

559 **Supporting Information**

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562 **A – Phylogenetic comparison of tmRNA sequences**

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<i>E. coli</i>	GGGGCUGAUUCUGGAUUCGACGGGAUUUGC--GAAACC--AAGGUGCAUGC CGAGG--GGCGGUUGGCCUCGUAAAA-----AGCCGCA----AAAAAUAGUC	89
<i>K. pneumoniae</i>	GGGGCUGAUUCUGGAUUCGACGGGAUUUGC--GAAACC--AAGGUGCAUGC CGAGG--GGCGGUUGGCCUCGUAAAA-----AGCCGCA----AAAAAUAGUC	89
<i>E. cloacae</i>	GGGGCUGAUUCUGGAUUCGACGGGAUUUGC--GAAACC--AAGGUGCAUGC CGAGG--GGCGGUUGGCCUCGUAAAA-----AGCCGCA----AAAAAUAGUC	90
<i>A. baumannii</i>	GGGGAUGUUAUUGGCUUCGACGCCGGUGAU--GAAACUCAUAGAUGC AUGCCGAGAG-CGCAUUUUUCUCGUAAAAUAA-----AAUUUGCA--UUUAAAUAGUC	95
<i>P. aeruginosa</i>	GGGGCCGAU-UAGGAUUCGACGCCGGU AAC--AAAACUU-GAGGGGCAUGC CGAGCU--GUUAGCAGAACUCGUAAAAUUCG--CUGCUGCA--AACUUUAGUU	94
<i>E. faecium</i>	GGGGACG-UUACGGAUUCGACAGGCACAGUC--GA-GCUUGAAUUGCGUUUCGUAG-GUUACGU---CUACGUAAAA-----ACGUUACAGUUAAAAUUAACU	90
<i>S. aureus</i>	GGGGACGUUCAUGGAUUCGACAGGGGUC CCCC-GA-GCUCAUUAAGCGUGUCGGAG--GGUUGU---CUUCGUCAUCAACACACACAGUU-----UAUAAUAACU	93
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<i>E. coli</i>	GCAAACGACGAAAAC-----UACGCUUUAGCAGCUUAA--UAACUCGUCUAGAGCCUCUCUCCUAGCCUCCGUCUUAAGGACGGGGA--UCAAGAGAGG--	181
<i>K. pneumoniae</i>	GCAAACGACGAAAAC-----UACGCUUUAGCAGCUUAA--UAACUCGUCUAGAGCCUCUCUCCUAGCCUCCGUCUUAAGGACGGGGA--UCAAGAGAGG--	182
<i>E. cloacae</i>	GCAAACGACGAAAAC-----UACGCUUUAGCAGCUUAA--UAACUCGUCUAGAGCCUCUCUCCUAGCCUCCGUCUUAAGGACGGGGA--UCAAGAGAGG--	183
<i>A. baumannii</i>	GCAAACGACGAAAACU-----UACGCUUAGCUGCCUAA-----GGGCCGCUUGUCGCUUCCUAGAAU--ACUUGUGGUCUGGGAA CCGGAC-----	175
<i>P. aeruginosa</i>	GCCAACGACGACAAC-----UACGCUUAGCUGCUUAA-----UGCGGCUAGCAGUCGCUAGGGGAU--GCGUUAACCCCGAAACGACUG-----	173
<i>E. faecium</i>	GCUAAAAACGAAAACAACUCUUAACGCUUUAGCUGCCUAAAAA--CAGUUAGCGUA--GAUCCUCUCGGCAUC--GCCCAU-GUGCUCGAGUAAGGGUCUUAACU	187
<i>S. aureus</i>	GCCAAUUCAAAACAACU--AAUUUCGACAGUCGCUUAAUC--GCACUCUGC-----AUCGCCUAACAGCAU--UCCUAU--AUGCUGUAACGCGAUUCAACCU	185
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<i>E. coli</i>	-----UCAAACCCAAAAGAGAUCCGCU--GGAAGCCCU--GCCUGGGGUUGAAGCGU--UAAAACUUAACAGGC--UA--GUUUGUAGUGGCGUGUCGUCGCG	271
<i>K. pneumoniae</i>	-----UCAAACCCAAAAGAGAUCCGCU--GGAAGCCCU--GCCUGGGGUUGAAGCGU--UAAAACU--CAUCAGGC--UA--GUUUGUAGUGGCGUGUCUUCGCG	271
<i>E. cloacae</i>	-----UCAAACCCAAAAGAGAUCCGCU--GGAAGCCCU--GCCUGGGGUUGAAGCGU--UAAAACU--AAUCAGGC--UA--GUACGUUAGUGGCGUUGUUGUCG	272
<i>A. baumannii</i>	---UGAAGCGCACGCACAAGUCCGUUAUAGAGUCA--GCCUCGGGGCUUUAUAC--CAAACUUA--GAGGUAUC--GCACUUUGU--ACCCU--GUUCGUC--	262
<i>P. aeruginosa</i>	-----UCA-GAUAGAACAGGAUCGCCGCCAAGUUC--GCGUAGACGUAACGGC--UAAAACUCA-UACAGC--UC--GCUCCA-AGCACCCUGCCACUCG	258
<i>E. faecium</i>	UUA-----GUGGGAUAC-GUUUCAACUUUCC--GUCUGU--AAGUUGAAAAAGAGAACAU--CAGAC--UAGCGAUACAGAA--UGCCU--GUCACUCG	268
<i>S. aureus</i>	UA-----AUAGGAUUAU-GCUAAACACUGCC--GUUUGAAGUCUGUUUAGAGAAACUUA--UCAAGC--UAGCAUCAUGU--UGGUU--GUUUUAUCA	267
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<i>E. coli</i>	CAGCUGGCAAGC-----GAAUGUAAA--GAC-UGA-CUAAAGCAUGUAGUACCGAGGAUGU--AGGAAUUUCGACGCGGGUUAACUCCCGCCAGCUCACCA	363
<i>K. pneumoniae</i>	CAGCUGGCAAGC-----GAAUGUAAA--GAC-UGA-CUAAAGCAUGUAGUACCGAGGAUGU--AGGAAUUUCGACGCGGGUUAACUCCCGCCAGCUCACCA	363
<i>E. cloacae</i>	CAGCUGGCGUGC-----GAAUGUAAA--GAC-AAA-CUAAAGCAUGUAGUACCGAGGAUGU--AGAAUUUCGGACGCGGGUUAACUCCCGCCAGCUCACCA	364
<i>A. baumannii</i>	GGGU-CACUUGGUGU--UAAAACAUA--GACGAUAUCUAAAGCAUGUAGUUAUCGAGCG-UAGUCUGCGGACGCGGGUUAACUCCCGCCAGCUCACCA	360
<i>P. aeruginosa</i>	GGCGGCGGGAGU-----UAACUCAGUA-GAGCUGG-CUAAAGCAUGUAGAACCGAUAGCGG--AGAGCUGCGGACGCGGGUUAACUCCCGCCAGCUCACCA	353
<i>E. faecium</i>	GCAAGCUGUAAAGU-GAAUCCUUAAA--GAGUUGA-CUAUGAAGCUGAG-AUUUUUUAAGUGGCGAUGUGUUUGGACGCGGGUUCGACUCCCGCCAGCUCACCA	367
<i>S. aureus</i>	CUUUUCAUGA-UGC-GAAACCUUUC---GA-UAAA-CUACACAGUAG-AAAGAUUGUUAUCAGGACCCUCUGACGCGGGUUAACUCCCGCCAGCUCACCA	362
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565 Adapted from the tmRNA Website by Kelly Williams and Corey Hudson (All tmRNA Alignment 08-  
 566 JUL-2014), with base-pairing color-coded (P1, P2, P3, P4, MLD, P5, P6, P7, P8, P9, P10, P11, and  
 567 P12). The predicted reading frame for the proteolysis-inducing peptide tag is underlined, and the  
 568 portions of the expected 3' CCA tail that are not encoded in the genome are lowercase.

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571 **B – Phylogenetic comparison of SmpB amino-acid sequences**

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<i>E. coli</i>	-MTKKKAHKPGSATIALNKRARHEYFIEEEFEAGLALQGWEVKSLRAGKANISDSYVLLR	59
<i>K. pneumoniae</i>	-MTKKKAHKPGSATIALNKRARHEYFIEDEYEAGLALQGWEVKSLRAGKANIGDSYVILK	59
<i>E. cloacae</i>	-MTKKKAHKPGSATIALNKRARHEYFIEEEFEAGLALQGWEVKSLRAGKANIGDSYVILK	59
<i>A. baumannii</i>	MAKATVVKKHNGGTIAQNKRARHDYFIEEKFEAGMSLLGWEVKSLRAGRMSLTESYVIFK	60
<i>P. aeruginosa</i>	---MAKQKKHPSGTIAQNKKALHDYFIEQRFEAGVALAGWEVKSLRAGKAQLVDSYVLLK	57
<i>E. faecium</i>	-----MPKGEGLIAQNKKARHDYSIIDTMEAGMVLQGTEIKSIRNSRINLKDGFIRVR	54
<i>S. aureus</i>	-----MAKKKSPGTLAENRKARHDYNIEDTIEAGIVLQGTEIKSIRRGSA NLKDSYAQVK	55
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<i>E. coli</i>	DGEAFLFGANITPMAVASTHVCDPTRTRKLLLLNQRELDLSYGRVNREGYTVVALSLYWK	119
<i>K. pneumoniae</i>	DGEAFLFGANFTPMAVASTHYVCDPTRTRKLLLLNQRELDLYGRINREGYTVVALSLYWK	119
<i>E. cloacae</i>	DGEAFLFGANFTPLTVASSHYVCDPTRTRKLLLLNKRELESYGRINREGFTVVALSLYWK	119
<i>A. baumannii</i>	NGEAFLFGAQQIQLLSASTHIVPEATRTRKLLLSRRELEKLMGAVNQKGYSCVPLACYWK	120
<i>P. aeruginosa</i>	DGEAWLLGSHITPLTTASTHVIADPVRTRKLLLHKRELKGLFGAVQQKGYACVALSMYWK	117
<i>E. faecium</i>	NGEAFLHNHVISPYEQG-NIFNHDPLRTRKLLLHKKQIIRLENELKNTGITVVPLKVYIR	113
<i>S. aureus</i>	NGEMYLNMHIAPIYEEG-NRFNHDPLRSRKLHLHKREI IKLGDQTREIGYSIVPLKLYLK	114
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<i>E. coli</i>	NAWCKVKIGVAKGKKQHDKRSDIKEREWQVDKARIMKNAHR-	160
<i>K. pneumoniae</i>	NAWCKVKIGVAKGKKQHDKRTDLKDREWALDKARIMKHAGR-	160
<i>E. cloacae</i>	NAWCKVKVGVAKGKKQHDKRTDLKEREWQVDKARIMKNAGR-	160
<i>A. baumannii</i>	GHLVKLEIALVKGKQLHDKRATKEDRWQRDKARIFHK----	158
<i>P. aeruginosa</i>	KHLVKCEIALAKGKDFDKRHTEKERDSREIQRAMRHGKDD	159
<i>E. faecium</i>	NGYAKVLIGLAKGKSYDKREDLKRKIDRQIDRTLKNFSR-	154
<i>S. aureus</i>	HGHCKVLLGVARGKKKYDKRQALKEKAVKRDVARDMKARY--	154
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574 Residue properties are color-coded: small [small+ hydrophobic (incl. aromatic -Y)]; acidic; basic – H;

575 Hydroxyl + sulfhydryl + amine + G, unusual amino or imino acids, etc. Key: \*, positions having a single

576 and fully conserved residue; ., weakly similar properties conserved between groups; :, strongly similar

577 properties conserved between groups.

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580 **Supplementary Figure 1. Phylogenetic comparison of SmpBs and tmRNAs from ESKAPE**

581 **pathogens and *E. coli*.** A) Shown here are the RNA sequences of tmRNAs and B) the SmpB amino

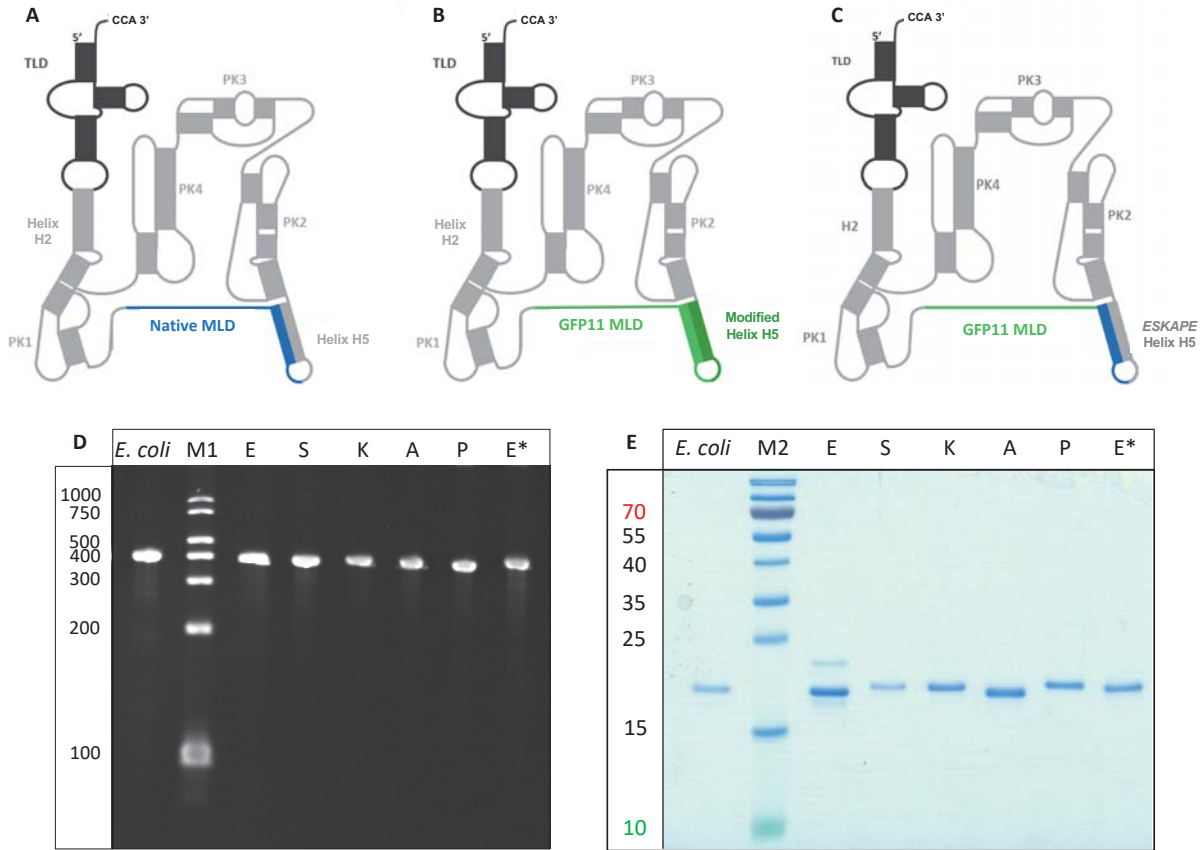
582 acid sequences (Clustal Omega) from ESKAPE and *E. coli* bacteria.

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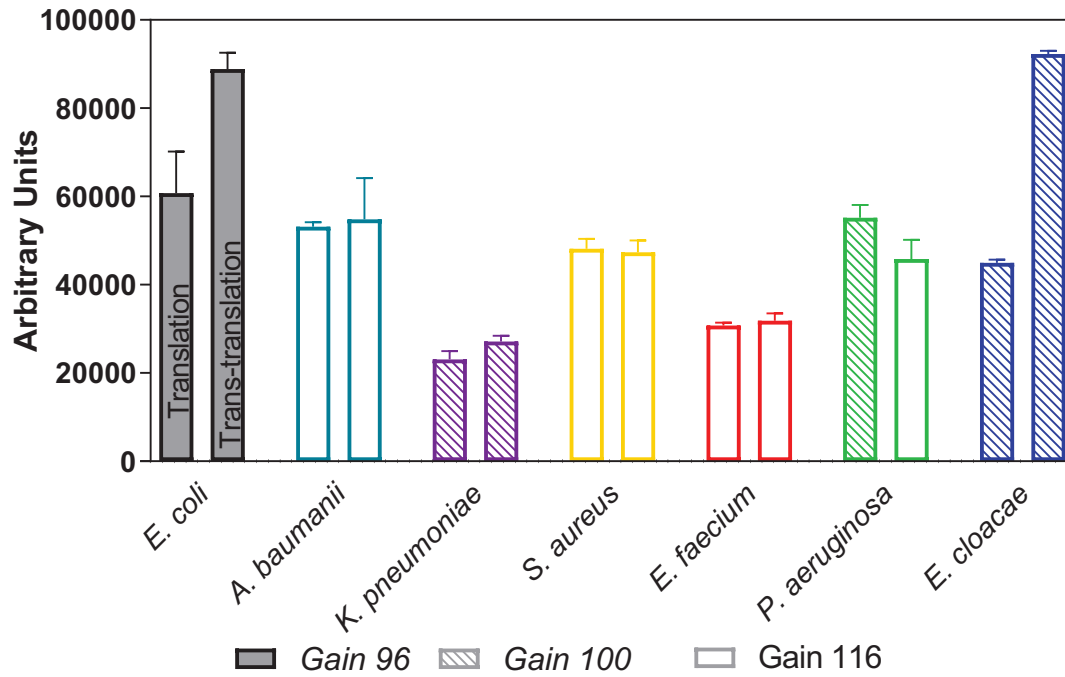
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588 **Supplementary Figure 2. Schematic illustrations of tmRNA secondary structures as well as**  
 589 **production patterns of tmRNA and SmpB variants.** (A) Wild-type tmRNA, with a black transfer-  
 590 like domain (TLD) and blue messenger-like domain (MLD). (B) Mutated tmRNA<sub>GFP11</sub> has an engineered  
 591 MLD (light green) that encodes the eleventh GFP beta-strand. Compensatory mutations (dark green)  
 592 maintain the base-pairing interactions of the H5 helix, and the 3'-ends for all species are CCA. (C)  
 593 Version 2 mutated tmRNA<sub>GFP11</sub>. An engineered MLD encoding the eleventh GFP beta-strand is green,  
 594 the WT 3'-end MLD is blue, and the WT H5 helix is gray. (D) Visualization of ESKAPE tmRNA<sub>GFP11</sub>  
 595 variants (5 pmol) on 8% urea-PAGE, with *E. coli* tmRNA<sub>GFP11</sub> used as a control. (E) As C, but showing  
 596 50 pmol *E. coli* and ESKAPE SmpB on 15% SDS-PAGE. Key: M1, RNA Century™-Plus Markers  
 597 (ThermoFisher Scientific), M2, PageRuler™ Prestained Protein Ladder, 10 to 180 kDa (ThermoFisher  
 598 Scientific), E, *Enterococcus faecium*; S, *Staphylococcus aureus*; K, *Klebsiella pneumoniae*;  
 599 A, *Acinetobacter baumannii*; P, *Pseudomonas aeruginosa*; E\*, *Enterobacter cloacae*.



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601 **Supplementary Figure 3:** Arbitrary units of fluorescence for the controls of the *E. coli* and ESKAPE  
 602 homologous systems translation assays. The smaller the gain unit is, the higher is the fluorescence  
 603 detected by the equipment.

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615 **Supplementary Table 1:** *E. coli* and ESKAPE tmRNA<sub>GFP11</sub> sequences for *trans*-translation *in vitro*. The  
 616 eleventh beta-strand is light green and the compensatory mutations forming the helix H5 are darker  
 617 green.

Species and Accession	tmRNA <sub>GFP11</sub> sequence
<i>E. faecium</i> <a href="#">NC_017960.1/1726235-1726601</a>	GGGGACGUUACGGAUUCGACAGGCACAGUCGAGCUUGAAUUGCGUUUCGUAGGUUACGUCUAC GUAAAAACGUUACAGUUAAAUAUAACU <b>GCACGUGACCACAUGGUCCUUC AUGAGUACGUAAAU</b> <b>GCUGCUGGGAUUACAUAUAACCUAAUCCUAGC</b> AUCCUCUCGGCAUCGCCAUGUCUCGAG UAAGGGUCCUAACUUUAGUGGGAUACGUUUCAACUUUCCGUCUGUAAAGUUGAAAAAGAGAACA UCAGACUAGCGAUACAGAAUGCCUGUCACUCGGCAAGCUGUAAAGUGAAUCCUAAAUGAGUU GACUAUGAACGUAGAUUUUUAAGUGGCGAUGUGUUUGGACGCGGGUUCGACUCCCGCCGUCUC CACCA
<i>S. aureus</i> <a href="#">NZ_GG774480.1/1107214-1106853</a>	GGGGACGUUCAUGGAUUCGACAGGGGUCCCCGAGCUCAUUAAGCGUGUCGGAGGGUUGUCUU CGUCAUCAACACACACAGUUUAUAUAACU <b>GCACGUGACCACAUGGUCCUUC AUGAGUACGUAA</b> <b>AAUGCUGCUGGGAUUACAUAUAACCUAAUCCUAGC</b> AUCGCCUAACAGCAUUUCCUUAUUGCU GUUAACGCGAUUCAACCUUAUAGGAUAUGCUAAACACUGCCGUUUGAAGUCUGUUUAGAAGA AACUUAUACAAGCUAGCAUCAUGUUGGUUUGUUUAUCACUUUCAUGAUGCGAAACCUUUCGAU AAACUACACACGUAGAAAGAUGUGUAUCAGGACCUCUGGACGCGGGUUCAAAUCCCGCCGUCU CCACCA
<i>K. pneumoniae</i> <a href="#">NC_017540.1/3743434-3743796</a>	GGGGCUGAUUCUGGAUUCGACCGGAUUUGCGAAACCCAAGGUGCAUGCCGAGGGGCGGUUGGC CUCGUAAAAAGCCGCAAAAAUAGUC <b>GCACGUGACCACAUGGUCCUUC AUGAGUACGUAAAUG</b> <b>CUGCUGGGAUUACAUAUAACCUAAUCCUAGC</b> CCUCUCUCCUAGCUUCCGUCUUAAGACGG GGAUCAAGAGAGGUCAAACCCAAAAGAGAUCGCGUGGAUGCCUUGCCUGGGGUUGAAGCGUU AAAUCUCAUCAGGCUAGUUUGUAGUGGCGUGUCUGUCCGCAGCUGGCAAGCGAAUGUAAAGA CUGACUAAGCAUGUAGUCCGAGGAUGUAGGAAUUUCGGACGCGGGUUCAAACUCCCGCCAGCU CCACCA
<i>A. baumannii</i> <a href="#">NZ_KB849843.1/1574036-1573677</a>	GGGGAUGUUAUUGGCUUCGACGCGGGUGAUGAAACUCAUAGAUGCAUGCCGAGAGCGCAUUUU CUCUCGUAAAUAUUUUUGCAUUUAUUUAGUC <b>GCACGUGACCACAUGGUCCUUC AUGAGUACG</b> <b>UAAAUGCUGCUGGGAUUACAUAUAACCUAAUCCUAGC</b> GUCCGCUUCCUAGAAUACUUGUGGU CUGGGAACCCGACUGAAGCGCACGCACACAAGUCCGUUAUAGAGUCAAGCCUCGGGGCUUUAUA CCAAACUUAGAGAUAGCACUUUGUACCCUGUUCGUCGGGUCACUUGGUGUUAUAAACAAUAGA CGAUUAUCUAAAGCAUGUAGUAUUCUGAGCGUAGUGCUGGCGGACGCGGGUUCAAACUCCCGCC UCUCACCA
<i>P. aeruginosa</i> <a href="#">NC_002516.2/901872-901520</a>	GGGGCCGAUUAGGAUUCGACGCCGGUAACAAAACUUGAGGGGCAUGCCGAGCUGGUAGCAGAA CUCGUAAAUUCGUCUGCUGCAAACUUAUAGUU <b>GCACGUGACCACAUGGUCCUUC AUGAGUACGU</b> <b>AAAUGCUGCUGGGAUUACAUAUAACCUAAUCCUAGC</b> CAGUCGCUAGGGGAUGCCUGUAAACC CGAAACGACUGUCAGAUAGAACAGGAUCGCCGCAAGUUCGUCUGUAGACGUAAACGGCUAAAAC UCAUACAGCUCGUCUCCAGCACCCUGCCACUCGGCGGCGCGGAGUUAAACUAGAGCUGG CUAAGCAUGUAGAACCGAUAGCGGAGAGCUGGCGGACGGGGUUCAAAUCCCGCCGUCAC CA
<i>E. cloacae</i> <a href="#">NC_016514.1/3580054-3580417</a>	GGGGCUGAUUCUGGAUUCGACGCGGAUUUGCGAAACCCAAGGUGCAUGCCGAGGGGCGGUUUGC CUCGUAAAAGCCGCAAAAAUAGUC <b>GCACGUGACCACAUGGUCCUUC AUGAGUACGUAAAU</b> <b>GCUGCUGGGAUUACAUAUAACCUAAUCCUAGC</b> CCUCUCUCCUAGCUUCCGUCUUAAGACG GGAUUC AAGAGAGGUCAAACCCAAAAGAGAUCGCGUGGAAGCCUUGCCUGGGGUUGAAGCGUU UAAAACUAAUCAGGCUAGUACGUUAGUGGCGUGUUUGUUCGCAGCUGGCGUGCGAAUGUAAAG ACAAACUAAAGCAUGUAGUACCGAGGAUGUAGAAUUUCGGACGCGGGUUCAAACUCCCGCCAGC UCCACCA
<i>E. coli</i> (Guyomar et al ; 2020) <a href="#">NC_000913.2/2753615-2753977</a>	GGGGCUGAUUCUGGAUUCGACGCGGAUUUGCGAAACCCAAGGUGCAUGCCGAGGGGCGGUUUGC CUCGUAAAAGCCGCAAAAAUAGUC <b>GCACGUGACCACAUGGUCCUUC AUGAGUACGUAAAUG</b> <b>CUGCUGGGAUUACAUAUAACCUAAUCCUAGC</b> CCUCUCUCCUAGCCUCCGUCUUAAGACGG GGAUCAAGAGAGGUCAAACCCAAAAGAGAUCGCGUGGAAGCCUUGCCUGGGGUUGAAGCGUUA AAACUUAUUCAGGCUAGUUGUAGUGGCGUGUCCGUC CCGAGCUGGCAAGCGAAUGUAAAGA CUGACUAAGCAUGUAGUACCGAGGAUGUAGGAAUUUCGGACGCGGGUUCAAACUCCCGCCAGCU CCACCA
	<b>tmRNA<sub>GFP11</sub>V2 sequence</b>
<i>P. aeruginosa</i>	GGGGCCGAUUAGGAUUCGACGCCGGUAACAAAACUUGAGGGGCAUGCCGAGCUGGUAGCAGAA CUCGUAAAUUCGUCUGCUGCAAACUUAUAGUU <b>GCACGUGACCACAUGGUCCUUC AUGAGUACGU</b> <b>AAAUGCUGCUGGGAUUACAUAUAACCUAAUCCUAGC</b> CUAGCUGCUUAAUGCGGCUAGCAGUCGCUAGGGGAUGCCUG UAAACCCGAAACGACUGUCAGAUAGAACAGGAUCGCCGCAAGUUCGUCUGUAGACGUAAACGGC UAAAACUCAUACAGCUCGUCUCCAAAGCACCCUGCCACUCGGGCGGCGGGAGUUAACUCAGUAG AGCUGGCUAAGCAUGUAGAAACCGAUAGCGGAGAGCUGGCGGACGGGGUUCAAAUCCCGCCG CUCCACCA

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620 **Supplementary Table 2:** *E. coli* and ESKAPE SmpB amino acid sequences

Species	Accession numbers	SmpB sequence
<i>E. faecium</i>	<a href="#">NC_017960.1/2002719-2002255</a>	MPKGEGKLI AQNKKARHDYSIIDTMEAGMVLQGT EIKSIR NSRINLKDGFIRVRNGEAF LHNVHISPYEQGNIFNHDPLR TRKLLLHKKQIIRLENELKNTGITV VPLKVYIRNGYAKVL IGLAKGKKS YDKREDLKRK DIDRQIDRTLKNFSR
<i>S. aureus</i>	<a href="#">NZ_GG774480.1/1107769-1107305</a>	MAKKKSPGTLAENRKARHDYNI EDTIEAGIVLQGT EIKSI RRGSANLKDSYAQVKN GEMYLNNMHIAPYEEGNRFNHDPL RSRKL LLLHKREI IKLGDQ TREIGYSIVPLKLYLKHGHCKV LLGVARGKKKYDKRQALKEKAVKRDVARDMKARY
<i>K. pneumoniae</i>	<a href="#">NC_017540.1/3742807-3743289</a>	MTKKKAHKPGSATIALNKRARHEYFIEDEYEAGLALQGWE VKSLRAGKANIGDSYVILKDGEAF LFGANFTPM AVASTHY VCDPTRTRKLLL NQRELD TLYGRINREGYTVVALS LYWKN AWCKVKIGVAKGKKQHDKRTDLK DREWALDKARIMKHAGR
<i>A. baumannii</i>	<a href="#">NZ_KB849843.1/1643614-1644090</a>	MAKATVVKKHNGGTIAQNKRARHDYFIEEKFEAGMSLLGW EVKSLRAGRMSLTESYVIFKNGEAF LFGAQIQPLLSASTH IVPEATRTRKLLL SRRELEKLMGAVNQKGYSCVPLACYWK GHLVKLEIALVKGKQLHDKRATEKERD WQRDKARIFHK
<i>P. aeruginosa</i>	<a href="#">NC_002516.2/5353783-5354262</a>	MAKQKKHPSGTIAQNKKALHDYFIEQRFEAGVALAGWEVK SLRAGKAQLVDSYVLLKDGEAWLLGSHITPLTTASTHVIA DPVRTRKLLLHKRELGK LFGAVQQKGYACVALSMYWKHL VKCEIALAKGKKDFDKRHTEKERDSDREIQRAMRHGKDD
<i>E. cloacae</i>	<a href="#">NC_016514.1/3579482-3579964</a>	MTKKKAHKPGSATIALNKRARHEYFIEEF EAGLALQGWE VKSLRAGKANIGDSYVILKDGEAF LFGANFTPLTVASSHY VCDPTRTRKLLL NKRELES LYGRINREGFTVVALS LYWKN AWCKVKVGVAKGKKQHDKRTDLKEREWQLDKARIMKNAGR
<i>E. coli</i>	<a href="#">NC_000913.2/2752918-2753400</a>	MTKKKAHKPGSATIALNKRARHEYFIEEF EAGLALQGWE VKSLRAGKANISDSYVLLRDGEAF LFGANITPM AVASTHV VCDPTRTRKLLL NQRELD SLYGRV NREGYTVVALS LYWKN AWCKVKIGVAKGKKQHDKRSDIKEREWQVDKARIMKNAHR

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623 **Supplementary Table 3:** Primers and antisense oligonucleotide sequences.

	Name	Letter/ Number	Sequence	Reference
Primers	GFPfold_for	#1	5' <u>CTCGATCCCGCGAAATTAATACG</u> 3'	Guyomar <i>et al.</i> , 2020
	GFP1-10nonSTOP_rev	#2	5' CTTTTTCGTTGGGATCTTTTCG 3'	
	alaGFPfold_rev	#3	5' CCGGCCTAGGTTATGTAATCCCAGCAGCATT ACGTACTCATGAAGGACCATGTGGTCACGTGC CTTTTTTCGTTGGGATCTTTTCGAAAG 3'	
Antisense oligonucleotide	Antisense tmRNA	A	5' GCTGCTAAAGCGTAGTTTTTCGTCGTT 3'	

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625 **Supplementary Table 4:** PCR product sequences for *in vitro trans*-translation. The T7 promoter is  
 626 underlined, the RBS sequence is green, the start and stop codons are red, and the tmRNA alanine resume  
 627 codon is orange.

Name	Sequence	Reference
sfGFP1-10	5' <u>CTCGATCCCGCGAAATTAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAATT</u> CCCCTCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGATATAACATATGGGTGGCACTAG TAGCAAAGGAGAAGAAGCTTTTCACTGGAGTTGTCCCAATCCTTGTGTAATTAGATGGTGA TGTTAATGGGCACAAATTTTCTGTCAGAGGAGAGGGTGAAGGTGATGCTACAATCGGAAA ACTCACCTTAAATTTATTTGCACTACTGGAAAACCTACCTGTTCCATGGCCAACACTTGT CACTACTCTGACCTATGGTGTTCATGCTTTTCCCGTTATCCGGATCACATGAAAAGGCA TGACTTTTTCAAGAGTGCCATGCCCGAAGGTTATGTACAGGAACGCACTATATCTTTCAA AGATGACGGGAAATACAAGACGCGTGTGTAGTCAAGTTTGAAGGTGATACCTTGTAA TCGTATCGAGTTAAAGGGTACTGATTTTAAAGAAGATGGAAACATTCTCGGACACAAACT CGAGTACAACCTTTAACTCACACAATGTATACATCACGGCAGACAAAACAAAAGAATGGAAT CAAAGCTAACTTCACAGTTCCGACACACGTTGAAGATGGTTCCGTTCAACTAGCAGACCA TTATCAACAAAATACTCCAATTGGCGATGGCCCTGTCTTTTACCAGACAACCATTACCT GTCGACACAAACTGTCTTTTCGAAAGATCCCAACGAAAAG3'	Guyomar <i>et al.</i> , 2020
sfalaGFP	5' <u>CTCGATCCCGCGAAATTAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAATT</u> CCCCTCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGATATAACATATGGGTGGCACTAG TAGCAAAGGAGAAGAAGCTTTTCACTGGAGTTGTCCCAATCCTTGTGTAATTAGATGGTGA TGTTAATGGGCACAAATTTTCTGTCAGAGGAGAGGGTGAAGGTGATGCTACAATCGGAAA ACTCACCTTAAATTTATTTGCACTACTGGAAAACCTACCTGTTCCATGGCCAACACTTGT CACTACTCTGACCTATGGTGTTCATGCTTTTCCCGTTATCCGGATCACATGAAAAGGCA TGACTTTTTCAAGAGTGCCATGCCCGAAGGTTATGTACAGGAACGCACTATATCTTTCAA AGATGACGGGAAATACAAGACGCGTGTGTAGTCAAGTTTGAAGGTGATACCTTGTAA TCGTATCGAGTTAAAGGGTACTGATTTTAAAGAAGATGGAAACATTCTCGGACACAAACT CGAGTACAACCTTTAACTCACACAATGTATACATCACGGCAGACAAAACAAAAGAATGGAAT CAAAGCTAACTTCACAGTTCCGACACACGTTGAAGATGGTTCCGTTCAACTAGCAGACCA TTATCAACAAAATACTCCAATTGGCGATGGCCCTGTCTTTTACCAGACAACCATTACCT GTCGACACAAACTGTCTTTTCGAAAGATCCCAACGAAAAGGACGTCGACCACATGGTCCT TCATGAGTACGTAAATGCTGCTGGGATTACATAAACCTAGGCCGG3'	Guyomar <i>et al.</i> , 2020

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630 **Supplementary Table 5: Plasmid list.** These synthesized sequences were cloned by GenScript in  
 631 pUC19 between the **HindIII** and **BamHI** restriction sites or in pET22b+ between the **NdeI** and **XhoI**  
 632 restriction sites. The T7 promoter is grey and underlined, the eleventh beta-strand is light green, and the  
 633 mutations making up for the formation of the H5 helix are dark green. BsmBI **or** EarI allow generation  
 634 of the 3' end of tmRNA. SmpB sequences are codon optimized for *E. coli*.

pUC19ESKAPetmRNA <sub>GFP11</sub>	pUC19 <i>E.faecium</i> tmRNA <sub>GFP11</sub>	<u>aagctT</u> AATACGACTCACTATAGGGGACGTTACGGATTTCGACAGGCACAGTCGAGCTTGAATTGCGTTTCGTAGGTTACGTCTACGTAAAAACGTTACAGTTAAATATAA CTGCACGTGACCACATGGTCCCTTCATGAGTACGTAATGCTGCTGGGATTACATA ATAACCTAATCCTAGCGATCCTCTCGGCATCGCCCATGTGCTCGAGTAAGGGTCC TAACTTTAGTGGGATACGTTTCAACTTTCCGTCTGTAAGTTGAAAAAGAGAACAT CAGACTAGCGATACAGAATGCCTGTCACTCGGCAAGCTGTAAAGTGAATCCTTAA ATGAGTTGACTATGAACGTAGATTTTAAAGTGGCGATGTGTTTGGACGCGGGTTC GACTCCCGC <u>CGTCTC</u> AccaCGAAGAGgatcc
	pUC19 <i>S.aureus</i> tmRNA <sub>GFP11</sub>	<u>aagctT</u> AATACGACTCACTATAGGGGACGTTTCATGGATTTCGACAGGGTCCCGG AGTTCATTAAGCGTGTGCGAGGGTGTCTTCGTCTCAACACACACAGTTTATAA TAACTGCACGTGACCACATGGTCCCTTCATGAGTACGTAATGCTGCTGGGATTAC ATAATAACCTAATCCTAGCATCGCCTAACAGCATTTCCTATATGCTGTTAACGCG ATTCAACCTTAATAGGATATGCTAAACACTGCCGTTTGAAGTCTGTTTAGAAGAA ACTTAATCAAGCTAGCATCATGTTGGTGTGTTATCACTTTTCATGATGCGAAACC TTTCGATAAACTACACAGTAGAAAGATGTGTATCAGGACCTCTGGACGCGGGTT CAAATCCCGC <u>CGTCTC</u> AccatCGAAGAGgatcc
	pUC19 <i>K.pneumoniae</i> tmRNA <sub>GFP11</sub>	<u>aagctT</u> AATACGACTCACTATAGGGGCTGATTCTGGATTTCGACGGGATTTGCGAA ACCCAAGGTGCATGCCGAGGGGCGGTTGGCCTCGTAAAAAGCCGCAAAAAATAGT CGCACGTGACCACATGGTCCCTTCATGAGTACGTAATGCTGCTGGGATTACATAA TAACCTAATCCTAGCCCTCTCTCCCTAGCTTCCGCTCTTAAGACGGGGATCAAAG AGAGGTCAAACCCAAAAGAGATCGCGTGGATGCCCTGCCTGGGGTTGAAGCGTTA AATCTCATCAGGCTAGTTTGTAGTGGCGTGTCTGTCCGACGCTGGCAAGCGAAT GTAAAGACTGACTAAGCATGTAGTCCCGAGGATGTAGGAATTTCCGACGCGGGTT CAACTCCCGCCAGCTCCAccaGGAGACgatcc
	pUC19 <i>A.baumannii</i> tmRNA <sub>GFP11</sub>	<u>aagctT</u> AATACGACTCACTATAGGGGATGTTATTGGCTTCGACGCCGGTGATGAA ACTCATAGATGCATGCCGAGAGCGCATTTTCTCTCGTAAATAAAAATTTGCATTTA AATAGTGCACGTGACCACATGGTCCCTTCATGAGTACGTAATGCTGCTGGGATT ACATAATAACCTAATCCTAGCGTCCGCTTCCTAGAATACTTGTGGTCTGGGAACC CGACTGAAGCGCACACACAAGTCCGTATAGAGTCAAGCCTCGGGCTTTATAC CAAACCTTAGAGGATCGCACTTTGTACCCTGTTCGTCGGGTCACTTGGTGTAAAA CAATAGACGATATCTAAGCATGTAGTATTCTCGAGCGTAGTGTGGCGGACGCGG GTTCAACTCCCGCCATCTCCAccaGGAGACgatcc
	pUC19 <i>P.aeruginosa</i> tmRNA <sub>GFP11</sub>	<u>aagctT</u> AATACGACTCACTATAGGGGCCGATTAGGATTTCGACGCCGGTAACAAAA CTTGAGGGGCATGCCGAGCTGGTAGCAGAACTCGTAAATTCGCTGCTGCAAACTT ATAGTTGCACGTGACCACATGGTCCCTTCATGAGTACGTAATGCTGCTGGGATT CATAATAACCTAATCCTAGCCAGTCCGCTAGGGGATGCCTGTAAACC CGAAACGAC TGTACAGATAGAACAGGATCGCCGCCAAGTTCGCTGTAGACGTAACCGCTAAAAC TATACAGCTCGCTCCAAGCACCTGCCACTCGGGCGGCGGAGTTAACTCAGTA GAGCTGGCTAAGCATGTAGAACCAGTATAGCGGAGAGCTGGCGGACGGGGTTCAA TCCCCCGGCTCCACCAGGAGACgatcc
	pUC19 <i>E.cloaca</i> tmRNA <sub>GFP11</sub>	<u>aagctT</u> AATACGACTCACTATAGGGGCTGATTCTGGATTTCGACGGGATTTGCGAA ACCCAAGGTGCATGCCGAGGGGCGGTTTGCCTCGTAAAAAGCCGCAAAAAATAG TGCACGTGACCACATGGTCCCTTCATGAGTACGTAATGCTGCTGGGATTACATA ATAACCTAATCCTAGCCCTCTCTCCCTAGCTTCCGCTCTTAAGACGGGGATTCAA GAGAGGTCAAACCCAAAAGAGATCGCGTGGAAAGCCCTGCCTGGGGTTGAAGCGTT AAAATAATCAGGCTAGTACGTTAGTGGCGTGTGTTTTCGACGCTGGCGTGC GAA TGTAAGACAAAATAAGCATGTAGTACCAGGATGTAGAAATTTCCGACGCGGGT TCAACTCCCGCCAGCTCCAccaGGAGACgatcc
	pUC19 <i>P.aeruginosa</i> tmRNA <sub>GFP11</sub> V2	<u>aagctT</u> AATACGACTCACTATAGGGGCCGATTAGGATTTCGACGCCGGTAACAAAA CTTGAGGGGCATGCCGAGCTGGTAGCAGAACTCGTAAATTCGCTGCTGCAAACTT ATAGTTGCACGTGACCACATGGTCCCTTCATGAGTACGTAATGCTGCTGGGATT CATAAActagctgcttaaTGCGGCTAGCAGTCCGCTAGGGGATGCCTGTAAACCCGA AACGACTGTACAGATAGAACAGGATCGCCGCCAAGTTCGCTGTAGACGTAACCGCT AAAATAATCAGCTCGCTCCAAGCACCTGCCACTCGGGCGGCGGAGTTAACT TCAGTAGAGCTGGCTAAGCATGTAGAACCAGTATAGCGGAGAGCTGGCGGACGGGG TTCAAATCCCCCGGCTCCACCAAGAGACgatcc

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pET22b+ESKAPESmpB	pET22b+ <i>E.faecium</i> SmpB	<b>CATATG</b> CCGAAGGGCGAGGGCAAACCTGATTGCGCAGAACAAGAAAGCGCGTCACG ACTACAGCATCATTTGATACCATGGAGGCGGGTATGGTGTGCAAGGCACCGAAAT CAAAAGCATTTCGTAACAGCCGTATCAACCTGAAGGACGGTTTTTCATTCGTGTGCGT AACGGCGAGGCGTTTTCTGCACAACGTTTACATCAGCCGTATGAACAGGGTAACA TTTTCAACCACGATCCGCTGCGTACCCGTAAACTGCTGTGCTGCACAAGAAAACAAT CATTCGTCTGGAGAACGAACTGAAAAACACCGGTATCACCGTGGTTCCGCTGAAG GTGTACATTTCGTAACGGCTATGCGAAGGTTCTGATCGGTCTGGCGAAAAGCAAGA AAAGCTACGACAAGCGTGAAGATCTGAAGCGTAAAGACATCGATCGTCAGATTGA CCGTACCCTGAAGAACTTTAGCCGT <b>CTCGAG</b>
	pET22b+ <i>S.aureus</i> SmpB	<b>CATATG</b> GCGAAGAAAAAGAGCCCGGGTACCCTGGCGGAGAACCCTAAAGCGCGTC ACGACTATAACATCGAGGATACCATTTGAAGCGGGTATCGTGCTGCAGGGCACCGA GATCAAGAGCATTTCGTGCTGGTAGCGCGAACCTGAAAAGACAGCTACGCGCAAGTT AAGAACGGCGAAATGTATCTGAACAACATGCACATTGCGCCGTACGAGGAAGGTA ACCGTTTTCAACCAGACCCGCTGCGTAGCCGTAAGCTGCTGCTGCACAAACGTGA GATCATTAAAGCTGGGTGATCAGACCCGTGAAATTGGCTACAGCATCGTGCCGCTG AAGCTGTATCTGAAAACCGCCACTGCAAAGTGCTGCTGGGTGTTGCGCGTGGCA AAAAGAAATATGATAAGCGTCAAGCGTGAAGGAAAAAGCGGTGAAACGTGACGT TGCCGTGATATGAAGGCGCGTTAC <b>CTCGAG</b>
	pET22b+ <i>K.pneumoniae</i> SmpB	<b>CATATG</b> ACCAAGAAAAAGGCGCACAAACCGGGTAGCGCGACCATCGCGCTGAACA AGCGTGCGCGTCACGAATACTTCATTGAGGACGAATATGAGGCGGGTCTGGCGCT GCAGGGTTGGGAAGTAAAAGCCTGCGTGCGGGCAAGGCGAACATCGCGACAGC TATGTTATTCTGAAAGATGGTGAAGCGTTTCTGTTGGCGCGAACTTTACCCCGA TGCGCGTGGCGAGCACCCACTACGTTTGGACCCGACCCGTACCCGTAAGCTGCT GCTGAACCAGCGTGAAGTGGATACCCTGTACGGTCTATCAACCCTGAGGGCTAT ACCGTGTTGCGCTGAGCCTGTACTGGAAAAACCGGTGGTGCAAAAGTGAAGATTG GTGTTGCGAAGGGCAAAAAGCAACACGACAAACGTACCGACCTGAAGGATCGTGA GTGGCGCTGGATAAAGCGCGTATCATGAAGCACGCGGGCCGT <b>CTCGAG</b>
	pET22b+ <i>A.baumannii</i> SmpB	<b>CATATG</b> GCGAAGGCGACCGTGGTTAAGAAACACAACGGTGGCACCATCGCGCAGA ACAAGCGTGCGCGTCACGACTACTTCATTGAGGAAAAATTTGAGGCGGGTATGAG CCTGCTGGGCTGGGAAGTGAAGAGCCTGCGTGCGGGTTCGTATGAGCCTGACCGAG AGCTATGTTATCTCAAAAACGGTGAAGCGTTCTGTTGGTGCGCAGATCCAAC CGCTGCTGAGCGCGAGCACCCACATTTGTGCCGGAGGCGACCCGTACCCGTAAACT GCTGCTGAGCCGTGCTGAGCTGGAAAAGCTGATGGGTGCGGTGAACCAAAAAGGC TACAGCTGCGTTCGCTGGCGTGTATTGGAAGGGTACCTGGTGAACCTGGAAA TCGCGCTGGTTAAGGGCAAAACAGCTGCACGATAAGCGTGCAGCCGAGAAAAGAAC TGACTGGCAACGTGATAAGGCGCGTATTTTTACAAA <b>CTCGAG</b>
	pET22b+ <i>P.aeruginosa</i> SmpB	<b>CATATG</b> GCGAAACAGAAAGAAACACCCGAGCGGTACCATCGCGCAAAACAAGAAAG CGTGCACGACTACTTCATTGAGCAGCGTTTTGAAGCGGGTGTGGCGCTGGCGGG TTGGGAAGTAAAAGCCTGCGTGCGGGCAAGGCGCAACTGGTGGACAGCTATGTT CTGCTGAAAGATGGTGAAGCGTGGCTGCTGGGTAGCCACATTACCCCGCTGACCA CCGCGAGCACCCACGTGATTGCGGATCCGGTTCGTACCCGTAAGCTGCTGCTGCA CAAACGTGAGCTGGCAAGCTGTTCCGGCGCGGTGCAGCAAAAGGGTTACGCGTGC GTTGCGCTGAGCATGTATTGGAAGAAAACCTGGTGAATGCGAGATCGCGCTGG CGAAGGGCAAGAAAGACTTTGATAAACGTCACCCGAGAAGAACGTGACAGCGA TCGTGAAATTTCAGCGTGCATGCGTACCGCAAGGACGAT <b>CTCGAG</b>
	pET22b+ <i>E.cloacae</i> SmpB	<b>CATATG</b> ACCAAGAAAAAGGCGCACAAACCGGGTAGCGCGACCATCGCGCTGAACA AGCGTGCGCGTCACGAATACTTCATTGAGGAAGAGTTTGAAGCGGGTCTGGCGCT GCAGGGTTGGGAAGTTAAAAGCCTGCGTGCGGGCAAGGCGAACATCGCGACAGC TACGTGATTCTGAAAGATGGCGAGGCGTTTCTGTTGGCGCGAACTTCACCCCGC TGACCGTTGCGAGCAGCCACTATGTGTGCGACCCGACCCGTACCCGTAACCTGCT GCTGAACAAGCGTGAAGTGGAGAGCCTGTACGGTCTATCAACCCTGAAGGCTTT ACCGTGTTGCGCTGAGCCTGTATTGAAAAACCGGTGGTGCAAAAGTGAAGGTTG GTGTTGCGAAGGGCAAAAAGCAGCACGACAAACGTACCGATCTGAAGGAGCGTGA GTGGCAACTGGATAAAGCGCGTATTATGAAGAACGCGGGCCGTCTCGAG <b>CTCGAG</b>