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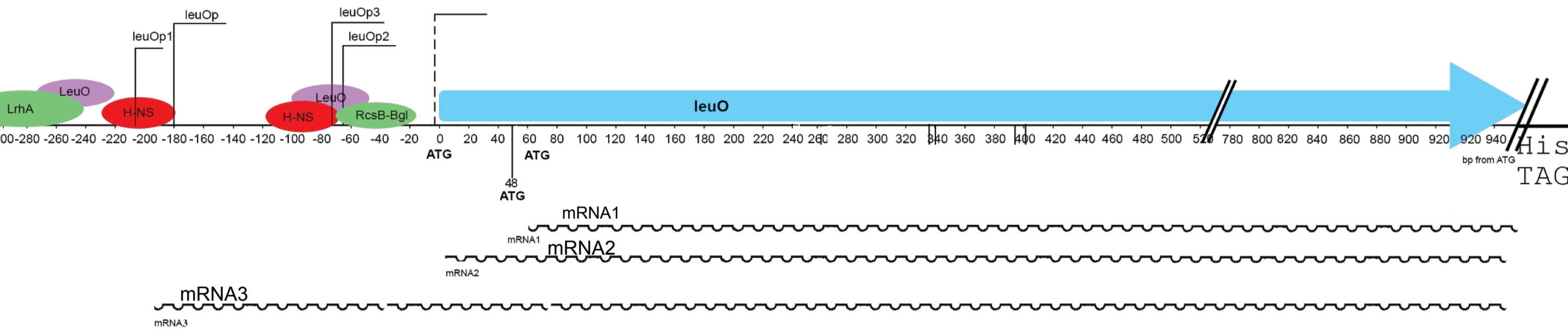Masha Tutukina

Where are the alternative LeuO ORFs?

LeuO – один из регуляторов бактериальной транскрипции, принадлежащий к суперсемейству LysR. У *Salmonella* LeuO является одним из факторов вирулентности. Показано, что при синтезе с плазмиды данный белок существует в двух изоформах, считывающихся с одного гена.

В прошлом году в нашей лаборатории изучали белок YjjM, который также является регулятором бактериальной транскрипции и имеет несколько изоформ, одна из которых регулирует образование биопленок, и, следовательно, является одним из факторов патогенности. В этом году целью нашего исследования было выявление и анализ совпадающих генов-мишеней у LeuO и YjjM, а также анализ возможности существования нескольких изоформ LeuO с хромосомной ДНК *Escherichia coli* K12 MG1655.
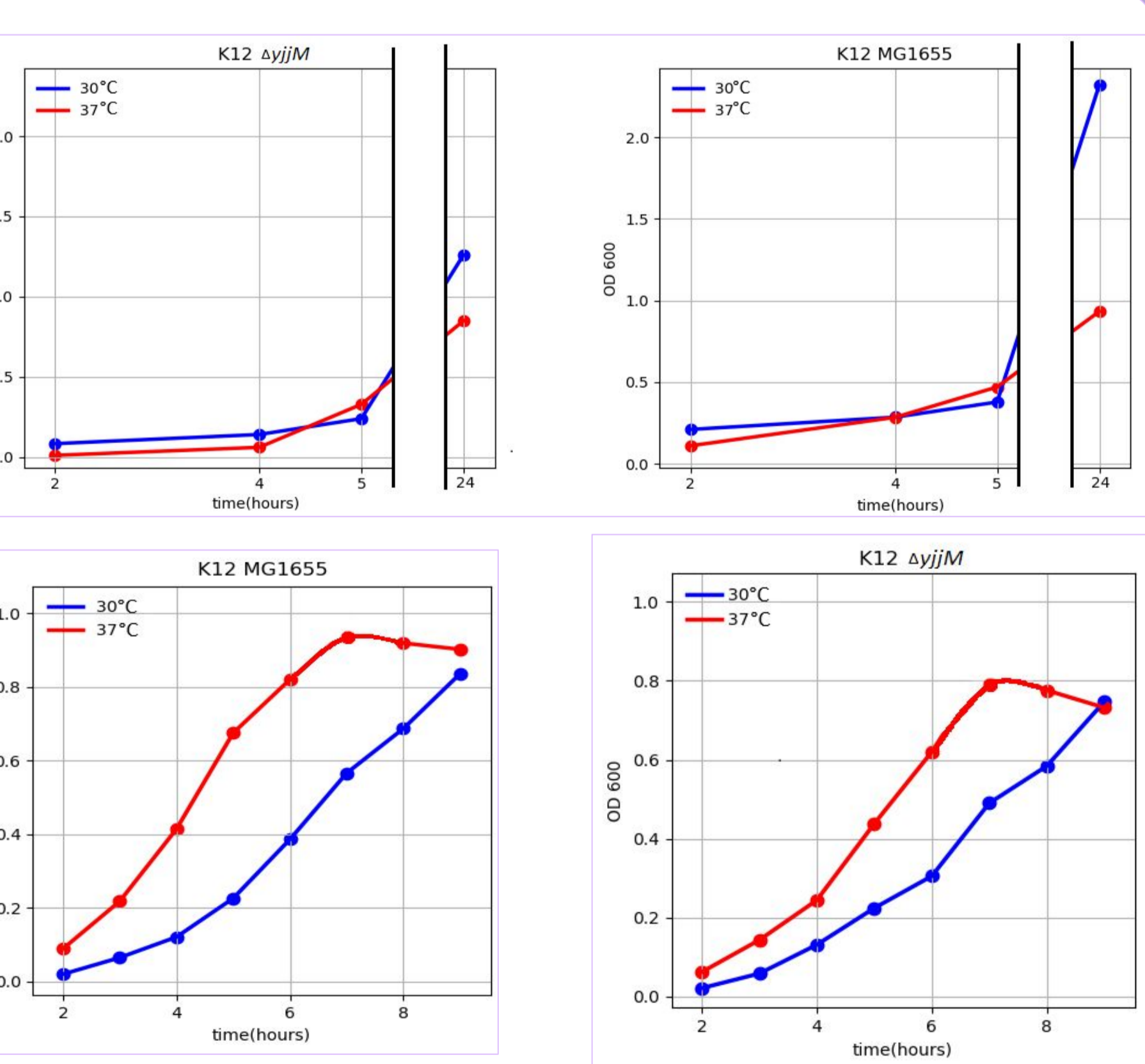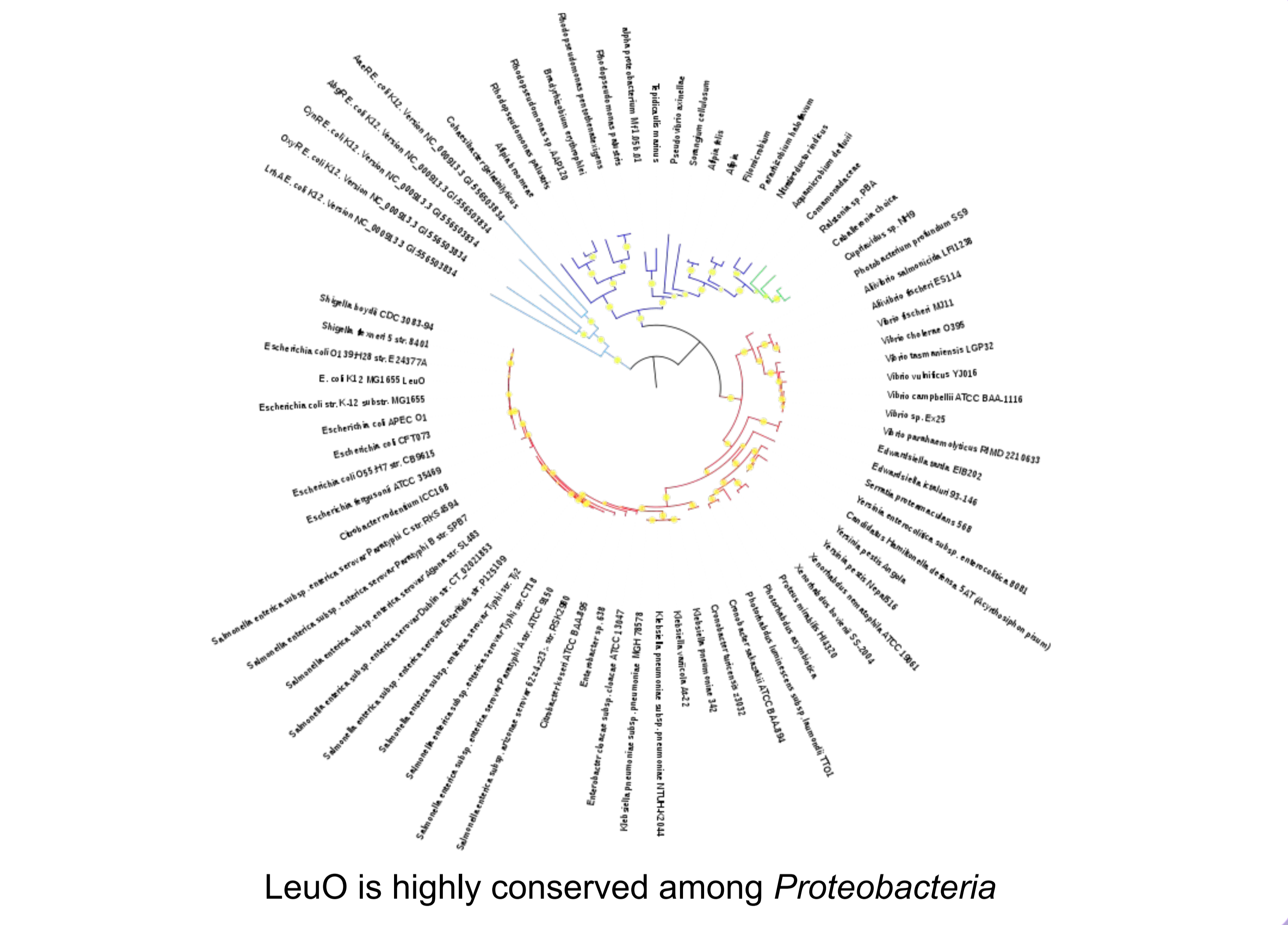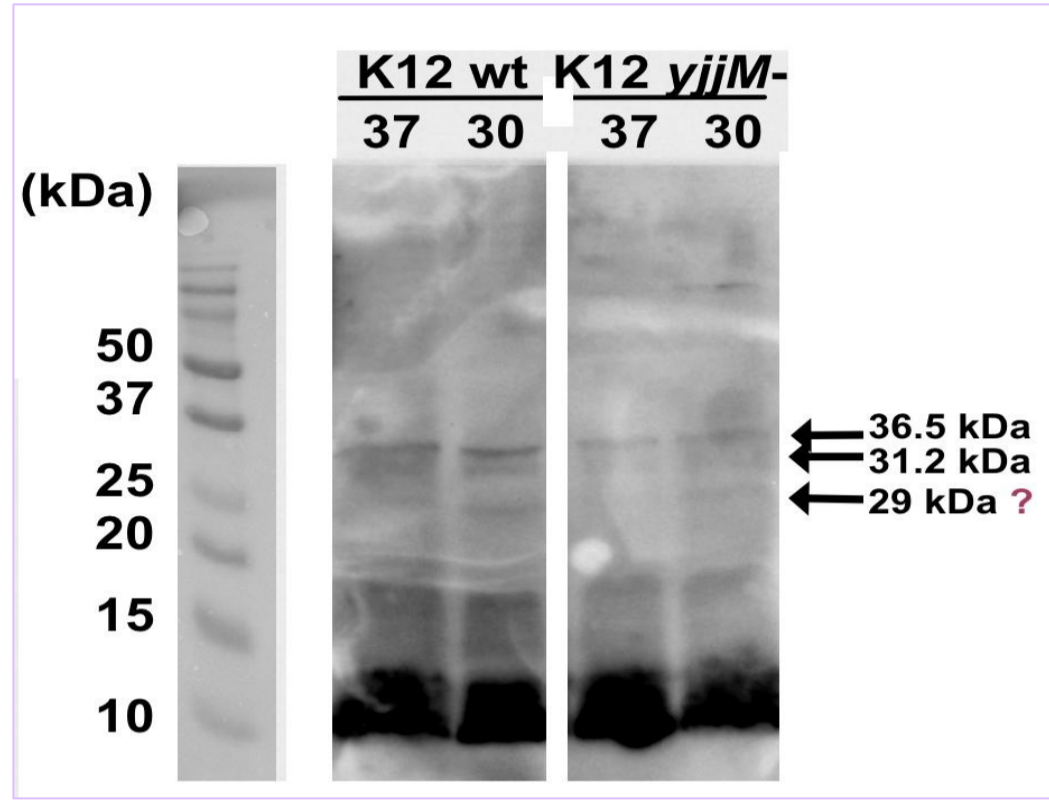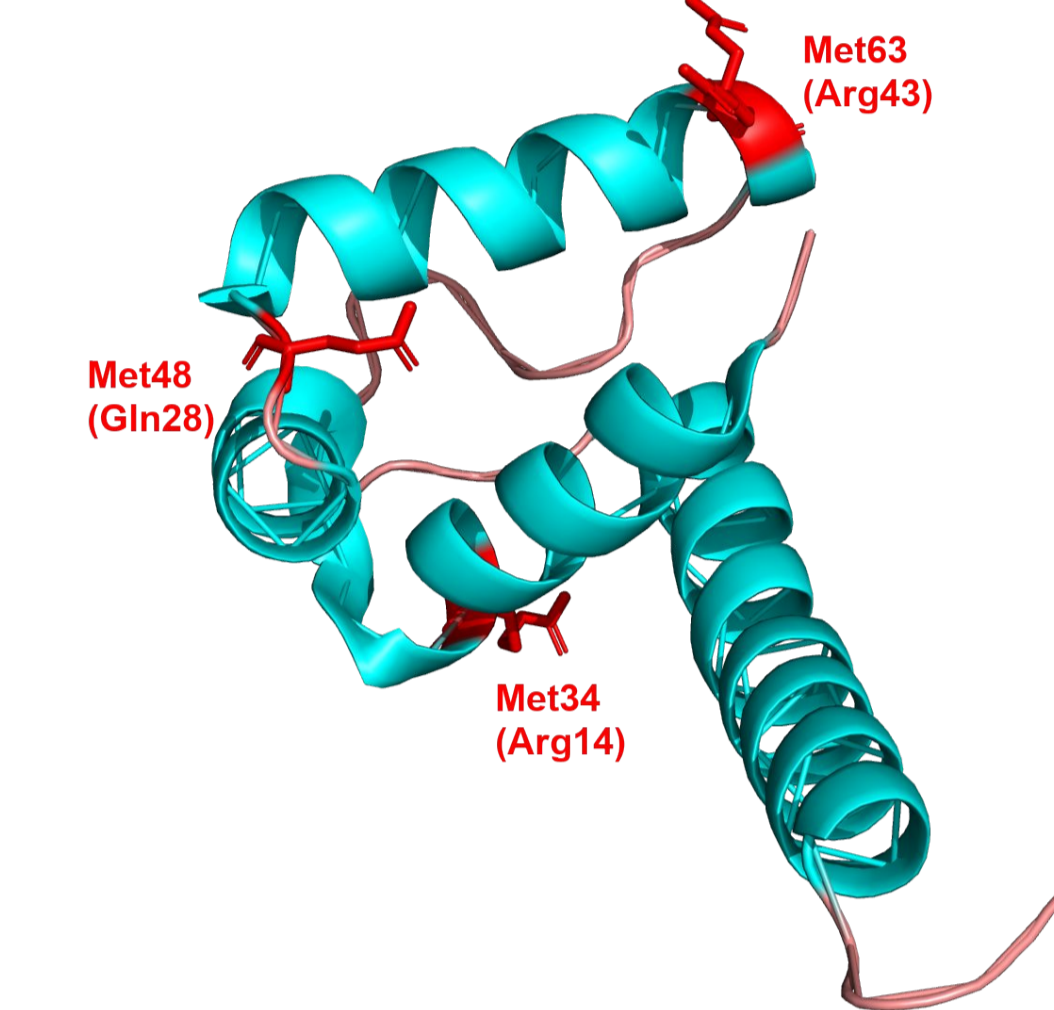
LeuO is one of the regulators of bacterial transcription, which belongs to the LysR superfamily. In *Salmonella*, LeuO is one of the factors controlling virulence. Earlier it was found that on the plasmid this protein has several isoforms translated from one gene.

Last year, in out lab we studied another transcriptional regulator, YjjM, that exists in several isoforms, one of which controls biofilm formation. Hence, it is one of the factors of pathogenicity. This year, our purpose was to identify and to analyze the coincident target genes for LeuO and YjjM, and to check if multiple LeuO isoforms can be synthesized from its gene on the chromosomal DNA of *Escherichia coli* K12 MG1655.

RNA isolation

1) Cell destruction
2) Elimination of proteins
3) Nucleic acid precipitation
4) DNAse I treatment

Control electrophoresis

Quality check of RNA integrity. There is a result

RNA electrophoresis

chromosome DNA
plasmid DNA
23S rRNA
16S rRNA

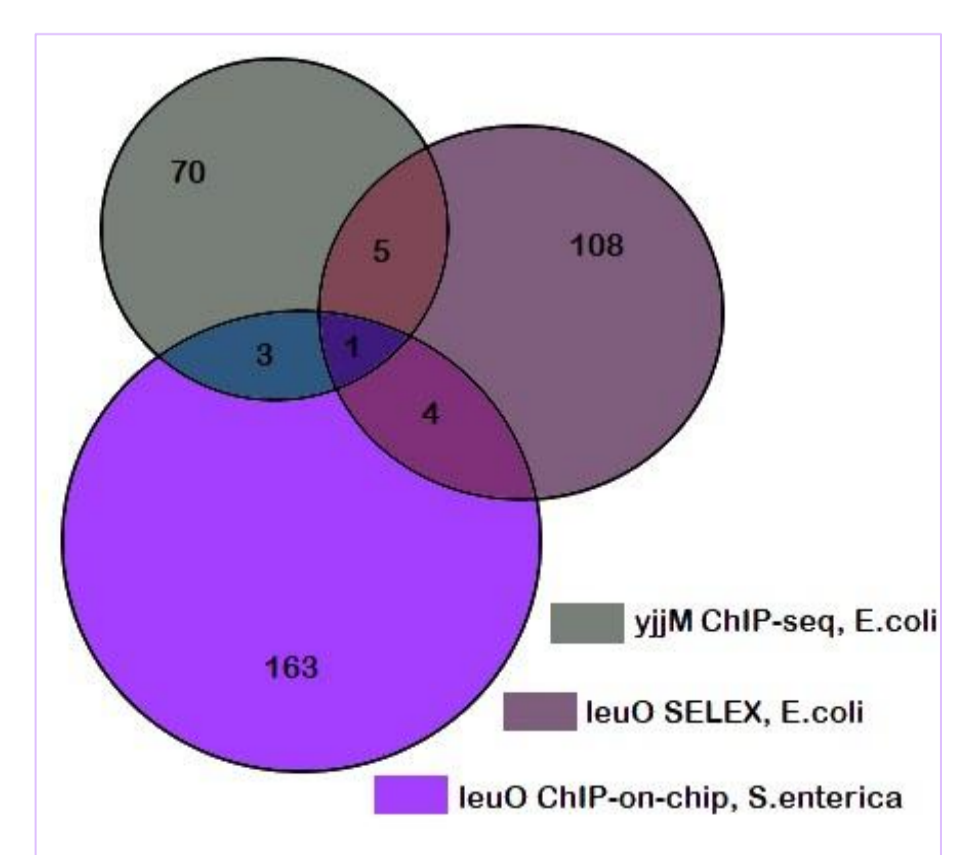K12 wt   K12 ΔyjjM
K12 +LeuO

Several proteins can be synthesized from the *leuO* gene on the *E. coli* chromosome, differing in their DNA-binding domain. YjjM might act as their activator.

MetR DNA-binding domain

Met63 (Arg43)
Met48 (Gln28)
Met34 (Arg14)

K12 wt   K12 *yjjM*-
37 30   37 30

(kDa)
50
37
25
20
15
10

36.5 kDa
31.2 kDa
29 kDa ?

Reverse transcription

1) Primer selection for target genes
2) Sample preparation with different RNAs
3) Obtaining cDNA

Gene	Description
ybeQ	Predicted to play a role in the oxidative stress response
sdiA	Controls transcription of the genes involved in cell division
fes	Enterochelin esterase; catalyzes hydrolysis of enterobactin and ferric enterobactin
fepA	Outer membrane protein that binds and transports ferric enterobactin, colicins B and D
tsr	The cytoplasmic domain of a monomeric methyl-accepting chemotaxis serine-sensing Tsr receptor
acrE	Membrane fuse pump protein; part of a drug efflux system
yjjQ	DNA-binding transcriptional repressor of genes required for flagellar synthesis, capsule formation and other genes related to virulence

qRT-PCR

LeuO is highly conserved among *Proteobacteria*

37, anaerobic growth

expression relative to K12 at 30, aerobic
yjjQ fepA fes sdiA tsr ybeQ acrE

■ K12 MG 1655   ■ leuO (overexpression)   ■ - yjjM

During **anaerobic growth at 37**, LeuO activates expression of *yjjQ* and *acrE*, and represses *tsr* and (moderately) *sdiA*.
YjjM, in contrast, activates *sdiA* and *tsr*, repressing *acrE*.

Under **aerobic conditions and low temperature**, LeuO also represses *sdiA* and activates *acrE*, but no effect was registered for *tsr*. However, it is strongly repressed by YjjM confirming a key role of YjjM in control of chemotaxis.

Thus, LeuO and YjjM share targets responsible for bacterial virulence and act either in the same or opposite directions.

30, aerobic growth

expression relative to K12 at 30, aerobic
fes sdiA tsr ybeQ acrE

■ K12 MG1655   ■ leuO (overexpression)   ■ - yjjM

Electrophoresis of PCR products

DNA electrophoresis

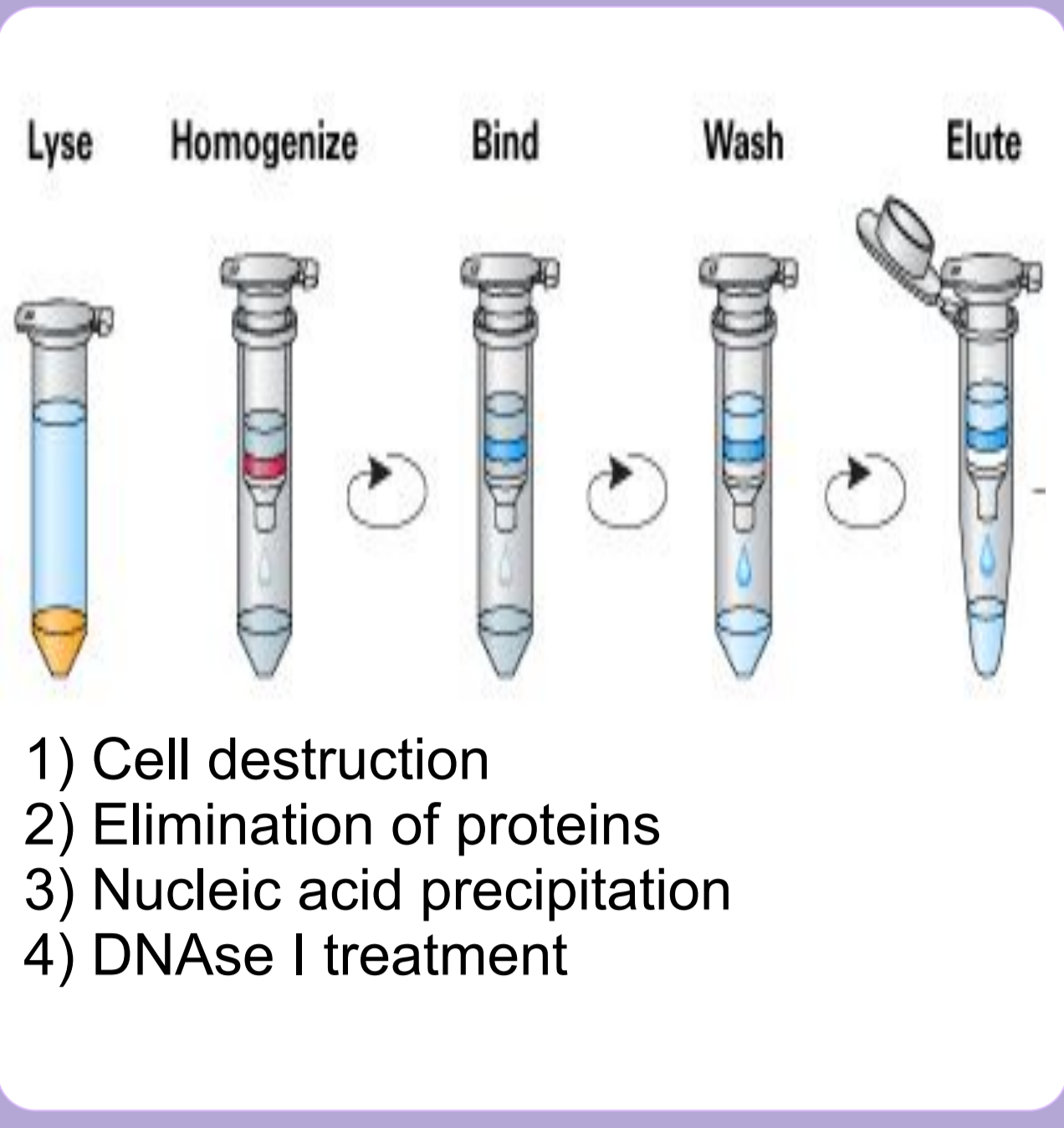*rpoB* gene

yjjQ gene
fepA gene

+ −
reverse transcriptase

To find genes with regulatory regions bound by LeuO, we analysed two datasets obtained by different techniques: *in vitro* SELEX for *Escherichia coli* and *in vivo* ChIP-on-chip for *Salmonella*. To check if LeuO has any shared targets with YjjM, we intersected these datasets with ChIP-seq data for YjjM. This picture shows how many target genes are common for considered datasets. As you can see, YjjM and LeuO have 6 common targets, and one target (the *sdiA* gene) is shared among all three datasets.
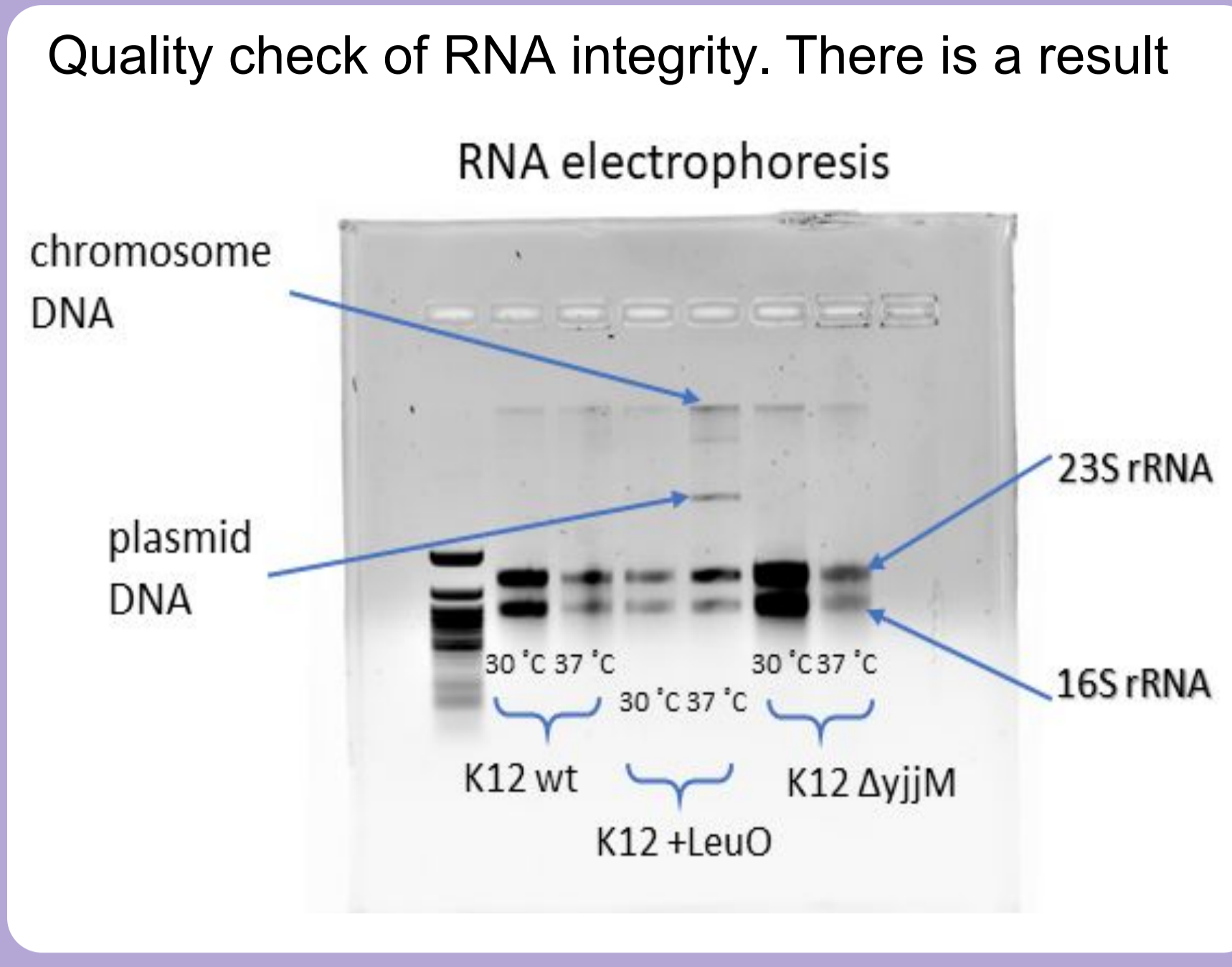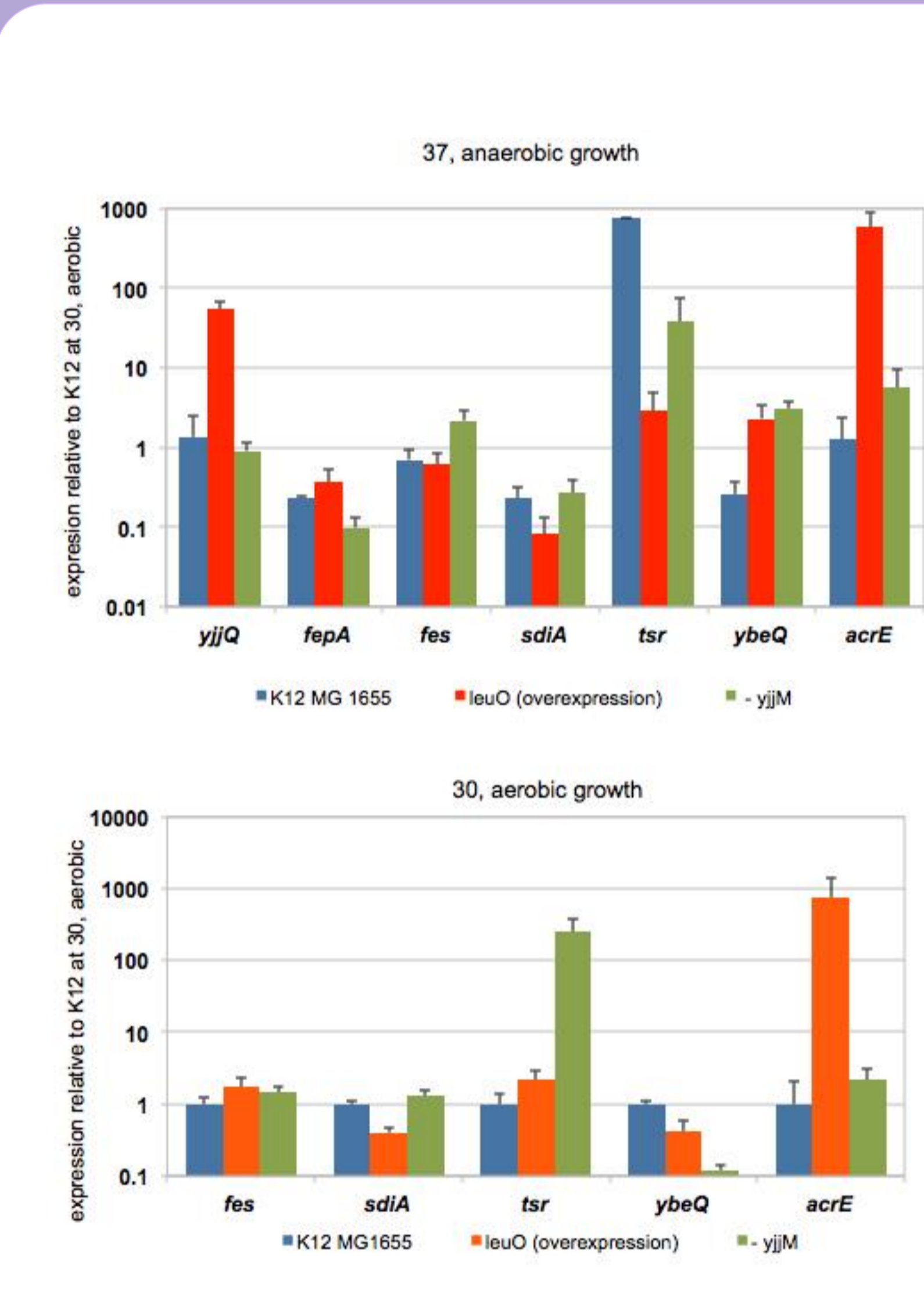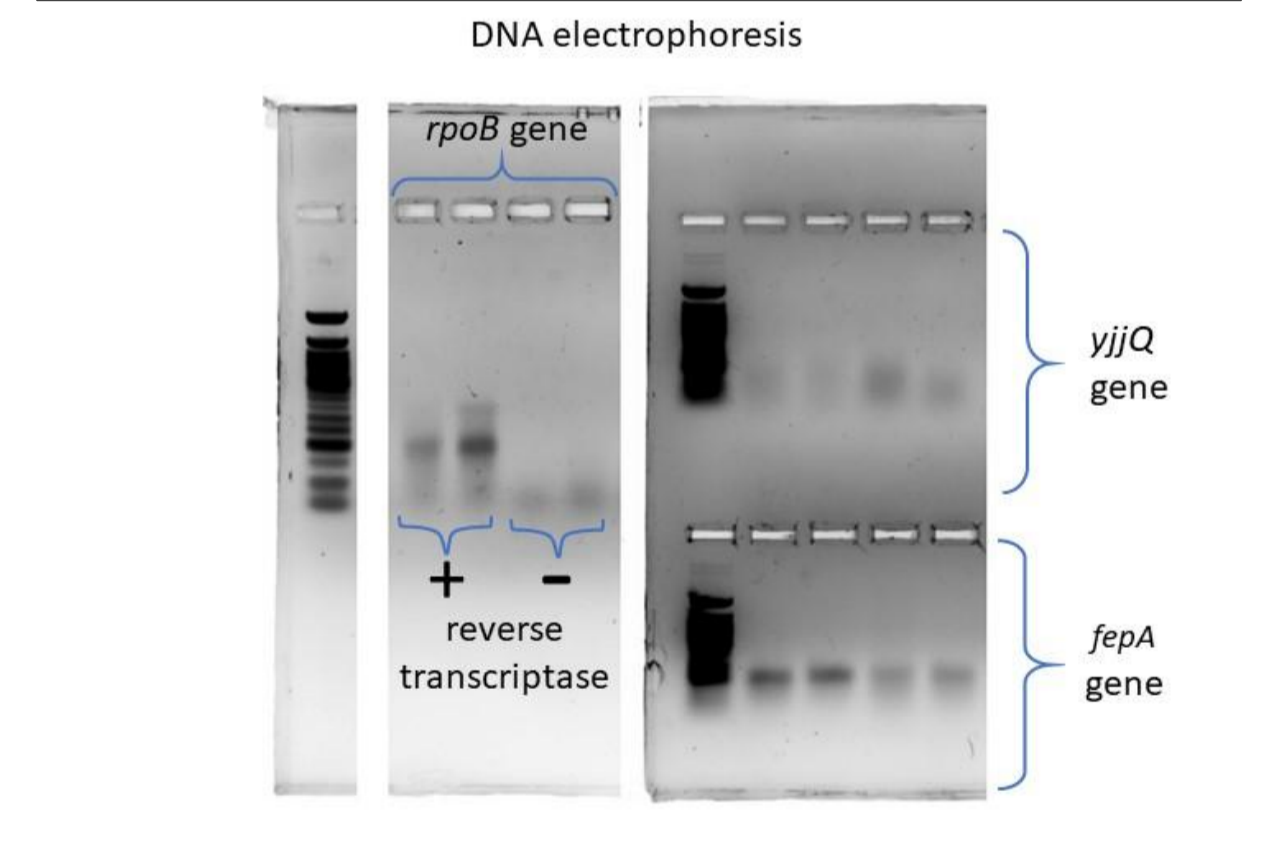

K12 ΔyjjM   K12 MG1655
30°C 37°C   30°C 37°C

K12 MG1655   K12 ΔyjjM
30°C 37°C   30°C 37°C

➔ Strain with deleted *yjjM* is not able to grow as fast as wild type.

➔ Both strains grow poorly in anaerobic conditions.

70
5
108
3
4
163

■ yjjM ChIP-seq, E.coli
■ leuO SELEX, E.coli
■ leuO ChIP-on-chip, S.enterica

Multiple alignment of LeuO-like proteins

helix-turn-helix motif

bacteria which have *LeuO*-like genes

— alfa-spirals   → beta-strands   aminoacids, contacting with the ligands

LysR family transcriptional regulator

48 M

Escherihia coli

out of Gammaproteobacteria

10 20 30 40 50 60 70 80 90 100   200   300