

· **Hypothesis:**

1. Area of GFP:

Null hypothesis = unc-43 degradation gives the same size phenotype as wildtype (and does not give the same size as the unc-43 loss of function).

Alternative hypothesis = unc-43 degradation does not give the same phenotype as wildtype (and does give the same size as the unc-43 loss of function).

2. Fluorescence Intensity of GFP:

Null hypothesis = unc-43 degradation gives the same fluorescence intensity of GFP as the wildtype (and does not give the same fluorescence intensity of GFP as the unc-43 loss of function).

Alternative hypothesis = unc-43 degradation does not give the same fluorescence intensity of GFP as the wildtype (and does give the same fluorescence intensity of GFP as the unc-43 loss of function).

3. Fluorescence Intensity of RFP:

Null hypothesis = unc-43 degradation gives the same fluorescence intensity of RFP as the wildtype (and does not give the same fluorescence intensity of RFP as the unc-43 loss of function).

Alternative hypothesis = unc-43 degradation does not give the same fluorescence intensity of RFP as the wildtype (and does give the same fluorescence intensity of RFP as the unc-43 loss of function).

· **Predictions:**

1. Auxin treated *C. elegans* gives similar puncta size of clk-1, the gene tagged with GFP, to the unc-43 loss of function. Meanwhile, a bigger puncta size of clk-1 compared with wildtype.
2. Auxin treated *C. elegans* gives fainter GFP intensity of clk-1 compared to wild type and similar to unc-43 loss of function.
3. Auxin treated *C. elegans* gives similar RFP intensity of rab-3, the vesicle protein for synaptic formation, to unc-43 loss of function. Meanwhile a fainter intensity of rab-3 compared with wildtype

- **Statistical test used:**

Kruskal-Wallis test, Dunn's test, and Permutation test will be used.

First, we will use the Kruskal-Wallis test to check the equality of means between different categories. Then Dunn's test will be used to make pairs between categories and see if there is difference between each paired group. Permutation tests will also be used to see if the pairs are different from one another. This test is used in addition to Dunn's test since it has less assumptions about the data. The result of Dunn's test agrees with the permutation test for all of the pairs in all of the hypotheses.

- **Parameter per hypothesis:**

In the permutation test conducted, difference of means of the treatment and control were used as a parameter to test the null hypotheses. Data is permuted multiple times in a loop (where permutation occurs by randomizing the labels of the numbers) and the mean of difference is then calculated for permuted data between control and treatment.

After many permutations the mean of difference of the permutations that were higher than the observed mean of difference were separated and divided by the total number of permutations conducted, thereby returning the p value for each pair, allowing us to reject (or fail to reject) the null hypothesis of equality. (More detailed walk-through is in the comments in the code)