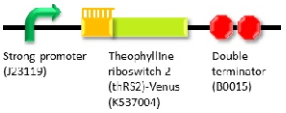
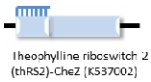
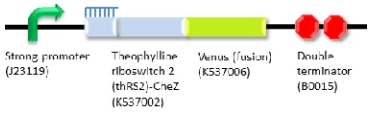
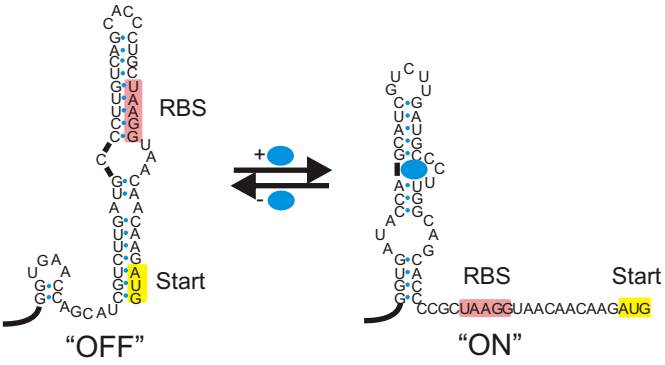
# DATASHEET: THEOPHYLLINE RIBOSWITCH 2

| Part Number | Part Name | Construct | Length |
|---|---|---|---|
| BBa_K537004 | Theophylline riboswitch 2-Venus |  | 781 bp |
| BBa_K537010 | Promoter-Theophylline riboswitch 2-Venus-Double terminator |  | 961 bp |
| BBa_K537002 | Theophylline riboswitch 2-CheZ N-fusion |  | 702 bp |
| BBa_K537012 | Promoter-Theophylline riboswitch 2-CheZ Venus-Double terminator |  | 1587 bp |

**Theophylline riboswitch 2: Summary**

The Gallivan lab developed an in vivo-based selection strategy aimed at selecting bacteria based on fluorescence activated cell sorting (FACS) (Lynch et al., 2009) (Fig.6). By randomizing the sequence encoding the RBS (+ Shine Delgarno/SD), clone 12.1 was identified, which displays a 96-fold expression activation in the presence of theophylline (1 mM) . This is a massive improvement over previous riboswitches. This riboswitch contains a strong (and longer) UAAGG SD which is found in a tight (inaccessible) duplex in the “OFF” (ie no theophylline) state. Moreover, the UAAGG sequence is spaced optimally, with 6 nt separating the 5’ A of the anti-SD sequence and the start codon of the translation (AUG) (Figure 1).

**12.1 Theophylline RS (Lynch&Gallivan NAR 2009)**




## Fluorometry

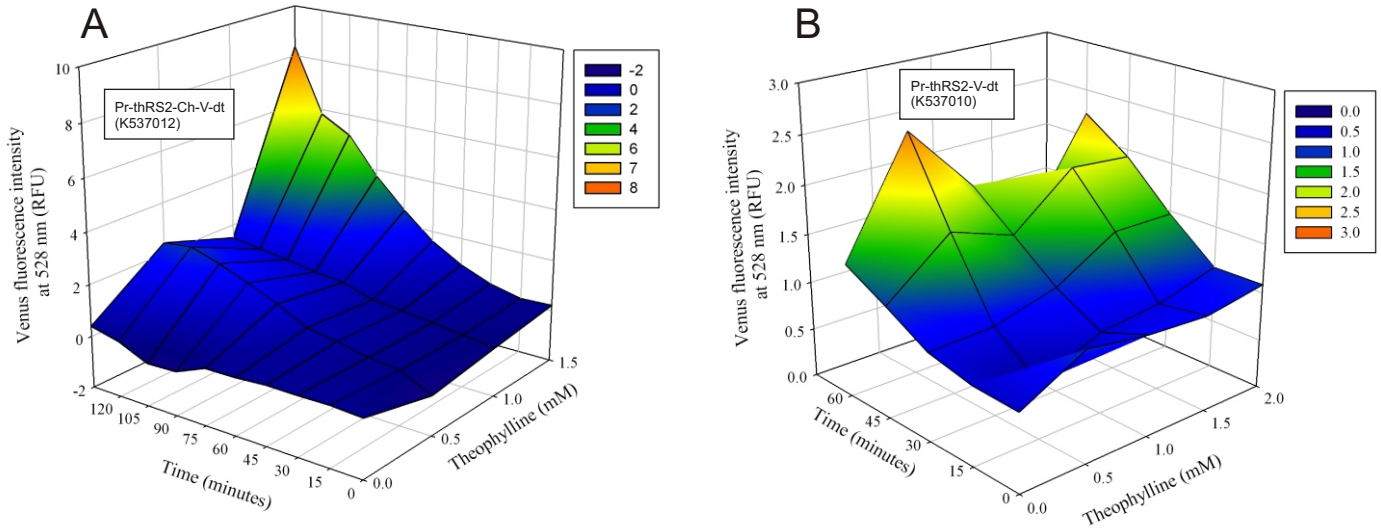



Figure 2: The fluorescence produced by Promoter-theophylline riboswitch 2-CheZ-Venus-double terminator (BBa_537012) (A) and Promoter-theophylline riboswitch 2-Venus-double terminator (BBa_537010) (B) at different theophylline concentrations over time. Bacterial cell cultures were transformed with the 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

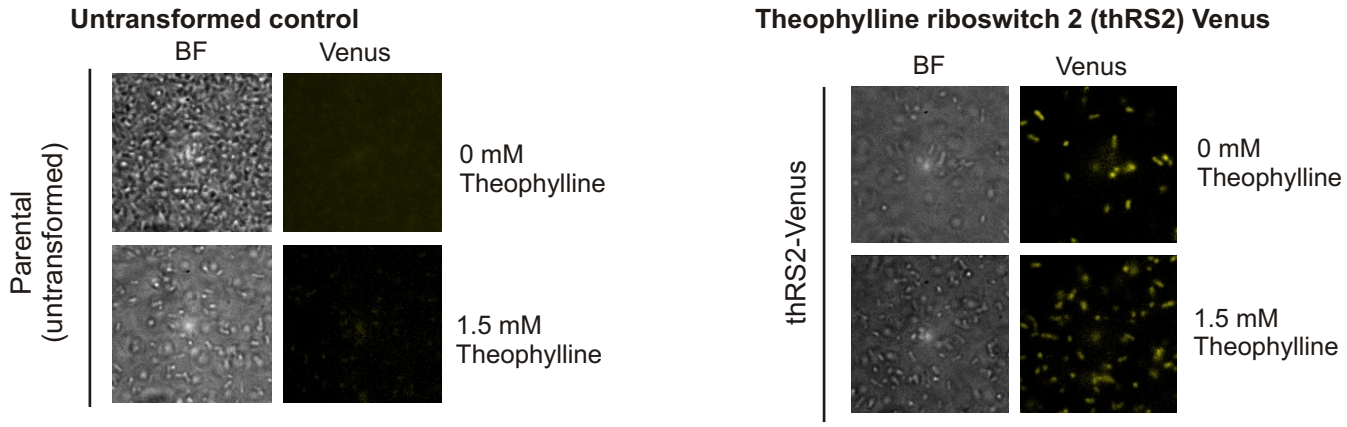



Figure 3: *E.coli* CheZ mutants which were transformed with the Promoter-ThRS2-venus-DoubleTerminator construct (BBa_537010). The bright field (BF) images in the left column depict all bacterial cells in the field. The Venus fluorescence images in the right column depict bacterial cells which emitted 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 2.

## Motility Assay

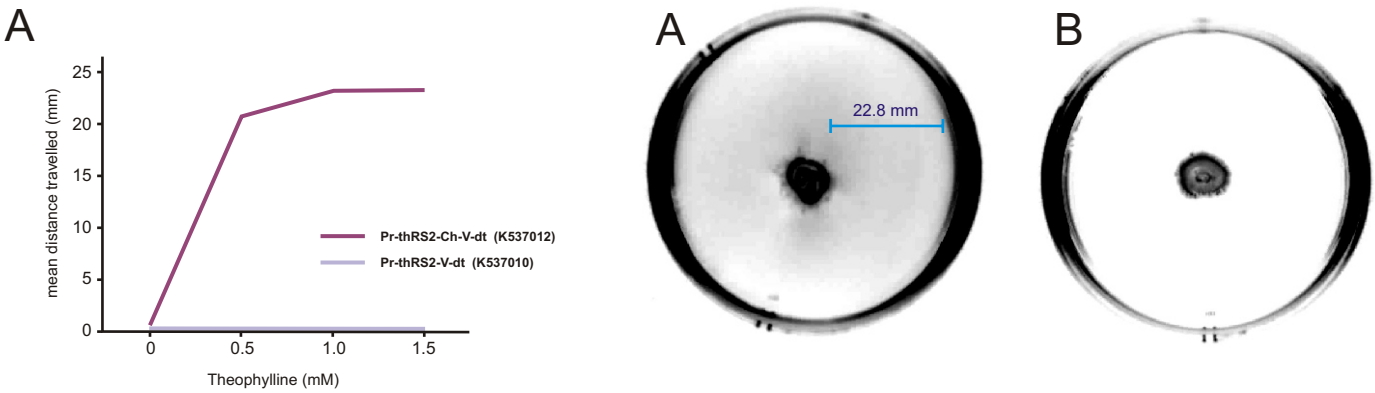



Figure 4. The distance travelled by *E. coli* containing either Promoter-theophylline riboswitch 2-CheZ-Venus-double terminator (BBa_537012) or Promoter-theophylline riboswitch 2-Venus-double terminator (BBa_537010) (A) at different theophylline concentrations over time. The observed trend in B can be seen on semi-solid agar plates containing 1.0mM theophylline with cells transformed with BBa_537012 (B) and BBa_537010 (C), where the halo surrounding the point of inoculum in B is indicative of regained motility. The distance travelled was 22.8 mm in 24 hours by *E. coli* containing the CheZ gene under the control of a theophylline riboswitch. In the absence of CheZ, motility is not restored (C).

## Chemotaxis

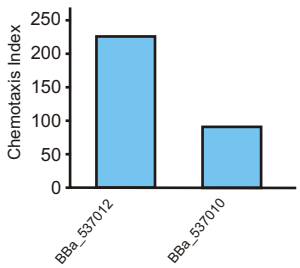



Figure 5. A graph to show the chemotaxis index of ΔCheZ *E. coli* transformed with Promoter-theophylline riboswitch 2-CheZ-Venus-double terminator (BBa_537012) and Promoter-theophylline riboswitch 2-Venus-double terminator (BBa_537010). The *Chemotaxis Index* of 100 for BBa_537010 indicates bacteria travelled equally towards 0mM theophylline as 2.0mM theophylline, suggesting no attraction towards theophylline. Conversely, the chemotaxis index of 220 for BBa_537012 increased movement towards 2.0mM theophylline compared with 0mM theophylline, strongly indicating riboswitch-controlled chemo-attraction.

## References

Lynch S.A. and Gallivan J.P., A flow cytometry based screen for synthetic riboswitches. 2009, Nucleic Acids Res. 37(1)184-192

**Authored by Wits_CSIR_SA iGEM 2011 Team**