

Supplementary materials for

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Manuscript Title: Constructing an efficient *Bacillus subtilis* spore display by using cohesin–dockerin interaction

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Table S1. Strains and plasmids used in this study

Name	Description	Source
Strains		
<i>E. coli</i>		
Top10	<i>F⁻ mcrA Δ(mrr-hsdRMS-mcrBC) φ80lacZΔM15 ΔlacX74 nupG recA1 araD139 Δ(ara-leu)7697 galE15 galK16 rpsL(Str^R) endA1 λ⁻</i>	Laboratory stock
BL21(DE3)	<i>F⁻ ompT gal dcm lon hsdSB (r_B m_B) λ(DE3 [lacI lacUV5-T7 gene 1 ind1 sam7 nin5])</i>	Laboratory stock
<i>Clostridium thermocellum</i> ATCC 27405	Template for encoding type-I and type-II cohesin genes	ATCC
<i>Clostridium cellulovorans</i> DSM 743B	Template for encoding type-I cohesin gene	DSMZ
<i>Clostridium cellulolyticum</i> ATCC 35319	Template for encoding type-I cohesin gene	ATCC
<i>Ruminococcus flavefaciens</i> FD-1	Template for encoding type-III cohesin gene	DSMZ
<i>B. subtilis</i>		
168	<i>trpC2</i>	BGSC
WB600	<i>ΔnprE, ΔnprB, aprΔ3, Δepr, Δbpf, ΔaprE, trpC2</i>	BGSC
GC01	WB600WT <i>cotG-Ctcoh-I</i>	This study

GC02	WB600WT <i>cotG-Ctcoh-II</i>	This study
GC03	WB600WT <i>cotG-Ccscoh-I</i>	This study
GC04	WB600WT <i>cotG-Ccmcoh-I</i>	This study
GC05	WB600WT <i>cotG-Rfcoh-III</i>	This study
BD01	BL21(DE3) <i>bgaB-Ctdoc-I</i>	This study
BD02	BL21(DE3) <i>bgaB-Ctdoc-II</i>	This study
BD03	BL21(DE3) <i>bgaB-Ccsdoc-I</i>	This study
BD04	BL21(DE3) <i>bgaB-Ccmdoc-I</i>	This study
BD05	BL21(DE3) <i>bgaB-Rfdoc-III</i>	This study
GBJ01	WB600WT <i>cotG-Ctcoh-I::bgaB-Ctdoc-I</i>	This study
GBJ02	WB600WT <i>cotG-Ctcoh-II::bgaB-Ctdoc-II</i>	This study
GBJ03	WB600WT <i>cotG-Ccscoh-I::bgaB-Ccsdoc-I</i>	This study
GBJ04	WB600WT <i>cotG-Ccmcoh-I::bgaB-Ccmdoc-I</i>	This study
GBJ05	WB600WT <i>cotG-Rfcoh-III::bgaB-Rfdoc-III</i>	This study
GB01	WB600WT <i>cotG-bgaB</i>	This study
PB01	WB600WT <i>P_{cry1Aa}-bgaB</i>	This study

Plasmids

pET-28a	pBR322 <i>ori lacI T7lac Kan^r</i>	Novagen
pET-28a- <i>bgaB</i>	pET-28a carrying <i>bgaB</i>	(Wang et al., 2016)
pET-28a- <i>bgaB-Ctdoc-I</i>	pET-28a carrying fusion gene <i>bgaB-Ctdoc-I</i>	This study
pET-28a- <i>bgaB-Ctdoc-II</i>	pET-28a carrying fusion gene <i>bgaB-Ctdoc-II</i>	This study
pET-28a- <i>bgaB-Ccsdoc-I</i>	pET-28a carrying fusion gene <i>bgaB-Ccsdoc-I</i>	This study
pET-28a- <i>bgaB-Ccmdoc-I</i>	pET-28a carrying fusion gene <i>bgaB-Ccmdoc-I</i>	This study
pET-28a- <i>bgaB-Rfdoc-III</i>	pET-28a carrying fusion gene <i>bgaB-Rfdoc-III</i>	This study
	<i>E. coli-B. subtilis</i> shuttle plasmid	(Xu et al., 2011)
pEB03- <i>cotG</i>	pEB03 carrying <i>cotG</i>	(Wang et al., 2016)
pEB03- <i>cotG-Ctcoh-I</i>	pEB03 carrying fusion gene <i>cotG-Ctcoh-I</i>	This study
pEB03- <i>cotG-Ctcoh-II</i>	pEB03 carrying fusion gene <i>cotG-Ctcoh-II</i>	This study
pEB03- <i>cotG-Ccscoh-I</i>	pEB03 carrying fusion gene <i>cotG-Ccscoh-I</i>	This study
pEB03- <i>cotG-Ccmcoh-I</i>	pEB03 carrying fusion gene <i>cotG-Ccmcoh-I</i>	This study
pEB03- <i>cotG-Rfcoh-III</i>	pEB03 carrying fusion gene <i>cotG-Rfcoh-III</i>	This study

pEB03-*cotG-bgaB*

pEB03 carrying fusion gene *cotG-bgaB*

This study

pEB03-*P_{cry1Aa}-bgaB*

pEB03 carrying *P_{cry1Aa}-bgaB*

This study

Table S2. List of all primers used in the study

Name	Sequence (5' → 3')	Restriction enzyme site
<i>cotG-F</i>	ATCGATAAGCTTGATATCGAATTCCTTTTCTAGAAGTGTCCT	<i>EcoR</i> I
<i>cotG-R</i>	AGAACTAGTGGATCCCTGCAGTTTGTATTCTTTTTGACTACC CAGCAATTG	<i>Pst</i> I
<i>coh I -1-F</i>	AAAAAGAAATACAAACTGCAGGGAGATACAACAGTACCTA C	<i>Pst</i> I
<i>coh I -1-R</i>	CGCTCTAGAACTAGTGGATCCTTACTTGTCGTCATCGTCTTT GT	<i>BamH</i> I
<i>coh II -1-F</i>	AAAAAGAAATACAAACTGCAGATTGAAATGGTTCTTGATAA	<i>Pst</i> I
<i>coh II -1-R</i>	CGCTCTAGAACTAGTGGATCCTTACTTGTCGTCATCGTCTT	<i>BamH</i> I
<i>coh I -2-F</i>	AAAAAGAAATACAAACTGCAGGTAACAGCTACAATTGGAA AAGT	<i>Pst</i> I
<i>coh I -2-R</i>	CGCTCTAGAACTAGTGGATCCTTACTTGTCGTCATCGTCTTT GTAGTCGATAGTTACTGTTCCCTGGGT	<i>BamH</i> I
<i>coh I -3-F</i>	AAAAAGAAATACAAACTGCAGGAAATATCAATCGGCAAAG T	<i>Pst</i> I
<i>coh I -3-R</i>	CGCTCTAGAACTAGTGGATCCTTACTTGTCGTCATCGTCTTT GTAGTCTGTTATCTCACCCCTCTGTGA	<i>BamH</i> I
<i>cohIII-1-F</i>	AAAAAGAAATACAAACTGCAGGGAGATACAACAGTACCTA C	<i>Pst</i> I
<i>cohIII-1-R</i>	CGCTCTAGAACTAGTGGATCCTTACTTGTCGTCATCGTCTTT GT	<i>BamH</i> I
<i>1-bgaB-F</i>	AAAAAGAAATACAAACTGCAGATGAATGTGTTATCCTCAAT TTGTTACGG	<i>Pst</i> I
<i>1-bgaB-R</i>	CGCTCTAGAACTAGTGGATCCTTACTTGTCGTCATCGTCTTT GTAGTCAACCTTCCCGGCTTCATCATGC	<i>BamH</i> I
<i>2-bgaB-F</i>	GTGCCGCGGGCAGCCATATGATGAATGTGTTATCCTCAATT TGTTACGG	<i>Nde</i> I
<i>2-bgaB-R</i>	ATCGCCCATGTAGGGAATTCACCTACCGCCACCTCCAACCT TCCCGGCTTCATCATGC	<i>EcoR</i> I
<i>doc I -1-F</i>	GGAGGTGGCGGTAGTGAATTCCTCCGAAAATTACCTATGGAGA	<i>EcoR</i> I
<i>doc I -1-R</i>	GTGGTGGTGGTGGTGCTCGAGAACGGGAAAACCTCGTTATTA	<i>Xho</i> I

<i>doc II -1-F</i>	GGAGGTGGCGGTAGT <u>GAATTC</u> AATAATGATGTGGGTAGGAGA	<i>EcoR I</i>
<i>doc II -1-R</i>	GTGGTGGTGGTGGTG <u>CTCGAG</u> TGCGTCGTAATCACTTGATG	<i>Xho I</i>
<i>doc I -2-F</i>	GGAGGTGGCGGTAGT <u>GAATTC</u> AATAATGATGTGGGTAGGAGA	<i>EcoR I</i>
<i>doc I -2-R</i>	GTGGTGGTGGTGGTG <u>CTCGAG</u> GCTAAGAAGTTTCTTTTTTAG AAGAG	<i>Xho I</i>
<i>doc I -3-F</i>	GGAGGTGGCGGTAGT <u>GAATTC</u> GTAATTGTATATGGAGATTA	<i>EcoR I</i>
<i>doc I -3-R</i>	GTGGTGGTGGTGGTG <u>CTCGAG</u> GCTTGAAGCTTACTTACCA	<i>Xho I</i>
<i>docIII-1-F</i>	GGAGGTGGCGGTAGT <u>GAATTC</u> CCTACATGGGGCGATACAAA	<i>EcoR I</i>
<i>docIII-1-R</i>	GTGGTGGTGGTGGTG <u>CTCGAG</u> GCTAAACGTCTGCGTTAACCTT AC	<i>Xho I</i>
