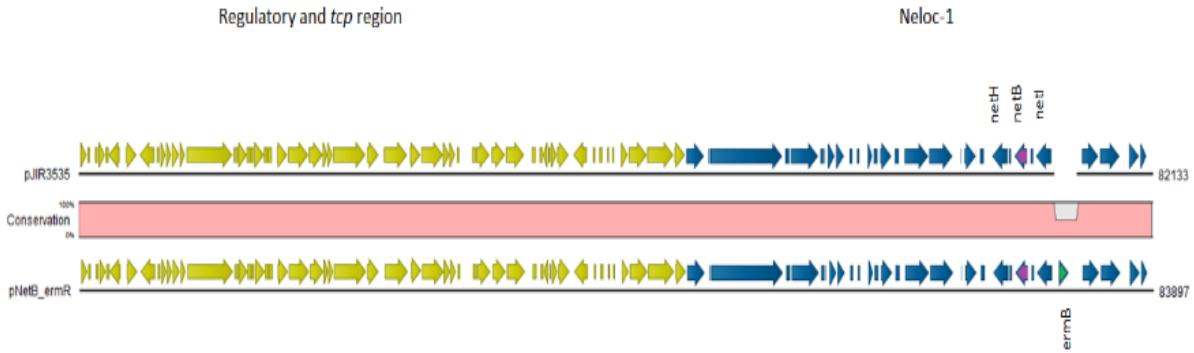


1 **Supplementary Material**

2 **Supplementary Table 1:** Conjugation frequencies from *in vitro*-constructed transconjugants

Strain Combination	Number of Donors in 1 ml (Rif, Erm)	Number of recipients in 1 ml (Nal)	Donor: Recipient Ratio	Number of Transconjugants in 1 ml (Nal Erm)	Efficiency of conjugation
NE18:PBD1	2.00×10^8	1.00×10^9	1:10	3.00×10^4	1.5×10^{-4}
NE18:NE33	7.00×10^8	1.30×10^9	1:10	1.80×10^4	2.57×10^{-4}

3



4

5 **Supplementary Figure 1:** Alignment of pJIR3535 and pNetB-Erm^R; there is 100% conservation
 6 across the plasmid, apart from the *ermB* gene TargetTron insertion. The yellow coloured cds
 7 represent genes in the regulatory and *tcp* conserved regions shared by most large *C. perfringens*
 8 plasmids, blue represents cds in NEloc-1 with *netB* gene coloured purple and the *ermB* gene in
 9 green.

10

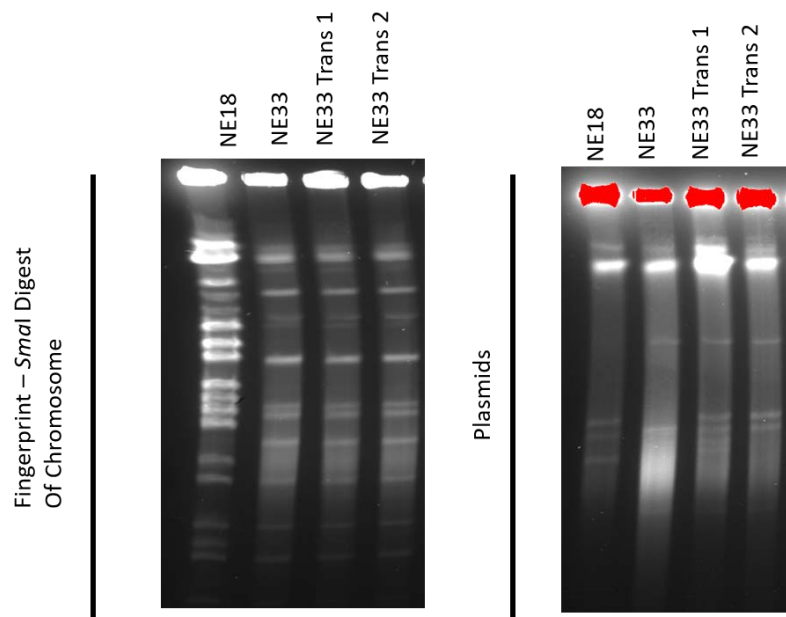
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17 **Supplementary Figure 2:** PFGE confirmation of *in vitro*-derived BER-NE33 transconjugants.

18 The panel on the left shows the PFGE *Sma*I chromosomal fingerprint of the donor strain EHE-

19 NE18 (Erm^R, Rif^R), recipient strain BER-NE33 (Nal^R, Tet^R) and two transconjugants NE33

20 Transconjugant 1 (Erm^R, Nal^R, Tet^R) and NE33 transconjugant 2 (Erm^R, Nal^R). The panel on the

21 right shows the presence of undigested plasmid(s) in a PFGE gel. The donor strain EHE-NE18

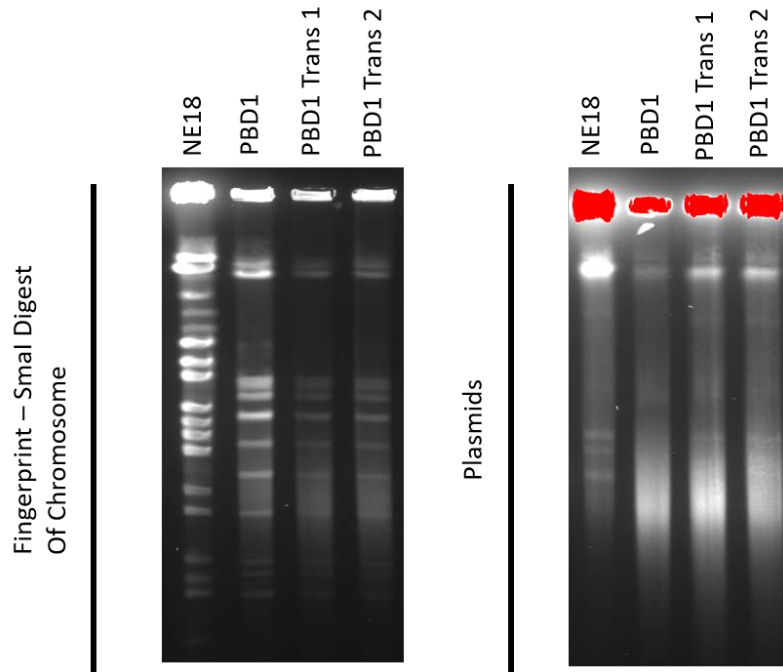
22 shows three bands corresponding to the three plasmids pJIR3536, pJIR3537 and pJIR3838. The

23 recipient strain shows a single band corresponding to the atypical tetracycline resistance plasmid.

24 The transconjugant BER-NE33 Transconjugant 1 (Erm^R, Nal^R, Tet^R) has 4 bands corresponding

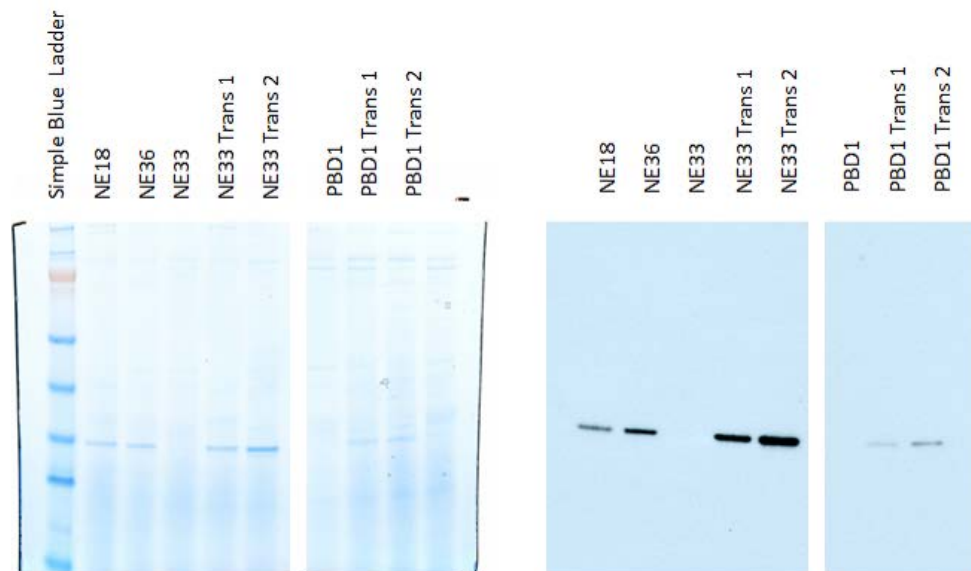
25 to all the plasmids in BER-NE33 and EHE-NE18, and BER-NE33 transconjugant 2 (Erm^R, Nal^R)

26 only contains two bands due to the absence of the tetracycline resistance plasmids.



27

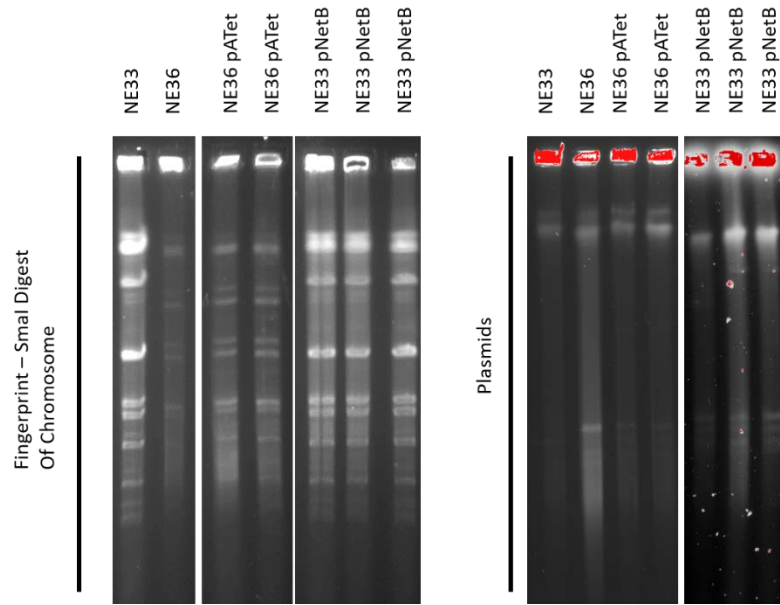
28 **Supplementary Figure 3:** PFGE confirmation of *in vitro*-derived PBD1 transconjugants. The
 29 panel on the on the left shows the PFGE *SmaI* chromosomal fingerprint of the donor strain EHE-
 30 NE18 (Erm^R, Rif^R), recipient strain PBD1 (Nal^R) and two transconjugants PBD1 Transconjugant
 31 1 (Erm^R, Nal^R, Tet^R) and PBD1 transconjugant 2 (Erm^R, Nal^R). The panel on the right shows the
 32 presence of undigested plasmid in a PFGE gel. The donor strain EHE-NE18 shows three bands
 33 corresponding to the three plasmids pJIR3536, pJIR3537 and pJIR3844. The recipient strain
 34 shows no bands as no plasmids are observed in this strain. The transconjugants PBD1
 35 transconjugant 1 (Erm^R, Nal^R,) has 2 bands due to the absence of the tetracycline resistance
 36 plasmid, while PBD1 transconjugant 2 (Erm^R Nal^R, Tet^R) contains all three plasmids from the
 37 EHE-NE18 donor.



38

39 **Supplementary Figure 4:** Simply blue stained SDS-PAGE protein gel of TCA precipitated
 40 culture supernatants from actively growing cultures ($OD_{600}=1.0$ in TPG broth) of *C. perfringens*
 41 strains; EHE-NE18, WER-NE36, BER-NE33, PBD1 and four transconjugant; 2 BER-NE33
 42 transconjugants (one with tetracycline resistance, one with tetracycline sensitivity) and 2 PBD1
 43 transconjugants (one with tetracycline resistance, one with tetracycline sensitivity). The most
 44 intense band is the NetB protein (33 kDa). Presence of NetB expression in supernatants was
 45 confirmed by Western Blot on the right using rabbit anti-NetB polyclonal IgG.

46



47

48 **Supplementary Figure 5:** PFGE confirmation of *in vivo*-derived transconjugants isolated from
 49 co-challenge with BER-NE33 (Nal^R, Tet^R) and WER-NE36 (Rif^R, Erm^R). The panel on the left
 50 shows the PFGE *Sma*I chromosomal fingerprint of the challenge strains and the isolated
 51 transconjugants. The panel on the right shows the presence of undigested plasmids on PFGE.

52