

**S1 Table. List of Plasmids and Primers**

Plasmid	Relevant genotype	Ori	Source or Reference
pBOMB4-Tet::L2	<i>bla Ptet::mCherry P<sub>Nm</sub>::gfp</i>	pUC19	1
pBOMB-cl <sub>s</sub> _6xH::L2	<i>bla Ptet::cls_6xH P<sub>Nm</sub>::gfp</i>	pUC19	This study
pBOMB-ΔN25_cl <sub>s</sub> _6xH::L2	<i>bla Ptet::ΔN25_cl<sub>s</sub>_6xH P<sub>Nm</sub>::gfp</i>	pUC19	This study
pBOMB-psdD_6xH::L2	<i>bla Ptet::psdD_6xH P<sub>Nm</sub>::gfp</i>	pUC19	This study
pTLR2- <i>mreB</i> _6xH	<i>bla Ptet::mreB_6xH P<sub>dnaKmut</sub>::mKate2</i>	ColE1	2
pBOMBmC::L2	<i>bla Ptet::mCherry P<sub>Nm</sub>::mCherry</i>	pUC19	3
pBOMBmC-cl <sub>s</sub> _TM_GFP::L2	<i>bla Ptet::cls_TM_GFP P<sub>Nm</sub>::mCherry</i>	pUC19	This study
pBOMB-cl1orf83_6xH::L2	<i>bla Ptet::cl1orf83_6xH P<sub>Nm</sub>::gfp</i>	pUC19	This study
pBOMB-cl <sub>s</sub> _TM_cl1orf83_6xH::L2	<i>bla Ptet::cls_TM_cl1orf83_6xH P<sub>Nm</sub>::gfp</i>	pUC19	This study
pBOMB-TM_cl1orf83_6xH::L2	<i>bla Ptet::TM<sub>Ec</sub>_OppB_cl1orf83_6xH P<sub>Nm</sub>::gfp</i>	pUC19	This study
pBOMB-TM_mCherry::L2	<i>bla Ptet::TM<sub>Ec</sub>_OppB_mCherry P<sub>Nm</sub>::gfp</i>	pUC19	This study
pSTM25	<i>aadA Plac::t25-TM<sub>Ec</sub>_OppB</i>	p15A	4
pSTM25-cl <sub>s</sub>	<i>aadA Plac::t25-TM<sub>Ec</sub>_OppB-cl<sub>s</sub></i>	p15A	This study

Primer name	Sequence	Features	Usage
cls/(pBOMB)/5'	gatctaaagaggagaaaggatctgcATGAAA ATGGCTTTTTTACG	lower case for plasmid overlap construction	For amplification of cls into pBOMB
cls_6xH/(pBOMB)/3'	tttgaatggtcgaccggtacctgcattaatggtgatggtgatggtgAGATGGCATGTATCTCTG TTC	lower case for plasmid overlap construction; adds 6xH sequence to cls	For amplification of cls into pBOMB
psdD/(pBOMB)/5'	gatctaaagaggagaaaggatctgcATGGCAG CGCGGGAAATG	lower case for plasmid overlap construction	For amplification of psdD into pBOMB
psdD_6xH/(pBOMB)/3'	tttgaatggtcgaccggtacctgcattaatggtgatggtgatggtgTGAAGAGAAACGTTTTCC TAACGATTG	lower case for plasmid overlap construction; adds 6xH sequence to cls	For amplification of psdD into pBOMB
cls/(pSTM25)/5'	acgcacaagggcctctagagATAGCTATTC CGGATGGAG	lower case for plasmid overlap construction	For amplification of cls into pSTM25

cls/(pSTM25)/3'	attcttagttacttaggtacttaAGATGGCATG TATCTC	lower case for plasmid overlap construction	For amplification of cls into pSTM25
N26- cls/(pBOMB)/5'	aaagaggagaaaggatctgcATGGCTATTC CGGATGGAGAC	lower case for plasmid overlap construction	For amplification of ΔN25_cls into pBOMB
cls_TM/(pBOMBm C)/5'	aaagaggagaaaggatctgcATGAAAATG GCTTTTTTACGG	lower case for plasmid overlap construction	For amplification of cls_TM fragment
cls_TM/(GFP)/3'	ctcctttactAGTGTGCGCAAACCAT TC	lower case for overlap with GFP	For amplification of cls_TM fragment
GFP/(cls_TM)/5'	tgcgcacactAGTAAAGGAGAAGCAC TTTTTC	lower case for overlap with cls_TM	For amplification of GFP
GFP/(pBOMB)/3'	tttgaatggtcgaccggtacTTATTTGTATA GTTTCATCCATGCCATG	lower case for plasmid overlap construction	For amplification of GFP
ct284 cls qPCR F	CACCTGTTGGCCGCTATTA		Transcript analysis
ct284 cls qPCR R	GCGCTGAAGAACTGTGGATA		Transcript analysis
ct446 euo qPCR F	CGAAGACTACTCGTTGGGAAAT A		Genomic DNA quantification
ct446 euo qPCR R	AACAGAAGCTCTCCTTGATAAGT		Genomic DNA quantification

gBlock name	Sequence	Features	Usage
c11orf83_6xH (pBOMB)	aaagaggagaaaggatctgcATGGACTCACT CCGTAAGATGTTAATATCCGTCGC TATGCTTGGAGCTGGTGCAGGTGT TGGCTACGCATTACTAGTTATCGT GACCCCGGCGAACGCAGAAAAC AGGAAATGCTCAAGGAAATGCCT TTACAGGACCCACGTTCAAGAGA AGAAGCGGCCCGAACGCAACAGT TACTCTTAGCAACCTTACAGGAAG CTGCTACAACACAGGAGAATGTT GCCTGGAGAAAAAATTGGATGGT AGGTGGGGAAGGCGGTGCAGGTG GAAGATCCCCGCACCATCACCAT <b>CACCATTAAgtaccggtcgaccattcaaa</b>	lower case for plasmid overlap, bolded is 6xH	Insert codon- optimized c11orf83_ 6xH into pBOMB
cls_TM_c11orf83 _6xH (pBOMB)	aaagaggagaaaggatctgcATGAAAATGG <u>CTTTTTTACGGAAAATATTTGTAT</u> <u>TTGTAGCTTGTGTTGTCTCGTTGA</u> <u>ATGGTTTTGCGCACACTGACTCAC</u> TCCGTAAGATGTTAATATCCGTCG	lower case for plasmid overlap, underlined sequence is cls_TM, bolded is 6xH	Insert codon- optimized cls_TM_c1 1orf83_6x

	CTATGCTTGGAGCTGGTGCAGGTG TTGGCTACGCATTACTAGTTATCG TGACCCCCGGCGAACGCAGAAAA CAGGAAATGCTCAAGGAAATGCC TTTACAGGACCCACGTTCAAGAG AAGAAGCGGCCCGAACGCAACAG TTACTCTTAGCAACCTTACAGGAA GCTGCTACAACACAGGAGAATGT TGCCTGGAGAAAAAATTGGATGG TAGGTGGGGAAGGCGGTGCAGGT GGAAGATCCCCGCACCATCACCA <b>TCACCATTA</b> Agtaccggtcgaccattcaaa		H into pBOMB
TM_c11orf83_6x H (pBOMB)	gatctaaagaggagaaaggatctgcATGTTAAA <u>ATTTATTCTACGTCGCTGTCTGGA</u> <u>AGCGATTCCGACGCTATTTATTCT</u> <u>TATTACTATTTCGTTCTTTATGATG</u> <u>CGCCTCGCGCCGGGAAGCCCTTTT</u> <u>ACCGGTGGATCATTACTAGTTATC</u> GTGACCCCCGGCGAACGCAGAAA ACAGGAAATGCTCAAGGAAATGC CTTTACAGGACCCACGTTCAAGAG AAGAAGCGGCCCGAACGCAACAG TTACTCTTAGCAACCTTACAGGAA GCTGCTACAACACAGGAGAATGT TGCCTGGAGAAAAAATTGGATGG TAGGTGGGGAAGGCGGTGCAGGT GGAAGATCCCCGCACCATCACCA <b>TCACCATTA</b> Agtaccggtcgaccattcaata tgt	lower case for plasmid overlap, underlined sequence is oppB TM from <i>E.</i> <i>coli</i> , italicized sequence is GGS linker, bolded is 6xH, c11orf83 sequence lacks any predicted membrane- targeting motifs (ΔN23)	Insert codon- optimized TM_c11or f83_6xH into pBOMB
Ec_oppB_TM (pBOMB)	gatctaaagaggagaaaggatctgcATGTTAAA <u>ATTTATTCTACGTCGCTGTCTGGA</u> <u>AGCGATTCCGACGCTATTTATTCT</u> <u>TATTACTATTTCGTTCTTTATGATG</u> <u>CGCCTCGCGCCGGGAAGCCCTTTT</u> <u>ACCGGTGGATC</u> Aggcatggtcttaagggeg aggaaga	lower case for plasmid overlap, underlined sequence is oppB TM from <i>E.</i> <i>coli</i> , italicized sequence is GGS linker	Insert <i>E.</i> <i>coli</i> OppB 1 <sup>st</sup> TM domain into pBOMB

- 1 Bauler, L. D. & Hackstadt, T. Expression and targeting of secreted proteins from *Chlamydia trachomatis*. *J Bacteriol* **196**, 1325-1334, doi:10.1128/JB.01290-13 (2014).
- 2 Lee, J., Cox, J.V., & Ouellette, S. P. Critical role for the extended N-terminus of chlamydial MreB in directing its membrane association and potential interaction with divisome proteins. *J Bacteriol* **202**, e00034-20 (2020).
- 3 Wood, N. A., Blocker, A. M., Seleem, M. A., Conda-Sheridan, M., Fisher, D. J., & Ouellette, S. P. The ClpX and ClpP2 orthologs of *Chlamydia trachomatis* perform discrete and essential functions in organism growth and development. *mBio* **11**, e02016-20 (2020).
- 4 Ouellette, S. P., Gauliard, E., Antosova, Z., & Ladant, D. A Gateway®-compatible bacterial adenylate cyclase-based two-hybrid system. *Environ Microbiol Rep* **6**: 259-267 (2014).

