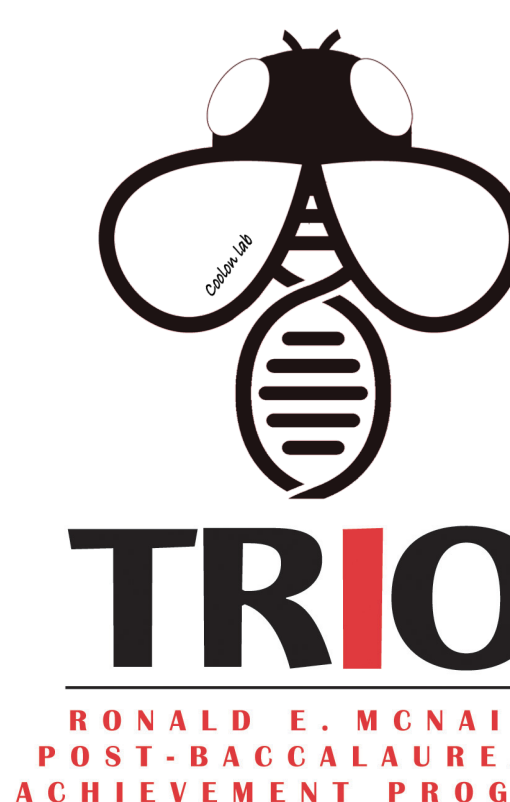


Genome Wide Effect of Doxycycline, Tetracycline, and 4-epidoxycycline in *Saccharomyces cerevisiae*

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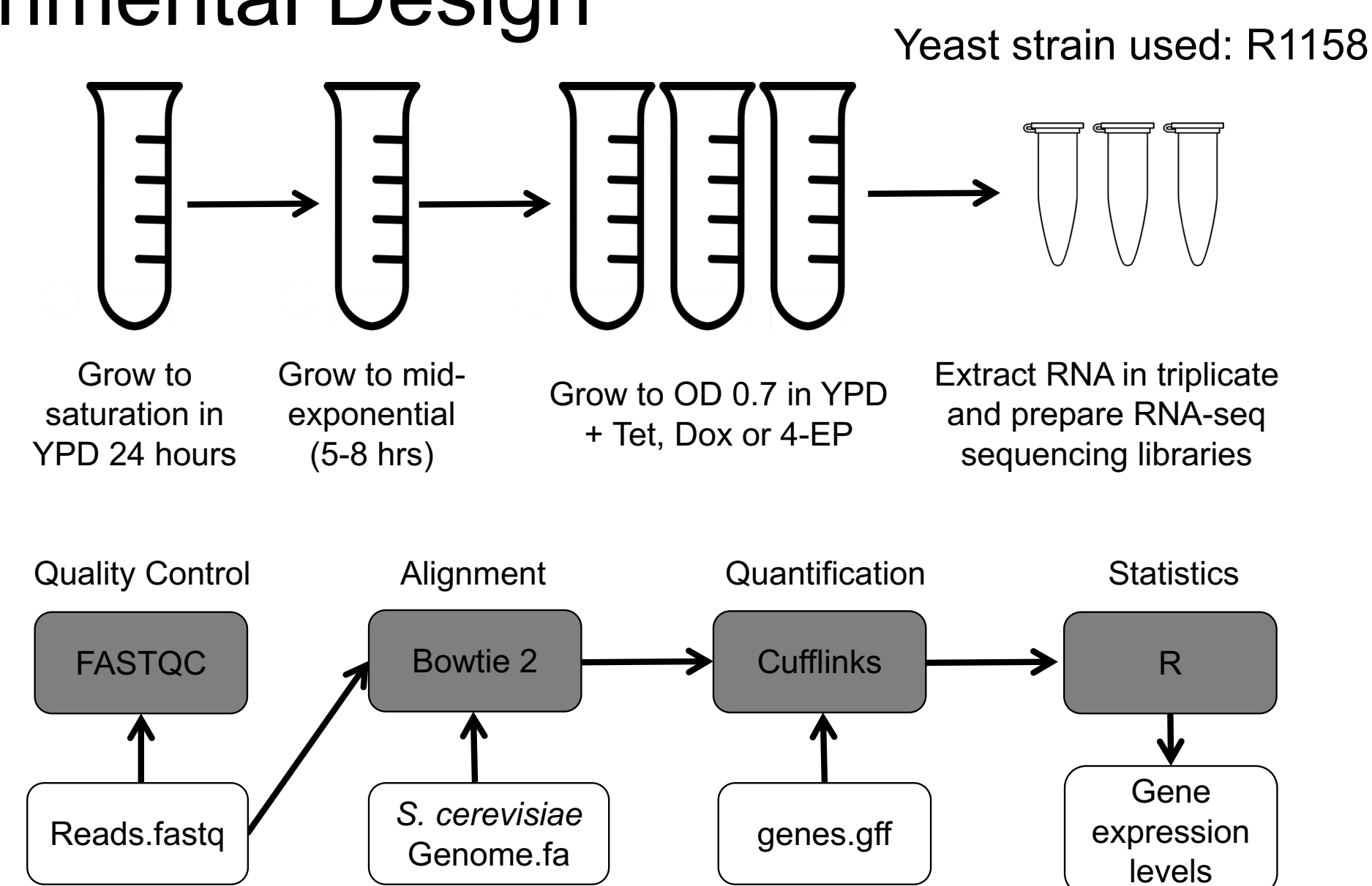
Email: gsanchez@wesleyan.edu Website: <http://coolonlab.research.wesleyan.edu>



Tetracyclines used to study gene expression in *S. cerevisiae*

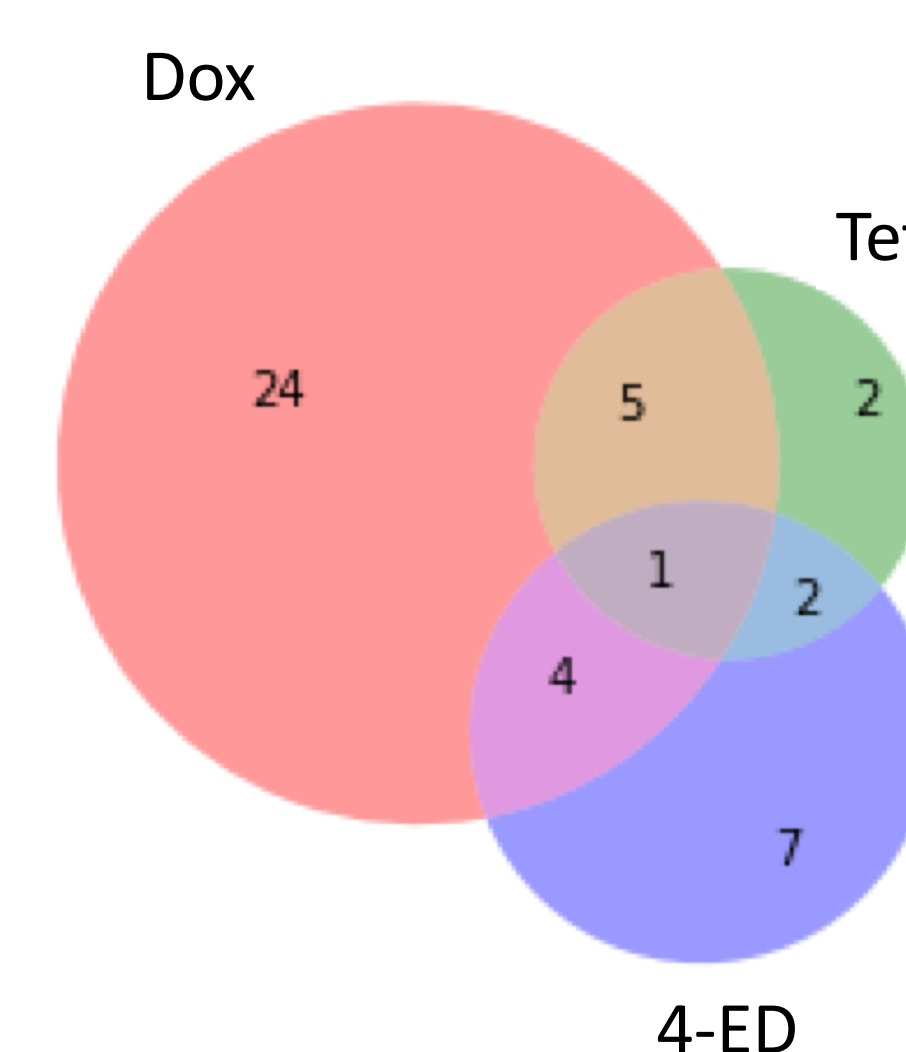
Tetracycline (Tet) and derivative chemicals have gained widespread recognition for their antibiotic properties but recent work with these chemicals in the lab has shifted to study gene expression. The most widely used Tet-modulated methodology is the Tet-On/ Tet-Off gene expression system. This system was initially developed for the use of Tet, but its derivative, Doxycycline (Dox) is now more commonly used because of its increased stability. However, a previous study showed that Dox results in mitochondrial protein imbalance. A new Tet derivative, 4-epidoxycycline (4-ED), was developed and was shown to have less off-targeted consequences on mitochondrial health. Here we use RNA sequencing to test the genome wide effects of all three chemicals in *S. cerevisiae* to determine the best use of these chemicals.

Experimental Design



Differentially Expressed Genes (DEGs)

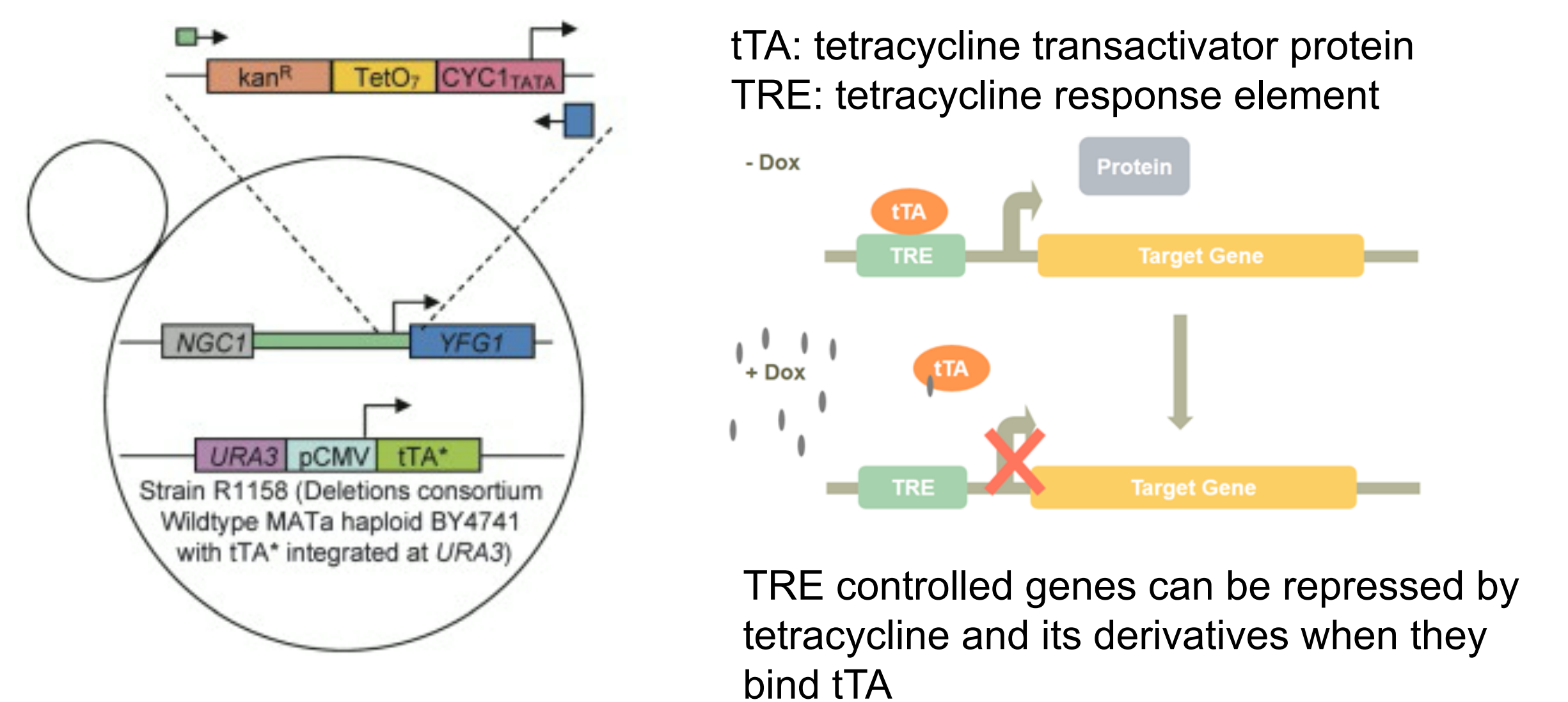
Significantly DEGs genes are genes in which the difference observed in expression level between two experimental conditions has a q value < 0.05



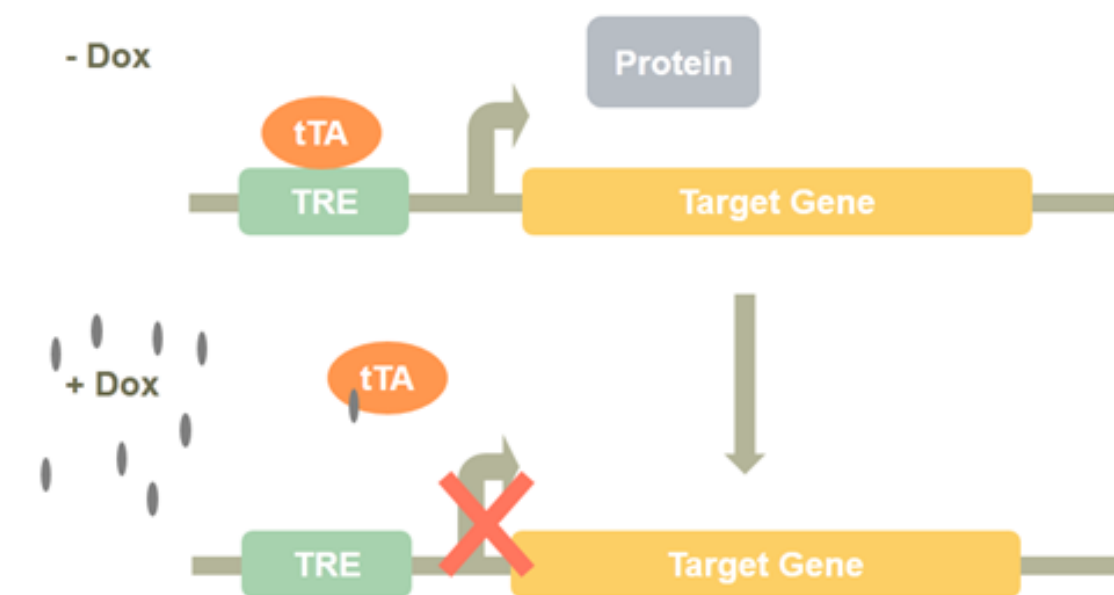
Genes in all three	Genes in Dox/Tet	Genes in Dox/4-ED	Genes in Tet/4-ED
<i>SFC1</i>	<i>VID24</i> , <i>GIN4</i> , <i>SMC1</i> , <i>ASF1</i> , <i>SFC1</i> , <i>KAR3</i>	<i>HOR2</i> , <i>SFC1</i> , <i>CAR2</i> , <i>PDE3</i> , <i>CAR1</i>	<i>SFC1</i> , <i>PCK1</i> , <i>FBP1</i>

Overlap of significantly DEGs in response to Tet, Dox, and 4-ED

Tet-On/Tet-Off System



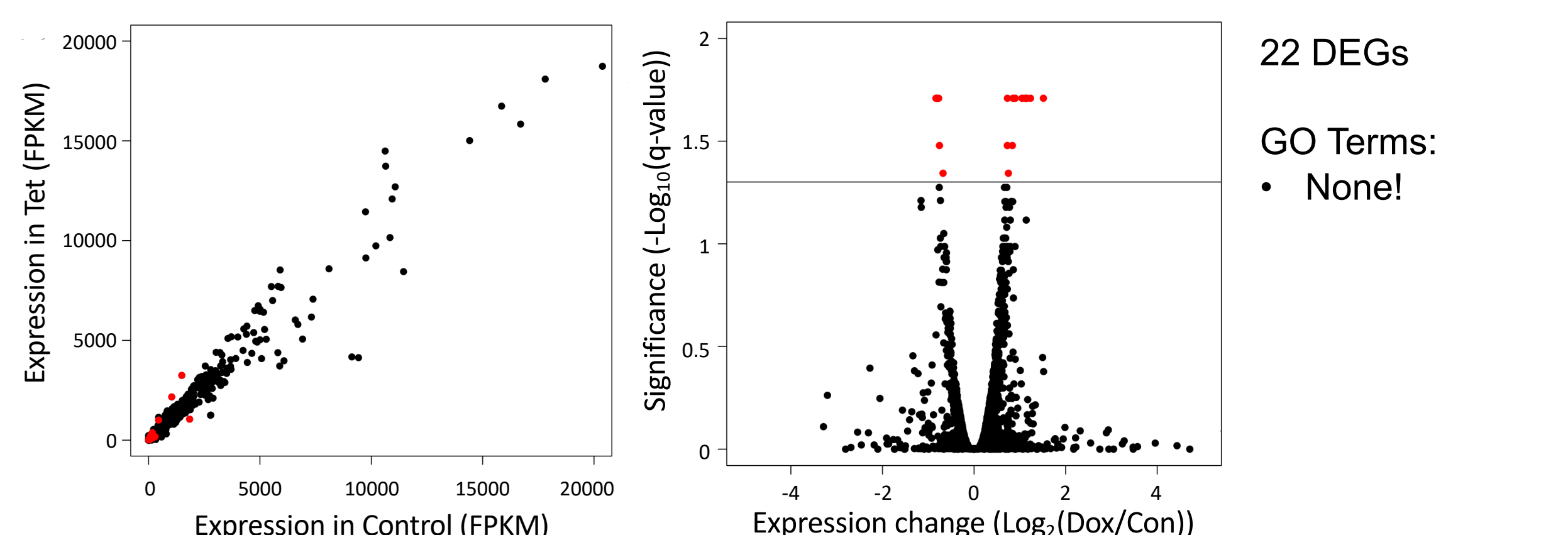
tTA: tetracycline transactivator protein
TRE: tetracycline response element



TRE controlled genes can be repressed by tetracycline and its derivatives when they bind tTA

Mnaimneh S, Davierwala AP, Haynes J, Moffat J, Peng W-T, Zhang W, Yang X, Pootoolal J, Chua G, Lopez A, et al. Exploration of Essential Gene Functions via Titratable Promoter Alleles. *Cell* 118:31-44.

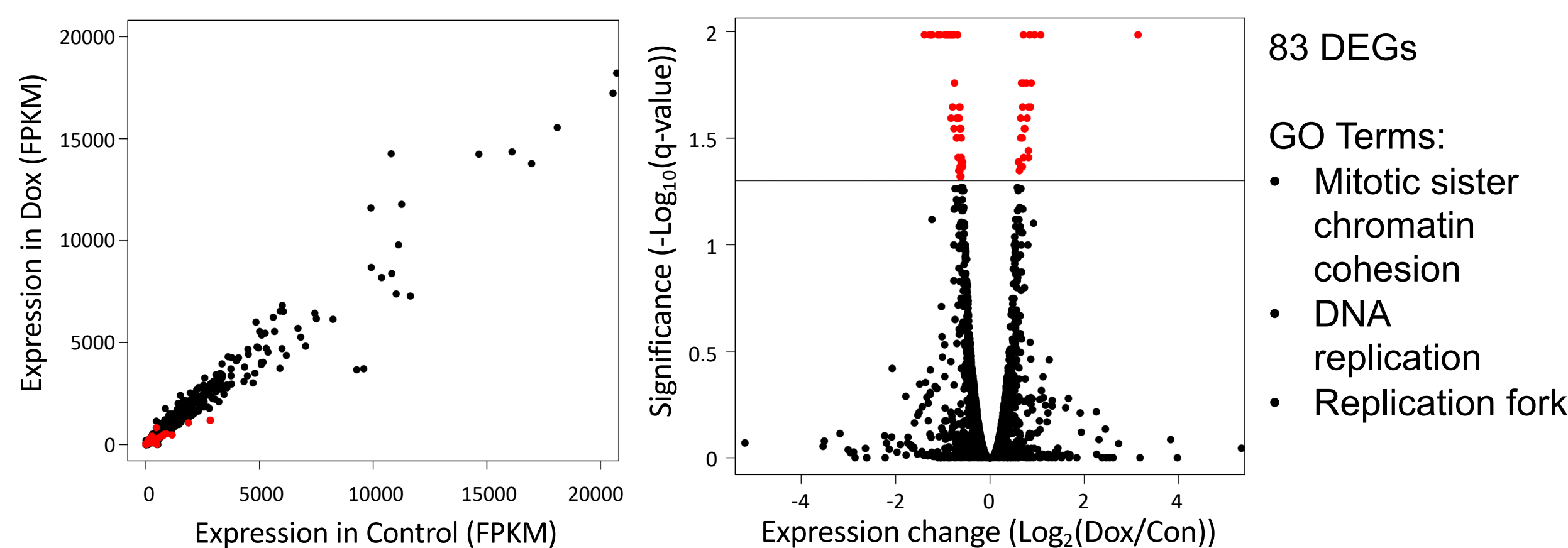
Tetracycline



22 DEGs

GO Terms:
• None!

Doxycycline

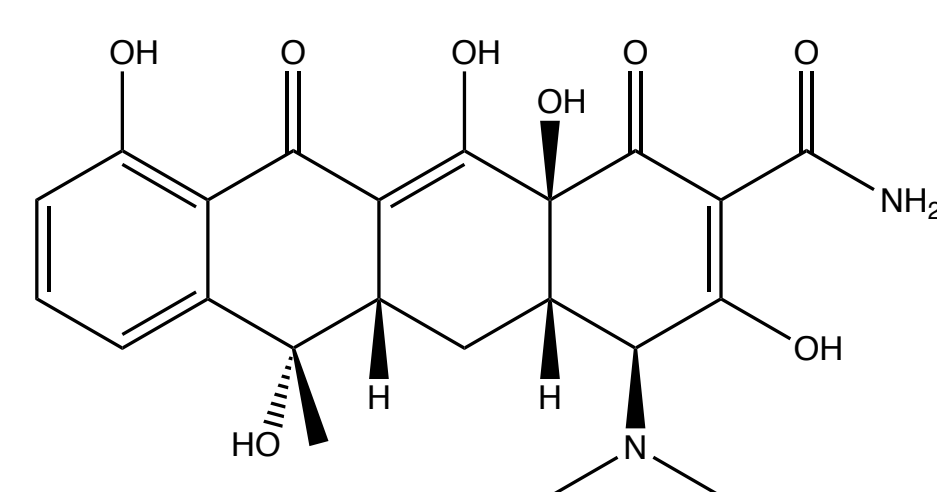


83 DEGs

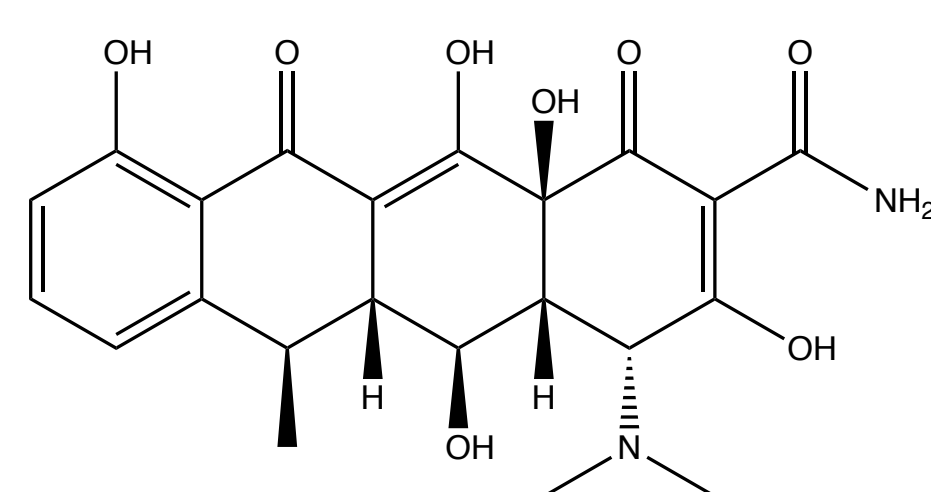
GO Terms:
• Mitotic sister chromatin cohesion
• DNA replication
• Replication fork

Our options...

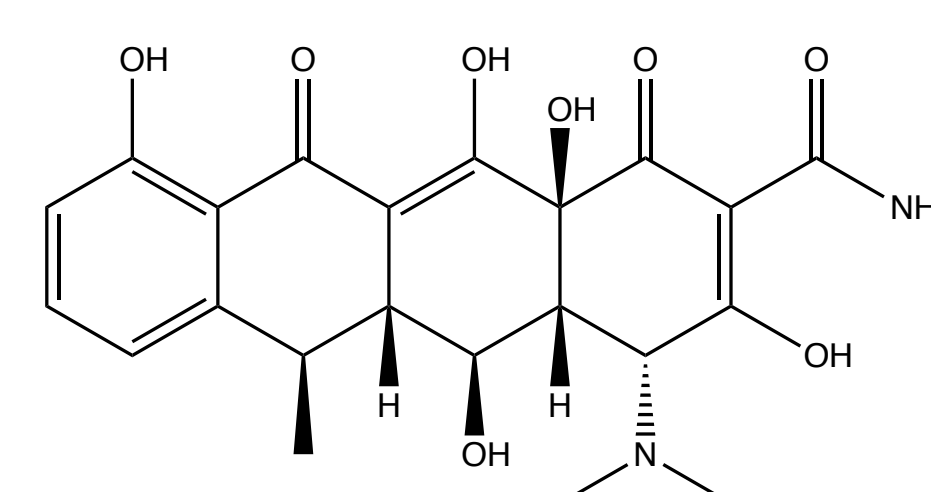
Tetracycline



Doxycycline

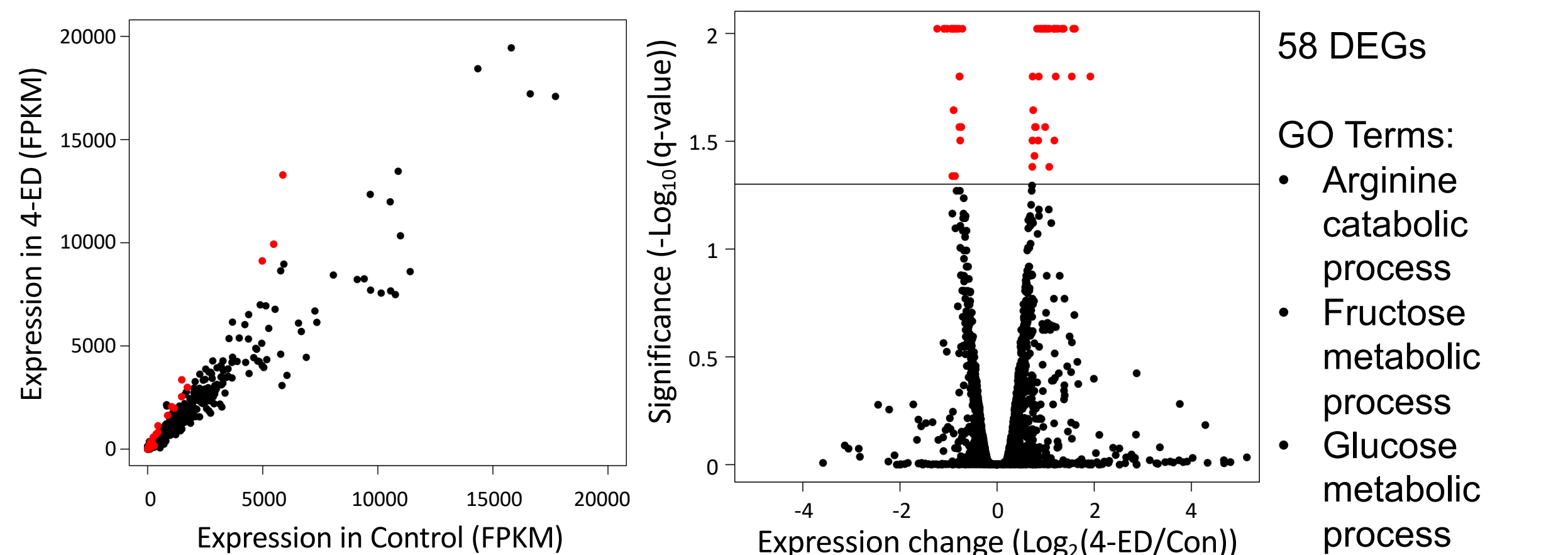


4-epidoxycycline



- Tet is the least stable of the three^{1,2}
- Dox results in mitochondrial imbalance³
- 4-ED has not been studied much⁴

4-epidoxycycline



58 DEGs

GO Terms:
• Arginine catabolic process
• Fructose metabolic process
• Glucose metabolic process

Conclusions

- Tetracycline use should be tailored for use in specific projects
- Caution should be taken when using Dox in studies focused on DNA replication, DNA repair, cell cycle, chromosome cohesion or any genes associated with or downstream of these processes
- 4-ED is not ideal for studies focused on metabolism or downstream of these processes
- Control experiments should be included in experimental designs to ensure that off target effects are not the source of any observed effects on gene expression

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