TWO-WAY ANALYSIS OF VARIANCE (TWO-WAY ANOVA)

Some experiments involve the investigations of two factors e.g., the effect of temperature on growth rate in wild-type and mutant *Sacharomyces*. One factor (A) is temperature and the other factor (B) is the type of *Sacharomyces* (wild type or mutant). You would have three sets of hypotheses:

Ho_1: Temperature has no effect on growth rate of *Sacharomyces*.
Ha_1: Temperature has an effect on growth rate of *Sacharomyces*

Ho_2: Presence of the mutation has no effect on growth rate of *Sacharomyces*.
Ha_2: Presence of the mutation has an effect on growth rate of *Sacharomyces*.

Ho_3: The effect of temperature on the growth rate of *Saccharomyces* is the same in wild type and mutant.
Ha_3: The effect of temperature on the growth rate of *Saccharomyces* is not the same in wild type and mutant.

If you test three replicates of the wild type and mutant your data table might look like this:
Table 1. Growth rate of *Sacharomyces* at three temperatures (the replicate for each temperature is a different sample of cells).

Cell type	Temperature 1	Temperature 2	Temperature 3	
Wild-type replicate1	Growth rate	Growth rate	Growth rate	Group mean: wild type
Wild-type replicate2	Growth rate	Growth rate	Growth rate	
Wild-type replicate3	Growth rate	Growth rate	Growth rate	
Mutant replicate1	Growth rate	Growth rate	Growth rate	Group mean: mutant
Mutant replicate1	Growth rate	Growth rate	Growth rate	
Mutant replicate1	Growth rate	Growth rate	Growth rate	
	Group Mean: T1	Group Mean: T2	Group Mean: T3	
	Overall mean: all 18 values			

How this statistical test works:

For hypothesis 1, the mean is calculated for all the values for growth rate in each of the Temperature columns (wild type and mutant are considered together) and the deviation of each value from the group mean (each temperature mean) is compared with the deviation of each value from the overall mean (all 18 values). This is similar to a one-way ANOVA for temperature; if there is no effect of temperature on growth rate, there should be little difference in the deviation of values from the group mean as compared with the overall mean. If the *p* value for this comparison is ≤ 0.05, that means there is a less than 5% chance that all the values are from the same population so you reject H_o and support H_a that temperature affects growth rate in *Sacharomyces.* You can examine the 95% confidence intervals (calculated separately) to determine which temperatures are most likely contributing to significant differences in growth rate and to determine if the direction of change was as predicted.

For Hypothesis 2: the means are calculated separately for all the values for wild type at all the temperatures and the mutant at all the temperatures (9 values for each). The deviation of each value from the group mean (wild type or mutant) is compared with the deviation of each value from the overall mean (all 18 values). This is similar to a one-way ANOVA for cell type; if there is no effect of cell type on growth rate, there should be little difference in the deviation of values from the group mean as compared with the overall mean. If the *p* value for this comparison is ≤ 0.05, that means there is a less than 5% chance that all the values are from the same population so you reject H_o and support H_a that cell type affects growth rate in *Sacharomyces.* You can examine the 95% confidence intervals (calculated separately) to determine which growth rates are most likely contributing to significant differences between wild type and mutant and to determine if the direction of change was as predicted.

For Hypothesis 3: The deviations from the means for each temperature and cell type for each value are compared separately with the deviation from the overall mean. If there is no effect of cell type on growth rate at each temperature, there should be little difference in the deviation of mean values for each cell type at each temperature from the overall mean. If the *p* value for this comparison is ≤ 0.05, you reject H_o and support H_a that the effect of temperature on the growth rate of *Saccharomyces* is not the same in wild type and mutant. You can examine the 95% confidence intervals (calculated separately) to determine which growth rates at which temperatures are most likely contributing to significant differences between wild type and mutant.

How to perform this test:

Instructions for PC users
Download the Excel file found on Connect: Resources → Statistics → Two-way ANOVA calculator.
Enter or copy and paste your data into the columns as set up on the calculator (this must be done exactly as set up; do not change the columns and rows). You can add or subtract columns for more/fewer treatments and extra rows for more replicates; all the wild-type rows must be grouped in order and all the mutant rows must also be grouped in order.
In the excel spread sheet click on data → data analysis → ANOVA: Two-Factor with Replication →OK. [If you do not have a data analysis icon to open, you can download it for free (for Excel 2010 and later): File → Options → Add-ins → Analysis tool pak → OK.]
Input range – highlight your entire data table including column and row names.
Rows per sample = number of replicates for each cell type for each treatment – in the above example this would be 3.
Alpha = 0.05 – this is the acceptable probability of rejecting H_o when H_o is true.
Output range – pick a cell below your data table; this is where your results will be displayed.
OK.

What the output means:

You will see a summary table with your column and row headings. Below that is your ANOVA summary. For our purposes the important column is the *p* value and the important rows are: sample = Ho_2, Column = Ho_1 and Interactions = Ho_3.
Examine the 95% confidence intervals (calculated separately) to determine which values are most likely contributing to the significant differences observed and if the direction is as predicted.

Note: if you change an entry in your data table you must repeat the test as the input range won't automatically be updated.

Instructions for Mac users

You have to download StatPlus separately.

Set up your data table as follows, i.e., all the wild type cells are grouped together for the treatments, followed by all the mutant cells:

Cell type	Treatment	Response
Wild type	Temperature 1	Growth rate
Wild type	Temperature 1	Growth rate
Wild type	Temperature 1	Growth rate
Wild type	Temperature 2	Growth rate
Wild type	Temperature 2	Growth rate
Wild type	Temperature 2	Growth rate
Wild type	Temperature 3	Growth rate
Wild type	Temperature 3	Growth rate
Wild type	Temperature 3	Growth rate
Mutant	Temperature 1	Growth rate
Mutant	Temperature 1	Growth rate
Mutant	Temperature 1	Growth rate
Mutant	Temperature 2	Growth rate
Mutant	Temperature 2	Growth rate
Mutant	Temperature 2	Growth rate
Mutant	Temperature 3	Growth rate
Mutant	Temperature 3	Growth rate
Mutant	Temperature 3	Growth rate

In Statplus go to 1. Statistics → 2. ANOVA → 3. Two-way ANOVA
For Response highlight the Response column (selection includes the title Response in the first row)
For Factor 1, highlight Cell Type column (A (selection includes the title Cell Type in the first row))
For factor 2, highlight Treatment column (B (selection includes title Treatment in the first row))

4. Hit OK and results should appear in a new window.
p values are listed in the *p* level column in the ANOVA results excel file
The first *p* value refers to Ho_2 and the second *p* value refers to Ho_1,