

# Fig. S1A. Map of pFD1045

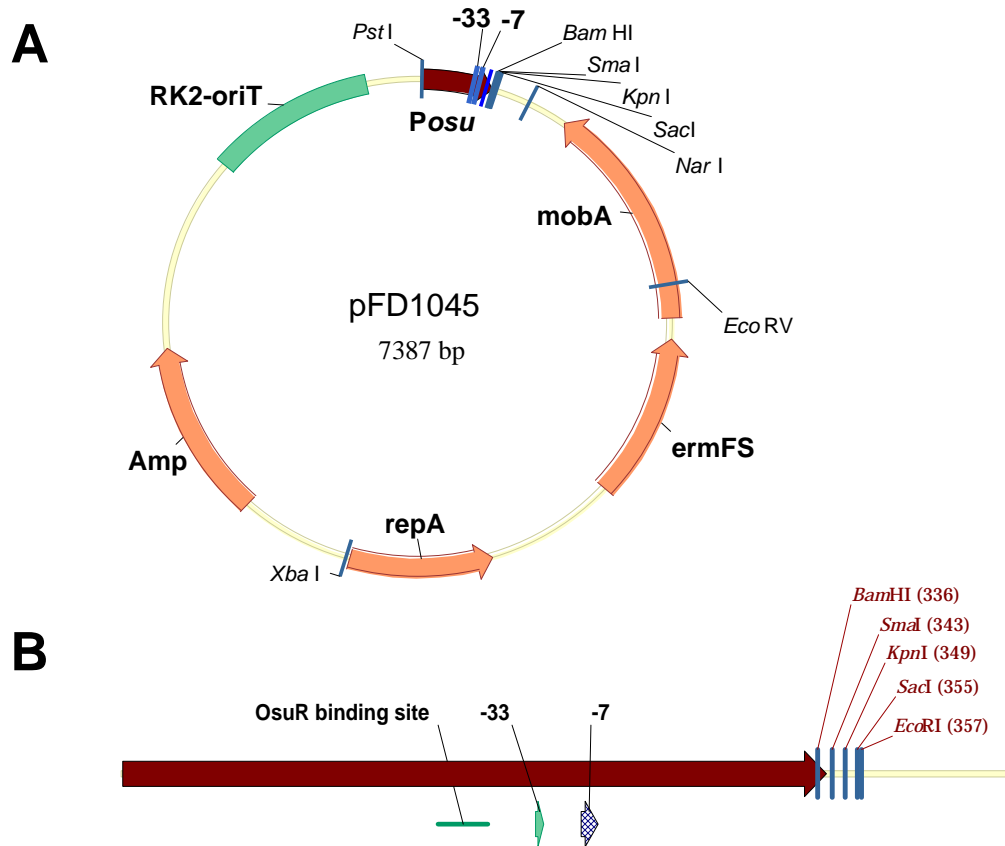


Fig. S1A. Maltose inducible expression plasmid pFD1045. A) map showing entire 7.4 kb plasmid and unique restriction sites. B) 400 bp from the promoter region of the *osuA* gene showing the *osu* promoter conserved -7 and -33 sites and the maltose responsive OsuR binding site. There is 107 bp from the transcription initiation site to the BamHI site.



# Fig. S2A. Map of pFD1146

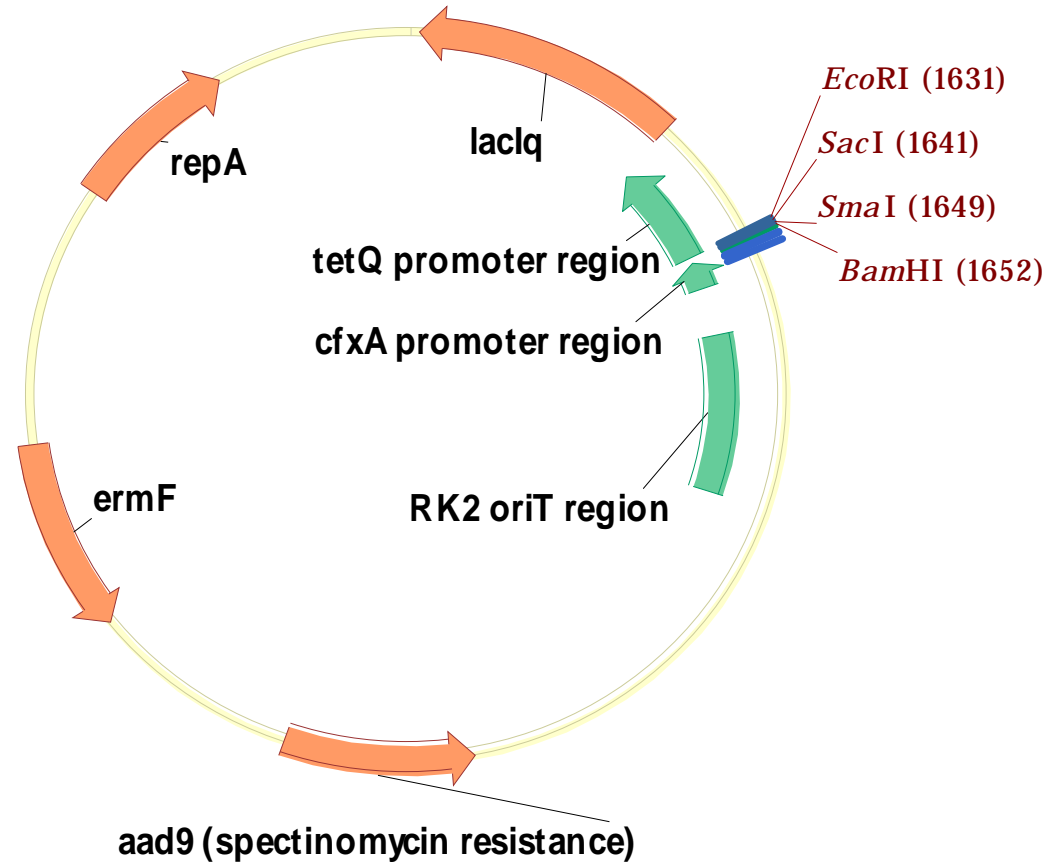


Fig. S2A. IPTG inducible expression plasmid pFD1146. Map showing the 9.4 kb plasmid and unique restriction sites that can be used for cloning target genes

# Fig. S2B. IPTG Inducible Promoter of pFD1146

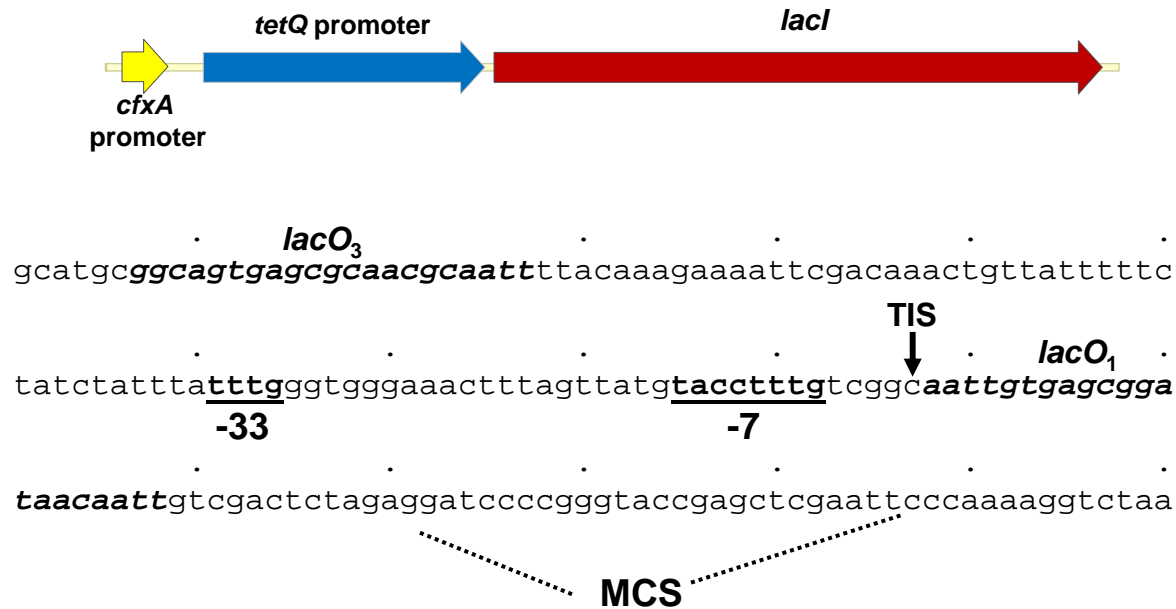
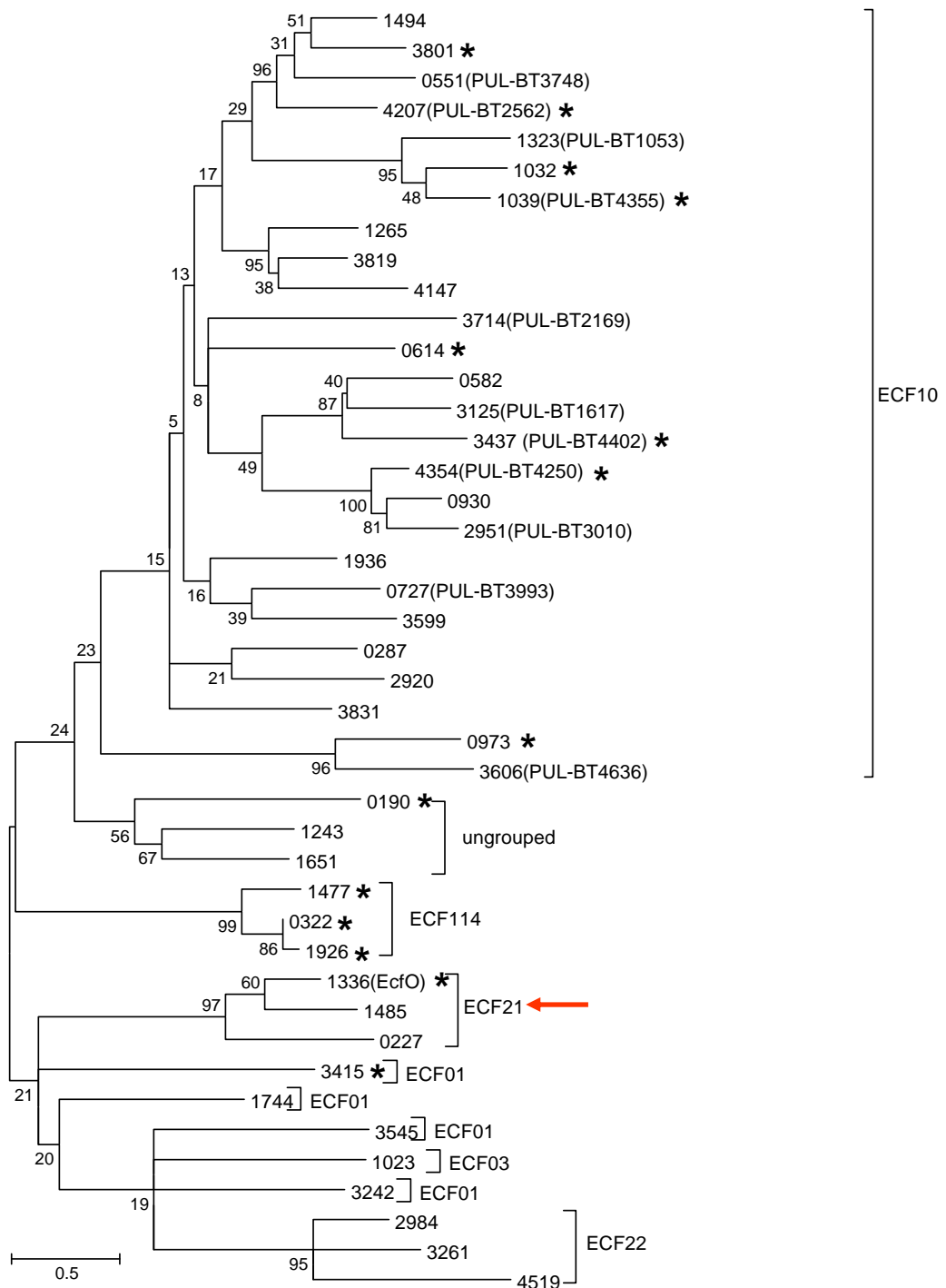


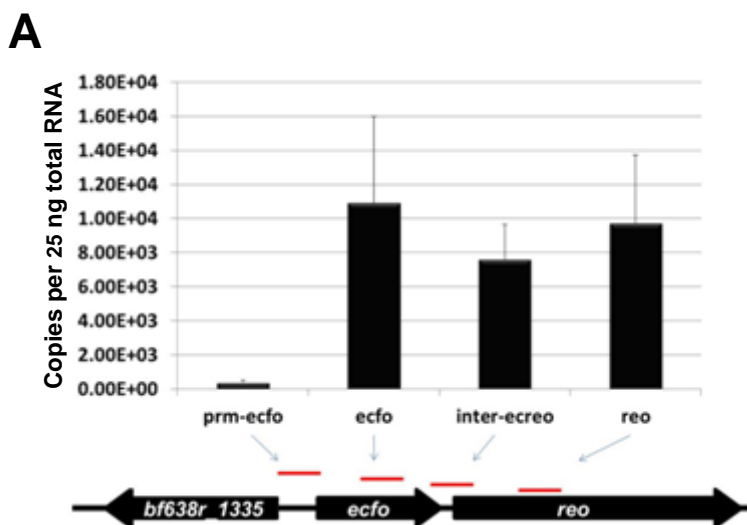
Fig. S2B. The *lacO* binding sites were engineered into the *Bacteroides cfxA* promoter shown with the conserved -7 and -33 sites. The *lacI* gene was cloned downstream under control of the *tetQ* promoter and engineered with a *Bacteroides* ribosome binding site to assure expression in the *Bacteroides* host. The DNA sequence shows the *cfxA* promoter region and multiple cloning site (MCS) with unique restriction sites.



**Fig. S3. Structural diversity of *B. fragilis* ECF sigma factors**

Primary amino acid sequences were aligned using MUSCLE and evolutionary history was inferred by using the Maximum Likelihood method as implemented with MEGA software. A bootstrap consensus tree was inferred from 500 replicates and the percentage of replicate trees in which the associated proteins clustered together are shown next to the branches. The analysis involved 43 amino acid sequences. Each ECF sigma factor protein is represented by its BF638R gene number. If the homologous gene for the ECF sigma factor or its associated anti sigma factor was induced or repressed by oxygen exposure as shown previously in the ATCC25285 strain (Sund et al, 2008) it is indicated by the asterisks. The ECF sigma factor families according to Staron et al, 2009 are shown by the brackets and EcfO is shown with red arrow.

# Co-transcription of *ecfO* and *reo*



**B**

Stock #	Primer Name	5'-3' Primer Sequence	Tm	GC%	Product Length (bp)
#289	prm-ecfo-qRT1	ATTGAGAAAATGCAGGAAGTGATG	58.0	37.5	157
#290	prm-ecfo-qRT2	TCCGACAGCTCTAATTCATTCA	57.3	40.9	
#291	ecfo-qRT1	TGGTACACGACGGTTTCCTG	60.0	55	147
#292	ecfo-qRT2	TGGTCTGGTTCATCACGTCG	60.0	55	
#293	inter-ecreo-qRT1	CGCGCAAAAAGTGATTGGC	59.3	50	181
#294	inter-ecreo-qRT2	CAGGCATCGGCTCCGAATAA	60.3	55	
#295	reo-qRT1	GGCAGTGACGGAGGAAAAGA	60.0	55	191
#296	reo-qRT2	TACCCACTCCCATCGACCAT	60.0	55	

Fig. S4. Evidence that the *ecfO* and *reo* genes are co-transcribed in an operon. **A)** Genetic map and RNA copy number results for 4 primer pairs located in the region of the *ecfO* locus. qRT-PCR reactions were performed using RNA obtained from the wild type strain, 638R, grown to mid-logarithmic phase cell in TYG medium under anaerobic conditions. Reactions were performed on two independent RNA samples. The locations of the amplicons produced by the primer pairs are shown by the red lines above the genetic map of the *ecfO* region. **B)** The sequence and properties of the corresponding primers is shown.

## Supplemental cloning procedures and construction of pHT1045 and pHT1146

A construct was designed to delete the sequences coding for amino acids 45-123 of the *ecfO* gene. A 881bp upstream PCR fragment containing 5'-*HindIII* and 3'-*Sall* modifications, was amplified from chromosomal DNA using the 5'-3'primers: *ecfO* Nterm del-*HindIII* and Fwd/sigOD Nterm del-*Sall*-Rev respectively. A 861bp downstream PCR fragment containing 5'- *Sall* and 3'- *HindIII* modifications, was amplified from chromosomal DNA using the 5'-3'primers: *ecfO* Cterm del-*Sall*-Fwd and *ecfO* Cterm del-*HindIII*- respectively. After sub-cloning into pUC19, fragments were ligated into the *Bacteroides* suicide vector pYT102 (1) to create a 237bp deletion. The plasmid was mobilized into *B. fragilis* ADB77, and following confirmation of the double-crossover allelic exchange, the mutant strain was reverted back to thymine prototrophy (1)

The BF638R1337 marked mutant was created by using a similar strategy, except that the deleted portion of the gene was replaced by a tetracycline resistance cassette, and the suicide vector used was pFD516. To delete *reo* (BF638R1337), an 875bp *PstI*-*BamHI* "upstream" fragment (amplified with primers: AntiODN-*PstI*\_FWD/ AntiODN-*BamHI*\_REV) and an 820bp *BamHI*-*EcoRI* "downstream" fragment (amplified with primers AntiODC-*BamHI*\_FWD/AntiODC-*EcoRI*\_REV) were cloned by three fragment ligation into the *PstI*/*EcoRI* multiple cloning site of pFD516. The *tetQ* containing cassette was cloned into the *BamHI* site of the construct to generate the suicide vector used to knock out the *reo* gene.

Recombinant constructs for affinity tagged protein expression in *E.coli* were generated after amplifying coding sequences for the respective genes using gene specific primers containing *BamHI* and *EcoRI* sites in the forward and reverse primers respectively. PCR products were cloned into the multiple cloning sites of either pET32a or pGEX4T-1. Sequence integrity was verified by gene sequencing, and protein expression was verified before and after IPTG induction of expression, by coomassie staining and/or western blotting.

Recombinant constructs for affinity tagged protein expression in *B. fragilis* were generated as follows: The primers pET32\_*SmaI*-Fwd and pET32-*SmaI*-Rev were used to amplify the coding region of  $\Delta ecfO$ , multiple cloning sites and sequences for the flanking tags from the his-tag expression vector pET32a-sigmat. This was sub-cloned into the *SmaI* site of pFD1045, in front of the *osu* promoter. To include an *ahpC* derived ribosome binding site, the resulting template was used to amplify another PCR product using the primers RBS-*BamHI*HIS-Fwd and *EcoRI*His-REV, and cloned into the *BamHI*/*SmaI* site of pFD1146 and pFD1045 to generate pHT1146 and pHT1045 (see supplemental data Figure S3) respectively. Dual his-tagged proteins were subsequently generated by cloning and replacing the fragments between *BglII* and *XhoI*.

Table S1: Primers used in this study

PRIMER	5'-3' - SEQUENCE	Purpose
1335-GSP1.1	CCCAATTGATTGGGGGAGTCATTAAAGGC	BF638R1335 - 5' RACE Gene specific primer
1335-GSP2	CTGTGACGTTGATACGGTCG	BF638R1335 - 5' RACE Gene specific primer
1335-GSP2.2	CCGTCAGTGGGATGATCTCTTTTCGTC	BF638R1335 - 5' RACE Gene specific primer
1355rbsBamHI Fwd	GACTGGATCCGCATTTTCTCAATACAATAAAGAATA AATCTCAATGG	BF638R1335 cloning and expression in Bacteroides
Hyp1335-BglII-Fwd(52.6)	GACTAGATCT TATGAAGAAATTTAATTTAATAACTTTTGCCG	BF638R1335 cloning and expression in Bacteroides
Hyp1335-XhoI-Rev(52.4)	GACTCTCGAGTTGAGAATGAAATGATTTTATTTTA AGTTTTTG	BF638R1335 cloning and expression in Bacteroides
1335 EcoRI Rev	GACTGAATTC GCCTGATTGTGTAGGTGTTTCTTGTTGTTTTA	BF638R1335 cloning and expression procedures
1355 BamHI Fwd	GACTGGATCC ATGAAGAAATTTAATTTAATAACTTTTGCCG	BF638R1335 cloning and expression procedures
1335 delCFwd	GACTGGATCCAACATCAGGATGACGGCAAAGTTA	BF638R1335 knockout construct procedures
<i>AntsgODN-EcoRI-R2</i>	GACTGAATTCTTAACGGATTTTCATCAGCTACCGGAC	BF638R1335 knockout/BF638R1337 N+TM cloning procedures
OxyR_BamHIFWD	GACTGGATCCATGACTATACAACAATTGGAATATA TTCTGGCTGT	BF638R1335 mutation procedures
OxyR-EcoRBamHRev	GACTGGATCCGAATTCTTATAACCAGACACTGAACG GCCTGCAGGG	BF638R1335 mutation procedures
F-sigOD-RBS- BamHI(atsOD)	GACTGGATCCTACTGAGGTGAACCTTGATTTTC	BF638R1337 cloning and expression in Bacteroides
AntiOC1-HindIII_Rev	TAGCAAGCTTTTAATAAGTGAATCGCAATCCTGCCT GC	BF638R1337 cloning procedures
AntiSigOD -BamHI_FWD	GACTGGATCCATGATGAAAGAAGACGAAAA	BF638R1337 cloning procedures
AntiSigOD-EcoRI_REV	GACTGAATTCATAAGTGAATCGCAATCCTGC	BF638R1337 cloning procedures
<i>AntSgOD-BamHI-F</i>	GACTGGATCCATGATGAAAGAAGACGAAAAATGG ATT	BF638R1337 cloning procedures
ATS-N-BamHI Rev	GACTGGATCCGTGAAGGAGCCGTTGCATAACGG	BF638R1337 cloning procedures
ATS-OD-SmaI_REV	GACTCCCGGGTTAATAAGTGAATCGCAATCCTGC	BF638R1337 cloning procedures



PRIMER	5'-3' - SEQUENCE	Purpose
ATS-OD-XhoI_REV	GACTCTCGAGATAAGTGAATCGCAATCCTGCCTGC A	BF638R1337 cloning procedures
ATS-OD-XhoI_stopREV	GACTCTCGAGTTAATAAGTGAATCGCAATCCTGCCT GCA	BF638R1337 cloning procedures
<i>Bam</i> HIR del_ASTM	GACTGGATCCCATTCTATTTTCATTTTTGAAATCCAA GGTTCAC	BF638R1337 cloning procedures
AntiSigODΔTM - BamHI_FWD	GACTGGATCCGCTGATGAAATCCGTTATGCAAC	BF638R1337 cloning procedures for C-term expression
AntiODC- <i>Bam</i> HI_FWD	GACTGGATCCACCATCCGAAAAGAAAGGC	BF638R1337 knockout construct procedures
AntiODC- <i>Eco</i> RI_REV	GACTGAATTCTATCACTTTTCCGGCGGAG	BF638R1337 knockout construct procedures
AntiODN- <i>Bam</i> HI_REV	GACTGGATCCAGCTTATCCTTGAATGCT	BF638R1337 knockout construct procedures
AntiODN- <i>Pst</i> I_FWD	GACTCTGCAGCAGGATGACGGCAAAAGTTA	BF638R1337 knockout construct procedures
<i>Antsg</i> ODN- <i>Eco</i> RI-R1	GACTGAATTCTACCGGCGATACGGATATATTCG	BF638R1337 N-TM cloning procedures
pET32_Smal-Fwd	GACTCCCGGGGCCGTTCTGGTTCTGGCCATATG	Generation of <i>Bacteroides</i> his-tagged plasmid
pET32-Smal-Rev	GACTCCCGGGCTTTCCGGCTTTGTTAGCAGCCG	Generation of <i>Bacteroides</i> his-tagged plasmid
RBS- <i>Bam</i> IHis-Fwd	GTACGGATCCTCTAAATAAGAAACAATTATGCACC ATCATCATCATCATTCTTC	Generation of <i>Bacteroides</i> his-tagged plasmid
SstI-His-REV	GGCCAGTGAATTCGAGCTCGGTACCCGG	Generation of <i>Bacteroides</i> his-tagged plasmid
RBS- <i>Bam</i> IHis-Fwd	GTACGGATCCTCTAAATAAGAAACAATTATGCACC ATCATCATCATCATTCTTC	pHT1045/1147 cloning-ahpc rbs creation in pHT-vector
<i>Eco</i> RIHis-REV	CAGTGAATTCGAGCTCGGTACCCGG	pHT1045/1147 cloning-Final creation of pHT-vector
pET32_Smal-Fwd	GACTCCCGGGGCCGTTCTGGTTCTGGCCATATG	pHT1045/1147 cloning-His tag cloning from pET32
pET32-Smal-Rev	GACTCCCGGGCTTTCCGGCTTTGTTAGCAGCCG	pHT1045/1147 cloning-His tag cloning from pET33
588ChipRT1	GGACCTGAAACAACCTTTGCCGT	qRT-PCR of BF638R0588 promoter after ChIP
588ChipRT2	ACCGGCAGAACAAACAACCTGCCA	qRT-PCR of BF638R0588 promoter after ChIP
743ChipRT1	CCGGCATGCAGCTCGTGA	qRT-PCR of BF638R0743 promoter after ChIP
743ChipRT2	TGCAATGCCGGCAGCATCAG	qRT-PCR of BF638R0743 promoter after ChIP
2478ChipRT1	TCGACCCTCGCTACCGCTC	qRT-PCR of BF638R2478 promoter after ChIP
2478ChipRT2	CCTGCAGCCGACAGCAGCAT	qRT-PCR of BF638R2478 promoter after ChIP
2785ChipRT1	ACTCCGCCTTCATCCCTCCC	qRT-PCR of BF638R2785 promoter after ChIP

<b>PRIMER</b>	<b>5'-3' - SEQUENCE</b>	<b>Purpose</b>
2785ChipRT2	ACCACTGAGAAAGCAATCGCA	qRT-PCR of BF638R2785 promoter after ChIP
4448ChIpRT1	GGTTGTTGCCGTTGATGCCCCG	qRT-PCR of BF638R4448 promoter after ChIP
4448ChIpRT2	GCGCCCCGATTACTGTATCGTAGCC	qRT-PCR of BF638R4448 promoter after ChIP
sigChip-RT-1	TGAGAAAATGCAGGAAGTGATG	qRT-PCR of sigOD/BF638R1335 promoter after ChIP
sigChip-RT-2	AATTCATTCATTCTCCTAAACACG	qRT-PCR of sigOD/BF638R1335 promoter after ChIP
588RT1	GGGGCAAATCCGTCTGACCCA	qRT-PCR transcript quantification of BF638R0588 expression
588RT2	GCCGGTTTAGCCGGTGTAAACGG	qRT-PCR transcript quantification of BF638R0588 expression
743RT1	GCCGATAGACGGGCAACGGG	qRT-PCR transcript quantification of BF638R0743 expression
743RT2	TCCCCGCCGATCCACATCA	qRT-PCR transcript quantification of BF638R0743 expression
1335RT-1	CGACCGTATCAACGTCACAG	qRT-PCR transcript quantification of BF638R1335 expression
1335RT-2	CAATTGATTGGGGGAGTCAT	qRT-PCR transcript quantification of BF638R1335 expression
antiSigOD-RT1	GCTGTCAACCCGGATGTATT	qRT-PCR transcript quantification of BF638R1337 expression
antiSigOD-RT2	CTCTTTTCTCCGTCCTACTGC	qRT-PCR transcript quantification of BF638R1337 expression
16SRT1-F	GATGCGTTCATTAGGTTGTTG	qRT-PCR transcript quantification of BF638R-16S expression
16SRT2-F	CACTGCTGCCTCCCGTAG	qRT-PCR transcript quantification of BF638R-16S expression
2478RT1	CGGTTGTTGCGTTTTCCCCGC	qRT-PCR transcript quantification of BF638R2478 expression
2478RT2	CCACCCGTTGCAGGCATGGT	qRT-PCR transcript quantification of BF638R2478 expression
2785RT1	TGCAGCCGTAGAAGGCCAGGA	qRT-PCR transcript quantification of BF638R2785 expression
2785RT2	AGTACCGCTACGCTGCCAGT	qRT-PCR transcript quantification of BF638R2785 expression
4448RT1	GGAGCTGTGCATACGGGCGA	qRT-PCR transcript quantification of BF638R4448 expression
4448RT2	TGCCACCTGTAAATCCACCGGC	qRT-PCR transcript quantification of BF638R4448 expression
1261 R7 Fwd (Gee)	GACGGTTTCTGAAGATTTT	qRT-PCR transcript quantification of sigOD
1261 RT Rev (Gee)	GGGGAGTTCATTAATAAATTGC	qRT-PCR transcript quantification of sigOD
sigOD-GSP2.2	GAATACGGTACGATATCCGGCGGG	sigOD - 5' RACE Gene specific primer
sigOD-GSP2/1261P	GAGCTCTAACGAAGACAGACACCAAG	sigOD - 5' RACE Gene specific primer
SigOD-RT2	AGCATCCGGTTCTTCATACG	sigOD - 5' RACE Gene specific primer
sigOD Cterm del-SalI-Fwd	GTCGACATTAATGAACTCCCCGCCG	sigOD 3' deletion-Forward primer
sigOD Cterm del-HindIII-Rev	AAGCTTATTACCGATACCCACTCCCA	sigOD 3' deletion-Reverse primer

<b>PRIMER</b>	<b>5'-3' - SEQUENCE</b>	<b>Purpose</b>
sigOD Nterm del-HindIII-Fwd	AAGCTTCAGGATGACGGCAAAGTTA	sigOD 5' deletion-Forward primer
sigOD Nterm del-Sall-Rev	GTCGACTTGTGCCATGTCCCTGTCTCC	sigOD 5' deletion-Reverse primer
F-sigOD-RBS-BamHI	GACTGGATCCTTTGCTGCATTTATAAGGTAAACAC G	sigOD cloning and expression of his-tagged protein in Bacteroides
SigOD BglIII-Fwd	GACTAGATCTAATGAATGAATTAGAGCTGTCGGAA CGTTG	sigOD cloning and expression of his-tagged protein in Bacteroides
SigOD-NotI-Rev	GACTGCGGCCGCACCATTGGTCACCAACCATTCTTT C	sigOD cloning and expression of his-tagged protein in Bacteroides
SigOD-BamHI_FWD	GACTGGATCCATGAATGAATTAGAGCTGTCTG	sigOD cloning and expression procedures
SigOD-EcoRI_REV	GACTGAATTCTCAACCATTGGTCACCAACC	sigOD cloning and expression procedures

Table S2. The most highly EcfO-induced genes

Gene	FUNCTION	Fold Induced	Log2 Expression	
			reo <sup>-</sup> + pEcfO	ecfO <sup>-</sup>
BF638R0588	conserved hypothetical protein	111.7442627	14.73195	7.92789
BF638R1335	conserved hypothetical protein	29.47000504	13.41907	8.5379
BF638R4448	hypothetical protein	24.5636692	12.33397	7.71551
BF638R4447	putative radical SAM-family protein	19.7859726	12.47337	8.16696
BF638R0743	conserved hypothetical protein	18.09102821	14.86485	10.68765
BF638R1336	putative RNA polymerase ECF-type sigma factor	11.48988056	11.68544	8.16315
BF638R1438	PSA-putative aminotransferase	9.70788002	12.88607	9.60691
BF638R2513	putative lipoprotein	9.347043991	13.33456	10.11005
BF638R1436	PSA-putative WxcM-like protein	9.10241127	11.49662	8.31037
BF638R1434	PSA-putative LPS biosynthesis related transcriptional regulatory protein	9.033407211	12.44713	9.27186
BF638R2785	putative exported protein	8.58799839	14.23393	11.13161
BF638R1459	PSA-putative DegT/DnrJ/EryC1/StrS family amino sugar synthetase	8.383256912	11.13367	8.06616
BF638R1435	PSA-putative UDP-GlcNAc 2-epimerase	7.93455267	11.33672	8.34857
BF638R1445	PSA-putative phosphoheptose isomerase	7.485687733	11.73781	8.83367
BF638R0780	PSG-putative LPS biosynthesis related DNTP-hexose dehydratase-epime	7.13273859	9.87614	7.04168
BF638R1433	PSA-putative LPS biosynthesis related transcriptional regulatory protein	6.900640488	9.50119	6.71446
BF638R3487	PSH-putative LPS biosynthesis related glycosyltransferase	6.819730282	9.86954	7.09982
BF638R1449;BF6	PSA-putative sugar epimerase (pseudogene)	6.801920414	12.70029	9.93434
BF638R1446	PSA-putative nucleotidyl transferease	6.478909492	10.22876	7.53301
BF638R1437	PSA-putative WbbJ-like protein	6.460772038	12.52453	9.83283
BF638R1435.2	PSA-putative WxcM-like protein	6.336722374	9.79084	7.1271
BF638R1442	PSA-putative GHMP kinase	6.333642483	10.72191	8.05888
BF638R3483	PSH-putative LPS biosynthesis related DNTP-hexose dehydratase-epime	6.19905138	9.33759	6.70554
BF638R1443	PSA-putative Nucleoside diphosphate sugar epimerase	6.13068676	10.15091	7.53486
BF638R0777	PSG-putative transcriptional regulator	6.020931244	9.34317	6.75319
BF638R2297	putative cation efflux-related lipoprotein	5.986685753	7.61941	5.03765
BF638R0778	PSG-putative UDP-glucose 6-dehydrogenase	5.869164944	8.88114	6.32798
BF638R0782	PSG-putative LPS biosynthesis related DNTP-hexose dehydratase-epime	5.82514286	8.3338	5.7915
BF638R1451	PSA-putative isomerase protein	5.800650597	9.08185	6.54564
BF638R1440	PSA-putative transmembrane protein	5.507408142	11.686	9.22463
BF638R1460	PSA-undecaprenyl-phosphate galactose phosphotransferase	5.330198288	10.55132	8.13714

BF638R1448	PSA-hypothetical protein	5.256542683	11.46113	9.06702
BF638R1447	PSA-putative glycosyl transferase	5.240895271	9.6069	7.21708
BF638R1867	PSB-putative LPS biosynthesis related phosphoenolpyruvate phosphom	5.175365925	11.23558	8.86392
BF638R1453	PSA-putative glycosyl transferase	5.100131512	8.98504	6.6345
BF638R1439	PSA-putative transmembrane protein	5.060304642	8.83592	6.49669
BF638R1458	PSA-putative 3-oxoacyl-[acyl-carrier-protein] synthase III	5.034759998	11.31067	8.97874
BF638R1868	PSB-putative LPS biosynthesis related phosphoenolpyruvate decarboxyl	4.949240685	11.01309	8.70588
BF638R1454	PSA-putative LPS biosynthesis related glucose-1-phosphate thymidyltr	4.942656994	10.19793	7.89264
BF638R3762	putative spore coat polysaccharide biosynthesis protein E	4.858386517	8.32597	6.04549
BF638R1444	PSA-putative histidine biosynthesis protein	4.782286167	9.13631	6.87861
BF638R1457	PSA-putative 3-oxoacyl-[acyl-carrier-protein] reductase	4.767580509	9.86133	7.60807
BF638R1869	PSB-putative LPS biosynthesis related 2-aminoethylphosphonate pyruva	4.712154388	10.94265	8.70626
BF638R0783;BF6	PSG-Nucleoside-diphosphate-sugar epimerases;pseudo Nucleoside-diph	4.614230633	9.47308	7.26699
BF638R1456	PSA-putative acyl carrier protein	4.583328724	9.5046	7.3082
BF638R3771	conserved hypothetical protein	4.550801277	8.0973	5.91118
BF638R1878	PSB-putative LPS biosynthesis related dehydratase	4.538176537	9.13338	6.95126
BF638R3766	putative LPS biosynthesis related aldo/keto reductase	4.447082043	7.67437	5.52151
BF638R1866	PSB-putative glucose-1-P-cytidyltransferase	4.381814003	7.87962	5.74809
BF638R3488	PSH-putative LPS biosynthesis related transcriptional regulatory protein	4.369565487	7.70001	5.57252
BF638R1441	PSA- hypothetical protein	4.310743809	9.892	7.78406
BF638R0779	PSG-putative LPS biosynthesis-related sugar-phosphate nucleotidyltrans	4.218164921	10.52475	8.44813
BF638R3473	PSH-putative LPS biosynthesis related dTDP-4-dehydrorhamnose 3,5-ep	4.218022346	9.93061	7.85405
BF638R0789	PSG-putative capsular polysaccharide biosynthesis protein	4.198000908	9.69671	7.62701
BF638R3489	PSH-putative LPS biosynthesis related transcriptional regulatory protein	4.137679577	9.10053	7.05171
BF638R1455	PSA-putative transmembrane protein	4.131970882	11.00367	8.95684
BF638R3482	PSH-DNTP-hexose dehydratase-epimerase	4.069438934	6.99655	4.97172
BF638R0781	PSG-conserved hypothetical protein	4.033638954	9.35381	7.34172
BF638R3774	putative transcriptional regulator	3.955482721	10.19313	8.20927
BF638R3484	PSH-putative LPS biosynthesis related DNTP-hexose dehydratase-epime	3.896668911	10.02625	8.06401
BF638R1088	putative LPS biosynthesis related dehydratase	3.76634407	9.44236	7.52919
BF638R3481	PSH-putative LPS biosynthesis related polysaccharide transporter/flippa	3.740711689	6.98784	5.08453
BF638R3767	putative DegT/DnrJ/EryC1/StrS aminotransferase family O-antigen relat	3.735872507	9.72285	7.8214
BF638R0792	PSG-putative glycosyltransferase	3.663678646	7.19932	5.32603
BF638R3763	putative LPS biosynthesis related Acetyltransferase	3.633581638	7.21743	5.35604

BF638R1880	PSB-putative LPS biosynthesis related reductase	3.613710403	8.61318	6.7597
BF638R3474	PSH-putative LPS biosynthesis related glucose-1-phosphate thymidyltr	3.560186625	9.18352	7.35156
BF638R0790	PSG-putative aminotransferase	3.514712572	9.78426	7.97086
BF638R3768	putative LPS biosynthesis related	3.501204491	10.14106	8.33321
BF638R1452	PSA-hypothetical protein	3.481672287	7.9716	6.17182
BF638R0810	putative DegT/DnrJ/EryC1/StrS family aminotransferase protein	3.425355911	9.42759	7.65134
BF638R0796;BF6	PSG-putative transmembrane polysaccharide modification protein;puta	3.345911026	6.65732	4.91493
BF638R1090	putative epimerase	3.34011364	9.37894	7.63904
BF638R1862	PSB- putative LPS biosynthesis related transcriptional regulatory proteir	3.303390741	7.44866	5.72472
BF638R0791	PSG-putative sugar-phosphate nucleotidyl transferase	3.289512634	8.01087	6.29299
BF638R0787	PSG-putative transmembrane protein	3.272656918	6.68375	4.97328
BF638R0121	putative RNA helicase dead-box protein	3.270205498	12.15492	10.44554
BF638R1576	hypothetical protein	3.263440132	8.97657	7.27018
BF638R1865	PSB-putative LPS biosynthesis related membrane protein	3.232635498	9.07723	7.38452
BF638R2298	putative cation efflux-related membrane protein	3.219130278	8.01012	6.32345
BF638R2681	anthranilate phosphoribosyltransferase	3.206250429	10.20176	8.52087
BF638R1857	putative GDP mannose 4,6-dehydratase	3.195005894	9.86048	8.18466
BF638R0397	putative exported protein	3.187184095	10.73887	9.06658
BF638R3751	putative epimerase/dehydratase	3.173950195	10.14584	8.47956
BF638R4446	putative two-component system sensor kinase/response regulator fusic	3.160017967	6.56584	4.90591
BF638R0398	putative TonB-dependent outer membrane exported protein	3.153612375	11.66213	10.00513
BF638R3750	putative LPS biosynthesis related glycosyl transferase	3.117562532	9.22284	7.58242
BF638R1670	putative ATP-dependent RNA helicase	3.093675137	9.90395	8.27463
BF638R1960	putative TonB-dependent outer membrane receptor protein	3.076897383	9.50805	7.88657
BF638R1863	PSB-putative LPS biosynthesis related transcriptional regulatory protein	3.074262619	7.86105	6.24081
BF638R3772	hypothetical protein	3.044889927	7.86415	6.25776
BF638R2911	putative uroporphirinogen biosynthesis-related protein	3.023969889	6.68034	5.0839
BF638R0793	putative polysaccharide biosynthesis protein	2.923807383	6.98472	5.43687
BF638R3755	putative UDP-GlcNAc 2-epimerase	2.913910389	6.37233	4.82937
BF638R1879	PSB-putative LPS biosynthesis related epimerase	2.909437418	7.3863	5.84556
BF638R3775	PSD-putative transcriptional regulator	2.907203674	11.00763	9.468
BF638R3472	PSH-putative LPS biosynthesis related conserved hypothetical protein	2.89193964	9.377	7.84497
BF638R3765	putative LPS biosynthesis related protein	2.884479523	7.17745	5.64914
BF638R3770	putative LPS biosynthesis related dehydratase	2.881490469	8.6009	7.07409

BF638R1864	PSB-putative glucose-1-phosphate thymidyl transferase	2.864048243	7.42402	5.90597
BF638R2718	putative cobalamin biosynthesis-related protein	2.863596916	12.28069	10.76286
BF638R1875	PSB-putative LPS biosynthesis related alpha-1,2-fucosyltransferase	2.840957642	8.5516	7.04522
BF638R1577	hypothetical protein	2.833425999	8.2959	6.79335
BF638R0745	putative transmembrane AAA-metalloprotease FtsH	2.831279993	12.09897	10.59752
BF638R3045	putative nitroreductase	2.828022003	10.94659	9.4468

**Table S3. Genes induced following ectopic expression of *ecfO* in three model systems.**

BFR638R Gene	Function	Fold Induction (rank) <sup>a</sup>		
		IPTG <sup>b</sup>	Maltose <sup>c</sup>	Constitutive <sup>d</sup>
<i>0588</i>	NigD family lipoprotein with secretion signal sequence	296 (1)	235 (2)	74 (1)
<i>1335</i>	NigD family lipoprotein with secretion signal sequence	86 (2)	258 (1)	27 (8)
<i>0743</i>	NigD family lipoprotein with secretion signal sequence	64 (3)	105 (3)	27 (7)
<i>2513</i>	lipoprotein with secretion signal sequence	63 (4)	6 (8)	50 (3)
<i>2785</i>	lipoprotein with possible secretion signal sequence	48 (5)	19 (4)	67 (2)
<i>4448</i>	putative lipoprotein, rSAM/lipoprotein system	32 (6)	4 (31)	17 (9)
<i>4447</i>	radical SAM family lipoprotein	22 (7)	8 (6)	29 (6)
<i>ecfO</i>	ECF sigma factor	6	<sup>e</sup> 1.5	71
<i>reo</i>	antisigma factor	NR	NR	12

<sup>a</sup> Rank is descending from the most highly expressed gene for a given experiment. The microarray expression data are available from the NCBI Gene Expression Omnibus under accession # GSE35539.

<sup>b</sup> IPTG induced expression of *ecfO* from pFD1146-*ecfO* in the *reo* mutant IB525. Induction is relative to empty vector in the *ecfO* mutant background IB524.

<sup>c</sup> Maltose induce expression of *ecfO* from pHT1045-*ecfO* in the *ecfO/reo* double mutant IB526. Induction is relative to the vector containing the  $\Delta$ *ecfO* gene in same IB526 background.

<sup>d</sup> Constitutive expression of *ecfO* during anaerobic, mid-logarithmic phase growth from pFD340-*ecfO* in the wild-type strain IB289 (ATCC25285). Fold induction is relative to empty vector in IB289. Orthologues of the IB289 genes to BFR638R genes *588*, *743*, *1335*, *ecfOD*, *rsoD*, *2513*, *2785*, *4447*, *4448* are respectively *540*, *700*, *1319*, *1320*, *1321*, *2551*, *2776*, *4287* and *4288*.

<sup>e</sup> Low induction values are misleading due to the high background resulting from expression of mRNA from the mutated  $\Delta$ *ecfO* gene which is detected by microarray probes (see text).