Supplementary Information

Predicting Selective RNA Processing and Stabilization operons and their protein stoichiometry via genome sequence

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1. Supplementary Methods.
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Supplementary Methods

Strains and growth conditions

Escherichia coli was used as the host strain for the routine cloning and incubated at 37 degree centigrade in Luria-Bertani (LB) medium. *C. cellulolyticum* ATCC 35319 (H10) was anaerobically cultured at 35 ºC in modified GS-2 medium (KH_2PO_4 1.5 g, $K_2HPO_4 \cdot 3H_2O$ 3.8 g, Urea 2.1 g, $MgCl_2 \cdot 6H_2O$ 1.0 g, $CaCl_2 \cdot 2H_2O$ 150 mg, $FeSO_4 \cdot 6H_2O$ 1.25 mg, cysteine-HCl 1.0 g, MOPS-Na 10 g, yeast extract 6.0 g, trisodium citrate$\cdot 2H_2O$ 3.0 g, resazurin 0.1 mg per liter, pH 7.4) (Johnson, et al., 1981) supplemented with 5.0 gL^{-1} cellobiose as carbon source. Erythromycin (20 µg ml^{-1} for *C. cellulolyticum*) or ampicillin (100 µg ml^{-1} for *E. coli*) was added into the medium as required.

RNA secondary structure prediction

The RNAMotif (Macke, et al., 2001) algorithm was used for motif discovery. It searches the RNA structure motif in nucleic acid sequences and the motif of interest were selected based on the parameters/constraints in the “descriptor” file provided with RNAMotif. Descriptor file contains the minimum and maximum length of stem and loop part in stem-loop. The minimal and maximal stem length was 6bp and 40bp, respectively, the loop length varied from 3 to 30nt and no restriction on bulged or mispaired base and GU-pairing was allowed in the stem; thus, RNAMotif predicted motif sequences on both strands. The RNAfold was used to calculate the secondary structure (stem-loop) and folding free energy (ΔG) of the predicted motifs. Single sequences were input to RNAfold with the default runtime parameters. Dotted positions are unpaired, whereas base-paring is represented by complementary parentheses. To remove the extended noise nucleotides from the stem-loops, dots before and after parentheses were discarded. Poly(U) tail and U-content of a SL were calculated by counting the number of

continuous U residues and number of all U residues respectively, present in 10 nts of downstream of SL.

Processing of the predicted SLs

The quality control step was used to remove the redundancy among sequences, which includes four constraints: (*i*) discarding completely overlapped sequences; (*ii*) removal of sequences having the same secondary structure; (*iii*) in the case of partially overlapped sequences (>75% similarity), sequence with high ΔG was discarded; (*iv*) sequences were required to have ΔG less than -5 kcal/mol.

Functional analysis of the stable SLs

To probe the functional role of the four different SL structures (**Fig. 2A**), a dual fluorescence reporter system was constructed using the *Ccel-E. coli* shuttle vector pMTC6, which harbours two reporter genes: (*i*) *fbfp* (encoding green fluorescence protein) coupled with the *pthl* promoter (Cui, et al., 2012), (*ii*) *mcherry* (encoding red fluorescence protein), which was inserted using *Eco*RI and *Bam*HI after *fbfp* gene. The resulted plasmid consisted of the green-fluorescence-encoding *fbfp* and the red-fluorescence-encoding *mcherry* were expressed in a single operon, with a *Bgl*II restriction site between the two genes for the introduction of the SLs (**Fig. 2C**). The recombinant plasmids were methylated *in vitro* with *Msp*I methyltransferase before electro-transformation of *Ccel* (Tardif, et al., 2001). The mutants were validated by colony PCRs (**Supplemental Data 1**). Positive colonies were inoculated into fresh medium supplemented with erythromycin.

Experimental validation of the classification rules and protein extraction

The derived classification rules were experimentally validated using the qRT-PCR analysis of the four different kinds of the SLs (with primer sets listed in **Supplemental Data 2**).

The qRT-PCR was performed using the SYBR Green I on LightCycler 480II using the FastStart Universal SYBR Green Master (Roche). The protein expression was extracted from the wild-type of *Ccel* in cellobiose medium using SDS-PAGE and LC-MS/MS.

To globally annotate the genes encoded by SRPS operons, COG annotation was performed using the eggNOG-mapper v1 (Huerta-Cepas, et al., 2017). The protein fasta-sequences of the genes of poly-cistronic operons were input to the eggnog-mapper with the HMMER mapping mode and other default parameters.

Ratio validation using experimentally measured abundance of transcripts and proteins

The gene expression data used from the cellulosome complex stoichiometry study (Xu, et al., 2015) and two protein expression data were used to validate the predicted ratio: (*i*) the LC/MS study described in this study and (*ii*) LC/MS data from cellulosome composition analysis of the *Ccel* study (Blouzard, et al., 2010). Gene expression for other bacteria was downloaded from Gene Expression Omnibus (Clough and Barrett, 2016; Edgar, et al., 2002) (GEO) using the following dataset series: GSE22426, GSE18471 and GSE80786 (for *Cthe*, *Cace* and *Bsub* respectively). The raw datasets were downloaded and normalized using the natural logarithm.

Supplementary Results

1. Prediction of stable SLs in the intergenic regions of *Ccel* genome for identifying SRPS operons

SLs were predicted across the *Ccel* genome using RNAMotif (**Fig. 1A**, **B**; **Methods**), which resulted in 432564 unique SL sequences. The secondary structure and corresponding minimal folding free-energy (ΔG, *i.e.*, representing the stability of SLs) were determined by RNAfold (Hofacker, 2003). The ΔG ranged from -49.00 kcal/mol to -0.10 kcal/mol. Since stable SLs have low ΔG, -5.00 kcal/mol was used as a threshold to remove the least stable SLs, which resulted in 124077 SLs. To eliminate redundant SLs, overlapping sequences were discarded (**Methods**). After these pre-processing steps, 87285 non-overlapping SLs remained.

The 87285 predicted SLs in the *Ccel* genome were grouped into five categories based on the relative position to corresponding gene (**Fig. 1D**): (*i*) 77551 intragenic SLs, i.e., located interior to a gene; (*ii*) 7163 intergenic SLs, i.e., flanked by two genes; (*iii*) 676 “overlapped_on_3’_end” SLs, i.e., located on the 3’ terminal of a gene; (*iv*) 1905 “overlapped_on_5’_end” SLs, i.e., located on the 5’ terminal of a gene; (*v*) 270 “overlapped_with_two_genes” SLs, i.e., either trailing one gene at the 3’ end and leading another gene at the 5’ end (when the two flanking genes are on the same strand) or trailing both flanking genes at the 3’ end (when the two genes are on the opposite strands).

2. Classification rules-based four stable SLs

To validate this hypothesis, *in-vivo* roles of four of these stable SLs, each 29-38 bp long and located in one of the four genomic regions below, were selected based on the classification scheme above (**Fig. 2A**): (*i*) SL_RS03710 (ΔG -13.5 kcal mol^{-1}), from the intergenic region between *Ccel_RS03710* and *Ccel_RS03715* in Operon 376, (*ii*) SL_RS07520 (ΔG -24.0 kcal mol^{-}

[1]), from the intergenic region between *Ccel_RS07520* and *Ccel_RS07525* in Operon 746, (*iii*) SL_RS05015 (ΔG -20.0 kcal mol^{-1}), from the intergenic region between *Ccel_RS05015* and *Ccel_RS05020* in Operon 495 and (*iv*) SL_RS01365 (ΔG -14.6 kcal mol^{-1}), from the 3'-UTR region of *Ccel_ RS01365* at Operon 142 (**Fig. 2B**). Based on the classification rules, these four SLs are from three distinct categories: SL_ RS07520 is a SSL due to the lack of poly(U) tail and the lower U content (≤4); SL_ RS03710 and SL_ RS05015 are STSLs, which harbor a poly(U) tail of 3 nt (U content = 5) and a discontinuous poly(U) tail of 4 nt (U content = 4), respectively; SL_ RS01365 is a TSL due to a poly(U) tail of 6 nt (U content = 7).

To probe their *in-vivo* role, each of these four SLs was inserted between the reporter genes of *fbfp* (encoding a green fluorescence protein) and *mcherry* (encoding a red fluorescence protein; **Fig. 2C**). The resulted four artificial operons, plus an operon where no SLs were inserted as the control, were then transformed into *Ccel*. Inside the bacterium, relative transcript abundance (TA) of SL_RS07520 is over 200% higher than SL_RS03710 and SL_RS05015 (i.e., the qPCR-determined transcript ratio of *fbfp* to *mcherry*; **Fig. 2C**). Moreover, the qPCR-based TA of the *fbfp* genes is strongly correlated (r = 0.88) with ΔG of their corresponding 3'-end inserted SLs (and with mRNA-Seq-based TA of the genes upstream of the SLs in the *Ccel* genome; r= 0.97; **Fig. 2D**), suggesting that these SLs can proportionally model the TA of their associated genes.

3. Validation of SRPS SLs using the dRNA-Seq data

The read-depths (number of reads associated with the gene) of the genes flanking the SLs were compared, and strong stabilization effect of the SL would be indicated by a high Normalized Read-depth Difference (NRD: difference in read-depth between the 5'-end and 3'-end flanking genes divided by read-depth of the 5'-end flanking gene; NRD is ranged from -1 to 1, where positive value indicates the SRPS-related SL, thus NRD > 0.5 was set as threshold to

minimize the risk of over-identification of SRPS SLs; **Fig. 3D**; **Methods**). In total, 44 out of the 59 active SRPS SLs (for seven SRPS SLs, read-depth of flanking genes is unavailable) showed NRD over 50%. For example, in Operon 42, SL_RS00440 (ΔG: -18.4) shows 97% NRD between its two flanking genes of *Ccel_RS00440* (at 5' region; read-depth: 3094) and *Ccel_RS00445* (at 3' region; read-depth: 74); in Operon 1000, SL_RS10060 (ΔG: -16.7) shows 87% NRD between its flanking *Ccel_RS10060* (at 5' region; read-depth: 18300) and *Ccel_RS10055* (at 3' region; read-depth: 2367). For example, *Ccel_RS03710* (read-depth: 550) and *Ccel_RS03715* (read-depth: 4232) in Operon-376 (*cip-cel*) are protected by SL_RS03710 (ΔG: -14.5) and SL_RS03715 (ΔG: -26.2) respectively, where the read-depth of these genes is in correspondence with the ΔG of associated SLs, *i.e.*, higher read-depth of a gene with the lower ΔG of an SL. Similarly, SL_RS10675 (ΔG: -16.8; operon 1052; read-depth: 8982) and SL_RS17245 (ΔG: -18.6; operon 1745; read-depth: 4635) are flanked (at 3' region) by genes associated with SLs SL_RS10670 (ΔG: -28.30; read-depth: 17873) and SL_RS17240 (ΔG: -19.60; read-depth: 5332) respectively, which are showing correspondence between the read-depths and ΔG of SLs (**Table S2**).

The predicted SLs thus provide a global landscape of SRPS operons in *Ccel* (**Fig. 3F, G**): (*i*) they are widely spread across the genome with ~60% and ~40% on sense (5'-3') and antisense (3'-5') strand, (*ii*) They tend to harbor more number of gene, *i.e.*, 73% and 50% operons with ≥3 and ≥4 genes, (*iii*) 14 out of 53 SRPS operons (27%) harbor two genes, *i.e.*, bi-cistronic operons. These SRPS operons are involved in different biological functions, such as cellulose degradation, membrane transport, energy production and flagellar biosynthesis. For example, operon 80, 495, 511, 569, 617, 622 and 693 are belong to ABC transporter and sugar-binding family; Operon 42, 142 (ATPase) and 716 represent phosphotransferase family; Operon 376 (*cip-cel*) and 746 are

involved in cellulose degradation and binding function; Operon 391 and 1018 belongs to ribosomal protein and flagellar biosynthesis respectively. This shows that SRPS operons contribute to diverse functions in *Ccel*.

4. SLOFE is applicable to a wider range of Gram-positive bacteria

To test its general applicability, SLOFE was expanded to a phylogenetically broader range of bacterial genomes (**Table S1**). Totally, 1007, 2158, 1829 and 177 stable SLs were predicted in the Gram-positive *Clostridium thermocellum* (*Cthe*), *Clostridium acetobutylicum* (*Cace*), and *Bacillus subtilis* (*Bsub*), plus the Gram-negative *Escherichia coli* (*Ecoli*) respectively. The number of stable SLs found appears linked to the phylogenetic distance, as closely related species have a similar number of stable SLs, e.g., *Cthe* (1007 SLs) and *Ccel* (1437 SLs), or in the case of *Cace* (2158 SLs) and *Bsub* (1829 SLs). In contrast, for *Ecoli*, only 177 stable SLs were predicted (including merely 3 inter-operonic stable SLs and 6 SRPS SLs), despite its relatively large genome size (**Table S1**). Thus at present SLOFE appears not applicable to *E.coli*.

To identify the SRPS operons in *Cthe*, *Cace* and *Bsub*, 71 (66 operons), 164 (133 operons) and 106 (93 operons) intergenic yet intra-operonic stable SLs, respectively, were extracted from the predicted stable SLs and categorized in a similar manner to *Ccel*. SLOFE revealed in *Cthe*, *Cace* and *Bsub* 33 (25 SSLs and 8 STSLs; 32 operons), 51 (24 SSLs and 27 STSLs; 45 operons) and 46 (29 SSLs and 17 STSLs; 42 operons) SRPS SLs, respectively, which correspond to 32, 45 and 42 SRPS operons (**Table S5B**, **S5C** and **S5D**).

5. SLOFE outperformed five existing methods in predicting stoichiometry in a wider range of Gram-positive bacteria

In *Cthe*, for the 32 predicted SRPS operons, SLOFE offered superior performance. Among the programs, SLOFE produced an *in silico* predicted ratios that is positively correlated with the

actual transcript-level ratio for the highest number of such operons (21; **Table S8**). On the other hand, for 16, 15, 13, 16, 13, and 11 of these operons (including bicistronic operons), CAI, RCBS, RCA, MELP, Gene-order and SLOFE actually produced a predicted ratio that is negatively correlated ratio with actual transcript-level ratio respectively, suggesting SLOFE generated the fewest errors (**Fig. 5F**). Remarkably, the average correlation between SLOFE and transcript level is ~70% higher than the top performer method (*i.e.*, Gene-order; **Table S6**).

In *Cace*, for the 45 predicted SRPS operons, CAI, RCBS, RCA, MELP, Gene-order and SLOFE produced a predicted ratio that is positively correlated with the actual ratio for 29, 22, 21, 20, 19 and 33 operons, and generated one that is negatively correlated for 16, 23, 24, 24, 26 and 12 operons respectively (**Fig. 5E**). In particular, SLOFE generated at least ~40% fewer errors than the other methods (**Table S9**). Notably, the average correlation between SLOFE and transcript level of *Cace* is ~25% higher (**Table S6**; **Table S9**).

In *Bsub*, the advantage of SLOFE is even more prominent (**Table S10**), as operons with their ratio positively correlated with transcript level numbered 23, 22, 23, 17, 22, and 35 for CAI, RCBS, RCA, MELP, Gene-order and SLOFE, respectively (**Fig. 5B; Table S10A**). At the protein level, for 26, 27, 9, 23, 22 and 32 of the operons, the predicted ratios are positively correlated in CAI, RCBS, RCA, MELP, Gene-order and SLOFE, respectively (**Fig. 5D**; **Table S10B**). Moreover, the average correlation for SLOFE is at least 30% higher than the other methods (**Table S6, 7, 8, 9, 10**). Thus, in each of the four Gram-positive bacteria tested here, SLOFE outperformed the five existing methods in predicting stoichiometry for SRPS operons.

Supplementary Figure

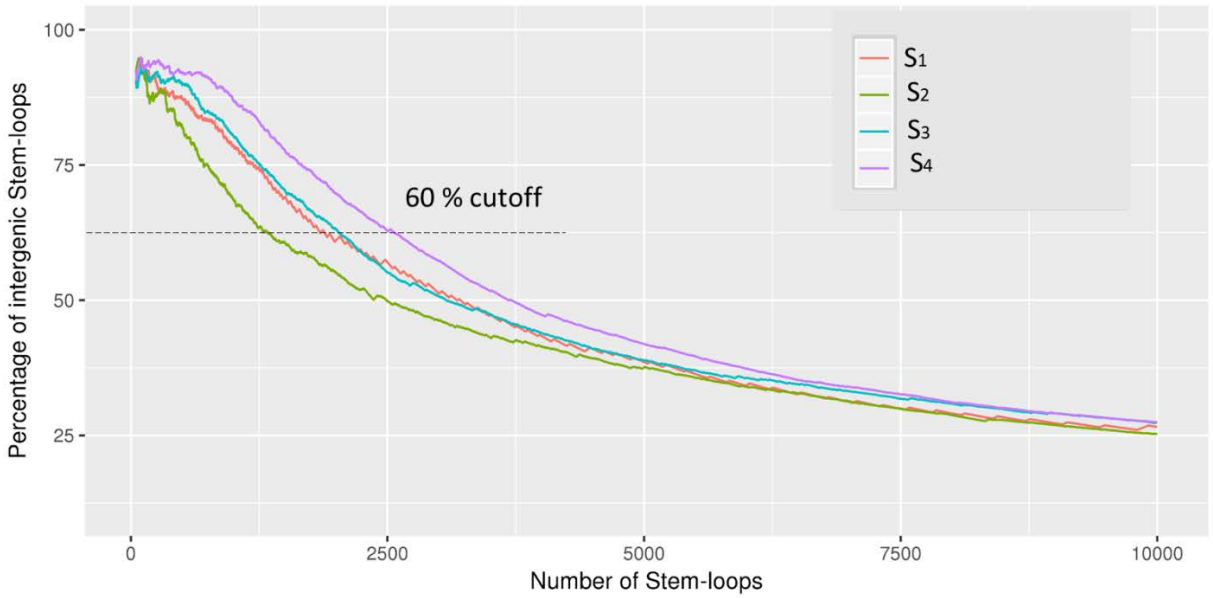


Figure S1: Selection of Stable stem-loops based on the four stability factors. Stable stem-loops were extracted from the millions of genome mapped stem-loops. Three stability factors for each stem-loop were calculated (**Materials and Methods**), and the stability factor which harbors the most number of intergenic stem-loops per 100 stem-loops was used with the 60% cutoff. Stability factor 4 (S_4) harbored the most number of intergenic stem-loops.

Supplementary Tables

Table S1. Bacterial genomes used in evaluating the SLOFE method.

Organism name	Genome size	RefSeq	Stable SLs	SRPS operons	Bi-cistronic operons
Ruminiclostridium cellulolyticum H10	4.07 mb	NC_011898.1	1437	53	11
Clostridium acetobutylicum ATCC 824	3.94 mb	NC_003030.1	2158	45	9
Clostridium thermocellum ATCC 27405	3.84 mb	NC_009012.1	1007	32	7
Bacillus subtilis Str. 168	4.22 mb	NC_000964.3	1829	42	11
Escherichia coli Str. K-12 substr. MG1655	4.64 mb	NC_000913.3	177	-	-

Table S2. Calculation of read-depth difference for the predicted SRPS SLs in *Ccel*. The Normalized Read-depth Difference (NRD) data in cellulose, cellobiose and glucose carbon substrates from the dRNA-Seq study was used to calculate the difference in the read-depth of two neighboring genes flanked around the SLs. “Bi” denotes the bi-cistronic operon.

			Cellulose			**Cellobiose**			**Glucose**				
Stem-loop	Operon	ΔG	5' gene read-depth	3' gene read-depth	NRD	5' gene read-depth	3' gene read-depth	NRD	5' gene read-depth	3' gene read-depth	NRD	Max NRD	Remarks
SL_RS00005	1	-19.7	175	159	0.091429	2136	1695	0.206461	1429	874	0.388383	0.388383	non-SRPS
SL_RS00055	4-Bi	-18	221	183	0.171946	118	58	0.508475	94	57	0.393617	0.508475	SRPS
SL_RS00075	6	-14.1	17	49	-0.65306	140	84	0.4	181	100	0.447514	0.447514	non-SRPS
SL_RS00440	42	-18.4	656	17	0.974085	3094	74	0.976083	5422	268	0.950572	0.976083	SRPS
SL_RS00755	80	-23.2	795	90	0.886792	92	13	0.858696	130	43	0.669231	0.886792	SRPS
SL_RS01335	142	-25.2	967	132	0.863495	5526	1370	0.752081	6277	2499	0.60188	0.863495	SRPS
SL_RS01350	142	-14.7	430	168	0.609302	3505	1799	0.486733	8393	3972	0.526748	0.609302	SRPS
SL_RS01680	170-Bi	-16.2	147	1	0.993197	554	13	0.976534	850	19	0.977647	0.993197	SRPS
SL_RS01850	190	-15	34	59	-0.42372	205	307	-0.33224	197	446	-0.55829	-0.33224	non-SRPS
SL_RS02130	216	-24.4	-	-	-	-	-	-	-	-	-	0	-
SL_RS02230	228-Bi	-21.2	1858	1709	0.080194	16161	14943	0.075367	5538	5244	0.053088	0.080194	non-SRPS
SL_RS02395	237	-16.8	1326	1568	-0.15433	2192	1744	0.20438	4662	3320	0.287859	0.287859	non-SRPS
SL_RS02895	288	-19.9	-	-	-	-	-	-	-	-	-	0	-
SL_RS02990	295-Bi	-16.2	246	0	1	1043	8	0.99233	1393	13	0.990668	1	SRPS
SL_RS03180	314	-18.6	208	21	0.899038	733	41	0.944065	1036	226	0.781853	0.944065	SRPS
SL_RS03695	376	-23.5	24668	8503	0.655302	28656	14389	0.497871	3968	2052	0.482863	0.655302	SRPS
SL_RS03700	376	-26.8	85031	351	0.995872	14389	331	0.976996	2052	153	0.925439	0.995872	SRPS
SL_RS03710	376	-14.5	547	5705	-0.90411	550	4232	-0.8700	203	950	-0.78631	-0.78631	SRPS

SL_RS03715	376	-26.2	5705	50	0.991236	4232	100	0.976371	950	33	0.965263	0.991236	SRPS
SL_RS03740	376	-16.3	73	4	0.945205	133	6	0.954887	48	5	0.895833	0.954887	SRPS
SL_RS03930	391	-17.3	499	744	-0.32930	1296	1894	-0.31573	1652	2377	-0.30500	-0.30500	non-SRPS
SL_RS03960	391	-20.7	2725	130	0.952294	6440	578	0.910248	5536	993	0.820629	0.952294	SRPS
SL_RS04310	432-Bi	23.7	18	12	0.333333	116	42	0.637931	276	45	0.836957	0.836957	SRPS
SL_RS05015	495	-20	84	12	0.857143	26	8	0.692308	61	24	0.606557	0.857143	SRPS
SL_RS05150	511	-18.5	2721	133	0.951121	940	118	0.874468	122	32	0.737705	0.951121	SRPS
SL_RS05250	514	-26.1	880	394	0.552273	346	133	0.615607	77	54	0.298701	0.615607	SRPS
SL_RS05495	545	-22	260	3267	-0.92041	148	1093	-0.86459	67	1273	-0.94736	-0.86459	non-SRPS
SL_RS05655	566-Bi	-28.4	169	139	0.177515	814	503	0.382064	1194	681	0.429648	0.429648	non-SRPS
SL_RS05685	569	-17.9	2045	128	0.937408	94	3	0.968085	72	14	0.805556	0.968085	SRPS
SL_RS06165	617	-16.6	7	4	0.428571	39	11	0.717949	83	80	0.036145	0.717949	SRPS
SL_RS06175	617	-14.4	28	5	0.821429	48	42	0.125	84	80	0.047619	0.821429	SRPS
SL_RS06180	617	-18.4	5	3	0.4	42	15	0.642857	80	34	0.575	0.642857	SRPS
SL_RS06215	617	-24.7	7	3	0.571429	19	19	0	71	103	-0.31067	0.571429	SRPS
SL_RS06275	622	-18.2	62	7	0.887097	175	9	0.948571	147	16	0.891156	0.948571	SRPS
SL_RS06525	632	-16.8	-	-	-	-	-	-	-	-	-	0	-
SL_RS07065	693	-16.7	55	14	0.745455	421	261	0.380048	940	246	0.738298	0.745455	SRPS
SL_RS07075	693	-28.7	450	10	0.977778	4738	248	0.947657	878	111	0.873576	0.977778	SRPS
SL_RS07235	716	-19.6	93	10	0.892473	436	44	0.899083	870	131	0.849425	0.899083	SRPS
SL_RS07520	746	-24	3253	650	0.800184	1729	357	0.793522	2562	764	0.701795	0.800184	SRPS
SL_RS07530	746	-17.8	546	183	0.664835	247	100	0.595142	271	182	0.328413	0.664835	SRPS
SL_RS08285	813	-20.3	16	2	0.875	104	26	0.75	61	21	0.655738	0.875	SRPS
SL_RS08610	849-Bi	-28	279	105	0.623656	1481	636	0.57056	4551	1413	0.689519	0.689519	SRPS
SL_RS08720	863	-19.7	2136	36	0.983146	6982	197	0.971785	5963	226	0.9621	0.983146	SRPS
SL_RS09085	898	-15.5	1879	129	0.931346	41895	1795	0.957155	19769	759	0.961607	0.961607	SRPS

SL_RS09255	915	-16.2	41	3	0.926829	339	25	0.926254	417	51	0.877698	0.926829	SRPS
SL_RS10060	1000	-16.7	6219	223	0.964142	18300	2367	0.870656	24934	4374	0.824577	0.964142	SRPS
SL_RS10050	1000	-26.3	430	24	0.944186	2589	248	0.90421	4609	287	0.937731	0.944186	SRPS
SL_RS10295	1018	-15.4	139	214	-0.35046	440	649	-0.32203	580	786	-0.26208	-0.26208	non-SRPS
SL_RS10685	1052	-20	41638	1242	0.970171	79521	8757	0.889878	1191	54	0.95466	0.970171	SRPS
SL_RS10675	1052	-16.8	1599	2026	-0.21076	8982	17873	-0.49745	71	199	-0.64321	-0.21076	SRPS
SL_RS10860	1073	-16.2	-	-	-	-	-	-	-	-	-	0	-
SL_RS11420	1135-BI	-17.9	1528	238	0.844241	5991	910	0.848105	4475	1483	0.668603	0.848105	SRPS
SL_RS12550	1247	-18	38	18	0.526316	77	7	0.909091	215	59	0.725581	0.909091	SRPS
SL_RS12610	1254-Bi	-27	304	293	0.036184	537	1081	-0.50323	955	615	0.356021	0.356021	non-SRPS
SL_RS13360	1341-Bi	-22.4	62	11	0.822581	73	6	0.917808	393	28	0.928753	0.928753	SRPS
SL_RS13485	1354	-35.4	230	147	0.36087	481	467	0.029106	446	952	-0.53151	0.36087	non-SRPS
SL_RS13510	1358-Bi	-16.2	3	0	1	5	1	0.8	16	6	0.625	1	SRPS
SL_RS13525	1359-Bi	-27.5	105	4	0.961905	178	6	0.966292	307	8	0.973941	0.973941	SRPS
SL_RS13720	1382	-23	-	-	-	13	29	-0.55172	24	43	-0.44186	-0.44186	non-SRPS
SL_RS14235	1435	-23.3	-	-	-	-	-	-	-	-	-	0	-
SL_RS14390	1445	-18.4	-	-	-	-	-	-	-	-	-	0	-
SL_RS14525	1466-Bi	-16.2	-	-	-	139	1	0.992806	259	2	0.992278	0.992806	SRPS
SL_RS14630	1477-Bi	-16.7	13	39	-0.66666	123	39	0.682927	105	26	0.752381	0.752381	SRPS
SL_RS15510	1560	-18.4	161	74	0.540373	669	389	0.418535	686	477	0.304665	0.540373	SRPS
SL_RS15870	1600	-25.9	-	-	-	-	-	-	-	-	-	0	-
SL_RS17245	1745	-18.6	889	1962	-0.54689	4635	5332	-0.13072	6528	8018	-0.18583	-0.13072	SRPS

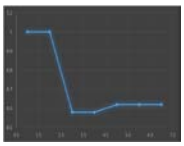
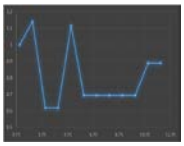
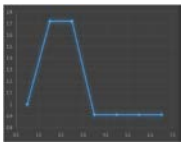
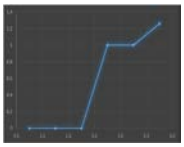
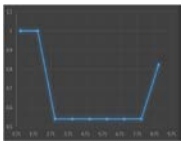
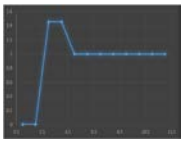
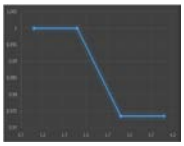
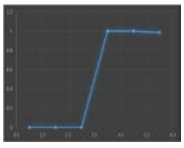
Table S3. Features of the identified poly-cistronic SRPS operons with the number of genes and their harbored SLs in *C. cellulolyticum*.

Operon	Polarity	Operon start	Operon end	# genes	# SLs	SL strand	SL start	SL end	Stem-loop structure	ΔG	Ratio	Annotation
1	+	27	4073	4	1	+	1367	1415	((((((((((((.....((((((....))))))...))))))))))))	-19.7	1:0:0:0	Chromosome replication
6	+	11177	12750	3	2	+	12440	12474	((((((((((((((.....))))))))))))))	-14.1	1:1:1.35	Haloacid
						+	12760	12800	((((((((((((((((.......))))))))))))))))	-19		
42	+	92021	101784	9	1	+	101327	101360	((((((((((((.((....)))))).))))))))	-18.4	1:1:1:1:1:1:1:1:0	ATP
80	+	172536	177484	4	2	+	174252	174285	((((((((((((((....))))))))))))))	-23.2	1:0.94:0.94:0.94	ABC transporter
						+	177505	177560	((((((((((((((((((((.....))))))).)))))))))))))	-21.8		
142	+	294975	301820	9	3	+	296220	296261	((((((((((((((((.........))))))))))))))))	-25.2	1:1:1:0.58:0.58:0.58:0.67: 0.67:0.67	ATP synthase
						+	298955	298996	((((((((((((.((((((....))))))))))).))))))))	-14.7		
						-	301825	301859	((((((((((((((.....))))))))))))))	-17		
190	+	422059	428303	4	2	+	424482	424505	((((((((((....))))))))))	-15	1:1:1.51:1.51	Phosphoglycerol
						+	428310	428359	((((((((((((((((((((.....))))))))))))))))))))	-22.7		
216	-	481263	484098	3	1	-	483076	483143	((((((((((((((((((((((.....))))))))))))))))))))))	-24.4	0:00:01	Unknown
237	+	544670	546728	3	1	+	546187	546215	((((((((((.....)))))).))))))	-16.8	1:01:00	Unknown
288	+	660532	661710	5	1	+	661594	661632	((((((((((((((((.....))))))))))))))))	-19.9	1:0:0:0:0	Unknown
314	+	723716	728092	3		+	726389	726424	((((((((((((((((....))))))))))))))))	-17.2	1:1.08:1.08	Diguanylate
						-	728110	728162	((((((((((((((.((..(((((.......))))..)).))))))))))))))	-18.6		
376	+	838275	864108	12	6	+	842933	842967	((((((((((((((.....))))))))))))))	-23.5	1.00:1.14:0.62:0.62:1.11: 0.69:0.69:0.69:0.69:0.69: 0.89:0.89	Cellulosome complex
						+	845233	845286	((((((((((((((..((..(((.........)))..)).))))))))))))))	-26.8		
						+	849002	849039	(((((((.(((((((((....))))))))).)))))))	-14.5		
						+	851740	851778	((((((((((((((((.....))))))))))))))))	-26.2		
						+	860347	860374	((((((((((.....))))))))))	-16.3		
						+	864130	864169	((((((((((((((.........)))))).))))))))	-20.9		
391	+	894101	906620	24	2	+	901607	901644	((((((((((((((((....))))))))))))))))	-17.3	1.00:1.00:1.00:1.00:1.00: 1.00:1.00:1.00:1.00:1.00: 1.00:1.00:1.00:1.00:1.00: 1.00:1.20:1.20:1.20:1.20: 1.20:1.20:0.00:0.00	Ribosomal protein
						+	905137	905172	((((((((((((((((....))))))))))))))))	-20.7		
495	+	1239924	1243099	3	2	+	1241316	1241346	((((((((((((.....))))))))))))	-20	1.00:1.25:1.25	ABC transporter

						+	1243174	1243217	((((((((((((((((((.....))))))..))))))))))))))	-25		
511	+	1277219	1286951	9	1	+	1278581	1278614	((((((((((((((....))))))))))))))	-18.5	1.00:0.00:0.00:0.00:0.00:0.00:0.00:0.00:0.00:0.00	ABC transporter
514	+	1294109	1304391	11	2	-	1301578	1301624	((((((((((.((.(((((.....)))))..)).)..))))))))))	-26.1	1.00:1.00:1.00:1.00:1.00:1.00:1.00:0.69:0.69:0.69:0.69	Pyridoxal-depen. decarboxylase
						+	1304400	1304434	((((((((((((((.....))))))))))))))	-18		
545	+	1346819	1352826	3	2	-	1348075	1348128	((((((((((((((((((..(((.....))).)).))))))))))))))))	-22	1.00:1.00:0.95	Unknown
						-	1352874	1352901	((((((((((....))))))))))	-20		
569	+	1393138	1396397	3	1	+	1394533	1394566	((((((((((((........))))))))))))	-17.9	1.00:0.00:0.00	ABC transporter
617	+	1505662	1537237	14	4	+	1509008	1509040	((((((((((((((...))))))))))))))	-16.6	1.00:1.00:0.87:0.87:1.11:1.49:1.49:1.49:1.49:1.49:1.49:1.49:0.00:0.00	Sugar-binding
						+	1512146	1512173	((((((((((....))))))))))	-14.4		
						-	1514433	1514464	((((((((((((......))))))))))))	-18.4		
						+	1531630	1531668	((((((((((((((.((.....)).))))))))))))))	-24.7		
622	+	1548963	1555274	4	1	+	1554284	1554322	((((((((((((((.....))))))))))))))	-18.2	1.00:1.00:1.00:0.00	Sugar ABC transporter
632	+	1604455	1606907	3	1	-	1606431	1606454	((.(((((((...)))))))))	-16.8	1.00:1.00:1.00:0.00	Unknown
693	+	1707355	1713745	6	3	+	1708114	1708140	((((((((((...))))))))))	-16.7	1.00:1.72:1.72:1.25:1.25:1.25	ABC transporter
						+	1710105	1710155	((((((((((((((...((((((..)))))).))))))))))))))	-28.7		
						+	1713761	1713796	((((((((((.(((....))).))))))))))	-20.8		
716	+	1743739	1745397	3	1	+	1745420	1745467	((((((((((.(((.((.....)).))))))))))))))	-19.6	1.00:0.00:0.00	ATPase
746	+	1806830	1820616	6	3	+	1813879	1813912	((((((((((((((......))))))))))))))	-24	1.00:0.74:0.74:0.79:0.79:0.79	Cellulose-binding
						+	1818549	1818575	((((((((((...))))))))))	-17.8		
						+	1820661	1820695	((((((((((.(((.....))).))))))))))	-18.9		
813	-	1975716	1982300	7	2	+	1975647	1975687	(((((((.(((((((((.....))))))))).)))))))	-17.2	0.85:0.85:0.85:0.85:1.00:1.00:1.00	Dihydroxyacetone kinase
						+	1978727	1978766	((((((((((..(((((.....))))).))))))))))	-20.3		
863	-	2075423	2076220	5	2	-	2075375	2075411	((((((((((((((.......))))))))))))))	-15.3	0.78:1.00:1.00:1.00:1.00	Chemotaxis protein
						+	2076297	2076334	((((((((((((((((....))))))))))))))))	-19.7		
898	-	2150635	2151774	7	1	+	2151880	2151904	((((((((((.....))))))))))	-15.5	0.00:0.00:0.00:0.00:1.00:1.00:1.00	Chemotaxis protein
915	+	2185763	2185999	3	2	+	2186035	2186071	(((((((((..(((((.....))))).)))))))))	-16.2	1.00:1.00:0.96	Unknown
						-	2187198	2187220	((((((((((...))))))))))	-15.6		
1000	-	2345505	2354089	6	3	-	2345421	2345477	((((((((((((((((.....(((...)))).....))))))))))))))))	-23.3	1.40:1.40:1.40:1.57:1.57:1.00	Two-component system
						-	2350047	2350099	((((((((((((.(((.(((((((.....))))))))))))))))))))))	-26.3		
						+	2352888	2352922	((((((((((.(((.....))).))))))))))	-16.7		
1018	-	2388471	2399850	13	1	-	2394967	2395006	((((((((((((((.....))))))...))))))))))	-15.4	0.00:0.00:0.00:0.00:0.00:0.00:0.00:1.00:1.00:1.00:	Flagellar biosynthesis

											1.00:1.00:1.00	
1052	-	2466969	2473329	4	3	-	2466885	2466933	((((((((((((.(((((((((.....)))))))).))))))))))))	-28.3	1.42:0.84:0.84:1.00	Unknown
						+	2469971	2470000	((((((((((.....))))))))))	-16.8		
						-	2471916	2471954	((((((.(((((((((.....)))))))))).))))))	-20		
1073	+	2515714	2518031	3	2	+	2516941	2516977	(((((((((..(((((.....))))).)))))))))	-16.2	1.00:1.00:0.96	Unknown
						-	2518104	2518126	((((((((((...))))))))))	-15.6		
1247	+	2998820	3001411	3	1	+	2999103	2999151	((((((((((((((..((.(((.....)))))))))))))))))))	-18	1.00:0.00:0.00	Membrane protein
1354	-	3216073	3216073	3	2	-	3215973	3216018	((((((((((((..(((((.....))))).))))))))))))	-20	0.56:0.56:1.00	Esterase
						-	3218722	3218784	((((((((((((((((((((((((.....)).))))))))))))))))))))))	-35.4		
1382	-	3268624	3277915	7	1	+	3269498	3269545	((((((((((.((((((((((....)))))))))).))))))))))	-23	0.00:0.00:1.00:1.00:1.00:1.00:1.00	Unknown
1435	-	3376013	3392707	17	1	-	3385225	3385259	(((((((((((((.....)))))))))))))	-23.3	0.00:0.00:0.00:0.00:0.00:0.00:0.00:1.00:1.00:1.00:1.00:1.00:1.00:1.00:1.00:1.00:1.00	Unknown
1445	-	3410668	3413578	5	2	-	3410617	3410657	(((((((((((((((((.....)))))))))))))))))	-29.3	1.59:1.59:1.00:1.00:1.00	Unknown
						-	3412329	3412376	((((((((((((...((.....)).....)))))).))))))	-18.4		
1560	-	3619722	3621210	3	2	+	3619672	3619713	((((((((((((((.((((...)).)).))))))))))))))	-16.3	0.89:1.00:1.00	Unknown
						-	3620051	3620090	(((((((...(((((((.....))))))).)))))))	-18.4		
1600	-	3695018	3699871	5	1	-	3695253	3695289	(((((((((((((((((...)))))))))))))))))	-25.9	0.00:1.00:1.00:1.00:1.00	Unknown
1745	-	3997446	4002355	4	2	+	3997405	3997434	((((((((((((.....))))))))))))	-19.4	1.05:1.00:1.00:1.00	Acetolactate synthase
						+	3999045	3999081	(((((((((((((((.....)))))))))))))))	-18.6		

Table S4. Correlation between SLOFE-predicted transcript ratio and those experimentally measured for selected operons from *Ccel*, *Cace*, *Cthe* and *Bsub*. These operons have skewed transcript ratios as predicted by SLOFE.

Operon ID	Organism	Predicted ratio	Correlation with transcript	Plot	Annotation
142	*Clostridium cellulolyticum*	1.00:1.00:0.58:0.58:0.58:0.62:0.62:0.62	0.698		ATP synthase
376	*Clostridium cellulolyticum*	1.00:1.14:0.62:0.62:1.11:0.69:0.69:0.69:0.69:0.69:0.88:0.88	0.751		Cellulosome
693	*Clostridium cellulolyticum*	1.00:1.72:1.72:0.91:0.91:0.91:0.91	0.556		ABC transporter
1000	*Clostridium cellulolyticum*	0:0:0:1.00:1.00:1.26	0.940		Two-component system
593	*Clostridium acetobutylicum*	1.00:1.00:0.53:0.53:0.53:0.53:0.53:0.53:0.53:0.53:0.53:0.82	0.701		Cellulosome
1068	*Clostridium acetobutylicum*	0:0:1.45:1.45:1.00:1.00:1.00:1.00:1.00:1.00:1.00:1.0	0.672		Cell division protein and lipoprotein
482	*Clostridium thermocellum*	1.00:1.00:0.97:0.97	0.672		Amino acid-binding protein
1135	*Clostridium thermocellum*	0:0:0:1.00:1.00:0.98	0.460		Restriction endonuclease Protein

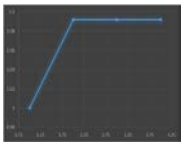
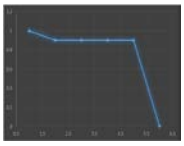
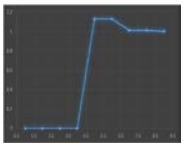
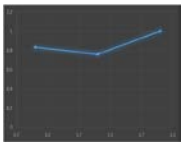
531	*Clostridium thermocellum*	1.00:1.09:1.09:1.09	0.802		Magnesium chelatase
679	*Bacillus subtilis*	1.00:0.90:0.90:0.90:0.90:0	0.773		ABC transporter
1491	*Bacillus subtilis*	0:0:0:0:1.12:1.12:1.01:1.0 1:1.00	0.762		Chaperone protein
1513	*Bacillus Subtilis*	0.8318:0.75:1.00	0.980		Mother cell lysis

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Table S5. SLOFE-predicted ratios of the SRPS operons from *Ccel* (A), *Cthe* (B), *Cace* (C) and *Bsub* (D). SLOFE predicted ratios for all the SRPS operons using ΔG of SLs.

(**A**) SLOFE-predicted ratios of the SRPS operons from *Ccel*.

# Operon	# of genes	Ratio
1	4	1.00:0:0:0
4	2	1.00:0.89
6	3	1.00:1.00:1.354
42	9	1.00:1.00:1.00:1.00:1.00:1.00:1.00:1.00:0
80	4	1.00:0.94:0.94:0.94
142	8	1.00:1.00:0.58:0.58:0.58:0.62:0.62:0.62
170	2	1.00:0.96
190	4	1.00:1.00:1.5:1.5
216	3	1.00:0:0
228	2	1.00:0.91
237	3	1.00: 1.00:0
288	5	1.00:0:0:0:0
295	2	1.00:0.85
314	3	1.00:0.989:0.989
376	12	1.00:1.14:0.62:0.62:1.11:0.69:0.69:0.69:0.69:0.69:0.88:0.88
391	24	1.00:1.00:1.00:1.00:1.00:1.00:1.00:1.00:1.00:1.00:1.00:1.00:1.00:1.00:1.00:1.00:1.00:1.00:1.00:1.00:1.00:1.2:1.2:1.2:1.2:1.2:1.2:0:0
432	2	1.00: 1.00
495	3	1.00:0.805:0.805
511	9	1.00:0:0:0:0:0:0:0:0
514	11	1.00:1.00:1.00:1.00:1.00:1.00:1.00:0.74:0.74:0.74:0.74
545	3	1.00: 1.00:0.95
566	2	1.00:0:0
569	3	1.00:0:0
617	14	1.00:1.00:0.86:0.86:0.97:1.48:1.48:1.48:1.48:1.48:1.48:1.48:0:0
622	4	1.00:1.00:1.00:0
693	6	1.00:1.72:1.72:0.91:0.91:0.91:0.91
716	3	1.00:0:0
746	6	1.00:0.74:0.74:0.78:0.78:0.78
813	7	1.00:1.00:1.00:1.00:1.17:1.17:1.17

849	2	0:1.00
863	5	0.845:1.00:1.00:1.00:1.00
898	7	0:0:0:0:1.00:1.00:1.00
915	2	1.00:0:0
1000	6	0.8859:0.8859:0.8859:1.00:1.00:1.26
1018	13	0:0:0:0:0:0:0:1.00:1.00:1.00:1.00:1.00:1.00:1.00
1052	4	0.84:0.64: 1.00
1073	3	1.00: 1.00:0.96
1135	2	0:1.00
1247	3	1.00:0:0
1254	2	0:1.00
1341	2	1.60:1.00
1354	3	0.5649:0.5649:1.00
1358	2	1.00:0.96
1359	2	0.57:1.00
1382	7	0:0:1.00:1.00:1.00:1.00:1.00
1435	16	0:0:0:0:0:0:0:1.00: 1.00: 1.00: 1.00: 1.00: 1.00: 1.00: 1.00: 1.00
1445	5	1.59:1.59: 1.00: 1.00: 1.00
1466	2	1.00:0.96
1477	2	1.00:0.76
1560	3	0.56: 1.00: 1.00
1600	5	0: 1.00: 1.00: 1.00: 1.00
1745	4	1.00:0.658:0.658:0.658

(**B**) SLOFE-predicted ratios of the SRPS operons from *Cthe*.

# operon	**# of genes**	**Ratio**
275	5	1:1:1:0.77:0.77
357	3	1:1:0.96
482	4	1:1:0.97:0.97
531	4	1:1.09:1.09:1.09
548	7	1:1:1:1:1:1:1.33
552	4	1:1:1:0.74
728	3	0:0:1
747	3	1:1:0.46
791	5	1:1:0.99:0.99:0.99

794	5	0:0:1:1:1
804	23	0:1:1:1:1:1:1:1:0.74:0.74:0.74:0.65:0.65:0.98:0.98:0.98:0.98:0.98:0.98:0.98:0.74:0.74:0.74
806	7	0:0:0:0:1:1:1
938	3	0:0:1
957	8	0:0:0:0:1:1:1:1
1135	6	0:0:0:1:1:0.98
1209	3	0:0:1
1228	5	1:1:1:0:0
1353	5	1:1:1:0:0
1359	4	1:1:1:0.82
1395	8	1:1:1:0.97:0.97:0.97:0.97:0.97
1465	3	1:1:0.65
1487	6	1:0.93:0.93:0.93:0.93:0.93
1522	3	1:1.52:1.52
1536	6	1:1:1:0:0:0

(**C**) SLOFE-predicted ratios of the SRPS operons from *Cace*.

# operon	**# of genes**	**Ratio**
120	5	1.00:1.00:1.21:1.21:0
205	6	1.00:1.00:0:0:0:0
216	2	1.00:0.58
239	5	1.00:1.00:1.00:0.77:0.77
244	10	1.00:1.00:1.00:1.00:1.00:0.83:0.83:0.83:0.83:0.83
304	7	1.00:0.90:0.90:1.14:1.14:1.14:0
317	2	1.00:0.48
356	3	1.00:1.00:1.36
362	6	1.00:1.00:1.29:1.29:1.29:1.29
401	3	1.00:1.2013:1.20
466	2	1.00:1.39
481	4	1.00:0.89:1.24:0
593	9	1.00:1.00:0.53:0.53:0.53:0.53:0.53:0.53:0.53:0.53:0.82
614	2	1.00:1.12
633	2	1.00:0:0
635	3	1.00:1.00:0.80

673	2	1.00:1.24
715	8	1.00:1.00:1.00:1.00:1.00:1.00:0:0
730	10	1.00:1.7:1.7:1.7:1.7:1.7:1.7:1.7:1.7:1.7
738	5	1.00:1.13:1.03:1.03:1.03
789	6	1.00:1.00:1.10:1.10:1.10:1.10
849	3	0:0:1.00
909	11	1.00:1.00:1.00:1.00:1.00:1.00:1.00:1.00:1.00:0:0
910	2	0:1.00
943	2	1.00:0:0
944	17	1.00:1.00:1.00:1.00:1.00:1.00:1.00:1.00:1.00:1.00:1.00:1.00:1.00:1.00:1.00:1.00:1.00:1.20
949	9	1.00:1.00:1.00:1.00:1.02:1.02:1.20:1.20:0.89
965	11	1.00:0.74:0.74:0.74:0.74:0.74:0.74:0.84:0.84:0.84:0.84
967	7	1.00:1.00:1.00:1.00:0:0:0
981	2	1.00:0.57
986	4	0:1.00:1.00:1.00
1008	26	1.00:1.16:1.16:1.16:1.16:1.16:1.16:1.16:1.16:1.16:1.16:1.16:1.16:1.14:1.14:1.14:1.14:1.14:1.14:1.14:1.14:1.14:1.14:1.14:1.14:1.14:1.14
1068	12	0:0:1.45:1.45:1.00:1.00:1.00:1.00:1.00:1.00:1.00:1.00
1090	6	0:1.02:1.21:1.21:1.38:1.00
1132	4	0:0:1.52:1.31
1248	9	0:0:1.00:1.00:1.00:1.00:1.00:1.00
1283	6	0:1.17:1.17:1.17:1.00:1.00
1336	3	1.00:1.00:1.08
1359	2	1.00:1.35
1362	2	1.00:1.01
1412	2	1.00:0:0
1454	5	1.00:1.09:1.09:0.82:0.82
1519	35	1.00:1.00:1.00:1.00:0.92:0.92:0.92:0.92:0.92:0.92:0.92:0.92:0.92:0.92:0.92:0.92:0.92:0.92:0.92:0.92:0.92:0.92:0.92:0.92:0.92:0.92:0.92:0.92:0.92:0.92:0.92:0.92:0.92:0.92:0.92:0.92:0.92:0.92:0.92:0.92:0.92:0.92:0.92:1.03:1.03:1.03:1.03:1.03:1.03
1522	4	0:0:1.00:1.00
1537	5	1.00:1.05:1.05:1.05:1.05
1553	9	0:0:0:0:0.66:0.78:0.78:0.78:1.00
1566	4	1.00:1.00:1.29:1.29
1684	3	1.00:1.00:0.75

1749	2	1.00:0.96
1784	2	1.00:2.07
1793	5	1.00:1.00:1.00:1.00:1.16

(**D**) SLOFE-predicted ratios of the SRPS s from *Bsub.*

#	#of genes	Ratio
40	6	1.00:1.00:1.00:1.00:0:0
44	5	1.00:1.00:1.00:1.00:1.23
47	7	1.00:1.00:0.64:0.64:0.64:0.64:0.71
49	31	1.00:1.00:1.00:1.00:1.00:0.91:0.91:0.91:0.91:0.91:0.91:0.91:0.91:0.91:0.91:0.91:0.9 1:0.91:0.91:0.91:0.91:0.91:1.85:1.85:1.85:1.85:1.85:1.85:1.85:1.32:1.32
130	3	1.00:0.93:0.93
136	3	1.00:1.00:0.90
200	4	1.00:1.00:1.00:0
341	3	1.00:1.07:1.07
361	12	1.00:1.00:1.00:1.06:1.06:1.06:1.06:1.06:1.06:1.06:1.06
394	4	1.00:0:0:0
406	3	1.00:0.99:0.78
460	3	1.00:0:0
679	6	1.00:0.90:0.90:0.90:0.90:0
692	7	1.00:1.00:1.00:1.00:1.00:1.00:0
744	10	1.00:0.89:0.89:0.89:0.89:0.89:0.89:0.89:0.89
836	5	1.00:1.00:1.00:1.12:1.12
925	11	1.00:1.10:1.10:1.10:1.10:0.91:0.91:0:0:0:0
934	6	1.00:1.00:1.00:1.00:1.19:1.19
961	11	1.00:1.00:1.00:1.00:1.00:1.00:1.00:0.81:0.81:0.81:0.83
964	3	1.00:0:0
1242	3	0:0:1.00
1461	5	1.02:1.02:1.02:1.02:1.00
1491	9	0:0:0:0:1.12:1.12:1.01:1.01:1.00
1492	3	1.19:1.19:1.00
1513	3	0.83:0.75:1.00
1602	4	1.00:1.00:0.97:0.97
1672	4	0.87:1.00:1.00:1.00
1693	3	1.00:1.00:1.31

1850	7	0:0.84:0.84:0.84:0.84:0.84:1.00
1894	5	0:0:0:0:1.00
1952	3	1.00:1.24:1.24
1956	4	0:0:0:1.00
1976	6	1.00:1.00:1.00:1.00:1.05:1.05
1990	13	1.00:1.00:1.00:1.00:1.00:0.80:0.80:0.80:0.80:0.92:0.92:0.92:0.92
2016	8	0:0:0:0:0:0:1.00:1.00
2030	3	1.00:2.11:2.11
2279	8	1.00:1.00:1.00:1.00:1.00:0.64
2358	3	0:1.00:1.00
2363	4	0:0:0:1.00

Table S6. Average Pearson correlation coefficients of the six methods for transcript and protein level prediction among the SRPS operons of *C. cellulolyticum*, *C. thermocellum*, *C. acetobutylicum* and *B. subtilis*.

Bacterial species		CAI	MELP	RCBS	RCA	Gene-order	SLOFE
C. cellulolyticum	Transcript level	0.364	-0.074	-0.004	0.333	0.414	0.587
	Protein level	0.383	-0.029	-0.075	0.324	0.408	0.621
C. thermocellum	Transcript level	-0.034	-0.148	0.032	0.106	0.044	0.342
C. acetobutylicum	Transcript level	0.230	-0.136	-0.062	0.016	-0.125	0.293
B. subtilis	Transcript level	0.082	-0.284	-0.084	0.095	0.147	0.464
	Protein level	0.298	0.055	0.301	-0.214	0.194	0.435

Table S7. Pearson correlation coefficients between predicted ratio and experimentally measured ratio for the SRPS operons of *Ccel*, for each of the six methods (CAI, RCA, RCBS, MELP, Gene-order and SLOFE). Correlations with the experimentally measured abundance of transcripts (**A**) and proteins (**B**) were both shown.

(**A**) Correlations with the experimentally measured abundance of transcripts.

#Operon	# of gene	CAI	MELP	RCBS	RCA	Gene-order	SLOFE
1	4	0.164	0.060	-0.221	0.441	0.726	0.488
6	3	0.863	-0.945	0.916	0.028	-0.341	0.914
42	9	0.121	-0.465	-0.547	0.042	0.496	0.676
80	4	0.901	0.546	0.930	0.845	0.892	0.975
142	8	0.817	-0.682	0.555	0.804	0.349	0.714
190	4	-0.261	-0.826	-0.274	0.768	0.387	0.166
237	3	0.168	-0.894	-0.711	-0.415	-0.360	0.423
376	12	0.667	0.472	0.508	0.565	0.820	0.752
391	24	0.159	0.226	-0.271	0.288	0.070	0.579
511	9	0.591	-0.439	0.519	0.533	0.813	0.901

514	11	-0.368	-0.522	-0.770	-0.132	0.342	0.412
545	3	0.953	0.826	-0.962	0.232	-0.091	-0.780
569	3	0.698	0.017	0.258	0.288	1.000	0.969
617	14	-0.184	-0.201	-0.321	-0.335	-0.513	0.036
622	4	0.768	0.312	0.510	0.620	-0.410	0.525
693	6	0.615	0.182	0.182	0.294	0.228	0.381
716	3	-0.264	0.672	-0.697	0.807	0.925	0.989
746	6	-0.157	-0.318	-0.449	-0.285	0.916	0.737
813	7	0.240	-0.297	0.151	0.583	0.045	0.232
863	5	-0.040	-0.421	-0.357	0.334	-0.166	0.547
898	7	0.806	-0.054	0.049	0.808	0.414	0.857
1000	6	0.973	0.539	0.884	0.931	0.926	0.972
1018	13	0.367	0.443	-0.165	0.128	0.375	0.400
1052	4	0.974	0.639	0.765	0.735	0.906	0.040
1247	3	0.998	0.159	0.652	0.977	-0.194	0.194
1354	3	-0.882	0.349	0.699	-0.829	0.612	0.784
1382	7	0.276	0.375	-0.929	0.750	0.670	0.886
1560	3	0.985	0.246	0.182	0.910	0.472	0.962
1745	4	0.249	-1.000	-0.924	-0.395	0.860	0.638
314	3	-0.915	-0.914	-0.991	-0.994	0.666	0.826
495	3	0.995	-0.388	0.967	0.999	0.992	0.993
Average		0.364	-0.074	0.004	0.333	0.414	0.587

(**B**) Correlations with the experimentally measured abundance of proteins. Dash (-) denotes no data available.

#	# of gene	CAI	MELP	RCBS	RCA	Gene-order	SLOFE
1	4	0.547	0.534	-0.659	-0.715	0.748	0.524
6	3	-	-	-	-	-	-

42	9	0.328	-0.361	-0.284	0.403	0.282	0.983
80	4	0.971	0.705	0.966	0.940	0.951	1.000
142	8	0.092	-0.439	-0.138	-0.115	0.178	0.060
190	4	0.531	0.420	0.242	-0.199	-0.903	0.507
237	3	-0.610	-0.278	0.035	0.388	0.442	0.953
363	3	0.734	-0.475	-0.515	0.987	0.971	1.000
376	12	0.584	0.771	0.419	0.322	0.576	0.283
391	24	-0.052	0.216	-0.168	0.146	0.236	0.076
511	9	-	-	-	-	-	-
514	11	-0.555	-0.352	-0.818	-0.138	0.405	0.822
545	3	0.444	0.683	-0.417	0.997	0.971	0.500
569	3	0.853	0.228	-0.012	0.515	0.971	1.000
617	14	-	-	-	-	-	-
622	4	-	-	-	-	-	-
693	6	0.292	0.634	0.624	-0.022	0.653	0.484
716	3	-0.118	-0.555	-0.583	0.885	0.971	1.000
746	6	0.185	-0.200	-0.516	-0.038	0.339	0.096
813	7	-0.189	-0.536	-0.214	0.170	0.743	1.000
863	5	0.306	-0.548	-0.514	0.505	-0.379	0.145
898	7	-0.008	-0.770	-0.659	0.482	0.475	0.626
1000	6	0.704	0.318	0.520	0.541	0.890	0.925
1018	13	0.382	0.392	0.401	-0.030	0.740	0.712
1052	4	0.957	0.391	0.568	0.528	0.791	0.333
1247	3	-	-	-	-	-	-
1354	3	-	-	-	-	-	-
1382	7	-	-	-	-	-	-
1560	3	0.995	0.500	-0.092	0.763	0.693	1.000
1745	4	0.764	-0.541	-0.162	0.529	-0.669	0.262

Average	0.354	0.032	-0.086	0.341	0.482	0.621

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Table S8. Pearson correlation coefficients between predicted ratio and experimentally measured ratio for the SRPS operons of *Cthe*, for each of the six methods (CAI, RCA, RCBS, MELP, Gene-order and SLOFE). Correlations with the experimentally measured abundance of transcripts were shown.

#	#of genes	CAI	MELP	RCBS	RCA	Gene-order	SLOFE
357	3	-0.818	-0.157	-0.018	-0.684	0.682	0.037
531	4	-0.423	-0.764	0.073	0.522	-0.832	0.803
548	7	0.127	-0.192	0.248	0.360	0.727	0.601
552	4	0.787	0.418	0.392	-0.532	-0.312	0.467
728	3	-0.582	-0.986	-0.612	-0.301	0.167	-0.075
747	3	-0.855	-0.515	-0.250	0.394	-0.780	0.907
791	5	0.219	-0.367	-0.234	0.216	0.053	-0.094
794	5	0.210	-0.109	0.634	0.307	0.798	0.711
804	23	-0.087	0.281	0.051	0.406	-0.366	0.171
806	7	-0.293	-0.441	-0.330	-0.248	-0.343	-0.359
938	3	-0.783	0.197	0.921	0.788	1.000	0.977
957	8	-0.059	0.147	0.392	-0.467	0.050	0.408
1135	6	-0.702	-0.527	-0.364	0.177	0.186	0.461
1209	3	0.999	0.019	0.283	0.648	-0.739	-0.556
1228	5	0.255	-0.824	-0.053	0.505	0.070	-0.021
1359	4	0.630	0.987	0.467	-0.291	0.300	-0.527
1395	8	-0.007	0.311	-0.132	-0.307	-0.890	0.886
1465	3	0.859	-0.900	-0.941	0.900	0.104	0.789
1487	6	0.035	0.161	0.118	0.157	0.587	0.387
1536	6	-0.193	0.308	0.002	-0.422	0.424	0.873
Average		-0.034	-0.148	0.032	0.106	0.044	0.342

Table S9. Pearson correlation coefficients between predicted ratio and experimentally measured ratio for the SRPS operons of *Cace*, for each of the six methods (CAI, RCA, RCBS, MELP, Gene-order and SLOFE). Correlations with the experimentally measured abundance of transcripts were shown.

#	#of genes	CAI	MELP	RCBS	RCA	Gene-order	SLOFE
205	6	0.367	-0.377	-0.566	-0.555	0.658	0.742
239	5	-0.217	-0.793	-0.487	0.376	0.639	0.970
304	7	0.271	-0.508	-0.420	-0.496	-0.451	0.101
356	3	0.277	0.146	0.396	0.894	-0.982	0.816
362	6	0.777	-0.352	-0.077	-0.132	-0.749	0.740
401	3	0.457	-1.000	-0.489	-0.010	-0.692	0.498
481	4	0.687	0.739	0.791	0.381	-0.998	-0.459
593	9	0.190	-0.327	0.039	-0.300	0.484	0.702
635	3	-0.511	-0.923	-0.979	0.409	0.907	0.932
715	8	-0.202	-0.617	-0.216	-0.031	-0.405	0.153
730	10	0.090	0.080	-0.684	-0.393	-0.877	0.615
738	5	-0.579	-0.069	-0.311	-0.109	0.076	0.923
849	3	0.904	-1.000	0.595	0.594	0.916	0.793
909	11	0.281	-0.179	-0.272	-0.184	-0.490	0.214
965	11	0.700	0.543	0.324	-0.617	0.590	0.224
967	7	0.677	-0.417	-0.466	0.048	0.876	0.464
1008	26	-0.269	0.589	-0.194	0.312	0.197	-0.019
1090	6	0.406	0.045	0.412	0.694	-0.155	0.191
1132	4	0.058	0.970	0.459	-0.718	0.721	0.331
1248	9	-0.238	0.308	0.111	-0.690	-0.173	0.217
1283	6	0.372	0.411	0.314	0.090	-0.651	-0.545
1336	3	0.998	-0.931	0.073	0.961	0.083	0.320
1454	5	-0.119	-0.217	-0.309	-0.890	-0.228	0.046
1519	35	0.220	0.161	0.213	0.219	-0.098	-0.451

1522	4	0.116	-0.252	-0.656	0.687	-0.776	0.598
1537	5	-0.220	-0.518	0.030	0.439	-0.167	0.117
1553	9	0.431	0.497	0.304	-0.678	-0.553	-0.199
1566	4	0.396	-0.159	0.047	0.462	-0.678	-0.166
1684	3	0.774	0.308	0.453	-0.358	-0.833	-0.179
1793	5	-0.180	-0.238	-0.315	0.073	0.040	0.106

Average	0.23048	-0.136	-0.0627	0.01606	-0.1256	0.2931

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Table S10. Pearson correlation coefficients between predicted ratio and experimentally measured ratio for the SRPS operons of *Bsub*, for each of the six methods (CAI, RCA, RCBS, MELP, Gene-order and SLOFE). Correlations with the experimentally measured abundance of transcripts (**A**) and proteins (**B**) were both shown.

(**A**) Correlations with the experimentally measured abundance of transcripts.

#	#of genes	CAI	MELP	RCBS	RCA	Gene-order	SLOFE
40	6	0.524	-0.435	-0.299	0.283	-0.356	0.325
47	7	0.094	-0.459	0.007	0.320	-0.027	0.800
49	31	0.077	-0.447	-0.586	0.077	0.064	0.011
130	3	0.848	-0.870	0.997	0.999	0.998	0.985
200	4	-0.814	-0.331	-0.208	0.171	0.334	0.725
361	12	0.678	-0.779	-0.582	0.695	-0.289	0.157
394	4	-0.488	-0.411	-0.045	0.042	0.958	0.975
406	3	0.263	0.227	0.806	0.622	-0.791	0.636
460	3	-0.913	-0.900	-0.999	-0.723	0.614	0.786
679	6	0.014	0.061	0.697	0.037	0.865	0.773
744	10	0.239	-0.553	-0.251	0.095	0.430	0.538
836	5	0.657	0.969	0.467	0.715	-0.635	0.904
925	11	-0.129	0.046	0.318	0.117	-0.706	-0.114
934	6	0.887	0.182	0.060	0.244	-0.124	0.353
961	11	0.482	-0.450	0.022	-0.233	-0.608	-0.378
964	3	-0.346	0.475	0.154	0.110	0.758	0.892
1513	3	0.695	-0.901	-0.878	0.903	0.739	0.980
1672	4	-0.170	0.573	0.448	-0.485	0.511	0.991
1693	3	0.934	0.970	0.780	-0.737	0.953	0.998
1850	7	-0.403	-0.772	-0.484	0.209	0.083	0.392
1894	5	-0.211	-0.886	-0.685	-0.364	0.934	0.759
1952	3	-0.985	-1.000	-0.928	0.437	-0.884	-0.950

1956	4	0.840	-0.860	-0.613	0.502	0.947	0.934
1976	6	0.685	0.766	0.693	-0.522	-0.755	-0.389
1990	13	-0.411	-0.600	-0.632	-0.301	0.389	0.322
2363	4	-0.921	-0.992	-0.442	-0.738	-0.570	-0.349
Average		0.082	-0.284	-0.084	0.095	0.147	0.464

(**B**) Correlations with the experimentally measured abundance of proteins.

#	#of genes	CAI	MELP	RCBS	RCA	Gene-order	SLOFE
40	6	0.643	-0.331	-0.170	0.402	-0.252	0.336
47	7	-0.900	0.061	0.516	-0.843	-0.567	0.933
49	31	0.177	0.230	0.405	0.057	0.009	0.450
130	3	0.804	-0.906	0.988	0.999	0.989	0.996
200	4	0.642	0.984	0.901	0.266	-0.346	0.353
361	12	-0.339	-0.172	0.554	-0.606	-0.140	0.140
394	4	-0.330	-0.630	-0.304	-0.373	0.630	0.833
406	3	0.983	-0.779	-0.184	0.975	0.208	-0.419
460	3	0.785	0.804	0.426	-0.276	0.414	0.183
679	6	-0.146	-0.267	0.485	-0.319	0.949	0.370
744	10	-0.169	0.166	-0.401	-0.468	-0.240	-0.074
836	5	0.866	0.181	0.980	-0.119	-0.478	0.659
925	11	0.716	-0.326	-0.257	-0.239	-0.127	0.320
934	6	0.857	0.312	0.237	0.422	-0.367	0.509
961	11	0.685	-0.412	-0.384	0.155	0.550	0.516
964	3	-0.270	0.544	0.232	-0.021	0.704	0.854
1513		-0.857	0.982	0.724	-0.983	-0.539	-0.896
1672	4	-0.280	0.780	0.723	-0.850	-0.105	0.757
1693	3	0.972	0.932	0.853	-0.647	0.983	0.998

1850	7	0.539	-0.966	0.577	-0.359	0.914	0.370
1894	5	-0.185	-0.822	-0.786	-0.512	0.949	0.851
1952	3	0.447	1.000	0.623	-0.987	-0.195	0.572
1956	4	0.794	-0.907	-0.980	-0.028	0.596	0.334
1976	6	0.719	0.766	0.934	-0.828	-0.251	0.241
1990	13	0.302	0.304	0.154	-0.210	-0.214	0.136
2363	4	0.287	-0.106	0.990	-0.179	0.957	0.996
Average		0.298	0.055	0.301	-0.214	0.194	0.435

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Table S11. Pearson correlation coefficients between predicted ratio and experimentally measured ratio for the bicistronic SRPS operons of *Ccel* (A), *Bsub* (B), *Cthe* (C), *Cace* (D) for each of the six methods (CAI, RCA, RCBS, MELP, Gene-order and SLOFE). Correlations with the experimentally measured abundance of transcripts and proteins were both shown for those SRPS operons where transcript and protein data are available, e.g., for *Ccel* and *Bsub*. Pearson correlation coefficients were calculated between the ratios predicted using these methods and the experimentally determined ratios at the transcript (or protein) level. NA denotes no data available.

#	# of genes	RCA		CAI		MELP		RCBS		Gene order		SLOFE	
		Gene	Protein	Gene	Protein	Gene	Protein	Gene	Protein	Gene	Protein	Gene	Protein
4	2	-	NA	-	NA	-	NA	-	NA	0.388225	NA	0.614764	NA
170	2	-0.051	-0.039	-0.010	-0.008	-0.096	-0.074	-0.050	-0.039	0.421	0.323	0.034	0.026
228	2	0.617	0.499	0.563	-0.459	0.106	-0.027	0.337	0.915	0.062	-0.016	0.308	-0.080
295	2	-0.078	-0.591	0.020	0.016	0.714	0.563	0.077	0.061	0.403	0.318	0.131	0.104
566	2	-0.067	-0.004	0.879	0.057	-0.463	-0.030	0.258	0.017	0.114	0.562	0.067	0.952
849	2	0.315	0.117	0.142	0.053	0.072	0.027	0.122	0.045	0.853	0.436	0.504	0.738
915	2	-0.093	NA	0.119	NA	0.731	NA	0.001	NA	0.533	NA	0.903	NA
1135	2	0.139	0.116	0.128	0.107	0.443	0.372	0.171	0.143	0.680	0.571	0.868	0.967
1254	2	-0.348	-0.070	-0.734	-0.033	0.101	0.242	0.255	0.096	0.033	0.728	0.020	0.810
1466	2	-0.063	NA	0.032	NA	-0.031	NA	0.009	NA	0.460	NA	0.037	NA
1477	2	0.198	NA	-0.021	NA	-0.472	NA	0.876	NA	-0.960	NA	0.531	NA
Average		0.052	**0.004**	0.102	**-0.038**	0.100	**0.153**	0.187	**0.177**	0.272	**0.417**	0.365	**0.502**

(**A**) Correlations with the experimentally measured abundance of transcripts and proteins for bi-cistronic operons in *Ccel*.

(**B**) Correlations with the experimentally measured abundance of transcripts and proteins for bi-cistronic operons in *Bsub*.

#	# of genes	RCA		CAI		MELP		RCBS		Gene order		SLOFE	
		Gene	Protein	Gene	Protein	Gene	Protein	Gene	Protein	Gene	Protein	Gene	Protein
1	2	-0.100	-0.039	-0.044	-0.017	0.753	0.516	0.212	0.083	-0.463	-0.839	-0.273	-0.703
280	2	0.104	0.025	-0.053	-0.013	0.195	0.816	0.588	0.141	-0.424	-0.565	-0.250	-0.957
620	2	-0.271	-0.704	-0.026	-0.066	-0.459	-0.177	-0.670	-0.575	0.463	0.178	0.844	0.456
893	2	-0.498	-0.111	0.006	0.001	-0.264	-0.843	-0.948	-0.235	0.412	0.541	0.921	0.205
1212	2	-0.374	-0.097	-0.246	-0.064	-0.220	-0.843	0.025	0.006	0.603	0.432	0.356	0.732
1411	2	-0.048	-0.108	-0.031	-0.070	-0.130	-0.289	-0.045	-0.099	0.955	0.472	0.454	0.993
1463	2	-0.083	-0.087	0.011	0.012	0.773	0.810	0.193	0.202	0.356	0.373	0.603	0.632
1635	2	-0.008	-0.004	-0.002	-0.001	0.203	0.097	0.151	0.072	0.891	0.538	0.526	0.911
1768	2	-0.026	-0.024	0.020	0.018	-0.053	-0.050	-0.095	-0.089	0.612	0.573	0.701	0.656
1934	2	-0.034	-0.036	0.035	0.037	-0.873	-0.821	-0.155	-0.165	0.513	0.546	0.869	0.924
2144	2	-0.190	0.240	-0.058	0.073	-0.964	0.762	-0.487	0.616	-0.755	0.597	0.227	-0.287
Average		-0.121	-0.071	-0.029	-0.004	-0.155	-0.055	-0.118	-0.023	0.297	0.280	0.472	0.370

(**C**) Correlations with the experimentally measured abundance of transcripts for bi-cistronic operons in *Cthe.*

#	# of gene	RCA	CAI	RCBS	MELP	Gene-order	SLOFE
236	2	-0.012	-0.009	-0.018	-0.145	0.669	0.883
569	2	-0.567	0.373	0.042	0.010	-0.029	0.990
699	2	0.540	-0.148	-0.221	0.078	-0.153	-0.090
1265	2	0.052	0.243	0.417	0.755	-0.347	0.301
1289	2	0.181	-0.021	0.930	0.225	-0.527	-0.311
1380	2	-0.146	0.194	-0.770	0.227	-0.595	-0.351
1518	2	0.486	-0.055	0.868	0.176	0.125	0.074
Average		0.076	0.082	0.178	0.189	-0.122	0.214

(**D**) Correlations with the experimentally measured abundance of transcripts for bi-cistronic operons in *Cace.*

# Operon	# of gene	Gene-order	MELP	RCA	CAI	RCBS	SLOFE
216	2	-0.104	0.069	0.418	0.817	0.318	-0.123
466	2	-0.758	0.356	-0.063	0.037	0.442	0.933
614	2	0.725	-0.829	-0.006	-0.079	-0.211	-0.200
633	2	0.877	0.381	-0.088	-0.160	0.067	0.518
943	2	0.061	-0.539	-0.321	0.101	0.313	0.036
981	2	-0.626	0.552	0.005	-0.051	0.098	0.721
1362	2	0.165	0.984	-0.144	0.553	-0.415	0.148
1412	2	-0.776	-0.355	0.025	-0.245	0.365	-0.458
1749	2	0.259	0.157	0.190	-0.353	0.963	0.330
Average		0.057	0.064	0.070	0.076	0.108	0.210

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