



# Development and Characterization of Synthetic Riboswitches for Small Molecule Metabolic Biomarkers



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## Background

- Creatinine is a small metabolic byproduct generated from creatine phosphate breakdown in muscle tissue. It is widely used as clinic biomarker for renal function because it's produced at a constant rate proportional to muscle mass and primarily eliminated through glomerular filtration in the kidneys.
- TMAO is a metabolite produced through the oxidation of trimethylamine (TMA), which is generated when dietary compounds (such as red meat, eggs, and dairy) are metabolized into trimethylamine (TMA), then subsequently oxidized into TMAO in the liver. Unlike many metabolites, TMAO is not only a byproduct but also an active participant in physiological processes, influencing vascular function.

## Methods

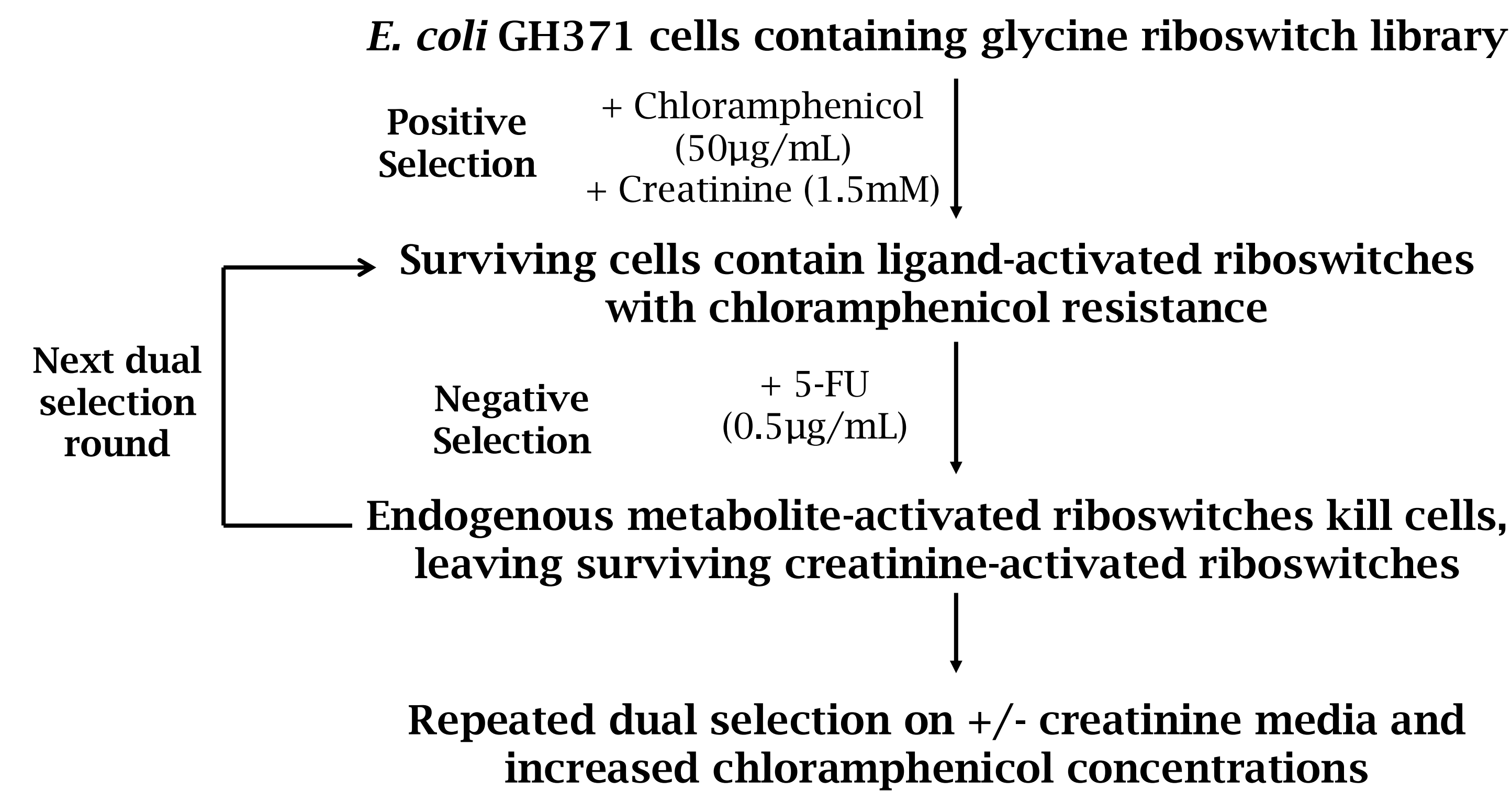
- A glycine riboswitch library was transformed into *E. coli* with a CAT-UPP reporter gene.
- The transformed plasmid sample was subjected to dual genetic selection.
- Cells grown in positive selection subjected to chloramphenicol allowed only ligand-activated riboswitches to survive.
- Cells grown in negative selection subjected to 5-FU killed activated riboswitches by endogenous ligands.

## Abstract

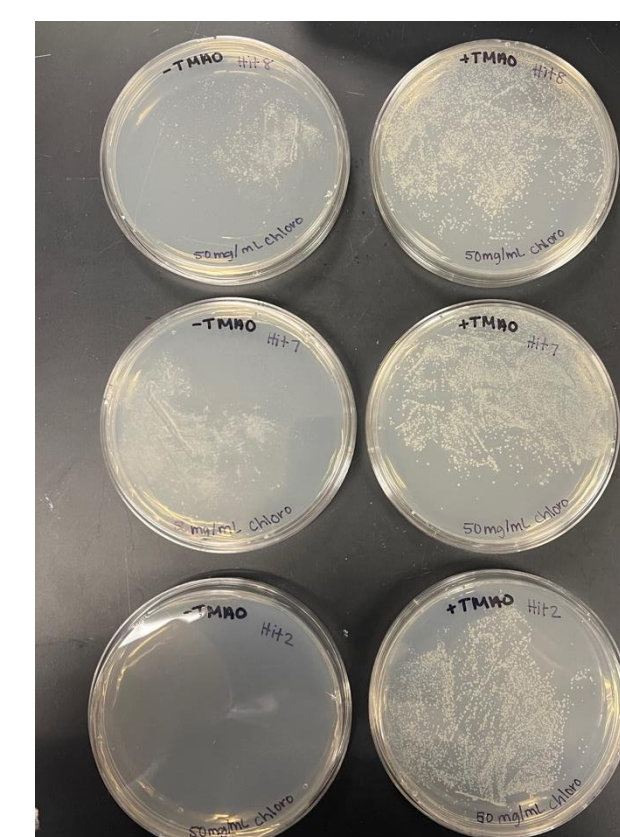
- This project aims to develop and characterize synthetic riboswitches for creatinine and trimethylamine N-oxide (TMAO), which are metabolic biomarkers for kidney and cardiovascular dysfunction.
- Riboswitches are structured RNA elements located in the 5'-untranslated regions (UTRs) of bacterial mRNAs that regulate downstream gene expression through ligand-induced conformational change with high affinity and selectivity.
- To select for the synthetic riboswitches specific to each metabolic biomarker, riboswitch libraries were subjected to dual genetic selection in the presence of chloramphenicol and 5-fluorouracil (5-FU).

## Results

- GH371 *E. coli* cells transformed with the pTrp-gcvT-CatUpp library plasmid was subjected to several rounds of the positive selection with chloramphenicol in the presence of creatinine and the negative selection with 5-fluorouracil in the absence of creatinine.



Surviving cells with activated riboswitches were tested on positive selection media with increased chloramphenicol concentration in the presence and absence of creatinine



TMAO riboswitch library samples were tested on LB media plates in the presence and absence of TMAO

## Conclusion

- After several repeated dual selection steps, including increased chloramphenicol and 5-FU concentrations, no glycine riboswitch variants were identified to show chloramphenicol resistance in the presence of creatinine.
- TMAO riboswitches are derived from the genetic selection of theophylline riboswitch library that showed chloramphenicol resistance only in the presence of TMAO.

## Future Directions

- Further testing on the TMAO riboswitch will be conducted by colorimetric or fluorescence assays using  $\beta$ -galactosidase and green fluorescence protein, respectively, in the presence of varying concentrations of TMAO

## References

- Gatarek, P.; Kaluzna-Czaplinska, J. Trimethylamine N-Oxide (TMAO) in Human Health. *EXCLI Journal* 2021, 20, 301-319. <https://doi.org/10.17179/excli2020-3239>.
- Guedez, A.; Sherman, M.; Ryu, Y. Dual Genetic Selection of the Theophylline Riboswitch with Altered Aptamer Specificity for Caffeine. *Biochemical and Biophysical Research Communications* 2021, 579, 105-109. <https://doi.org/10.1016/j.bbrc.2021.09.058>.
- Hosten, A. O. *BUN and Creatinine*. National Library of Medicine. <https://www.ncbi.nlm.nih.gov/books/NBK305/>.