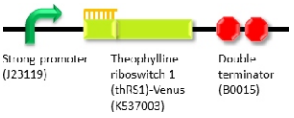
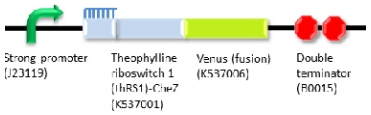
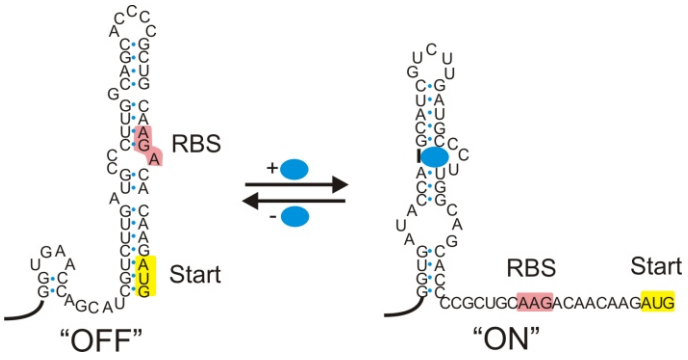
# DATASHEET: THEOPHYLLINE RIBOSWITCH 1

| Part Number | Part Name | Construct | Length |
|---|---|---|---|
| BBa_K537003 | Theophylline riboswitch 1-Venus |  | 780 bp |
| BBa_K537009 | Promoter-Theophylline riboswitch 1-Venus-Double terminator |  | 960 bp |
| BBa_K537001 | Theophylline riboswitch 1-CheZ N-fusion |  | 698 bp |
| BBa_K537011 | Promoter-Theophylline riboswitch 1-CheZ Venus-Double terminator |  | 1583 bp |

**Theophylline riboswitch 1: Summary**

Using a high-throughput screen(Lynch et al., 2007) and a high-throughput selection (Topp and Gallivan, 2008) in *E.coli*, the Gallivan lab has been able to develop a powerful theophylline-dependent riboswitch. It is worth noting that the motility-based screen (Topp and Gallivan, 2008) made use of a theophylline riboswitch library activating the expression of CheZ, the motility factor in *E.coli* which allows for running (as opposed to tumbling) movement. Therefore, activation of CheZ allows bacteria to move along the theophylline gradient. The best riboswitch identified in these earlier screens was the theophylline riboswitch clone 8.1 (Figure 1) which displays 36-fold expression activation ratio.

**8.1 Theophylline RS (Topp&Gallivan JACS 2007)**




## Fluorometry

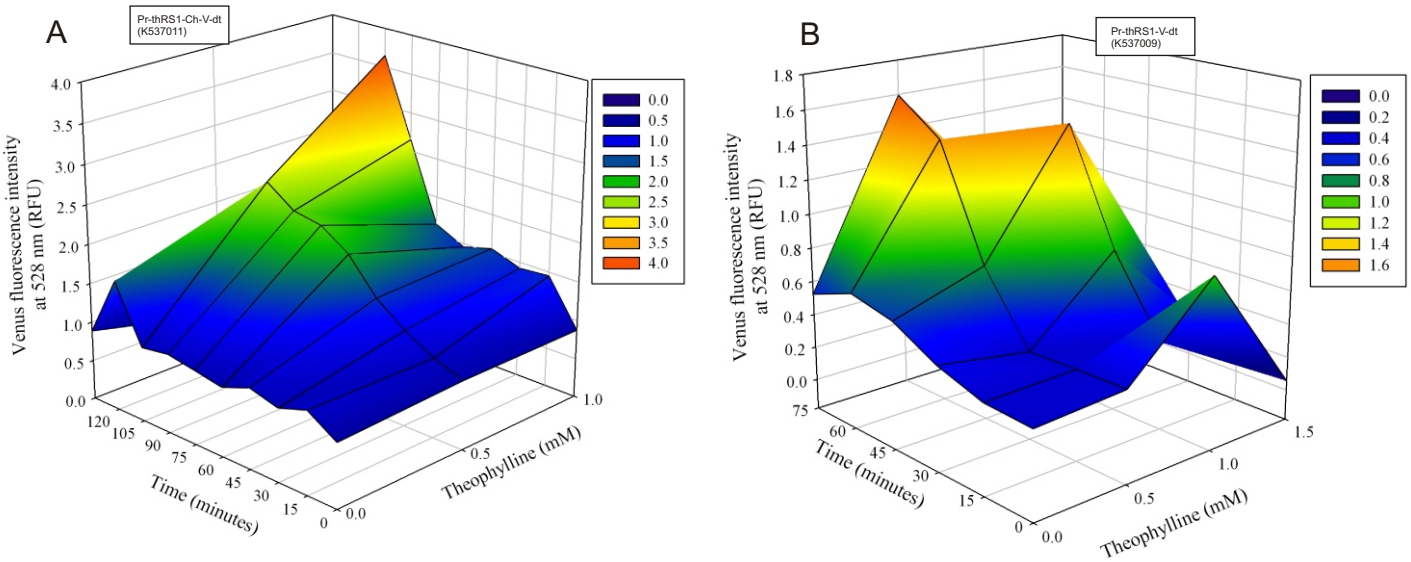



Figure 2: The fluorescence produced by Promoter-theophylline riboswitch 1-CheZ-Venus-double terminator (BBa_537011) (A) and Promoter-theophylline riboswitch 1-Venus-double terminator (BBa_537009) (B) at different theophylline concentrations over time. Bacterial cell cultures were transformed with the respective constructs and grown to the mid-log phase of growth 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 may confer 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

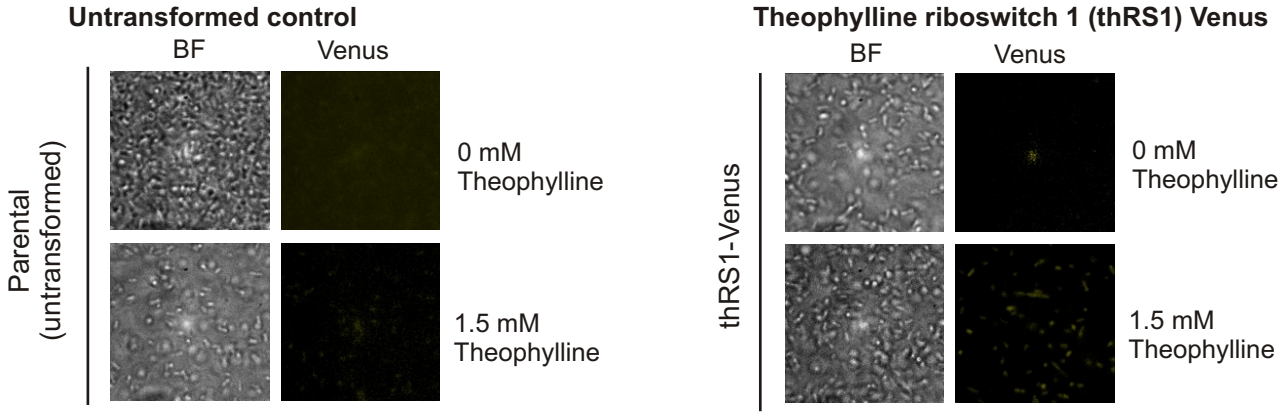



Figure 3: *E.coli* CheZ mutants which were transformed with the Promoter-ThRS1-venus-DoubleTerminator construct (BBa_537009). The bright field (BF) images in the left column depict all bacterial cells in the field. The Venus fluorescence images in the right column depict bacterial cells which emitted fluorescence. In the absence of theophylline, almost no fluorescence occurred (as with parental controls - left panels). Upon the addition of theophylline at a concentration of 1.5mM, many of the cells emitted fluorescence showing activation of the theophylline riboswitch 1.

## Motility Assay

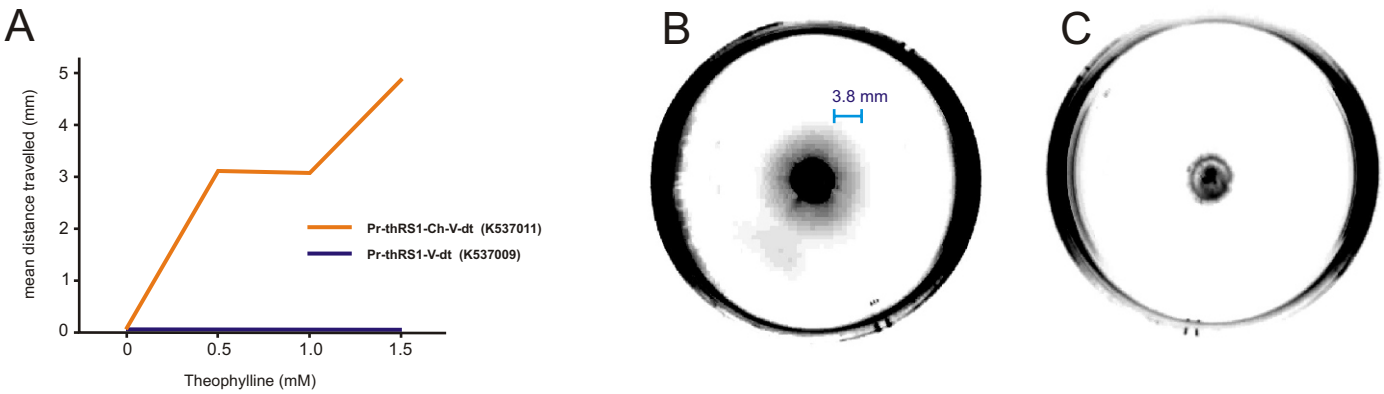



Figure 4. The distance travelled by E. coli containing either Promoter-theophylline riboswitch 1-CheZ-Venus-double terminator (BBa_537011) or Promoter-theophylline riboswitch 1-Venus-double terminator (BBa_537009) (C) at different theophylline concentrations over time. The observed trend in A can be seen on semi-solid agar plates containing 1.0mM theophylline with cells transformed with Promoter-theophylline riboswitch 1-CheZ-Venus-double terminator (B) and Promoter-theophylline riboswitch 1-Venus-double terminator (C), where the halo surrounding the point of inoculum in B is indicative of regained motility.

## Chemotaxis

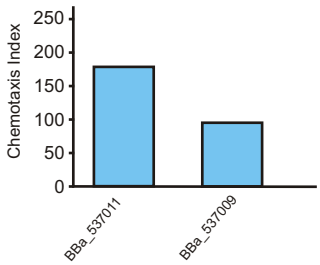



Figure 5. A graph to show the chemotaxis index of ΔCheZ *E. coli* transformed with Promoter-theophylline riboswitch 1-CheZ-Venus-double terminator (BBa_537011) and Promoter-theophylline riboswitch 1-Venus-double terminator (BBa_537009). The *Chemotaxis Index* of 100 for BBa_537009 indicates bacteria travelled equally towards 0mM theophylline as 2.0mM theophylline, suggesting no attraction towards theophylline. Conversely, the chemotaxis index of 180 for BBa_537011 increased movement towards 2.0mM theophylline compared with 0mM theophylline, strongly indicating riboswitch-controlled chemo-attraction.

## References


Lynch S.A. Desai S.K.,Sajja,H.K., and Gallivan J.P.A high-trhoughput screen for synthetic riboswitches reveals mechanistic insight into their function. 2007, ChemBiol 14:173-18

Topp S. and Gallivan J.P. Random walks to synthetic riboswitches – a high throughput selection based on cell motility. 2008, ChemBiochem 9:210-213



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