Supplementary Method 1: pET28b cloning

PCR primers

Primer	Direction	Sequence[1]	Res. site
cotA			
CotA-NcoI-F	forward	GAT*CCATGG*CTGTGGAAAATAATAAATG	NcoI
CotA-XhoI- R	reverse	ATC*CTCGAG*TGCAATATAATCTATAGAATCTACA CATAC	XhoI
cotB			
CotB-NcoI-F	forward	GAT*CCATGG*CTATAGATAATCAAAAATATG	NcoI
CotB-XhoI-R	reverse	ATC*CTCGAG*CATGTTTTTATAACTCTC	XhoI
cotC			
CotC-NcoI-F	forward	GAT*CCATGG*CTTGGATTTATCAAAAAAC	NcoI
CotC-XhoI-R	reverse	ATC*CTCGAG*AAACTGATGCTTGCACTC	XhoI
cotD			
CotD-NcoI-F	forward	GAT*CCATGG*CTTGGATATATCAGAAAAC	NcoI
CotD-XhoI-R	reverse	ATC*CTCGAGG*AACATTTTTTGAGATTC	XhoI
cotEΔ			
CotEC-NcoI-F	forward	GAT*CCATGG*CTCCAATTGTAGCAG	NcoI
CotEC-XhoI-R	reverse	ATC*CTCGAGG*AATTGCCCATAAATAC	XhoI

[1] 5'-3', restriction site is in italics

Construction of pET expression vectors.
pET28b expression vectors that express the *cot* gene ORFs were constructed by amplifying the respective DNA by PCR from *C. difficile* 630 chromosomal DNA and ligating to cleaved pET28b using the forward and reverse primers shown above.