***Clostridium difficile* surface proteins are anchored to the cell wall using CWB2 motifs that recognise the anionic polymer PSII**

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SUPPLEMENTARY INFORMATION

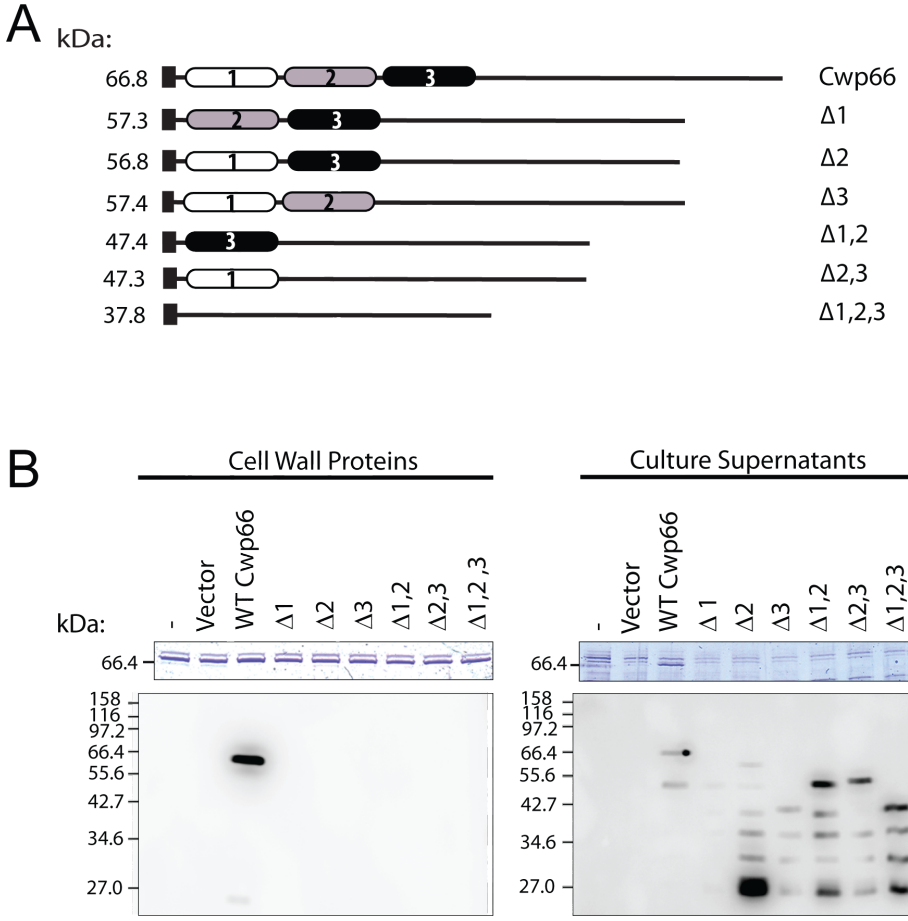

Fig. S1. Deletion of the CWB2 repeats in Cwp66 prevents cell wall attachment in *C. difficile*. A. Deletion derivatives of Cwp66 constructed. **B.** Cell wall extracts and culture supernatants from *C. difficile cwp66*::*erm* containing plasmids expressing the deletion derivatives of Cwp66 visualised by Coomassie blue staining (top) and western blotting using anti-Cwp66 antibody (bottom).

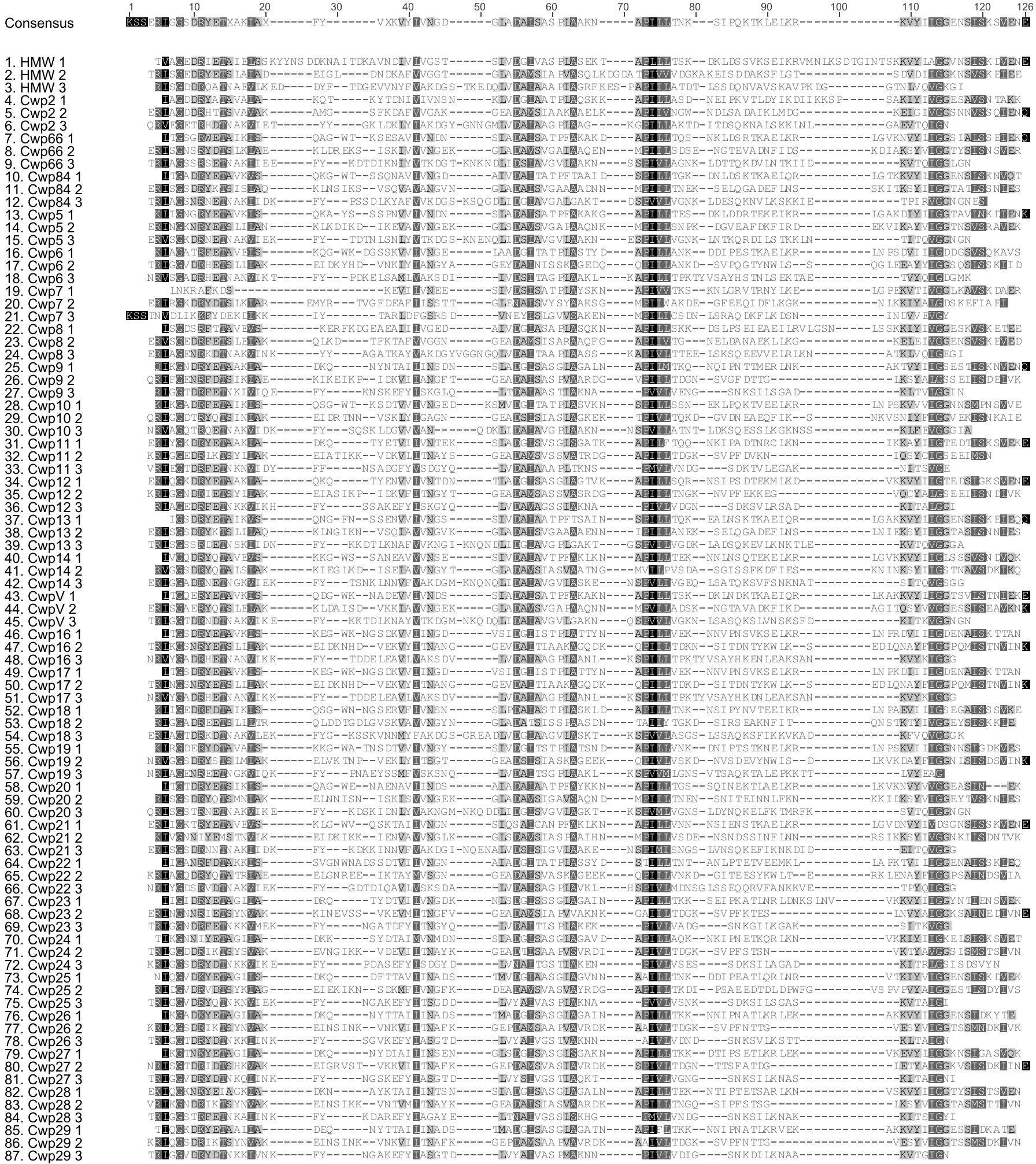
Consensus
1. HMW 1
2. HMW 2
3. HMW 3
4. Cwp2 1
5. Cwp2 2
6. Cwp2 3
7. Cwp66 1
8. Cwp66 2
9. Cwp66 3
10. Cwp84 1
11. Cwp84 2
12. Cwp84 3
13. Cwp5 1
14. Cwp5 2
15. Cwp5 3
16. Cwp6 1
17. Cwp6 2
18. Cwp6 3
19. Cwp7 1
20. Cwp7 2
21. Cwp7 3
22. Cwp8 1
23. Cwp8 2
24. Cwp8 3
25. Cwp9 1
26. Cwp9 2
27. Cwp9 3
28. Cwp10 1
29. Cwp10 2
30. Cwp10 3
31. Cwp11 1
32. Cwp11 2
33. Cwp11 3
34. Cwp12 1
35. Cwp12 2
36. Cwp12 3
37. Cwp13 1
38. Cwp13 2
39. Cwp13 3
40. Cwp14 1
41. Cwp14 2
42. Cwp14 3
43. CwpV 1
44. CwpV 2
45. CwpV 3
46. Cwp16 1
47. Cwp16 2
48. Cwp16 3
49. Cwp17 1
50. Cwp17 2
51. Cwp17 3
52. Cwp18 1
53. Cwp18 2
54. Cwp18 3
55. Cwp19 1
56. Cwp19 2
57. Cwp19 3
58. Cwp20 1
59. Cwp20 2
60. Cwp20 3
61. Cwp21 1
62. Cwp21 2
63. Cwp21 3
64. Cwp22 1
65. Cwp22 2
66. Cwp22 3
67. Cwp23 1
68. Cwp23 2
69. Cwp23 3
70. Cwp24 1
71. Cwp24 2
72. Cwp24 3
73. Cwp25 1
74. Cwp25 2
75. Cwp25 3
76. Cwp26 1
77. Cwp26 2
78. Cwp26 3
79. Cwp27 1
80. Cwp27 2
81. Cwp27 3
82. Cwp28 1
83. Cwp28 2
84. Cwp28 3
85. Cwp29 1
86. Cwp29 2
87. Cwp29 3

Fig. S2. Alignment of CWB2 motifs from *C. difficile*. The 87 CWB2 motifs from the 29 CWPs from *C. difficile* 630 were aligned using ClustalW and displayed using Geneious software. Highly conserved residues are shaded based on the Blosum 62 matrix; 100% similar, 80-100% similar, 60-80% similar and less than 60% similar. The motifs are numbered according to their location within their parent protein from N –> C terminus.

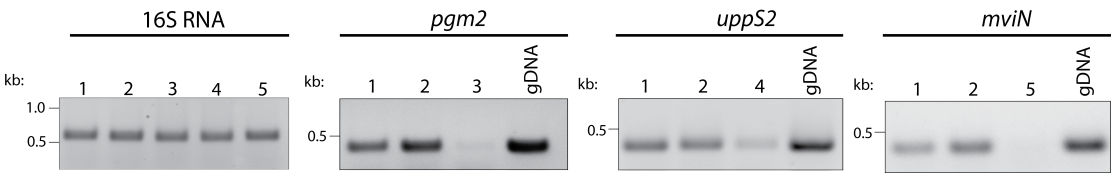

Fig. S3 Semi-quantitative RT-PCR of gene knock-down by expression of anti-sense RNA. Overnight cultures were diluted to an OD_{600} of 0.025 in BHIS broth. With the exception of 630, all cultures were supplemented with 500 ng/ml anhydrotetracycline. Cultures were grown to an OD_{600} of approximately 0.3 and cDNA prepared by reverse transcription of 1 µg of RNA. mRNA levels were then determined by semi-quantitative PCR. The strains are indicated as follows: 1 – 630; 2 – vector control, 3 – pSEW036 (*pgm2);* 4 - pSEW037 – (*uppS2);* 5 – pSEW038 (*mviN*). 25 cycles of PCR were used for amplification of 16S RNA, 35 cycles for amplification of *pgm2*, 32 cycles for amplification of *uppS2* and 28 cycles for amplification of *mviN.*

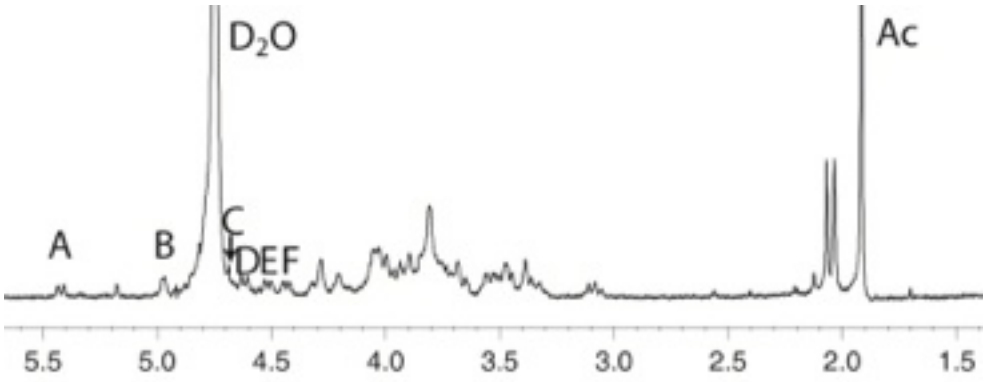

Fig. S4. ^{1}H NMR spectrum of the PS fraction purified from PG-PS. The ^{1}H NMR spectrum profile is similar to that of PSII (Ganeshapillai *et al* 2008). No other singlet, except the singlet between 1.9 and 2 which corresponds to acid acetic (Ac), was detected. The six anomeric resonances (designated A-F) and the two singlet resonances between 2.0 and 2.1 ppm were identified by Ganeshapillai *et al* 2008 as belonging to two D-Gal*p*NAc units.

Table S1 Plasmids created in this study

Plasmid	Relevant characteristics	Primers
pHAS002	pRFP144 based shuttle plasmid; expresses Cwp2	NF1304, NF1305
pZLS002	pRFP144 based shuttle plasmid; expresses Cwp66	NF1302, NF1303
pSEW002	Expression of Cwp2 lacking CWB_2 repeat 1	NF1552, NF1553
pSEW003	Expression of Cwp2 lacking CWB_2 repeat 2	NF1554, NF1555
pSEW004	Expression of Cwp2 lacking CWB_2 repeat 3	NF1556, NF1557
pSEW005	Expression of Cwp2 lacking CWB_2 repeat 1 and 2	NF1552, NF1555
pSEW006	Expression of Cwp2 lacking CWB_2 repeat 2 and 3	NF1557, NF1554
pSEW007	Expression of Cwp2 lacking CWB_2 repeat 1, 2 and 3	NF1557, NF1554
pSEW030	Expression of Cwp66 lacking CWB_2 repeat 1	NF1756, NF1757
pSEW031	Expression of Cwp66 lacking CWB_2 repeat 2	NF1758, NF1759
pSEW032	Expression of Cwp66 lacking CWB_2 repeat 3	NF1760, NF1761
pSEW040	Expression of Cwp66 lacking CWB_2 repeat 1 and 2	NF1756, NF1759
pSEW041	Expression of Cwp66 lacking CWB_2 repeat 2 and 3	NF1761, NF1758
pSEW042	Expression of Cwp66 lacking CWB_2 repeat 1, 2 and 3	NF1756, NF1761
pSEW056	Expression of Cwp66 with PILL residues of CWB_2 repeat 1mutated to AAAA	NF2163, NF2164
pSEW057	Expression of Cwp66 with PILL residues of CWB_2 repeat 2mutated to AAAA	NF2165, NF2166
pSEW058	Expression of Cwp66 with PILL residues of CWB_2 repeat 3mutated to AAAA	NF2167, NF2168
pSEW059	Expression of Cwp66 with PILL residues of CWB_2 repeat 1, 2 and3 mutated to AAAA	NF2163, NF2164, NF2165, NF2166, NF2167, NF2168
pSEW067	Expression of Cwp66 with PILL residues of CWB_2 repeat 1 and 2mutated to AAAA	NF2163, NF2164, NF2165, NF2166
pSEW068	Expression of Cwp66 with PILL residues of CWB_2 repeat 2 and 3mutated to AAAA	NF2165, NF2166, NF2167, NF2168
pSEW069	Expression of Cwp66 with PILL residues of CWB_2 repeat 1 and 3mutated to AAAA	NF2163, NF2164, NF2167, NF2168
pSEW085	Expression of Cwp66 with P of CWB_2 repeat 1 mutated to A	NF2664, NF2665

pSEW086	Expression of Cwp66 with P of CWB_2 repeat 2 mutated to A	NF2666, NF2667
pSEW087	Expression of Cwp66 with P of CWB_2 repeat 3 mutated to A	NF2668, NF2669
pSEW091	Expression of Cwp66 with P of CWB_2 repeat 1 and 2 mutated to A	NF2664, NF2665, NF2666, NF2667
pSEW092	Expression of Cwp66 with P of CWB_2 repeat 1 and 3 mutated to A	NF2664, NF2665, NF2668, NF2669
pSEW093	Expression of Cwp66 with P of CWB_2 repeat 2 and 3 mutated to A	NF2666, NF2667, NF2668, NF2669
pSEW096	Expression of Cwp66 with P of CWB_2 repeat 1, 2 and 3 mutated to A	NF2664, NF2665, NF2666, NF2667 NF2668, NF2669
pSEW079	Expression of Cwp2 with CWB_2 repeat 2 replaced with CWB_2repeat 1	NF2541, NF2542
pSEW080	Expression of Cwp2 with CWB_2 repeat 3 replaced with CWB_2 repeat 1	NF2543, NF2544
pSEW081	Expression of Cwp2 with CWB_2 repeat 1 replaced with CWB_2repeat 2	NF2545, NF2546
pSEW082	Expression of Cwp2 with CWB_2 repeat 3 replaced with CWB_2repeat 2	NF2547, NF2548
pHAS003	Retargeted vector for construction of *erm::cwp2* mutant	
pZLS003	Retargeted vector for construction of *erm::cwp66* mutant	

Table S2 Oligonucleotides used in this study

Name	Sequence (5' to 3')	Description
NF1552	CAATTTAACCTTTGAATTACCTTCTAATG	To remove CWB_1 of Cwp2 by inverse PCR
NF1553	CAATTAGAATCAGTAACTAAGAATATTG	To remove CWB_1 of Cwp2 by inverse PCR
NF1554	AATATTCTTAGTTACTGATTCTAATTGC	To remove CWB_2 of Cwp2 by inverse PCR
NF1555	CTTGCCGATATTGATAAAGATAGAAAAG	To remove CWB_2 of Cwp2 by inverse PCR
NF1556	AACTTTTCTATCTTTATCAATATCGGC	To remove CWB_3 of Cwp2 by inverse PCR
NF1557	GGAGTTGAATTGACAGTAATACAAAAG	To remove CWB_3 of Cwp2 by inverse PCR
NF1756	TGTATCTTTTGTAGATGCATTAGATG	To remove CWB_1 of Cwp66 by inverse PCR
NF1757	TTAAATGCAGAAAATATAAATTTTG	To remove CWB_1 of Cwp66 by inverse PCR
NF1758	AAAATTTATATTTTCTGCATTTAATTG	To remove CWB_2 of Cwp66 by inverse PCR
NF1759	AGTTTACCAAATGCAACAAGAATAGC	To remove CWB_2 of Cwp66 by inverse PCR
NF1760	TGCATTTGGTAAACTTCTTTCTACAG	To remove CWB_3 of Cwp66 by inverse PCR
NF1761	AATGAAAATGTTGTAGAAGATATAC	To remove CWB_3 of Cwp66 by inverse PCR
NF2163	GCTGCTGCTGCTACTCAAAGTAATAAATTGGATAG	To mutate PILL residues of CWB_2 repeat 1 of Cwp66 to AAAA by inverse PCR
NF2164	CGCATCTTTTGCTTTAGCAAATGG	To mutate PILL residues of CWB_2 repeat 1 of Cwp66 to AAAA by inverse PCR
NF2165	GCTGCTGCTGCTTCTGATTCAGAGAATGG	To mutate PILL residues of CWB_2 repeat 2 of Cwp66 to AAAA by inverse PCR
NF2166	CATGTTTTCTTGAGCAGCTATAGCTCC	To mutate PILL residues of CWB_2 repeat 2 of Cwp66 to AAAA by inverse PCR
NF2167	GCTGCTGCTGCTGCAGGAAATAAGCTTGATAC	To mutate PILL residues of CWB_2 repeat 3of Cwp66 to AAAA by inverse PCR
NF2168	AGAACTATTTTTAGCTGCTAATAC	To mutate PILL residues of CWB_2 repeat 3 of Cwp66 to AAAA by inverse PCR
NF2664	GCTATATTATTAACTCAAAGTAATAAATTGGATAGTAG	To mutate P of PILL residues of CWB_2 repeat 1 of Cwp66 to AAAA by inverse PCR
NF2665	CGCATCTTTTGCTTTAGCAAATG	To mutate P of PILL residues of CWB_2 repeat 1 of Cwp66 to AAAA by inverse PCR
NF2666	GCTATAATACTTTCTGATTCAGAGAATGGAAC	To mutate P of PILL residues of CWB_2 repeat 2 of Cwp66 to AAAA by inverse PCR
NF2667	CATGTTTTCTTGAGCAGCTATAGCT	To mutate P of PILL residues of CWB_2 repeat 2 of Cwp66 to AAAA by inverse PCR
NF2668	GCTATAGTATTAGCAGGAAATAAGCTTG	To mutate P of PILL residues of CWB_2 repeat 3 of Cwp66 to AAAA by inverse PCR
NF2669	AGAACTATTTTTAGCTGCTAATACACC	To mutate P of PILL residues of CWB_2 repeat 3 of Cwp66 to AAAA by inverse PCR
NF2541	aatcagtaactaagaatattATAGCTGGAGATGATAGATATG	Replace CWB_2 repeat 2 with CWB_2 repeat 1 in Cwp2 using Gibson's assembly
NF2542	tttatcaatatcggcaagCTTTTTAGCTGTATTTGATACTGC	Replace CWB_2 repeat 2 with CWB_2 repeat 1 in Cwp2 using Gibson's assembly
NF2543	ttgataaagatagaaaagttATAGCTGGAGATGATAGATATG	Replace CWB_2 repeat 3 with CWB_2 repeat 1 in Cwp2 using Gibson's assembly
NF2544	actgtcaattcaactccCTTTTTAGCTGTATTTGATACTGC	Replace CWB_2 repeat 3 with CWB_2 repeat 1 in Cwp2 using Gibson's assembly
NF2545	gtaattcaaaggttaaattgGAAAGACTAGCTGGAGATG	Replace CWB_2 repeat 1 with CWB_2 repeat 2 in Cwp2 using Gibson's assembly
NF2546	tagttactgattctaattgTTGATTTTCAATTTGGCTAGATAC	Replace CWB_2 repeat 1 with CWB_2 repeat 2 in Cwp2 using Gibson's assembly
NF2547	ttgataaagatagaaaagttGAAAGACTAGCTGGAGATG	Replace CWB_2 repeat 3 with CWB_2 repeat 2 in Cwp2 using Gibson's assembly
NF2548	actgtcaattcaactccTTGATTTTCAATTTGGCTAGATAC	Replace CWB_2 repeat 3 with CWB_2 repeat 2 in Cwp2 using Gibson's assembly

NF2549	gtaattcaaaggttaaattgCAAAGAGTTGAAGGAGAAAC	Replace CWB_2 repeat 1 with CWB_2 repeat 2 in Cwp2 using Gibson's assembly
NF2550	tagttactgattctaattgATTACCTATTTGAGTTACTTCTGC	Replace CWB_2 repeat 1 with CWB_2 repeat 2 in Cwp2 using Gibson's assembly
NF2551	aatcagtaactaagaatattCAAAGAGTTGAAGGAGAAAC	Replace CWB_2 repeat2 with CWB_2 repeat 3 in Cwp2 using Gibson's assembly
NF2552	tttatcaatatcggcaagATTACCTATTTGAGTTACTTCTGC	Replace CWB_2 repeat2 with CWB_2 repeat 3 in Cwp2 using Gibson's assembly
NF1810	gatc ggatcc AATTACCTTGAAATTTTAAATTAG	To amplify DNA from 630 gDNA for antisense RNA knock down of CD630_2780 (BamHI)
NF1811	gatc gagctc CTCTAAATTTTTATAAAATC	To amplify DNA from 630 gDNA for antisense RNA knock down of CD630_2780 (SacI)
NF1812	gatc ggatcc GATAATAATACCTATTG	To amplify DNA from 630 for antisense RNA knock down of CD630_2762 (BamHI)
NF1813	gatc gagctc CTCTACATAATCTATAAG	To amplify DNA from 630 for antisense RNA knock down of CD630_2762 (SacI)
NF1841	gatc ggatcc AGTTCTACTTTTAGAGTTTAAATTAG	To amplify DNA from 630 for antisense RNA knock down of CD630_2781 (BamHI)
NF1842	gatc gagctc CATACCTGTCCCATAGAAATTTG	To amplify DNA from 630 for antisense RNA knock down of CD630_2781 (SacI)
NF2734	CTGTAACAAATTGCTCTGTAAATAAAAGAC	To determine mRNA levels of CD630_2781 by RT PCR
NF2735	CTAAAGCAGCATTGTGGATTATG	To determine mRNA levels of CD630_2781 by RT PCR
NF2736	TGCTTTTATTTGACCTTCTTTGGCTTTAATTC	To determine mRNA levels of CD630_2762 by RT PCR
NF2737	TAGAAGGTGGGCAAGTCAAAATGGTATGAC	To determine mRNA levels of CD630_2762 by RT PCR
NF2738	CAAAAATATAAGCCTTTACACCATAAGCAC	To determine mRNA levels of CD630_2780 by RT PCR
NF2739	GAACAAACGAAAAATGAGCTATTAAGTATAAAAG	To determine mRNA levels of CD630_2780 by RT PCR
NF408	TCTTGAATATCAAAGGTGAGCCAGTACA	To amplify 16 S RNA of C. difficile
NF409	TACAGCGTGGACTACCAGGGTATCTAAT	To amplify 16 S RNA of C. difficile