Supplementary Note: Input and output files.

Algorithm for reconstruction of mathematical frame models of bacterial transcription regulation was implemented as Java program called “Operon equations”.

Input file is the plain text with tab as separator. The columns order is next: name of the operon, name of the TF regulating operon in a same row, left and right positions of TFBS (absolute positions in genome of the sites) and the TF regulation type (activator, repressor or dual role). The example of input file is shown in Figure S1.

aceEF	ArcA	0	0	repressor
aceEF	FNR	0	0	repressor
ada-alkB	Ada	0	0	repressor
ada-alkB	Ada	2308477	2308504	activator
agaR	AgaR	3276705	3276728	repressor
agaR	AgaR	3276735	3276758	repressor
alaS	AlaS	2820102	2820135	repressor
araBAD	AraC	70109	70126	activator
araBAD	AraC	70130	70147	dual
araBAD	AraC	70184	70201	repressor
araBAD	AraC	70204	70221	repressor
araBAD	AraC	70342	70359	repressor
araBAD	CRP	70158	70179	activator
betIBA	ArcA	328584	328598	repressor
betIBA	ArcA	328652	328666	repressor
betIBA	BetI	328605	328625	repressor

Figure S1. Typical input file. 1^{st} column –operon name, 2^{nd} column – TF name, 3^{rd} and 4^{th} columns – left and right TFBS positions, and 5^{th} column – regulation type of the TF. The tab is used as column separator. The "dual" value in regulation type is used when role of the TF is known both activator and repressor. The “regulation” value is used if you have no information TF function. When TFBS positions are also unknown the zero value is used (first 3 rows in an example). It means that there will be no intersections of selected TF with other TFs of the operon.

The output file includes information about generated or blocked states and the number of variables. The file also contains parameter description and equation (4) from the main text. Example of the output file is presented in Figure S2.

Mathematical model transcription of the operon: aceEF
Generated states: 3
Blocked states: 0
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variables:
1 ArcA ; 0 0 repressor
2 FNR ; 0 0 repressor
parameters: 12
V_0 ; Initial rate
a_basal ; Basal activity
k_1 ; ArcA activity
k_2 ; FNR activity
n_11 ; ArcA(#1) Hill parameter
level_1 ; ArcA(#1) rate activity parameter
n_1h1 ; ArcA(#1) Hill parameter
n_2h1 ; ArcA(#1) FNR(#2) Hill parameter
w12 ; cooperation parameter
level_12 ; ArcA(#1) FNR(#2) activity parameter
n_21 ; FNR(#2) Hill parameter
level_2 ; FNR(#2) rate activity parameter
Numerator =
a_basal+(level_1*((ArcA/k_1)^n_11))+(level_12*w12*((ArcA/k_1)^n_1h1)*((FNR/k_2)^n_2h1))+(level_2*((FNR/k_2)^n_21))
Denominator = 1+((ArcA/k_1)^n_11)+(w12*(ArcA/k_1)^n_1h1*(FNR/k_2)^n_2h1)+((FNR/k_2)^n_21)
f = V_0*Numerator/Denominator

Figure S2. Example of output file. Mathematical model for the *aceEF* operon is shown. For this case TFBS positions are unknown so it is no positional intersections (blocked states) between TFBS and all states are generated and added to equation.

We also tested the program with different input data. We have considered how the computation time depends on the number of TFs that can bind to their binding sites in a single operon. We also considered when TFs can overlap and when they cannot. Detailed information is presented in Table S1. As you can see, as TF increases, the number of parameters generated increases, and as a consequence the computation time increases. Therefore, we have set the upper limit in the program for the number of TFs per operon to 15.

Table S1. Program running time depending on the number of TFs and positions of binding sites per operon

Number of TF	Calculation time	Number of parameters	Blocked states
1	< 1 min	5	no
2	< 1 min	12	no
3	< 1 min	28	no
4	< 1 min	64	no
5	< 1 min	144	no
6	< 1 min	320	no
7	< 1 min	704	no
8	< 1 min	1536	no
9	< 1 min	3328	no
10	~ 1-2 min	7168	no
10	< 1 min	767	yes
11	~ 13 min	15360	no
11	~ 1-2 min	1151	yes
12	~ 16 min	16986	yes
13	> 84 h	50189	yes

The most of parameters denote the degree of Hill, because that have to unique in each case.

Commented code, as well as test data are available in the Github repository at github.com/tlakhova/Operon_equations.