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Genetics Lab

Yeast Lab Write-Up

Simple Cross:

The goal of the Simple Cross experiment is to observe the life cycle of the yeast cell by inducing its life stages. At the beginning of the experiment, the parents- HA2 and HBT- are haploid mating cells of type A and α. A sketch of them can be seen in Figure 1.

Figure 1

The parents in this experiment are HA2 and HBT. They are defined as:

HA2: MATA, ADE1, ade2, TRP5; red colored, will not grow on an MV plate

HBT: MATα, ADE1, ADE2, trp5; cream colored, will not grow on an MV plate

Their one expected cross is: HA2xHBT

MATA/MATα, ADE1/ADE1, ADE2/ade2, TRP5/trp5; cream colored, will grow on an MV plate

Because all of the mutations act recessively, the offspring would be a cream color on a YED plate. This is what is observed on the plate after the experiment is done. When looking under a microscope, after a cell is mixed with its opposite mating type it becomes a shmoo- a haploid cell prepared to mate, caused by the pheromones of the other mating type. After a couple of hours unbudded and budded zygotes can also be seen when looking at the sample under a microscope. A sketch of these cells can be seen in Figure 2.

Figure 2

The strains of yeast are then transferred to an MV plate, which is nutritionally poor and will not provide the yeast with any adenine or tryptophan. The purpose of this part of the experiment is to select the diploids. If the yeast strain has a mutation that causes it to not be able to synthesize adenine or tryptophan, it will not be able to grow on the MV plates. So the parent strains- HA2 and HBT- should not grow on the MV plates while the offspring- diploid cells with at least one functional copy of these genes- will grow because they are able to synthesize adenine and tryptophan. By putting the cells on the MV plate there will be a purified diploid colony. After completing the experiment and observing the MV plate, the results are as predicted. The offspring can grow on the plate and is cream colored because it is heterozygous for the color trait- a red homozygous ade2/ade2 would not be able to grow on the plate. At this point, when looking under a microscope, one should be able to see the diploid yeast cells. These can be seen in Figure 3.

Figure 3

To see the other half of the yeast life cycle, it is necessary to sporulate the diploid. The YEKAC plate is the perfect place to force the diploids to sporulate because it contains no nitrogen, which will force the diploids to go through meiosis. Before sporulating the diploid, the cells are moved to a YED to speed up the growth to ensure their sporulation when moved again. Then the cells are moved to the YEKAC plate. After letting them sporulate, it is possible to see the ascus- four cells enclosed in a sack resulting from meiosis. This sketch can be seen in Figure 4.

Figure 4

When streaking the plates, it is expected that some of the cells will become red because they will have completed an entire life cycle. After completing the experiment, there are no red cells in the streaking. There must have been some error or contamination in the process of plating and replating the cells.

Two Genes/One Cross:

The purpose of this experiment is to cross four red strains- HA1, HA2, HB1, and HB2- and observe their color phenotypes to see how many genes are involved in the expression of color. There are only four possible crosses: HA1xHB1, HA1xHB2, HA2xHB1, HA2xHB2. Cream diploids are expected because some offspring will receive only one recessive allele from their parents for each gene. Therefore, their genotype will be heterozygous and their phenotype will be cream. The parents and their cross in this experiment are listed in Table 1.

|  |  |  |  |
| --- | --- | --- | --- |
| **Cross** | **Genotype** | **Expected Phenotype** | **Actual Phenotype** |
| HA1 | MATA, ade1, ADE2, TRP5 | Red | Cream |
| HA2 | MATA, ADE1, ade2, TRP5 | Red | Red |
| HB1 | MATα, ade1, ADE2, TRP5 | Red | Cream |
| HB2 | MATα, ADE1, ade2, TRP5 | Red | Red |
| HA1xHB1 | MATA/MATα, ade1/ade1, ADE2/ADE2, TRP5/TRP5 | Red | Cream |
| HA1xHB2 | MATA/MATα, ade1/ADE1, ADE2/ade2, TRP5/TRP5 | Cream | Cream |
| HA2xHB1 | MATA/MATα, ade1/ADE1, ADE2/ade2, TRP5/TRP5 | Cream | Cream |
| HA2xHB2 | MATA/MATα, ADE1/ADE1, ade2/ade2, TRP5/TRP5 | Red | Red |

Table 1: The genotypes and expected/observed phenotypes of the parents and offspring done in the first part of the experiment on a YED plate.

Comparing the expected to the observed phenotypes of the parents and offspring, there are a few errors. HA1 and HB1 are both expected to be red since the recessive ade1 gene should make them red. Their cross would also be red because the diploid cell would be homozygous recessive for the ade1 gene. There must have been some error or contamination at some point when setting up the experiment after examining these phenotypes.

The second part of the experiment uses an MV plate, which does not provide any adenine or tryptophan to the cells. When moving the cells to the MV plate, it is expected that any cell with out the capability of synthesizing adenine or tryptophan will not be able to grow on the plate. The cream diploid cells from the first part of the experiment should be able to grow on the MV plate because they are able to synthesize all of the needed nutrients with out the plate. Table 2 lists the expected and observed crosses of the second part of the experiment.

|  |  |  |  |
| --- | --- | --- | --- |
| **Cross** | **Genotype** | **Expected Phenotype** | **Actual Phenotype** |
| HA1 | MATA, ade1, ADE2, TRP5 | No growth | Cream, little growth |
| HA2 | MATA, ADE1, ade2, TRP5 | No growth | Red, little growth |
| HB1 | MATα, ade1, ADE2, TRP5 | No growth | Cream, little growth |
| HB2 | MATα, ADE1, ade2, TRP5 | No growth | Red, little growth |
| HA1xHB1 | MATA/MATα, ade1/ade1, ADE2/ADE2, TRP5/TRP5 | No growth | Cream, little growth |
| HA1xHB2 | MATA/MATα, ade1/ADE1, ADE2/ade2, TRP5/TRP5 | Cream | Cream |
| HA2xHB1 | MATA/MATα, ade1/ADE1, ADE2/ade2, TRP5/TRP5 | Cream | Cream |
| HA2xHB2 | MATA/MATα, ADE1/ADE1, ade2/ade2, TRP5/TRP5 | No growth | Red, little growth |

Table 2: The genotypes and expected/observed phenotypes of the parents and offspring done in the second part of the experiment on an MV plate.

Again, there was some error. Although there was less growth than on a YED plate where no growth was expected, the yeast should not have grown. There must have been some error made or some contamination that led to the yeast growing. Although the cream colors for the cross of HA1xHB2 and HA2xHB1 are correct, the colors of HA1, HB1, and HA1xHB1- although they should not have appeared at all- are incorrect. The red color signifies that the strain in this case will not grow on an MV plate.

A Dihybrid Cross:

During this dihybrid cross, each yeast strain of mating type A is crossed with a strain of mating type α. There are two known genes that are being examined in this experiment: one that looks at the production of adenine and one that looks at the production of tryptophan. The known genotypes of these strains are controlled to observe the phenotypes when they are crossed and to interpret the results. There are eight strains that are being looked at:

ART (HA0): MATA, CREAM, TRP5; mating type a, cream colored, grows on MVA

ARt (HAT): MATA, CREAM, trp5; mating type a, cream colored, does not grow on MVA

ArT HAR): MATA, cream, TRP5; mating type a, red colored, grows on MVA

Art (HART): MATA, cream, trp5; mating type a, red colored, does not grow on MVA

BRT (HB0): MATα, CREAM, TRP5; mating type α, cream colored, grows on MVA

BRt (HBT): MATα, CREAM, trp5; mating type α, cream colored, does not grow on MVA

BrT (HBR): MATα, cream, TRP5; mating type α, red colored, grows on MVA

Brt (HBRT): MATα, cream, trp5; mating type α, red colored, does not grow on MVA

In the first part of the experiment, the crosses are occurring on a YED plate. All of the parents and offspring will grow on the YED plate because it provides plenty of nutrients even for the strains that cannot produce their own tryptophan. Therefore, it will be easy to observe the crosses for the color-controlling gene. Table 2 depicts the expected phenotypes of the mating grid.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| YED Plate | **ART (Cream)** | **ARt (Cream)** | **ArT (Red)** | **Art (Red)** |
| **BRT (Cream)** | RRTT (Cream) | RRTt (Cream) | RrTT (Cream) | RrTt (Cream) |
| **BRt (Cream)** | RRTt (Cream) | RRtt (Cream) | RrTt (Cream) | Rrtt (Cream) |
| **BrT (Red)** | RrTT (Cream) | RrTt (Cream) | rrTT (Red) | rrTt (Red) |
| **Brt (Red)** | RrTt (Cream) | Rrtt (Cream) | rrTt (Red) | rrtt (Red) |

Table 3: The expected growth on a YED plate for the crosses of each of the strains of yeast, the underlined genotypes are unable to grow on MVA.

After completing the first cross, the plate looked very similar to Figure 1. There were some red spots in the crosses with BrT, Brt, ArT, and Art because the cross was not fully mixed when the plate was set up. However, other than that the results mirrored the predictions made before the start of the experiment. These crosses make sense because any initially pink haploid yeast will cross with either a pink or a cream colored yeast. If crossed with a cream colored yeast, the recessive r allele will make the genotype Rr and therefore heterozygous for the cream colored gene. Any parent- no matter the color- crossed with a cream-colored parent will produce a cream offspring because the cream colored parent will always give its dominant allele to the offspring.

During the second part of the experiment, the initial strains were moved to a second plate- an MVA plate. However, on the MVA plate- a plate that is adenine rich, but provides no tryptophan- the strains that cannot synthesize tryptophan will not grow. This includes the offspring that inherited the gene. Table 3 represents the expected genotypes and phenotypes of the offspring- the highlighted sections are those that are not expected to grow on an MVA plate.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| MV Plate | ART (Cream) | ARt (No Growth) | ArT (Pink) | Art (No Growth) |
| BRT (Cream) | RRTT (Cream) | RRTt (Cream) | RrTT (Cream) | RrTt (Cream) |
| BRt (No Growth) | RRTt (Cream) | No Growth | RrTt (Cream) | No Growth |
| BrT (Pink) | RrTT (Cream) | RrTt (Cream) | rrTT (Pink) | rrTt (Pink) |
| Brt (No Growth) | RrTt (Cream) | No Growth | rrTt (Pink) | No Growth |

Table 4: The expected growth on an MVA plate for the crosses of each of the strains of yeast.

After looking at the plate after the cross was completed, the outcome is not as obvious as hoped. There is little growth in the areas where no growth was expected, however in the areas where the offspring was expected to be pink, it is almost completely cream, with only a little pink. It is unclear if this was caused by error or not enough mixing of the parent strains. Looking at the offspring of the parents who cannot synthesize tryptophan- only those that are homozygous recessive for the trait- having received it from both parents- were unable to grow on the plate. This makes sense because their inability to synthesize tryptophan does not allow them to grow on the MVA plate.