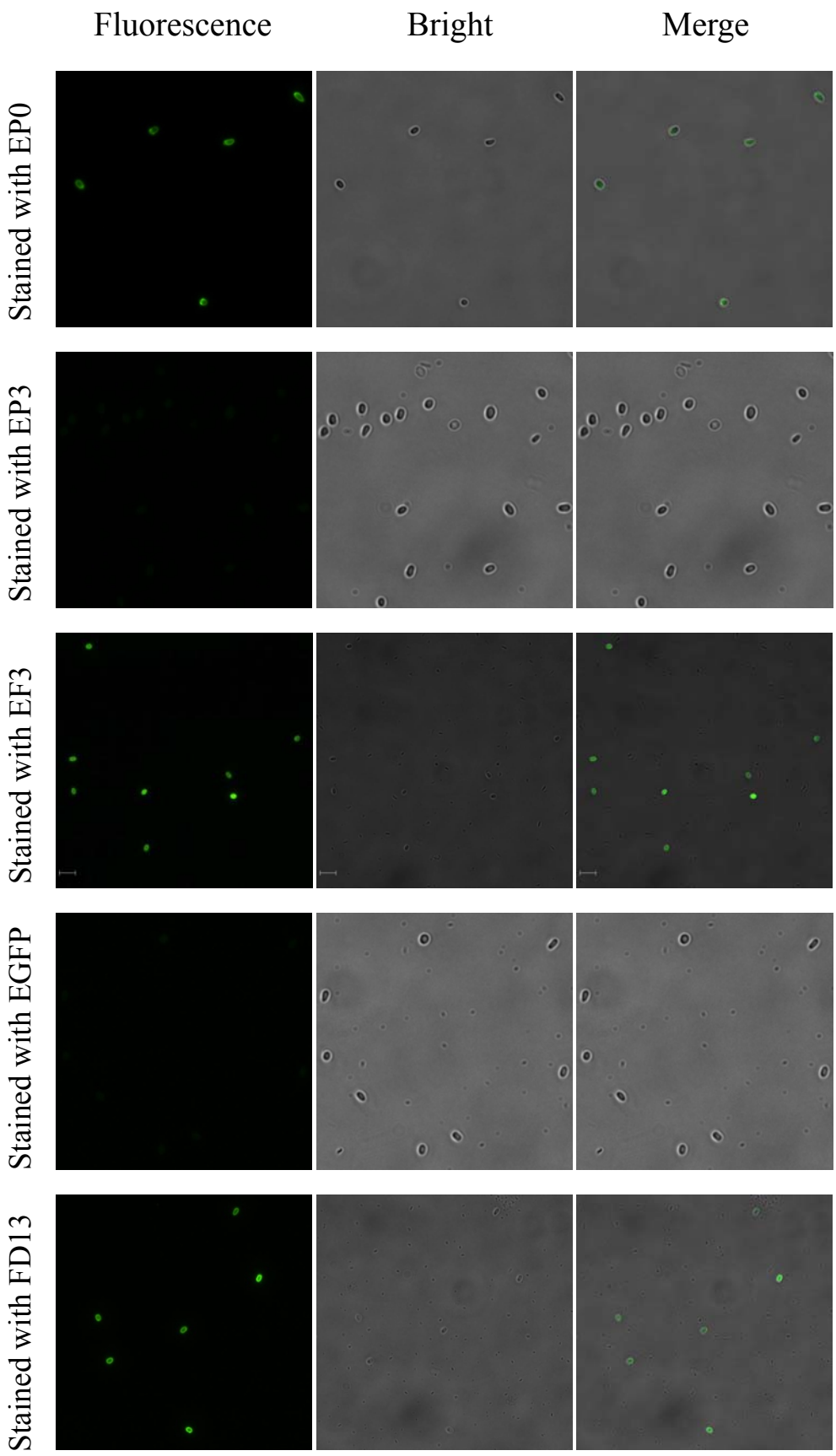


Fig. S2. Confocal analysis of *B. anthracis* vegetative cells stained with EP0. The cells conjugated with EP0 were washed three times with PBST buffer before imaging.

Figure S3. Binding difference of the truncated proteins and the peptides to *B. anthracis* spores.



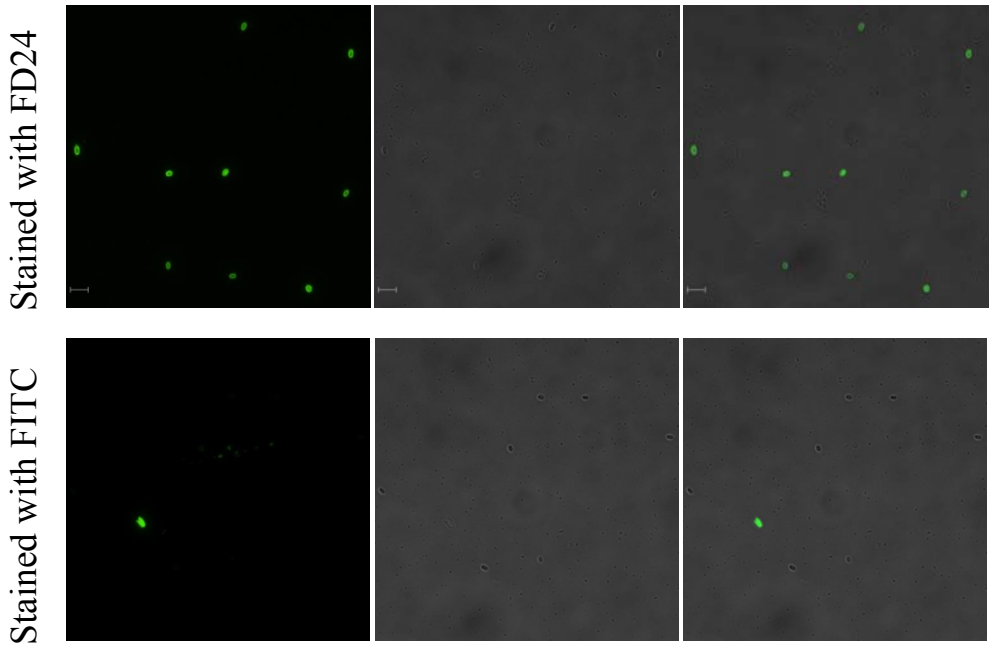


Fig. S3. Binding difference of the truncated proteins and the peptides to *B. anthracis* spores. All the images were captured under the same instrument conditions. Bar, 5 μm.

Figure S4. Kinetics of EP0, EP3 and EC5 interaction with *B. anthracis* spores.

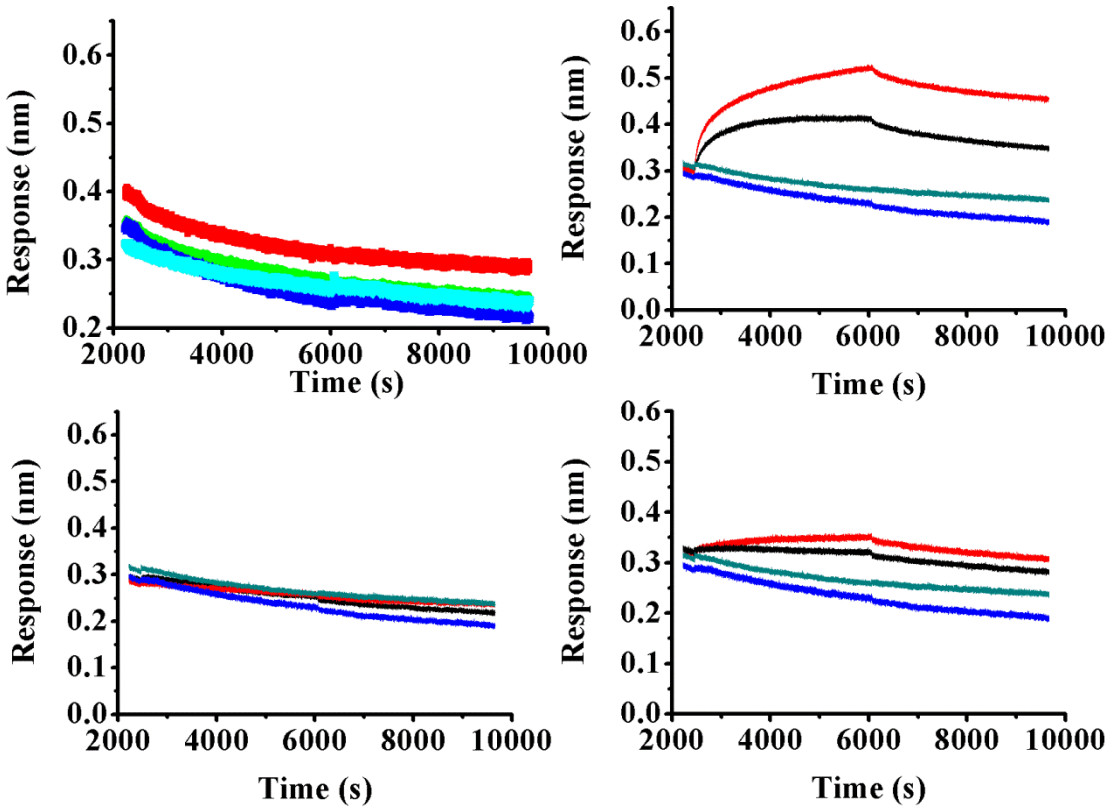


Fig. S4. Kinetics of EP0, EP3 and EC5 interaction with *B. anthracis* spores. (A) EC6 interaction with *B. mycoides* spores. Biotinylated *B. mycoides* spores were immobilized on the surface of the SA sensors. EC6 at concentrations of 674.6 nM (red line) and 337.3 nM (green line) were tested. (B) *B. anthracis* spores interaction with EC5 at a concentration of 681.2 nM (red line) and 340.6 nM (black line). (C) *B. anthracis* spores interaction with EP3 at a concentration of 776.3 nM (red line) and 388.1 nM (black line). (D) *B. anthracis* spores interaction with EP0 at a concentration of 611.1 nM (red line) and 305.6 nM (black line). EGFP (304.4 nM, pale blue line) and PBS (blue line) were used as the control and the blank in all the assays.

Figure S5. The relative absorption of N21-AuNPs and bare AuNPs.

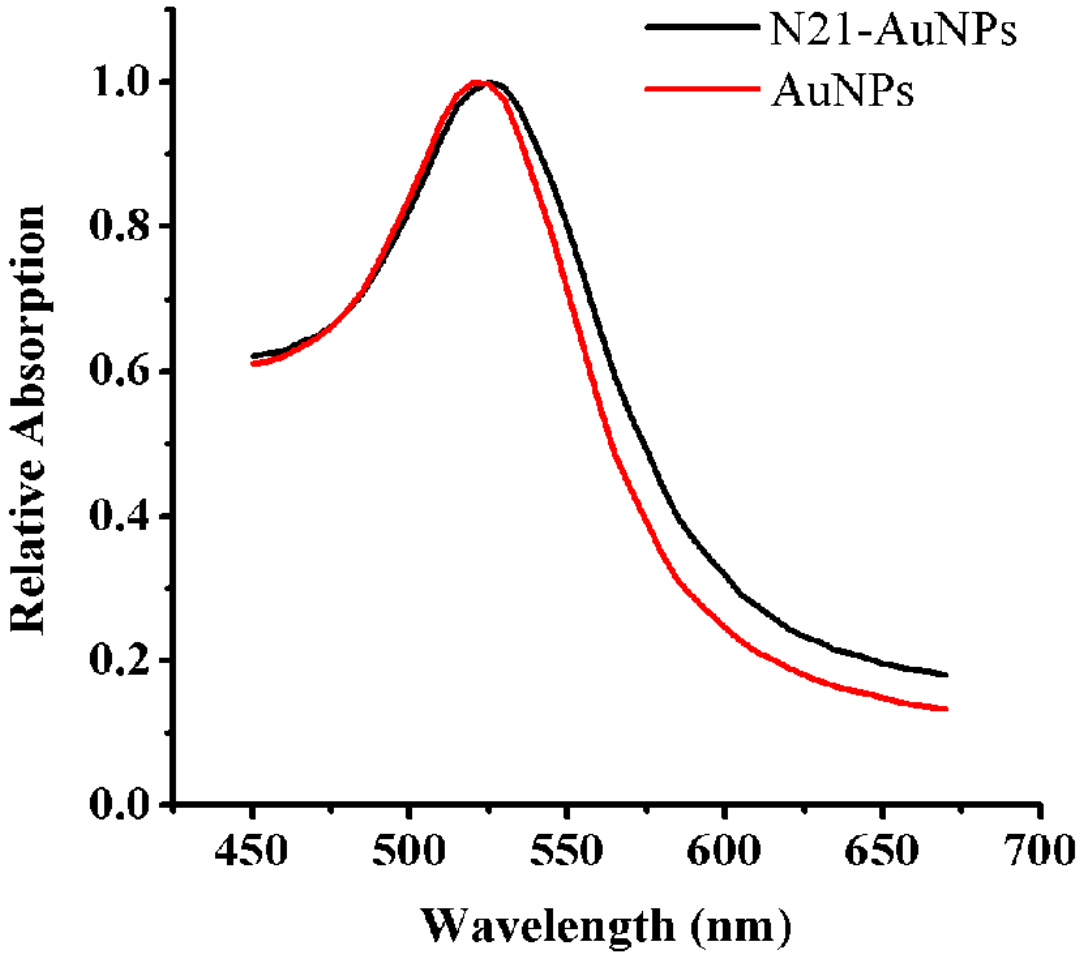


Fig. S5. The relative absorption of N21-AuNPs and bare AuNPs. The absorption of N21-AuNPs and bare AuNPs were monitored by a Synergy H1 spectrophotometer (BioTek, USA), the data was shown after normalization.

Table S1. *E. coli* strains, plasmids, peptides and oligonucleotides used in this work

Strain/plasmid	Relevant characteristics	Source
E. coli strain		
BL21(DE3)	*omp*T *hsd*S B ($rB^- mB^-$) (λ DE3)	Invitrogen
DH5α	Host strain for pUC57-plyG	Songon
plasmid		
pUC57	Cloning vector for chemical synthetic plyG	Songon
pUC57-plyG	Amp^R plyG gene cassette vector	Songon
pEGFP-C1	Amp^R *egfp* cassette vector	Invitrogen
pET28a(+)	Kan^R expressing vector with a T7 promoter	Novagen
pBAD24	Amp^R expressing vector with a BAD promoter	(1)
pB-plyG	plyG cloned into the *EcoR*I and *Nco*I sites of pBAD24 with primers plyG-F and plyG-R.	this work
pET-GFP	pEGFP-C1 egfp gene cloned into the *Nde*I and *BamH*I sites of pET28(+) with primers GFP-F and GFP-R.	this work
pET-EP9	CBD90[a] from plyG cloned into the *EcoR*I and *Xho*I sites of pET-GFP with primers P90-F and CBD-R	this work
pET-EP0	CBD106[a] from plyG cloned into the *EcoR*I and *Xho*I sites of pET-GFP with primers P106-F and CBD-R	this work
pET-EP3	CBD136[a] from plyG cloned into the *EcoR*I and *Xho*I sites of pET-GFP with primers P136-F and CBD-R	this work
pET-EC3	The EC3-F[b] and EC3-R annealed and cloned into the *EcoR*I and *Xho*I sites of pET-GFP	this work
pET-EC5	The fragment from 106 to 155 of plyG amplified and cloned into the *EcoR*I and *Xho*I sites of pET-GFP	this work
pET-EC6	The fragment from 106 to 165 of plyG amplified and cloned into the *EcoR*I and *Xho*I sites of pET-GFP	this work
pET-EC8	The fragment from 106 to 189 of plyG amplified and cloned into the *EcoR*I and *Xho*I sites of pET-GFP	this work
pET-EG1	plyG amplified and cloned into the *EcoR*I and *Xho*I sites of pET-GFP	this work
pET-EF3	The fragment from 125 to 145 of plyG amplified and cloned into the *EcoR*I and *Xho*I sites of pET-GFP	this work
peptide	**Sequence (N–C)**	
FD13[c]	DNAVDVVRQLMSMYNIPIENVRTHQSWSGKYCPHRMLAEG	
FD24[c]	NIPIENVRTHQSWSGKYCPHRMLAEGRWGAFIQKVK	
FD23[c]	NVRTHQSWSGKYCPHRMLAEG	
N21	CSGSG NVRTHQSWSGKYCPHRMLAEG	
Oligonucleotide	**Sequence (5'–3')**	
P90-F	AAAAGAATTCGAAATCTGTTATTCAAAATCAGGAG	
P106-F	AAAAGAATTCGATAATGCTGTTGATGTTGTACG	
P136-F	AAAAGAATTCTATTGTCCGCATAGAATGTTAGCTG	

plyG-F	AAAAGAATTCATGGAAATCCAAAAAAAATTAG
PlyG-R	AAACCATGGTTATTTAACTTCATACCACCAAC
CBD-R	AAACTCGAGTTTAACTTCATACCACCAACC
GFP-F	AATCCATATGGTGAGCAAGGGCGAG
GFP-R	GGCCGGATCCCTTGTACAGCTCGTC
EC-F	TAAAGAATTCGATAATGCTGTTGATGTTG
EC5-R	AAATCTCGAGTTACTTAACCTTCTGAATG
EC6-R	TAATCTCGAGTTATGTTGGTGAAGTAGTCG
EC8-R	ATGTCTCGAGTTATGACGTTAATGCTCC
EG1-F	AAAGAATTCGAAATCCAAAAAAAATTAG
EG1-R	AAACTCGAGTTATTTAACTTCATACCACC
EC3-F	AATTCGGTGGGGGAGGATCAGGTGGGGGAGGATCAGATAAT GCTGTTGATGTTGTACGACAACTTATGTCTATGTACAATATTC CGATTGAAAATGTTCGAACTCATCAATCCTGGTCAGGTAAAT AAC
EC3-R	TCGAGTTATTTACCTGACCAGGATTGATGAGTTCGAACATTTT CAATCGGAATATTGTACATAGACATAAGTTGTCGTACAACATC AACAGCATTATCTGATCCTCCCCCACCTGATCCTCCCCCACC G

[a]CBD90, [a]CBD106, [a]CBD136: the arabic numbers 90, 106 and 136 represent the sites where the recombinant proteins were truncated in the amino sequence of plyG.

[b]EC3-F: a chemical synthesized olignucleotide which contains a $(G4S)_2$ encoding sequences tandem with a nucleotide sequence from site 316 to 405 of plyG.

[c]FD13, [c]FD24 and [c]FD23: a N-terminal FITC modification were exploited.

Reference

1. Li H, Zhang X, Bi L, He J, & Jiang T (2011) Determination of the crystal structure and active residues of FabV, the enoyl-ACP reductase from Xanthomonas oryzae. (Translated from eng) *PLoS One* 6(10):e26743 (in eng).

Table S2. Binding selectivity of the truncated proteins to *B. anthracis* vegetative cells.

isolate or strain	EP9	EP0	EP3	Source or reference
B. anthracis				
A16	+	+	+	Lab collection
B.cereus	-	-	-	
NC7401/2455	-	-	-	Lab collection
AND 1315R	-	-	-	Lab collection
4810/72	-	-	-	Lab collection
ATCC 33018R	-	-	-	Lab collection
IS 195	-	-	-	Lab collection
B.thuringiensis	-	-	-	
sylvestriensis H61	-	-	-	Lab collection
bolivia H63	-	-	-	Lab collection
inensis H70	-	-	-	Lab collection
tenebrionis 1765	-	-	-	Lab collection
pulsiensis H65	-	-	-	Lab collection
BMB 171	-	-	-	Lab collection
GBJ 001	-	-	-	Lab collection
B. subtilis				
subsp.	-	-	-	Lab collection
B. mycoides				
subsp.	-	-	-	Lab collection
B. licheniformis				
subsp.	-	-	-	Lab collection
B. pumilus				
subsp.	-	-	-	Lab collection
X.oryzae				
ks-1-21	-	-	-	Lab collection
P. aeruginosa				
PA01	-	-	-	Lab collection
E. coli				
BL21(DE3)	-	-	-	Lab collection