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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. *elegan* 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. *elegan* gives fainter GFP intensity of clk-1 compared to wild type and similar to unc-43 loss of function.

3. Auxin treated C. *elegan* 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 will be used for all of the hypotheses. Permutation test is only conducted for the 1st hypothesis which is regarding the equality of puncta area between auxin degradation treatment and wild type.

First, the Kruskal-Wallis test is used to check the equality of means between different categories. Initially however we aimed to conduct an ANOVA test however since two of the assumptions failed of ANOVA (Normal distribution and Equal variance failed for all of the hypotheses), we conducted Kruskal-Wallis test. This test was conducted since one of the variables is discrete (environments under which the data points where collected i.e. Wild type, Loss of function and Auxin degradation) and the other variable is continuous (i.e. the area of GFP or the light intensity of GFP or that of RFP). The p values returned for all hypothesis in this test were extremely small, therefore the equality of

- Area of GFP in 3 environments
- Light intensity of GFP in 3 environments
- Light intensity of RFP in 3 environments

were all rejected.

Then Dunn's test will be used to make pairs between categories and see if there is difference between each paired group. In the 1st hypothesis (Area of GFP) the equality of the area of GFP between auxin and wild type is the only pair that can not be rejected.
In the 2nd and 3rd hypotheses, the equality between each pair is strongly rejected.

Permutation tests will also be used in the 1st hypothesis to see if the pairs are different from one another. This test is used in addition to Dunn's test since in this test p value is gained by differences in a small group of all possible pairs. Additionally, Dunn's test is

also very conservative and has a very low power which can make the results somewhat questionable. In the permutation test, on the other hand, all of the existing data is used only with randomized labels. The result of Dunn's test agrees with the permutation test for all of the pairs in the 1st hypothesis.

- **Parameter per hypothesis:**

In all hypotheses 95% Confidence interval of the mean is calculated as a parameter estimate. The 95% Confidence interval overall (i.e. the 95 CI across all categories) and the 95% CI for each category (control and treatment) are also included per hypothesis.

1. **Area of GFP:**

   The 95% CI of Wild type and Auxin below are extremely close which suggests that the null hypothesis that they are the equal in area of GFP can have merit. We can also see that the 95% CI of loss of function is considerably far away from auxin and wild type.

   95% CI Overall: **[0.081 , 0.091]**

   95% CI Wild type: **[0.090, 0.107]**
   95% CI Auxin: **[0.087 , 0.108]**
   95% CI Loss of function: **[0.067, 0.079]**

2. **Fluorescence Intensity of GFP:**

   In this set of 95% CI intervals although the ranges do not overlap, the difference is very small compared to the value (i.e. difference of 1000 among values in the 300,000 range). Despite the small difference, it can be seen that the values do not overlap at all. So this estimation does not help with our null hypothesis that auxin and wild type have the same fluorescence intensity. The loss of function is still away from wild type and auxin although the difference is not much. However, the range of loss of function is considerably smaller than the other two categories.

   95% CI Overall: **[356,370.01 , 356,914.64]**

   95% CI Wild type: **[357,350.25 , 358,338.49]**
   95% CI Auxin: **[359,502.98 , 360,420.10]**
   95% CI Loss of function: **[354,046.60 , 354,538.18]**

3. **Fluorescence Intensity of RFP:**

   Auxin and loss of function have very small intervals and are smaller compared to wild types. The differences among categories remain marginal. Given the closeness of the values the null hypothesis of equality of fluorescence intensity of RFP does not seem agreeable. Additionally, we can see that unlike the other two sets of hypotheses the loss of function has closer values to that of wild type.

95% CI Overall:	**[353,511.16 , 353,826.65]**
95% CI Wild type:	**[353,666.76 , 354,201.89]**
95% CI Auxin:	**[355,346.69 , 355,923.01]**
95% CI Loss of function:	**[352,176.95 , 352,470.37]**

- **Permutation test:**

In the permutation test conducted, difference of means was used as a parameter to test the null hypotheses. Data is permuted multiple times in a loop (where permutation occurs by randomizing the labels / treatment 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).

The permutation test conducted for the 1st hypothesis (Equality of Area of GFP between auxin and wild type) shows that it cannot reject the null hypothesis (p-value = 0.43). The permutation test was also conducted for equality of the area between auxin-loss of function and loss of function - wild type. Both of which were strongly rejected due to extremely low p value (p value is approximately 0 for both) in the 10,000 permutations conducted. 1,000,000 permutations were also conducted to ensure the correctness of p values and that sufficient tests were conducted. Fortunately the values in 10,000

permutations conducted are close to that of 1,000,000 permutations. Given this, the default number of permutations is set back on 10,000.