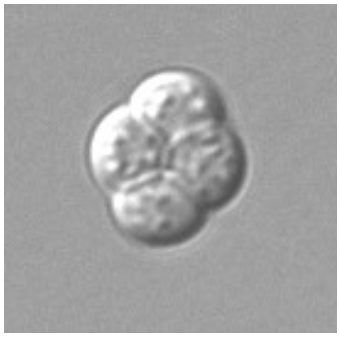
**Yeast Tetrad Analysis**

The following questions deal with the yeast *Saccharomyces cerevisiae*. Each part is independent.



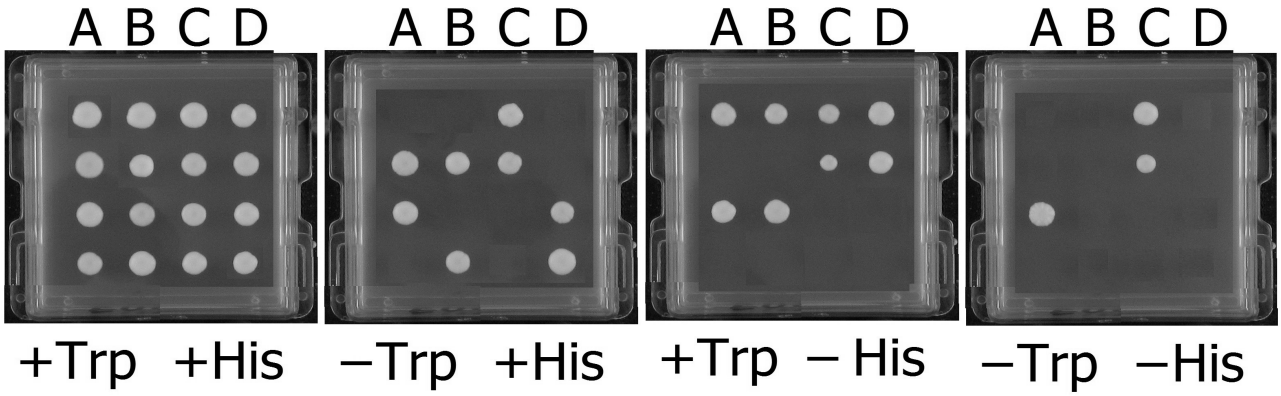
*A Saccharomyces tetrad.*

*Saccharomyces cerevisiae* is haploid. During mating, two haploid cells of opposite mating types fuse and form a diploid zygote, which undergoes meiosis to form a tetrad of four spores. Mating type is regulated by the *mat* locus, with alleles **a** and **α**.

Wild-type *Saccharomyces cerevisiae* can synthesize all amino acids it needs. ***trp3*** and ***his2*** are specific mutations in the *trp* and *his* genes, which block native pathways for synthesis of tryptophan and histidine, respectively. Wild-type alleles for these genes will be referred to as ***trp***$^+$ and ***his***$^+$.

Part i: Determining Genotypes of Tetrads

You perform a cross between **a *trp3 his***$^+$ and **α *trp***$^+$ ***his2*** strains, and obtain many tetrads. You dissect four of these tetrads (named A, B, C, and D) into four spores per tetrad and plate them on complete media containing histidine and tryptophan. After the complete media plate has incubated for a while, you use replica plating to copy colonies onto 3 plates with media lacking tryptophan, histidine, or both. Photographs of the results of this experiment are shown on the next page, along with what media each plate contained. Each column in the photographs is a single tetrad.




Identify the genotypes of tetrads A, B, C, and D. Write I, II, or III (see below) for each tetrad. It is possible that choices might be repeated or not used.

| Tetrad | Choice |
|---|---|
| A | _____ |
| B | _____ |
| C | _____ |
| D | _____ |

Choices:

| I. Parental Ditype | II. Tetratype | III. Non-parental Ditype |
|---|---|---|
| trp3 his+ | trp3 his+ | trp3 his2 |
| trp3 his+ | trp+ his+ | trp3 his2 |
| trp+ his2 | trp3 his2 | trp+ his+ |
| trp+ his2 | trp+ his2 | trp+ his+ |

Part ii: Genetic Mapping and Calculating Recombination Frequencies
*Note: A chi-squared table is provided at the end of this exam.*

a. Again, you perform a cross between **a *trp3 his*$^+$** and **α *trp*$^+$ *his2*** strains, and analyze the genotypes in each tetrad. The results are shown below.

| | Parental Ditype<br>trp3 his+<br>trp3 his+<br>trp+ his2<br>trp+ his2 | Tetratype<br>trp3 his+<br>trp+ his+<br>trp3 his2<br>trp+ his2 | Non-parental Ditype<br>trp3 his2<br>trp3 his2<br>trp+ his+<br>trp+ his+ |
|---|---|---|---|
| Count | 174 | 26 | 0 |

Are *trp* and *his* linked? (yes/no) _____

If the genes are linked, calculate the recombination frequency between the two loci. (Hint: Consider what proportion of spores in each category is recombinant?)
If the genes are unlinked, perform a $\chi^2$ test for goodness-of-fit against the genotype ratio you'd expect from no linkage.

b. Next, you perform a separate experiment and make cross between **a *trp3*** and **α *trp*$^+$** strains, and analyze the genotypes in each tetrad. The results are shown below.

| | Parental Ditype<br>a trp3<br>a trp3<br>α trp+<br>α trp+ | Tetratype<br>a trp3<br>a trp+<br>α trp3<br>α trp+ | Non-parental Ditype<br>a trp+<br>a trp+<br>α trp3<br>α trp3 |
|---|---|---|---|
| Count | 157 | 0 | 143 |

Are *trp* and *mat* linked? (yes/no) _____

If the genes are linked, calculate the recombination frequency between the two loci. (Hint: Consider what proportion of spores in each category is recombinant?)
If the genes are unlinked, perform a $\chi^2$ test for goodness-of-fit against the genotype ratio you'd expect from no linkage.

c. It is unusual that in that last cross there were no tetratypes. What does this imply about the distances between *trp* and centromere, and *mat* and centromere? Give your answer in centimorgans.

Using what linkage(s) you calculated on the previous page, draw a genetic map of the *trp*, *mat*, and *his* loci. Include centromere(s) and linear chromosome(s), and write in the centimorgan distances between pairs of loci and between centromere(s) and loci.

Part iii: Protocol analysis

Below is a protocol for sporulation and tetrad dissection in *Saccharomyces cerevisiae* crosses.

1. Grow diploid cells overnight at 30C on YPD complete media.
2. Inoculate cells heavily into 5mL of 2% potassium acetate.
3. Add 20uL of 1% amino acid solution for each amino acid the strain is auxotrophic for.
4. Incubate at room temperature overnight on a roller or with shaking.
5. Transfer tubes to 30C and incubate overnight on a roller or with shaking.
6. Verify microscopically that you have tetrads.
7. Spin down 200uL of the tetrads-and-potassium-acetate solution.
8. Wash 1X in 500uL of water.
9. Resuspend in 200uL of zymolyase solution.
10. Incubate for 10-20 min in a 37C water bath.
11. Add 1mL water and place on ice.
12. Aliquot 25-50 uL of spores onto a YPD complete media plate.
13. Allow the solution to dry into the plate.
14. Under a microscope, find tetrads that you are able to pick up individually with the needle. Deposit one on a predefined position on the plate. Set the needle lightly on the tetrad and tap the side of the scope.
15. Using a micromanipulator, move the 4 spores apart.
16. Repeat for many tetrads.
17. Place the plate at 30C.

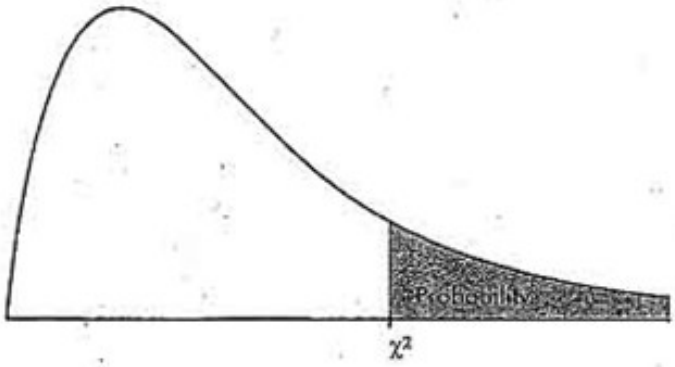
Answer the following questions about this lab protocol.

a. Which step(s) will not work correctly if you add water instead of zymolyase during step 9?

b. All tetrads you dissect produce strains that are able to survive in media lacking any or all of the original auxotrophies present. Which step did you omit?

c. Why is there shaking during incubation steps 4 and 5, but not during incubation step 17?

d. What is the result of "Set the needle lightly on the tetrad and tap the side of the scope" in step 14?




## TABLE C: $\chi^2$ CRITICAL VALUES

| | Tail probability $p$ | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| df | .25 | .20 | .15 | .10 | .05 | .025 | .02 | .01 | .005 | .0025 | .001 |
| 1 | 1.32 | 1.64 | 2.07 | 2.71 | 3.84 | 5.02 | 5.41 | 6.63 | 7.88 | 9.14 | 10.83 |
| 2 | 2.77 | 3.22 | 3.79 | 4.61 | 5.99 | 7.38 | 7.82 | 9.21 | 10.60 | 11.98 | 13.82 |
| 3 | 4.11 | 4.64 | 5.32 | 6.25 | 7.81 | 9.35 | 9.84 | 11.34 | 12.84 | 14.32 | 16.27 |
| 4 | 5.39 | 5.99 | 6.74 | 7.78 | 9.49 | 11.14 | 11.67 | 13.28 | 14.86 | 16.42 | 18.47 |
| 5 | 6.63 | 7.29 | 8.12 | 9.24 | 11.07 | 12.83 | 13.39 | 15.09 | 16.75 | 18.39 | 20.51 |
| 6 | 7.84 | 8.56 | 9.45 | 10.64 | 12.59 | 14.45 | 15.03 | 16.81 | 18.55 | 20.25 | 22.46 |
| 7 | 9.04 | 9.80 | 10.75 | 12.02 | 14.07 | 16.01 | 16.62 | 18.48 | 20.28 | 22.04 | 24.32 |
| 8 | 10.22 | 11.03 | 12.03 | 13.36 | 15.51 | 17.53 | 18.17 | 20.09 | 21.95 | 23.77 | 26.12 |
| 9 | 11.39 | 12.24 | 13.29 | 14.68 | 16.92 | 19.02 | 19.68 | 21.67 | 23.59 | 25.46 | 27.88 |
| 10 | 12.55 | 13.44 | 14.53 | 15.99 | 18.31 | 20.48 | 21.16 | 23.21 | 25.19 | 27.11 | 29.59 |
| 11 | 13.70 | 14.63 | 15.77 | 17.28 | 19.68 | 21.92 | 22.62 | 24.72 | 26.76 | 28.73 | 31.26 |
| 12 | 14.85 | 15.81 | 16.99 | 18.55 | 21.03 | 23.34 | 24.05 | 26.22 | 28.30 | 30.32 | 32.91 |
| 13 | 15.98 | 16.98 | 18.20 | 19.81 | 22.36 | 24.74 | 25.47 | 27.69 | 29.82 | 31.88 | 34.53 |
| 14 | 17.12 | 18.15 | 19.41 | 21.06 | 23.68 | 26.12 | 26.87 | 29.14 | 31.32 | 33.43 | 36.12 |
| 15 | 18.25 | 19.31 | 20.60 | 22.31 | 25.00 | 27.49 | 28.26 | 30.58 | 32.80 | 34.95 | 37.70 |
| 16 | 19.37 | 20.47 | 21.79 | 23.54 | 26.30 | 28.85 | 29.63 | 32.00 | 34.27 | 36.46 | 39.25 |
| 17 | 20.49 | 21.61 | 22.98 | 24.77 | 27.59 | 30.19 | 31.00 | 33.41 | 35.72 | 37.95 | 40.79 |
| 18 | 21.60 | 22.76 | 24.16 | 25.99 | 28.87 | 31.53 | 32.35 | 34.81 | 37.16 | 39.42 | 42.31 |
| 19 | 22.72 | 23.90 | 25.33 | 27.20 | 30.14 | 32.85 | 33.69 | 36.19 | 38.58 | 40.88 | 43.82 |
| 20 | 23.83 | 25.04 | 26.50 | 28.41 | 31.41 | 34.17 | 35.02 | 37.57 | 40.00 | 42.34 | 45.31 |
| 21 | 24.93 | 26.17 | 27.66 | 29.62 | 32.67 | 35.48 | 36.34 | 38.93 | 41.40 | 43.78 | 46.80 |
| 22 | 26.04 | 27.30 | 28.82 | 30.81 | 33.92 | 36.78 | 37.66 | 40.29 | 42.80 | 45.20 | 48.27 |
| 23 | 27.14 | 28.43 | 29.98 | 32.01 | 35.17 | 38.08 | 38.97 | 41.64 | 44.18 | 46.62 | 49.73 |
| 24 | 28.24 | 29.55 | 31.13 | 33.20 | 36.42 | 39.36 | 40.27 | 42.98 | 45.56 | 48.03 | 51.18 |
| 25 | 29.34 | 30.68 | 32.28 | 34.38 | 37.65 | 40.65 | 41.57 | 44.31 | 46.93 | 49.44 | 52.62 |
| 26 | 30.43 | 31.79 | 33.43 | 35.56 | 38.89 | 41.92 | 42.86 | 45.64 | 48.29 | 50.83 | 54.05 |
| 27 | 31.53 | 32.91 | 34.57 | 36.74 | 40.11 | 43.19 | 44.14 | 46.96 | 49.64 | 52.22 | 55.48 |
| 28 | 32.62 | 34.03 | 35.71 | 37.92 | 41.34 | 44.46 | 45.42 | 48.28 | 50.99 | 53.59 | 56.89 |
| 29 | 33.71 | 35.14 | 36.85 | 39.09 | 42.56 | 45.72 | 46.69 | 49.59 | 52.34 | 54.97 | 58.30 |
| 30 | 34.80 | 36.25 | 37.99 | 40.26 | 43.77 | 46.98 | 47.96 | 50.89 | 53.67 | 56.33 | 59.70 |
| 40 | 45.62 | 47.27 | 49.24 | 51.81 | 55.76 | 59.34 | 60.44 | 63.69 | 66.77 | 69.70 | 73.40 |
| 50 | 56.33 | 58.16 | 60.35 | 63.17 | 67.50 | 71.42 | 72.61 | 76.15 | 79.49 | 82.66 | 86.66 |
| 60 | 66.98 | 68.97 | 71.34 | 74.40 | 79.08 | 83.30 | 84.58 | 88.38 | 91.95 | 95.34 | 99.61 |
| 80 | 88.13 | 90.41 | 93.11 | 96.58 | 101.9 | 106.6 | 108.1 | 112.3 | 116.3 | 120.1 | 124.8 |
| 100 | 109.1 | 111.7 | 114.7 | 118.5 | 124.3 | 129.6 | 131.1 | 135.8 | 140.2 | 144.3 | 149.4 |

Points are for relative weighting between parts. There are 17 points total; resize weight accordingly.

Answers
for Part i **[4 points]**
**[1]** A : II (tetratype)
**[1]** B: I (parental ditype)
**[1]** C: III (non-parental ditype)
**[1]** D: I (parental ditype)

for Part ii-a **[3 points]**
**[1]** yes; linked
# recombinants = 13
# tetrads = 200
**[2]** recombination frequency = 6.5%

for Part ii-b **[3 points]**
**[1]** no; unlinked
original table: 157 143 total: 300
expected table: 150 150 total: 300
$\chi^2 = \Sigma(o-e)^2/e = 49/150 + 49/150 = 98/150 = 0.6533$
df = 1 (also accept 2)
p-value = more than 0.05 → no significant difference → it fits the distribution
**[1]** for $\chi^2$
**[1]** for accurate interpretation of p-value

for Part ii-d **[3 points]**
**[1]** for:
0 cM, 0 cM
**[1]** for these properties:
- mat and trp on separate chromosomes
**[1]** for this property:
- trp and his are 6.5 cM apart

for Part iii **[4 points]**
a. "14", "15", or "14 and 15" are all OK
b. "3"
c. only necessary for liquid culture, not for plate culture; to prevent the cells from settling. Any answer comparing liquid to plate culture is OK, and any answer mentioning settling or clumping of cells is OK.
d. the tetrad splits into 4 individual spores