**Worksheet Example**

**STEP 1: Observations**

1. Move to a table with a tank of fish. Work with your group (the other students at the same table as you) to do the following steps.

2. Observe the fish and identify all of the phenotypes present. Refer to the laboratory handout for descriptions of the possible phenotypes and the genes associated with them.

3. Decide on the phenotypes that are important for understanding the genetics.

4. Count the number of fish that have each phenotype and record in columns 1 and 2 below.

|  |
| --- |
| **Dihybrid Cross Key** |
| **Phenotype**  | **Fish ID#** |
| *Golden*/wild-type | 1 |
| *Golden*/purple  | 11 |
| Not *golden*/wild-type | 15, 10, 8 |
| Not *golden*/purple  | 2, 3, 4, 5, 6, 7, 9, 12, 13, 14 |

**STEP 2: Generate a Hypothesis**

1. Compare the numbers of wild-type versus transgenic mutant fish in the clutch. It may be easier to consider each phenotype separately (striped vs. not striped, yellow vs. not yellow, etc.)

2. Form a hypothesis about what transgenes and mutant genes are present, and what type of inheritance they have (recessive, dominant, etc.-see lab handout for more information). Punnett squares will likely help you come up with a good hypothesis.

Hypothesis for clutch1:

**The purple fluorescent protein is dominant over the wild-type.**

**The *golden* mutation is recessive to the wild-type.**

|  |  |  |
| --- | --- | --- |
|   | GloPFP | Glo- |
| GloPFP | GloPFP/GloPFP | GloPFP/Glo- |
| Glo- | GloPFP/Glo- | Glo-/Glo- |

|  |  |  |
| --- | --- | --- |
|   | + | *gol* |
| + | +/+ | +/*gol* |
| *gol* | +/*gol* | *gol*/*gol* |

**STEP 3: Analysis**

1. Use your hypothesis to calculate the expected values for each of the phenotypes. Fill in your calculated values in column 3 of the clutch 1 table.

**How to Calculate Expected Values**

1. List the phenotypes of progeny
2. Multiply the 2 fractions that relate to the specific phenotype
3. Multiply the result from #2 by the total number of progeny

Calculate here:

|  |  |
| --- | --- |
| **Phenotype** | **Expected Number of GloFish** |
| Purple/not *golden* | ¾ \* ¾ \*15 = 8.44 ---> 8 |
| Wild-type/not *golden* | ¼ \* ¾ \* 15 = 2.8  ---> 3 |
| Purple/*golden* | ¾ \* ¼ \* 15 = 2.8  ---> 3 |
| Wild-type/*golden* | ¼ \* ¼ \* 15 = 0.93 --->1 |

2. Chi-square Test

**How to do a Chi-squared test:**

A. Determine d and d2  from the observed and expected numbers you have gathered/calculated. Enter these numbers in columns 4 and 5 of the clutch 1 table.

B. Calculate d2/e for each phenotype.

C. Count the number of phenotypes you have listed in column 1. The number equals n. (if you observe two different phenotypes then n = 2)

D. The chi-squared value, X2, is the total of all the values in column 6. Place this number in the total row of column 6 and in row 7.

E. Calculate the degrees of freedom (df). df= n-1. Place this number in row 8.

F. Use the chi square table, the chi-squared value, and the df to calculate your P value. You will probably end up with a range, such as 1.0 > P > 0.5. Place this P value in row 9.

G. Make a conclusion-what does your P value tell you about your hypothesis? Place

this statement in row 9.

Clutch 1 Table:

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  (1) |  (2) |  (3) |  (4) |  (5) |  (6) |
| Phenotype | Observed Number,o | Expected Number,e |  d =(o – e) |  d2 |  d2/e |
| **Purple/not golden** | **10** | **8** | **2** | **4** | **0.5** |
| **Wild-type/not golden** | **3** | **3** | **0** | **0** | **0** |
| **Purple/golden** | **1** | **3** | **-2** | **4** | **1.33** |
| **Wild-type/golden** | **1** | **1** | **0** | **0** | **0** |
| Total | **15** | **15** |  |  | **1.83** |

(7) X2 = the sum of all of the numbers in column 6 = **1.83**

(8) Degrees of freedom (df) = n-1 = **4-1=3**

**Conclusion:**

(9) P-value and conclusion about your hypothesis:

**Using the Chi-Square Table on the front page of the packet, the p-value is between 0.65 and 0.7. This shows that the hypothesis is supported and significant.**