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Supplemental information

Human gut Actinobacteria boost drug absorption by secreting P-glycoprotein ATPase inhibitors

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SUPPLEMENTAL INFORMATION

SUPPLEMENTAL FIGURES AND LEGENDS

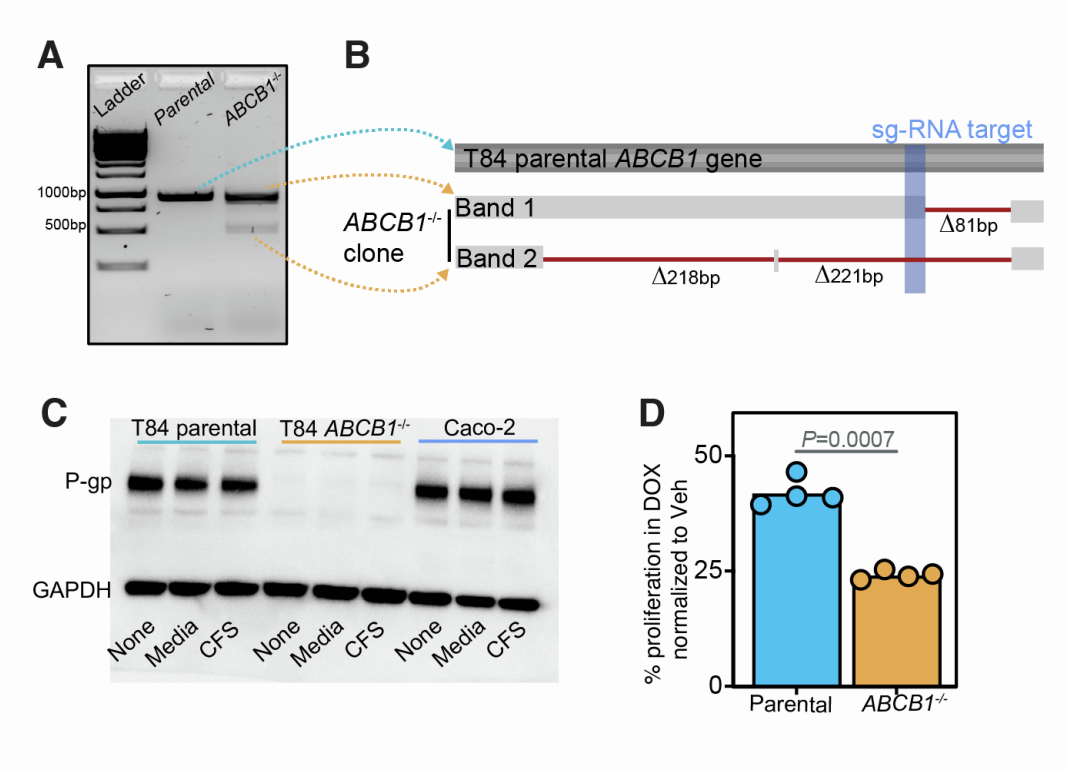

Figure S1, related to Figure 2. Validation of the *ABCB1* knockout monoclonal cell line. **(A)** T84 cells were transduced with lentiviral particles carrying cas9 gene and small guide RNA (sg-RNA) against *ABCB1* gene. The transfected cells were sorted into a single cell per well and expanded to generate a monoclonal cell line. DNA was extracted and PCR amplified using primers flanking the sg-RNA target site. **(B)** The two visible bands in the gel were extracted, sequenced, and aligned to the parental T84 PCR product using NCBI multiple alignments tool. **(C)** Western blot of untreated (none), BHI^{+} media, or *E. lenta* CFS treated *ABCB1*$^{-/-}$ cell lysates. T84 parental cells and Caco-2 cells were included as controls. **(D)** P-gp effluxes doxorubicin (DOX) rendering cells with functional P-gp more resistant. Cells without functional P-gp are therefore more sensitive to DOX. Using these principles, P-gp function in *ABCB1*$^{-/-}$ cells was tested in a DOX sensitivity assay and growth was normalized to DMSO vehicle (Veh) control (n=4/condition, Wilcoxon test).

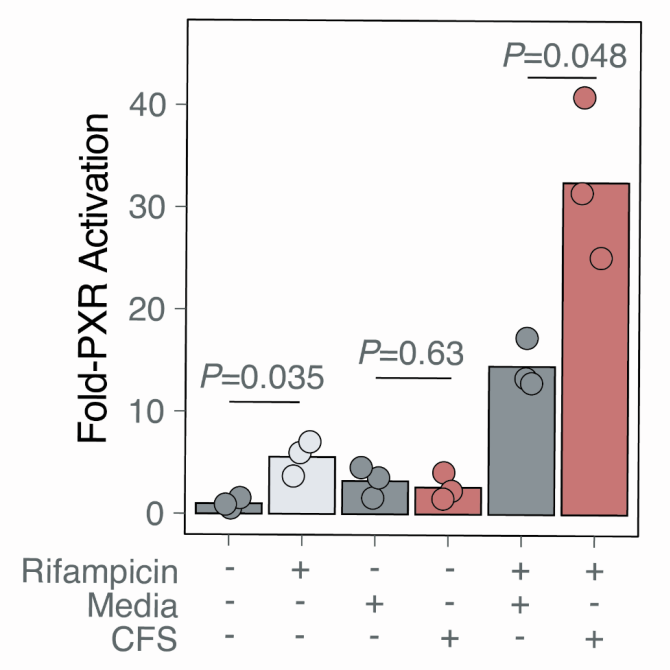

Figure S2, related to Figure 3. *E. lenta* CFS synergizes with PXR agonists to activate PXR. To test how P-gp transcription is induced, pregnane X receptor (PXR) luminescence reporter cell line was treated with *E. lenta* CFS cultured in BHI^{+}. PXR agonist rifampicin or DMSO vehicle control was added to test for synergistic activity. Luminescence measurements were normalized to the untreated control to calculate the fold change.

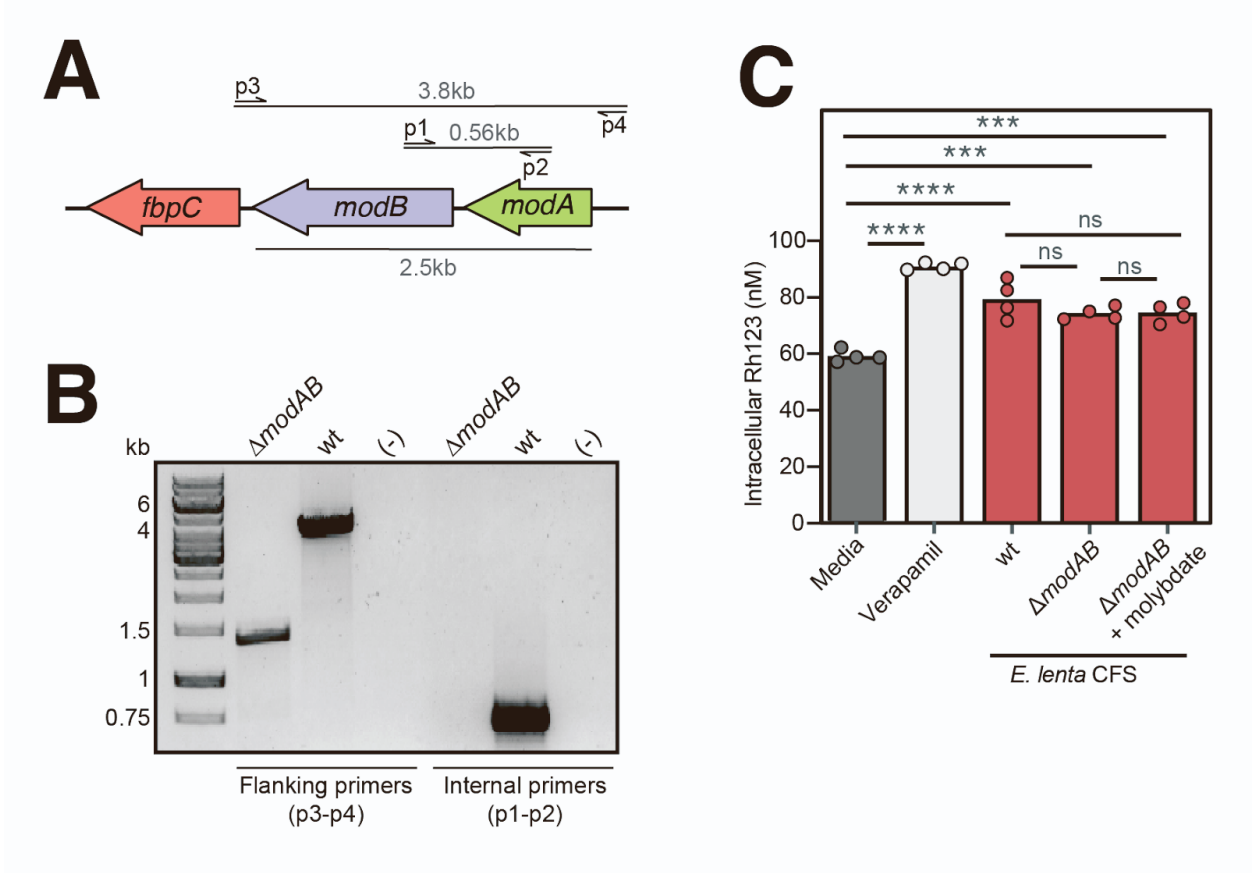

Figure S3, related to Figure 4. The *modAB* cluster in *E. lenta* is not required for P-gp inhibition. **(A)** Scheme of primer design to confirm *modAB* gene deletion in *E. lenta* DSM 2243: flanking primers p3 and p4 yield a 3.80-kb fragment from wildtype (wt) and a 1.38-kb fragment from the Δ*modAB* mutant, respectively. Internal primers P1 and P2 anneal only to the *modAB* genes of wt and do not anneal to the Δ*modAB* mutant, yielding a 0.56-kb fragment from wt. Primer sequences are listed in **Table S7**. **(B)** Colony PCR confirmed deletion of *modA* and *modB* genes from *E. lenta* DSM 2243 wt. **(C)** wt and Δ*modAB* strains were cultured in BHI^{+} media with and without 2 mg/L ammonium molybdate supplement. CFS was tested for P-gp inhibitory activity in our T84 cell-based assay. Verapamil (10 μM) was used as a positive control. n=4/condition and ANOVA with Tukey's correction. ***p<0.001, ****p<0.0001, ns, p>0.05.

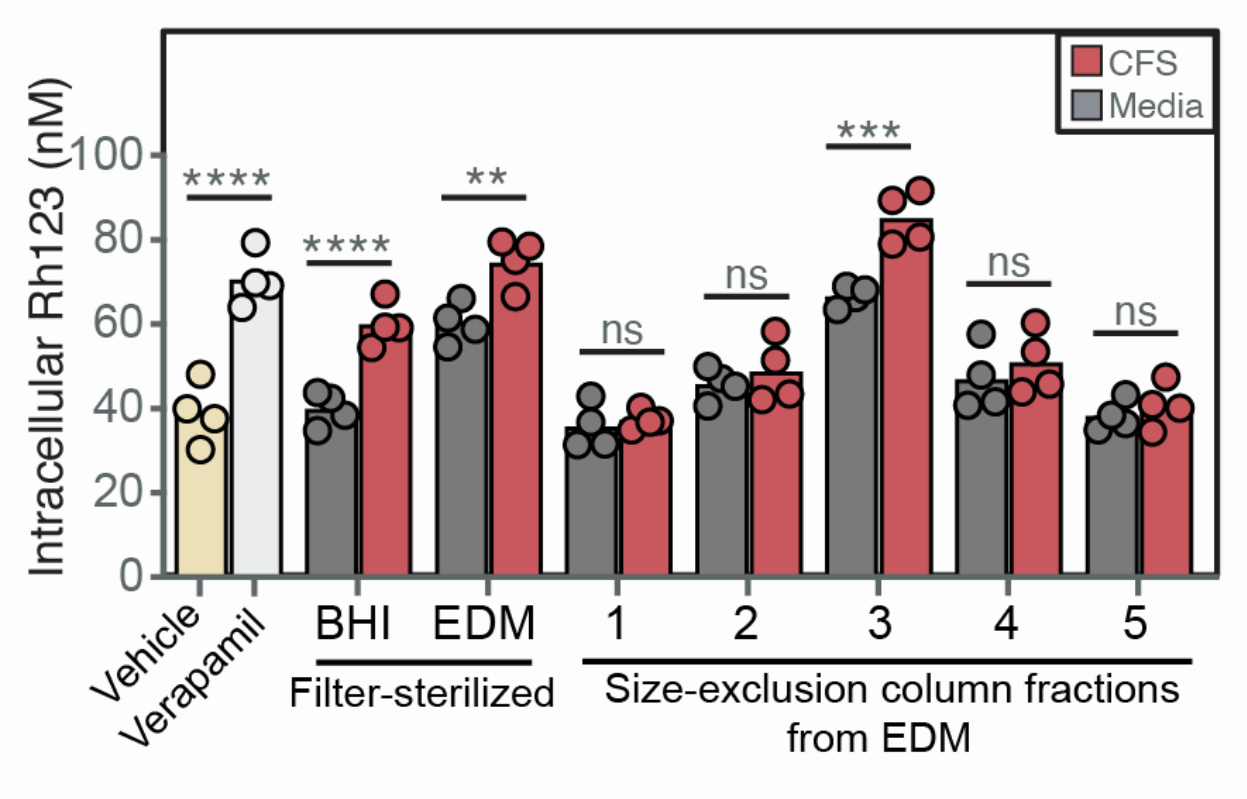

Figure S4, related to Figure 5. P-gp inhibitory activity is detected in *E. lenta* CFS cultured in EDM. *E. lenta* DSM2243 CFS from EDM cultures was processed through the activity-guided biochemical fractionation pipeline shown in **Fig. 5A** (n=4/group; two-way ANOVA with Tukey's correction). Vehicle (0.02% DMSO) and 10 μM verapamil were included as controls. Filter-sterilized CFS cultured in EDM and BHI^{+} were included as controls for fractionations. **p<0.01 ***p<0.001, ****p<0.0001, ns, p>0.05.

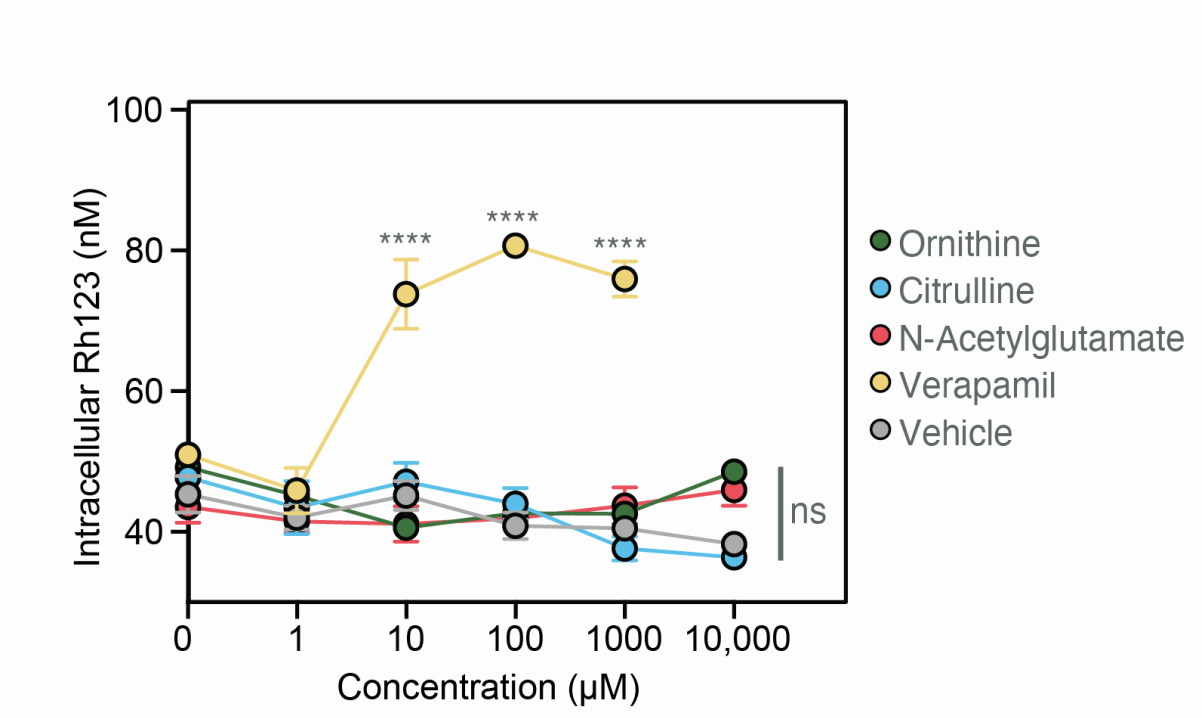

Figure S5, related to Figure 6. Citrulline, ornithine, and N-acetylglutamate do not inhibit P-gp at high concentrations. Citrulline and ornithine, byproducts of arginine metabolism in *E. lenta*, and *N*-acetylglutamate were tested for P-gp inhibitory activity using T84 Rh123 assay at a wide range of concentrations. Verapamil and vehicle were used as positive and negative controls, respectively. We observed cell death for 10mM verapamil incubation. n=4/condition, mean±SEM, ANOVA with Tukey's correction. ****p<0.0001, ns >0.05.

SUPPLEMENTAL TABLES

Table S1. Literature review of mechanisms of P-gp inhibition, related to Figure 3.

Mechanism	Literature evidence (PMID)
Decreased P-gp expression	23822562, 23621869, 23956061, 23967153
Impaired localization to the cell surface	23261525, 20460432, 27840996
Increased ATPase activity	9073309, 27531061, 24853187
Decreased ATPase activity	10510451, 28283574
Increased P-gp degradation	27452236, 15322230

Table S3. Comparative genomics hit annotations and gene expression in *E. lenta* DSM2243, related to Figure 4.

Orthologous gene cluster number	Annotation (*E. lenta* DSM2243)	Gene (*E. lenta* DSM2243)	PSIBLAST Annotation	Mean RPKM (*E. lenta* DSM2243)
264	hypothetical protein	DSM2243REF_00393	YerC/YecD family TrpR-related protein	227.578649
296	ATP synthase subunit b	DSM2243REF_01054	F0F1 ATP synthase subunit B	1545.93271
360	hypothetical protein	DSM2243REF_02238	molybdate ABC transporter permease subunit	38.7108297
362	Molybdate-binding protein ModA	DSM2243REF_02239	molybdate ABC transporter substrate-binding protein	142.585188
536	Iron-dependent repressor IdeR	DSM2243REF_00584	metal-dependent transcriptional regulator	776.663859
556	hypothetical protein	DSM2243REF_01460	nucleoid-associated protein	147.534337
770	hypothetical protein	DSM2243REF_02036	chloride channel protein	82.4496332

Table S4. Media composition of *E. lenta*-specific defined media (EDM), related to Figure 5.

Component	Amount per L
Calcium chloride	8mg
Sodium chloride	1.5g
Ferrous sulfate heptahydrate	1.5mg
Copper(II) sulfate anhydrous	0.088mg
Cobalt(II) chloride hexahydrate	0.19mg
Ammonium molybdate tetrahydrate	0.19mg
Boric acid	0.65mg
Zinc(II) sulfate heptahydrate	4mg
Potassium sulfate	23mg
Magnesium chloride	0.386g
Sodium bicarbonate	0.4g
ATCC mineral mix	10mL
Potassium phosphate monobasic	13.6g
Potassium phosphate dibasic	6.48g
Uracil	23mg
Citric acid	1.56g
Hematin	1.72mg
ATCC vitamin mix	10mL
Ascorbic acid	0.56g
NAD	2mg
Pyridoxamine 2HCl	5mg
Menadione	1mg
Arginine	10g
Cysteine	0.5g
Histidine	0.15g
L-isoleucine	0.24g
L-leucine	1g
L-lysine	0.5g
L-methionine	0.125g
L-phenylalanine	0.4g
L-serine	0.5g
L-threonine	0.5g
L-tryptophan	0.2g
L-tyrosine	0.3g
L-valine	0.7g
Proline	0.7g
Potassium acetate	0.9g
Water	Fill to 1L total

Table S7. List of primers used in this study, related to Figures 1 and 3.

Primer target	Primer name	Primer sequence (5' → 3')
mACTB (NM_007393)	mACTB_f	AGATCAAGATCATTGCTCCTCCT
	mACTB_r	CAGGTAAGCAAACTTTCTGG
mRpl13A (NM_009438)	mRpl13A_f	CCTATGACAAGAAAAAGCGG
	mRpl13A_r	CAGGTAAGCAAACTTTCTGG
mB2m (NM_009735)	mB2m_f	GTATGCTATCCAGAAAACCC
	mB2m_r	CTGAAGGACATATCTGACATC
mAbcb1a (NM_011076)	mAbcb1a_f	ACGTGAGGTCGTGATGGAAC
	mAbcb1a_r	CTGTCCAGCCAACCTGCATA
mCyp3a11 (NM_007818)	mCyp3a11_f	CCTGGGTGCTCCTAGCAATC
	mCyp3a11_r	TGTCGAATTTCCATAAACCCTTGT
hGapdh (NM_002046)	hGapdh_f	GCTCTCTGCTCCTCCTGTTC
	hGapdh_r	GACCAAATCCGTTGACTCCG
hACTB (NM_001101)	hACTB_f	ATGATGATATCGCCGCGCTC
	hACTB_r	CCACCATCACGCCCTGG
hRplp0 (NM_001002)	hRplp0_f	TCCTCGTGGAAGTGACATCG
	hRplp0_r	TGTCTGCTCCCACAATGAAAC
hAbcb1 (NM_000927)	hAbcb1_f	CCATGCTCAGACAGGATGTGA
	hAbcb1_r	ATCATTGGCGAGCCTGGTAG
E. lenta (*elnmrk1*)	elnmrk1_f	GTACAACATGCTCCTTGCGG
	elnmrk1_r	CGAACAGAGGATCGGGATGG
	elnmrk1_p	[6FAM]TTCTGGCTGCACCGTTCGCGGTCCA[BHQ1]
modAB upstream repair template	modABUPRT_f	aatgaccttccctcgctctc
	modABUPRT_r	ggatacgatcgtccgcagaa
modAB downstream repair template	modABDNRT_f	cttcaagtgcgcgtcgag
	modABDNRT_r	gcgtaatgggccctgtcat
pLRH3 shuttle plasmid backbone	pLRHbb_f	cccttaacgatctaaaacaattcatcc
	pLRHbb_r	ctgtcagaccaagtttactc
bridging oligo for pLRH3 backbone and modAB repair template	BO_1	aatcaatctaaagtatatatgagtaaacttggtctgacagcttcaagtgcgcgtcgagagccgagaacggctcgtccagc
	BO_2	gcgctgcgcgctgcgctcaggatgacagggcccattacgcaatgaccttccctcgctctcttttcgatggagcccgaacc
pLRHmodABKOs1 screening primer	pLRHmodABKOs1_f	cttttctacggggtctgacg
	pLRHmodABKOs1_r	gtatgacattgccttctgcgtccg
pLRHmodABKO CRISPR array gblock1	gblock1_f1	TTCCTCGGTCTCCgtcgaaacgattcggatcctgtattact
	gblock1_r1	aggccgcaacactcgtac
	gblock1_r2	TTCCTCGGTCTCCcgtacaaacagaccagattgtctgtt
pLRHmodABKO CRISPR array gblock2	gblock2_f	TTCCTCGGTCTCGtacgagtgttgcggcctaac
	gblock2_r1	TTCCTCGGTCTCCacccatcacgaggtcgttcttg
	gblock2_r2	TTCCTCGGTCTCCacccacgagcaactgcttga
pLRHmodABKO CRISPR array gblock3	gblock3_f	TTCCTCGGTCTCGgggtcactccccgcatggg
	glbock3_r	TTCCTCGGTCTCGgaatcaactttgggagagagttcaaa
pLRHmodABKOs1 backbone	s1_f	tacaggatccgaatcgtttcccctaacgatctaaaacaattcatccagt
	s1_r	aactctctcccaaagttgatggatacgatcgtccgcagaaag
ibson assembly for CRISPR gBlock and pLRHmodABKOs1 ligati	crisprgblock_f	ttctgcggacgatcgtatccatcaactttgggagagagttcaaaattg
	crisprgblock_r	ttgttttagatcgttaaggggaaacgattcggatcctgtattactatt
pLRHmodABKO screening primer	pLRHmodABKO_f	gacgctcagtggaacgaaaactcac
	pLRHmodABKO_r	gtatgacattgccttctgcgtccg
E. lenta DSM 2243 ΔmodAB screening primer, internal	ΔmodABi_f	taacctgcatagacgccgtt
	ΔmodABi_r	gacgacacgcacaagaacat
E. lenta DSM 2243 ΔmodAB screening primer, flanking	ΔmodABf_f	aacaggctgacgaggttctg
	ΔmodABf_r	gacttcgacttggccgtc