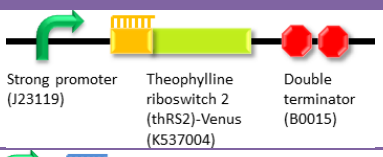
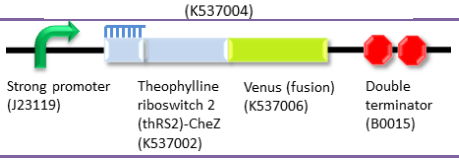
Theophylline riboswitch 2 datasheet

Part Number	Part Name	Construct	Length
BBa_K537010	Promoter-Theophylline riboswitch 2-Venus-Double terminator		961 bp
BBa_K537012	Promoter-Theophylline riboswitch 2 - CheZ-Venus-Double terminator		1587 bp

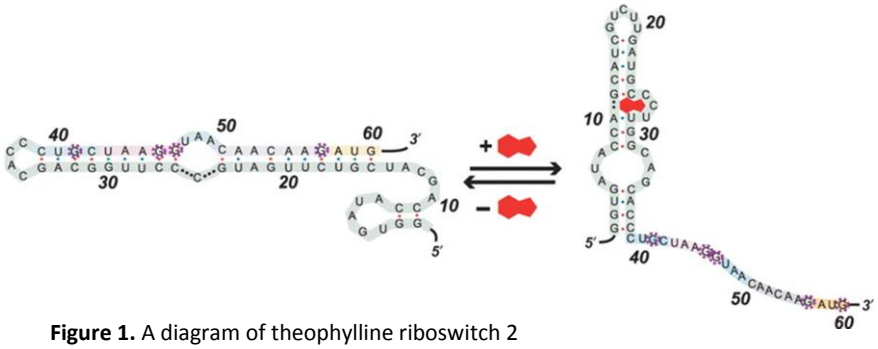
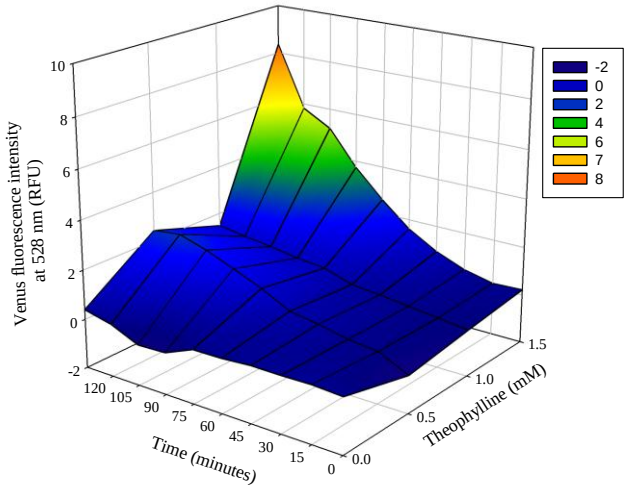


Figure 1. A diagram of theophylline riboswitch 2

In the absence of theophylline, the RBS is sequestered and downstream genes cannot be translated. Should theophylline interact with the riboswitch, a conformational change is ensued and the RBS is exposed resulting in the translation of downstream genes (Lynch and Gallivan, 2009)

Fluorometry Assay

A



B

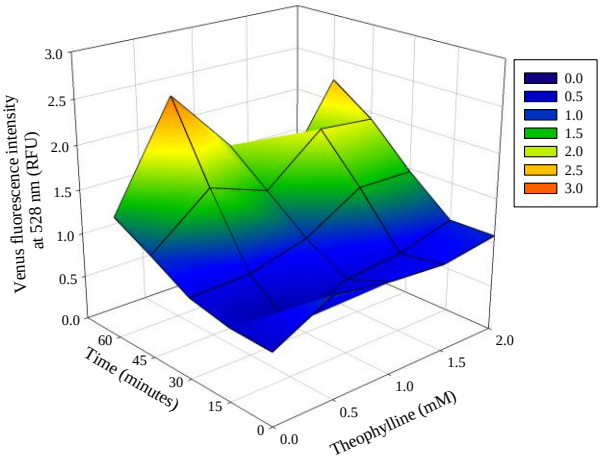


Figure 2: The fluorescence produced by Promoter-theophylline riboswitch 2-CheZ-Venus-double terminator (BBa_537012) **(A)** and Promoter-theophylline riboswitch 2-Venus-double terminator (BBa_537010) **(B)** at different theophylline concentrations over time. Bacterial cell cultures were transformed with the respective constructs and grown to mid-log phase from a seeding culture. The cultures were excited at 514 nm and emission intensity was detected at 528 nm using a Jasco FP-6300 spectrofluorometer. Activation of the riboswitch can be seen in the presence of theophylline. The presence of the CheZ gene confers some structural stability that enhances the activation when compared to the construct with venus alone. The activation seen may be sufficient to restore motility in CheZ deficient *E. coli* cells.

Fluorescence Microscopy

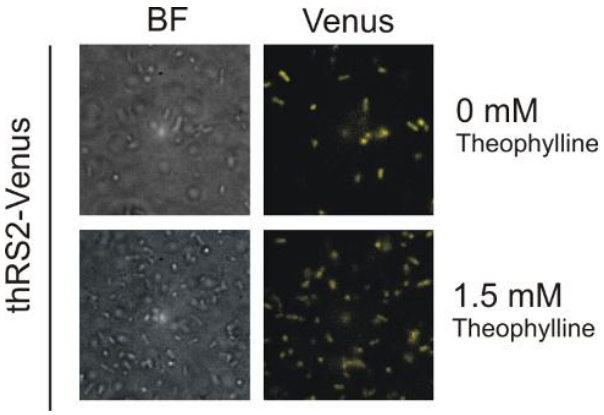
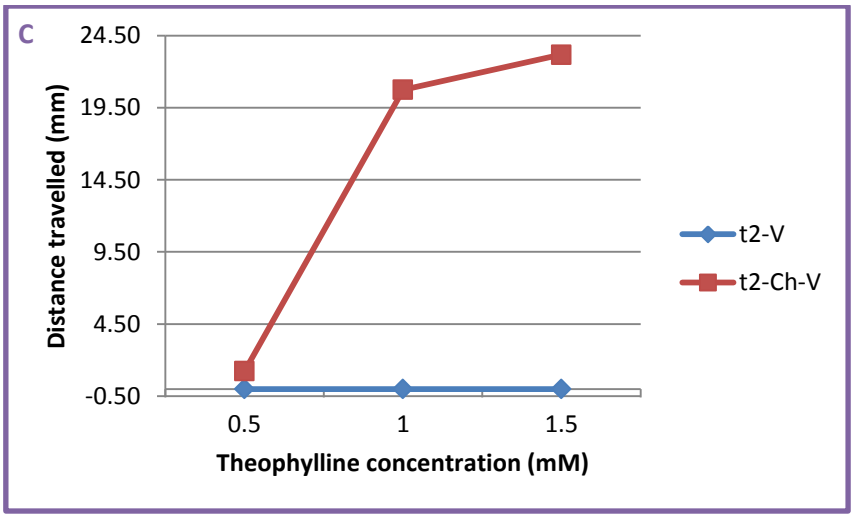


Figure 3: E.coli CheZ mutants which were transformed with the Promoter-ThRS2-venus-DoubleTerminator construct in pSB1A3 plasmid backbone. (BBa_K537010). The brightfield images in the left column depict all bacterial cells. The venus images in the right column depict bacterial cells which emitted fluorescence. In the absence of theophylline, some fluorescence was observed. This result showed the leakiness of the riboswitch. A substantial amount of venus translation is permitted because of the flexible conformation of this riboswitch. Upon the addition of theophylline at a concentration of 1.5mM, much more fluorescence was detected. More venus was expressed in these cells due to the activation of the riboswitch via theophylline.

Motility Assay



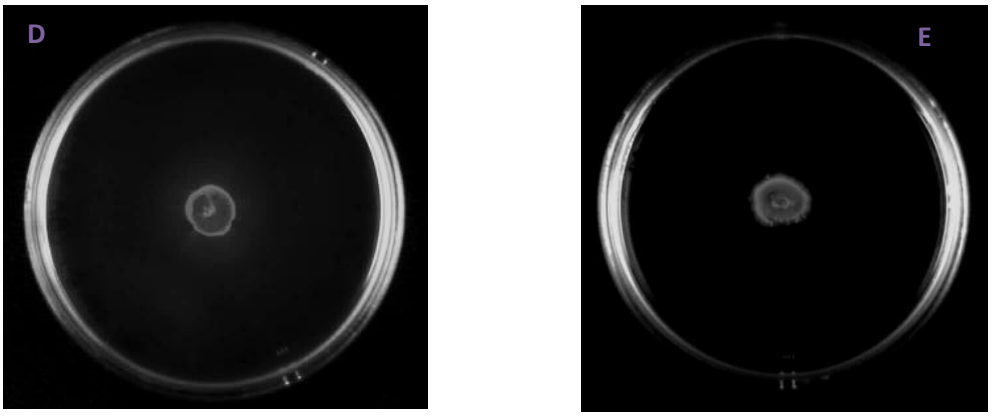


Figure 4. The distance travelled by *E. coli* containing either Promoter-theophylline riboswitch 2-CheZ-Venus-double terminator (BBa_537012) or Promoter-theophylline riboswitch 2-Venus-double terminator (BBa_537010) **(C)** at different theophylline concentrations over time. The observed trend in **C** can be seen on semi-solid agar plates containing 1.0mM theophylline with cells transformed with Promoter-theophylline riboswitch 2-CheZ-Venus-double terminator **(D)** and Promoter-theophylline riboswitch 2-Venus-double terminator **(E)**, where the halo surrounding the point of inoculum in **D** is

indicative of regained motility. The distance travelled was 24mm in 24 hours by *E. coli* containing the CheZ gene under the control of a theophylline riboswitch. The control (E) indicates that in the absence of CheZ, motility is not restored.

Chemotaxis Assay

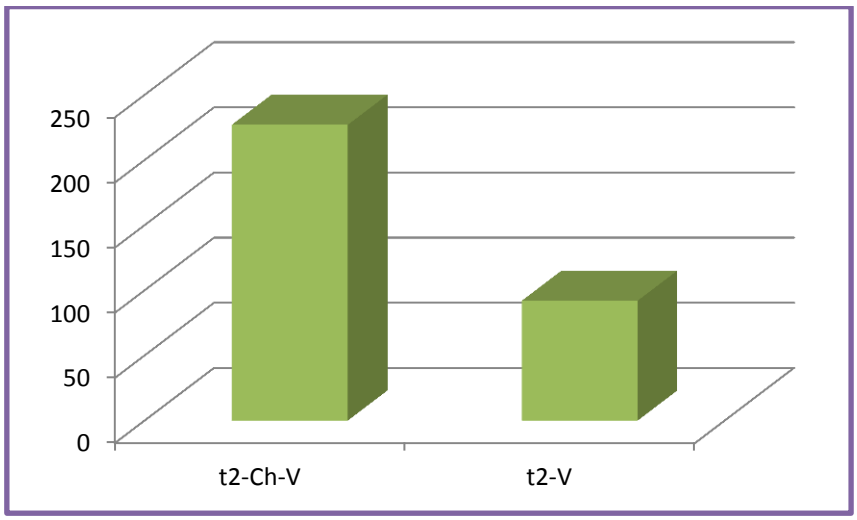


Figure 5. A graph to show the chemotaxis index of *E.* coli transformed with Promoter-theophylline riboswitch 2-CheZ-Venus-double terminator (BBa_537012) **(t2-Ch-V)** and Promoter-theophylline riboswitch 2-Venus-double terminator (BBa_537010) **(t2-V)**. The chemotaxis index of 100 presented by the Promoter-theophylline riboswitch 2-Venus-double terminator indicates that the same number of bacteria travelled towards 0mM theophylline and 2.0mM theophylline, and as such this transformant shows no attraction towards theophylline. Conversely, the chemotaxis index of 228 displayed by Promoter-theophylline riboswitch 2-CheZ-Venus-double terminator shows more bacteria travelled to 2.0mM theophylline compared with 0mM theophylline strongly indicating chemoattraction towards this chemical.

References

LYNCH, S. A. & GALLIVAN, J. P. 2009. A flow cytometry-based screen for synthetic riboswitches. Nucleic Acids Res, 37, 184-92.