For RNAi we used 1.0 × 10^10 cells to create each well (96-well plate) with 18 μl master mix (DNA forward and reverse primers, nucleic-acid-free water and 2x SYBR Green Supermix, Bio-Rad) using qPCR conditions of 40 cycles, 60°C annealing temp, melt curve added.

- qPCR assay optimization performed on CG8399 cDNA and GAPDH primers using GFP-treated cDNA in 10-fold dilution series, then cDNA from RNAi with CG8399 was analyzed.
- Data analyzed using ∆∆CT method. For our experiment, Average C_T (GAPDH primers): Average C_T (8399 primers); ∆ΔC_T (test)-∆ΔC_T (control) where the test is 8399 RNAi treatment and the control is either GFP treated cells or untreated cells.

\[2^{-\Delta\Delta C_T} = \text{relative gene expression fold change}\]

**Results**

**Quantitative PCR (qPCR)**

**A**

- Normalized Gene Expression
- **TRENT-27 RNAi**
- **Drosophila**
- **RNAi**
- **Control**
- **GFP**

**B**

- Relative Gene Expression
- **TRENT-27 RNAi**
- **Drosophila**
- **RNAi**
- **Control**
- **GFP**

**Discussion**

The differences in knockdown and fold change of CG8399 between the newly developed and previously used dsRNA primers need to be compared. After determining which regions are more successful in RNA interference, we will select the one which provides the best knockdown for use in future RNAi experiments. It is important that we are able to effectively and consistently knockdown the potential putative ferric reductase so that we can then accurately analyse the iron content of the cells following RNAi. At that point we will be able to test our hypothesis that the level of iron will be decreased in the CG8399 RNAi treated cells.

**Conclusion and Future Plans**

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