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Fundamentals of Genetics
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CONTENTS

Practical 1: Monohybrid Cross: Mendel's experiment, Procedure & Conclusion

https://www.youtube.com/watch?v=xTOMgXeGizU

Definition

A monohybrid cross is the hybrid of two individuals with homozygous genotypes which result in the opposite phenotype for a certain genetic trait."

Monohybrid cross is responsible for the inheritance of one gene. It can be easily shown through a Punnett Square. Monohybrid cross is used by geneticists to observe how homozygous offspring express heterozygous genotypes inherited from their parents.

Procedure:

Steps of Monohybrid Cross?

The ratios of the phenotype and the genotype that are estimated are only probabilities. Listed below are steps that can be used to calculate a monohybrid cross:

1. Indicate the alleles using characters – recessive alleles can be indicated by lower case letters while dominant alleles can be indicated by upper case letters
2. Note down both the phenotype and the genotype of the parents or the parental generation that are being crossed
3. Jot down the genotype of the gametes from the parental generation – As a result of meiotic division, the gametes will be haploid
4. Tabulate a Punnett square to get the probable combinations of the gametes – Any combination is possible if the process of fertilization is random
5. The phenotype and the genotype ratios of the prospective offspring can be written. The outcome hence obtained is known as the F1 generation. The F2, F3 etc generations form the subsequent generations.

Performing a Punnett Square

Monohybrid crosses can be calculated according to the following steps:

Step 1: Designate characters to represent the alleles
- Capital letter for dominant allele, lower case letter for recessive allele

Step 2: Write down the genotype and phenotype of the parents
- This is the P generation (parental generation)

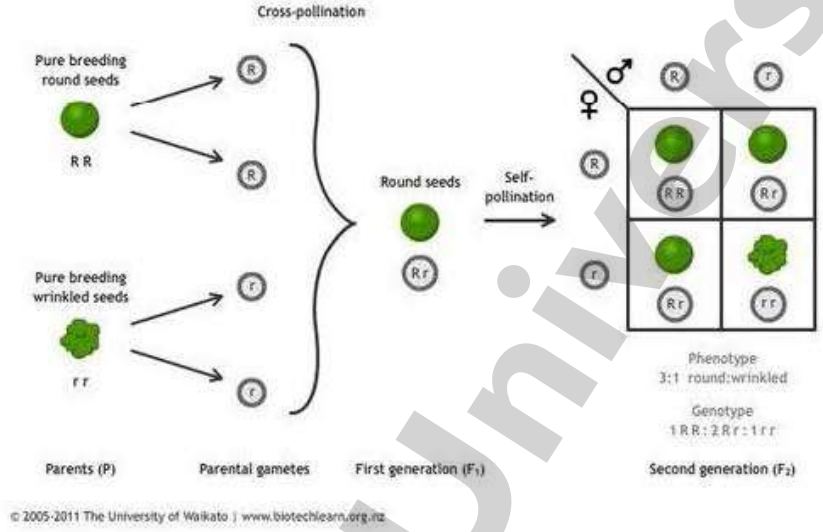
Step 3: Write down the genotype of the parental gametes
- These will be haploid as a result of meiotic division

Step 4: Use a Punnett grid to work out the potential gamete combinations
- As fertilisation is random, all combinations have an equal probability

Step 5: Write out the genotype and phenotype ratios of potential offspring
- This is the F_1 generation (first filial generation)

- Subsequent generations through interbreeding labeled F_2, F_3, etc.

Note: The genotypic and phenotypic ratios calculated are only probabilities

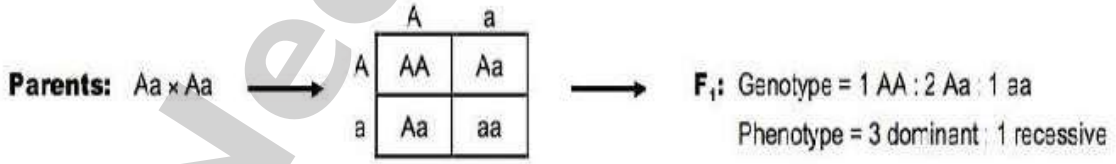


Examples of Monohybrid Crosses

Genotype and phenotype ratios can be determined for different patterns of inheritance using a monohybrid cross. It is important to note that these ratios reflect probabilities and do not guarantee actual proportions in offspring

Autosomal Dominance / Recessive

- Choose a letter where the upper and lower case forms are easily distinguishable (e.g. E/e, A/a, B/b)
- Use the capital letter for the dominant allele and the lower case letter for the recessive allele
- Example:



Solve the Problems:

1. In Snapdragon red is not completely dominant over white. What coloured flower would you expect, if you cross a red flower with a white one? What would be the phenotypic and genotypic ratio of the off springs?

Trait:

Parent 1:

Parent 2:

Punnett Square:

Phenotype of Offspring:

Genotype of Offspring:

2. In pea plants the short trait (tt) is recessive to tall trait (TT). If a homozygous tall is crossed with a heterozygous tall plant, What would be the phenotypic and genotypic ratio of the off springs?

Trait:

Parent 1:

Parent 2:

Punnett Square:

Phenotype of Offspring:

Genotype of Offspring:

Additional Details:

Practical 2: Dihybrid Cross: Mendel's experiment, Procedure & Conclusion

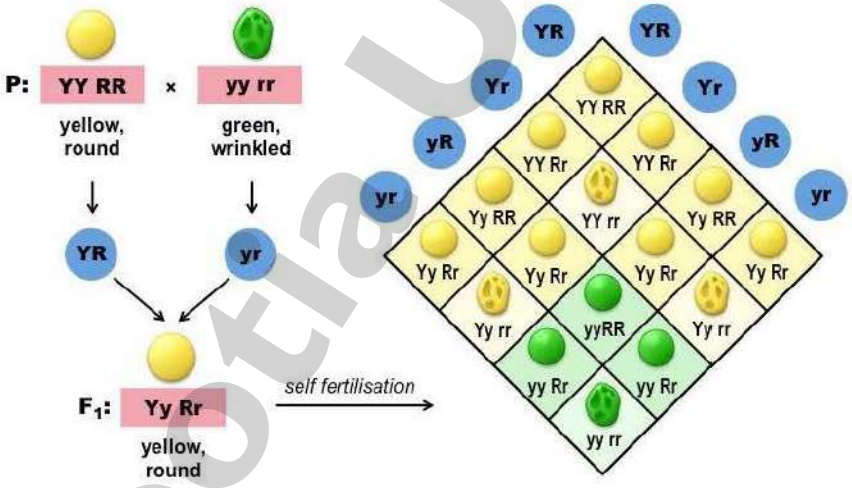
https://www.youtube.com/watch?v=xTOMgXeGizU

Definition: Dihybrid cross is the cross between two different genes that differ in two observed traits."

Procedure:

A dihybrid cross is a breeding experiment between two organisms which are identical hybrids for two traits. In other words, a dihybrid cross is a cross between two organisms, with both being heterozygous for two different traits. The individuals in this type of trait are homozygous for a specific trait. These traits are determined by DNA segments called genes.

In a dihybrid cross, the parents carry different pair of alleles for each trait. One parent carries homozygous dominant allele, while the other one carries homozygous recessive allele. The offsprings produced after the crosses in the F1 generation are all heterozygous for specific traits.



Examples of dihybrid Crosses

Mendel took a pair of contrasting traits together for crossing, for example colour and the shape of seeds at a time. He picked the wrinkled-green seed and round-yellow seed and crossed them. He obtained only round-yellow seeds in the F1 generation. This indicated that round shape and yellow colour of seeds are dominant in nature.

Meanwhile, the wrinkled shape and green colour of seeds are recessive traits. Then, F1 progeny was self-pollinated. This resulted in four different combinations of seeds in the F2 generation. They were wrinkled-yellow, round-yellow, wrinkled-green seeds and round-green in the phenotypic ratio of 9:3:3:1.

During monohybrid cross of these traits, he observed the same pattern of dominance and inheritance. The phenotypic ratio 3:1 of yellow and green colour and of round and wrinkled seed shape during monohybrid cross was retained in dihybrid cross as well.

Solve the Problems:

1. In summer squash, white fruit color (W) is dominant over yellow fruit color (w) and disk-shaped fruit (D) is dominant over sphere-shaped fruit (d).. If a squash plant true-breeding for white, disk-shaped fruit is crossed with a plant true-breeding for yellow, sphere-shaped fruit, what will the phenotypic and genotypic ratios be for:a. the F_1 generation? b. the F_2 generation?

Trait:
Parent 1: Parent 2:
Punnett Square:

	Gamete	Offspring	Offspring	Offspring	Offspring
Offspring					
Offspring					
Offspring					
Offspring					

Phenotype of Offspring:

Genotype of Offspring:

2. In mice the running (R) is a dominant trait over waltzing, and black hair colour (B) is dominant over brown (b). a. Cross of a homozygous running and homozygous black haired mouse produced heterozygous running, brown mouse, b. Cross a waltzing brown with a waltzing brown mouse

Trait:
Parent 1: Parent 2:

Punnett Square:

	Gamete	Offspring	Offspring	Offspring	Offspring
Offspring					
Offspring					
Offspring					
Offspring					

Phenotype of Offspring:

Genotype of Offspring:

Solve:

Trait:

Parent 1:

Parent 2:

Punnett Square:

	Gamete	Offspring	Offspring	Offspring	Offspring
Offspring					
Offspring					
Offspring					
Offspring					

Phenotype of Offspring:

Genotype of Offspring:

Additional Details:

Practical 3: Trihybrid Cross: Mendel's experiment, Procedure & Conclusion

https://www.youtube.com/watch?v=AzF0naZNu8k

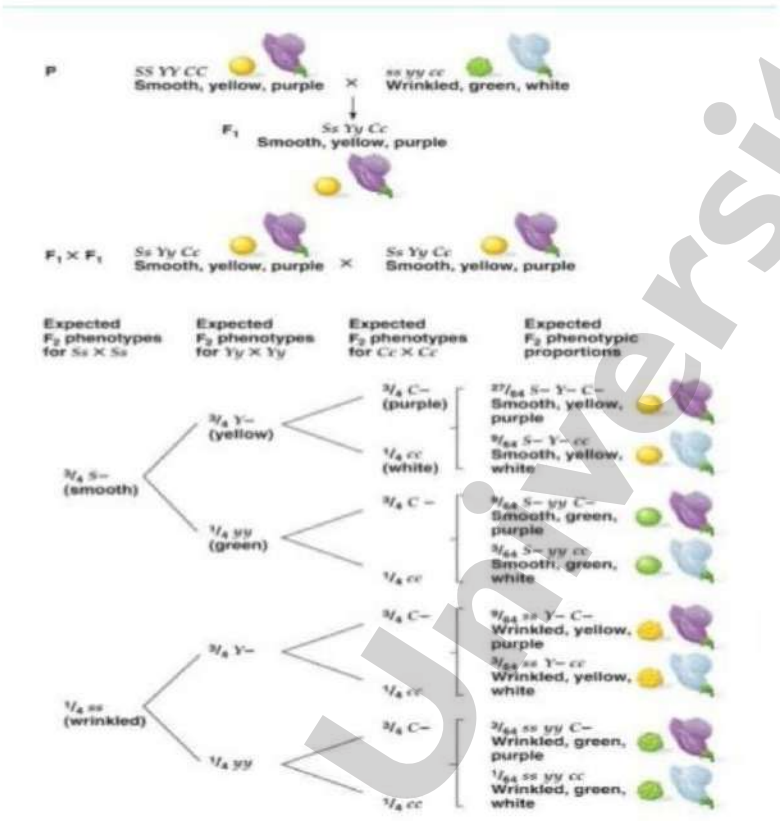
Definition: It is the **cross** between the two individuals of a species for studying inheritance of three pairs of factors or alleles belonging to three different genes.

Procedure:

Mendel extended his observations to tri-hybrid crosses involving three pairs of contrasting characters. The characters he considered were: seed shape—smooth (S) vs. wrinkled (s); colour of cotyledons—yellow (Y) vs. green (y); and flower colour—violet (V) vs. white (v). The F_1 hybrid produces 8 types of gametes. These on selfing have equal chances to combine with any of the 8 types of gametes produced by the other parent resulting in 64 different combinations. All the dominant phenotypes are expressed.

Trihybrid Corss Example:

In this way it is possible to predict genotypes and phenotypes in F_2 of crosses involving more than 3 genes (multi-hybrid crosses).In each case number of gametes formed by F_1 heterozygote is determined by the formula 2^n, where n represents the number of characters. Thus in a tri-hybrid cross $2^3 = 8$ gametes result.In a cross involving 4 characters, $2^4 = 2 \times 2 \times 2 \times 2 = 16$ gametes must result. The number of F_2 phenotypes resulting from selfing F_1 hybrid is a square of the number of gametes. Thus in a tri-hybrid cross there are $8 \times 8 = 64$ phenotypes, in a tetra-hybrid cross $16 \times 16 = 144$ phenotypes, and so on.



Courtesy: https://www.biology.iupui.edu/biocourses/N100/practiceproblems.html

For a **trihybrid cross**, the F_2 **phenotypic ratio** is 27:9:9:9:3:3:3:1.

Solve the Problem:

In a trihybrid cross of a tall, purple-flowered pea plant with round seeds (TtPpRr) with a tall, white-flowered pea plant with wrinkled seeds (Ttpprr), what is the probability:

a) that a tall, white-flowered plant with wrinkled seeds will be produced?_____

b) that a short, purple-flowered plant with round seeds will be produced? _____

c) that a short, white-flowered plant with wrinkled seeds will be produced? _____

d) that a tall, purple-flowered plant with round seeds will be produced? _____

e) that a tall, white-flowered plant with round seeds will be produced?_____

Traits:

Use Fork-line Method and deduce the phenotype:

Problem 1:

Problem 2:

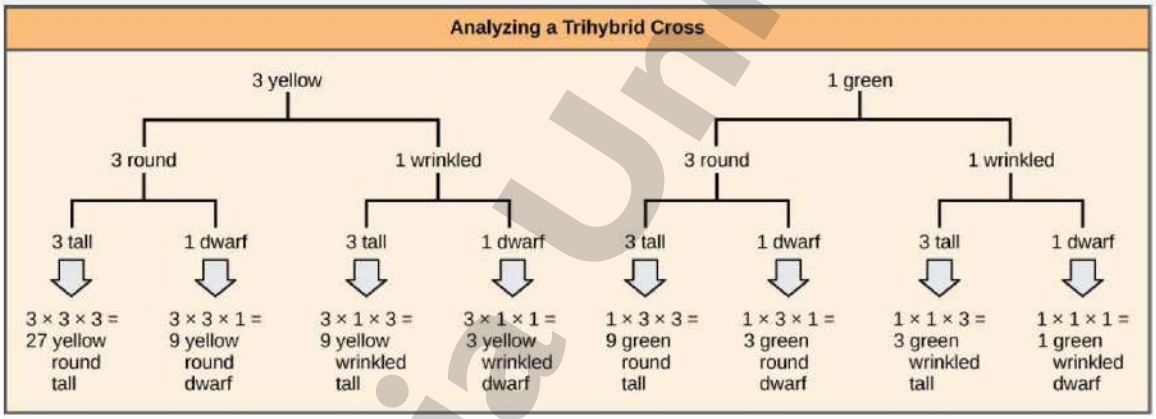
Additional Details:

Practical 4: Fork-line Method for Multi-gene Cross

https://www.youtube.com/watch?v=nBTX2h2GYYw

When more than two genes are being considered, the Punnett-square method becomes unwieldy. For instance, examining a cross involving four genes would require a 16 × 16 grid containing 256 boxes. It would be extremely cumbersome to manually enter each genotype. For more complex crosses, the forked-line and probability methods are preferred.

To prepare a forked-line diagram for a cross between F_1 heterozygotes resulting from a cross between *AABBCC* and *aabbcc* parents, we first create rows equal to the number of genes being considered, and then segregate the alleles in each row on forked lines according to the probabilities for individual monohybrid crosses (Figure). We then multiply the values along each forked path to obtain the F_2 offspring probabilities. Note that this process is a diagrammatic version of the product rule. The values along each forked pathway can be multiplied because each gene assorts independently. For a trihybrid cross, the F_2 phenotypic ratio is 27:9:9:9:3:3:3:1.



*https://www.oercommons.org/courseware/module/14996/student/?task=5

Solve the Problems:

Tabulate the phenotype of a cross between PPQQRRSS and ppqqrrss in F2 using fork-line diagram

Parent1: Parent2:

F1 Offspring:

Problem to be solved:

Solving Multi-Gene Genetic Crosses Using the Forked Line or Branch Diagram Method

Think you need to draw out massive Punnett squares in order to solve tri-hybrid or tetra-hybrid crosses? Think again!
Thanks to our mathematical ability to compound probabilities, you only need to do single-gene crosses (2x2 Punnett squares)!

Take this cross, for example: AaBBCc x aaBbCc (solve for the expected genotypic ratios)

STEP ONE: Break down multi-gene cross into its constituent single-gene crosses

Aa x aa

	A	a
a	Aa	aa
a	Aa	aa

½ Aa, ½ aa

BB x Bb

	B	B
B	BB	BB
b	Bb	Bb

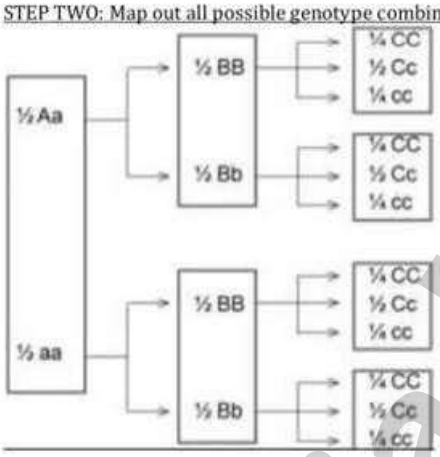
½ BB, ½ Bb

Cc x Cc

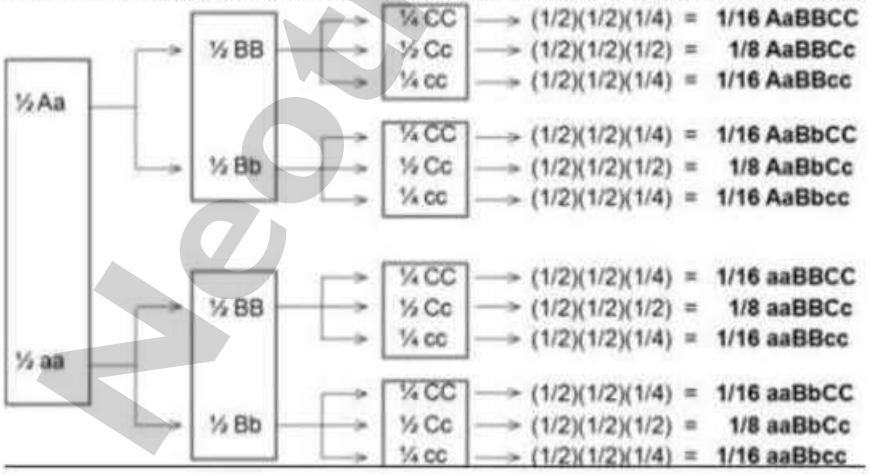
	C	c
C	CC	Cc
c	Cc	cc

¼ CC, ½ Cc, ¼ cc

STEP TWO: Map out all possible genotype combinations using forked lines/branches



STEP THREE: Compound probabilities for each path through the lines/branches (each possible genotype)



VARIATION: Finding the probability of one specific genotype
When asked for the probability of one specific genotype, you do not need to draw out all the branches.
Simply compound the applicable probabilities as you would for one of the paths above.

Probability of AaBbcc offspring = (1/2)(1/2)(1/4) = 1/16

Additional Details:

Practical 5: Back Cross: Mendel's experiment, Procedure & Conclusion

https://www.youtube.com/watch?v=AP4C3AGGmk8

Definition: Backcross is the mating of a hybrid organism (offspring of genetically unlike parents) with one of its parents or with an organism genetically similar to the parent. The backcross is useful in genetics studies for isolating (separating out) certain characteristics in a related group of animals or plants. In animal breeding, a backcross is often called a topcross. Grading usually refers to the mating of average, or "grade," females to a superior male, then backcrossing the female offspring to the same or a similar sire.

Procedure: When F_1 individuals are crossed with one of the two parents (either CC—red flowered or cc—white flowered) from which they have been derived, then such a cross is called back cross.

(A) When F_1 (Cc) is crossed to the parent with dominant phenotype i.e., homozygous for red colour (CC). In such a cross plants will be 100% red.

(B) When F_1 plant (Cc) is crossed to the parent with pure recessive (cc) white flowered plant. In such a cross 50% plants will be red flowered and 50% plants will be white flowered.

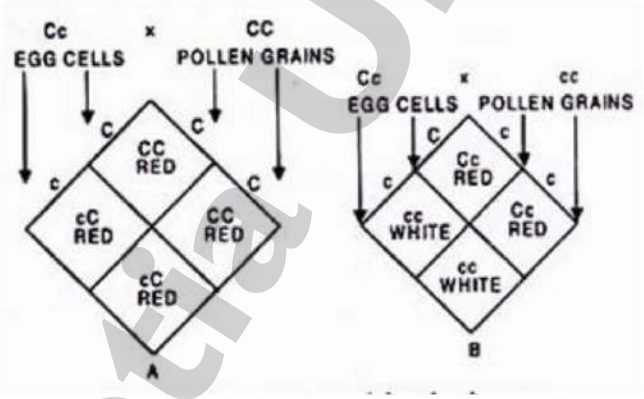


Fig: Monohybrid Backcross

Example of Dihybrid Backcross:

	[Double heterozygous F_1 Dihybrid]	[Double homozygous recessive parent]
Test cross Genotypes	*Yellow Round* YyRr	*Green Wrinkled* yyrr
Types of Gametes	YR Yr yR yr	yr

Test cross Progeny	yr	YR	Yr	yR	yr
		YyRr *Yellow Round*	Yyrr *Yellow Wrinkled*	yyRr *Green Round*	yyrr *Green Wrinkled*

1 : 1 : 1 : 1

25% 25% 25% 25%

Courtesy:

https://www.google.com/url?sa=i&url=http%3A%2F%2Fpinkmonkey.com%2Fstudyguides%2Fsubjects%2Fbiology-edited%2Fchap7%2Fb0707901

Solve the Problems:

1. In maize purple colour (P) is dominant over the green colour (p) and crinkled or cut character (c) is recessive to normal character (C) of leaves. What will be the phenotype and genotype of F_1 and F_2 generation if heterozygous purple normal maize is crossed with homozygous purple normal plant?

Trait:

Parent 1:

Parent 2:

F1 Offspring:

Punnett Square:

	Gamete	Offspring	Offspring	Offspring	Offspring
Offspring					

Phenotype of Offspring:

Genotype of Offspring:

2. In guinea pig black coat (BB) is dominant to white coat (bb) and short hair (SS) is dominant to long hair (ss). When a heterozygous black short haired male guinea pig is crossed with a white long haired female, what would be the result in F1 and F2?

Trait:

Parent 1:

Parent 2:

F1 Offspring:

Punnett Square:

	Gamete	Offspring	Offspring	Offspring	Offspring
Offspring					

Phenotype of Offspring:

Genotype of Offspring:

Additional Details:

Practical 6: Test Cross: Mendel's experiment, Procedure & Conclusion

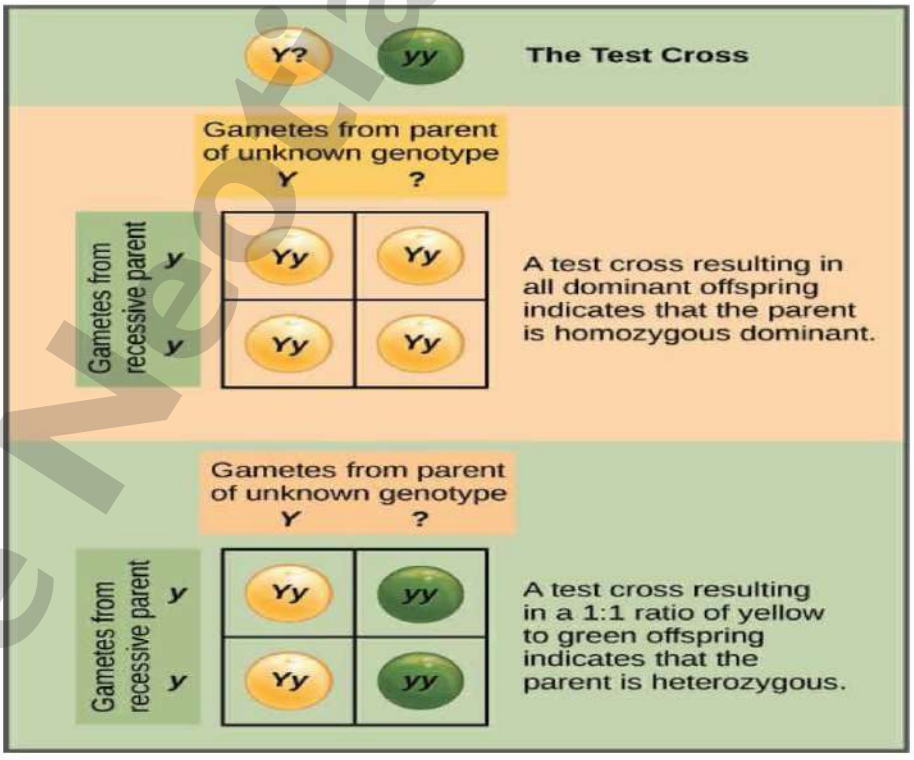
https://www.youtube.com/watch?v=8y_SLtToUOA

Definition: Crossing an organism with dominant genotype to a recessive homozygote for a specific phenotype in order to determine dominance/recessiveness of the unknown genotype and the pattern of inheritance based on the phenotype of each progeny. Test cross is first introduced by Gregor Mendel, the Father of Modern Genetics. He made use of the test cross to identify the genotypes responsible for the phenotypes of the progenies after a cross. This means that a test cross is made between an organism exhibiting the dominant trait (and thus carrying the unknown dominant genotype) and another organism displaying the recessive trait. If the recessive trait is manifested in any of their progenies it means that the unknown genotype is heterozygous dominant. In contrast, if the dominant trait is observed in all progenies the unknown genotype is homozygous dominant.

Procedure:

The test cross is another fundamental tool devised by Gregor Mendel. In its simplest form, a test cross is an experimental cross of an individual organism of dominant phenotype but unknown genotype and an organism with a homozygous recessive genotype (and phenotype). In order to understand how test crosses work, it helps to consider several examples, including those that involve just one gene of interest, as well as those that involve multiple genes.

Examples: Mendel bred the unknown yellow pea (Y?) with a green pea, being homozygous recessive (yy). The chart below shows the two possible outcomes of the test.



Courtesy: https://www.google.com/url?sa=i&url=http%3A%2F%2Ftreat.tier3.xyz%2Ff6cb-dihybrid-test-cross-genotypic-ratio

Either the offspring would be all yellow, or around half of them would be green. This is based on the results of the two *Punnett squares* shown. The top square shows the results if the unknown yellow pea is (YY). In this case, the pea has no recessive allele to pass to the offspring. Therefore, 100% of the offspring receive one Y allele and one y allele, making them all yellow.
In the second case, if the unknown yellow pea has the genotype Yy, half of the offspring will receive this allele. The other allele will be from the green pea, and will also be a green allele (y). In this case, half of the offspring will produce green peas. The test cross itself occurs when the two plants are bred together, by taking pollen from the recessive plant, and carefully placing it on the flowers of the yellow pea plant. Mendel would then carefully rear all of the beans produced (which would be yellow) into plants of their own. The color of peas that these plants produced would determine the genetics of the original plant, which produced the yellow (Y?) seeds.

Solve the Problems:

1. In the fruit fly *Drosophila melanogaster*, and recall that the ebony-body allele (e) is recessive to the normal yellow-body allele (E), while the brown-eye allele (b) is recessive to the normal red-eye allele (B). If you are given a male with a yellow body and red eyes, how can you determine its genotype?

Trait:

Parent 1:

Parent 2:

Punnett Square:

	Gamete	Offspring	Offspring	Offspring	Offspring
Offspring					

Phenotype of Offspring:

Genotype of Offspring:

2. A female guinea pig is heterozygous for both fur colour and coat texture was crossed with a male with light fur colour and smooth coat texture. What would be the F2 test result?

Trait:

Parent 1:

Parent 2:

F1 Offspring:

Punnett Square:

	Gamete	Offspring	Offspring	Offspring	Offspring
Offspring					

Phenotype of Offspring:

Genotype of Offspring:

3. In man, assume that spotted skin (S) is dominant over non-spotted skin (s) and that wooly hair (W) is dominant over non-wooly hair (w). Cross a marriage between a heterozygous spotted, non-wooly man with a homozygous non-wooly-haired, non-spotted woman. Give genotypic and phenotypic ratios of offspring

Trait:

Parent 1:

Parent 2:

F1 Offspring:

Punnett Square:

	Gamete	Offspring	Offspring	Offspring	Offspring
Offspring					

Phenotype of Offspring:

Genotype of Offspring:

Additional Details:

Practical 7: Probability Statistics and its application in Agriculture

https://www.youtube.com/watch?v=CfZa1daLjwo

Definition: Probabilities are mathematical measures of likelihood. In other words, it's a way of quantifying (giving a specific, numerical value to) if an event could happen.

Procedure:

- A Punnett's square is actually a statistical table—a device which allows you to "do" statistics without doing the calculations. But genetics is a statistical science, and problems can also be solved using statistics.
- When solving an agricultural genetics problem, you are calculating probabilities. The probability of a particular event is the "chance" that event will occur. It's a prediction.
- Probabilities are expressed as decimals.
- Probability values range from 0 to 1.0. A probability of 1.0 is a certainty - it's equivalent to a chance of 100%. The probability that, if you toss a coin into the air, it will come back down (given that we are on the surface of the Earth and that there are no obstacles to prevent its descent) would be 1.0.
- A probability of zero means the event will never happen. The probability of tossing a penny into the air and have it come down magically changed into a quarter is zero.

The product (multiplication) rule of probability:

One probability rule that's very useful in genetics is the **product rule**, which states that the probability of two (or more) independent events occurring together can be calculated by multiplying the individual probabilities of the events. For example, if you roll a six-sided die once, you have a 1/6, 1/6 chance of getting a six. If you roll two dice at once, your chance of getting two sixes is: (probability of a six on die 1) x (probability of a six on die 2) = (1/6) x(1/6) = 1/36(1/6)·(1/6)=1/36th.

The sum rule of probability:

In some genetics problems, you may need to calculate the probability that any one of several events will occur. In this case, you'll need to apply another rule of probability, the sum rule. According to the **sum rule**, the probability that any of several mutually exclusive events will occur is equal to the sum of the events' individual probabilities.

1. In a cross of two heterozygous brown cats, what is the probability of getting brown cats? What is the probability that the baby cats will be homozygous?

Additional Details:

Practical 8: Chi-square test and its application in Genetics

https://www.youtube.com/watch?v=WXPBoFDqNVk

Definition: The chi-square test was used to test that alleles segregate on Mendelian principles. It is required a comparison of expected and observed numbers. It is used in statistics for judging the significance of the sampling data. Prof. Fisher developed chi-square test. Symbolically written as X^2 (pronounced as Ki-square).

It is a statistical measure with the help of which it is possible to assess the significance of the difference between the observed genotypic numbers (frequencies) and the expected numbers (frequencies) obtained from some hypothetical universe.

Calculation: This can be calculated by the following formula:

$X^2 = \sum = (\text{observed value} - \text{expected value})^2/\text{expected value}$

Let us take the example of ratio of phenotype and genotype of experiments conducted by Mendel. By applying X^2, results show that the observed frequencies are in agreement with the predicted ratios. The data of Mendel's actual experiments are given in the following table. The variations in the expected and predicted ratio are due to experimental error alone.

Fisher and Yates (1963); probability associated with values of the Chi-square Statistics

d.f.	0.90	0.50	0.10	0.05	0.01
1	0.016	0.46	2.71	3.84	6.64
2	0.21	1.39	4.61	5.99	9.25
3	0.58	2.37	6.25	7.82	11.35
4	1.06	3.36	7.78	9.49	13.28
5	1.61	4.35	9.24	11.07	15.09
6	2.2	5.35	10.65	12.59	16.81
7	2.83	6.35	12.02	14.07	18.48
8	3.49	7.34	13.36	15.51	20.09
9	4.17	8.34	14.68	16.92	21.67
10	4.87	9.34	15.99	18.31	23.21
11	5.58	10.34	17.28	19.68	24.73
12	6.3	11.34	18.35	21.03	26.22
13	7.04	12.34	19.81	22.36	27.69
14	7.79	13.34	21.06	23.69	29.14
15	8.35	14.34	22.31	25.00	30.58
20	12.44	19.34	28.41	31.41	37.57
25	16.47	24.34	34.38	37.67	44.31

The hypothesis is never agreed or disagreed by the P value. The results of the investigator which are acceptable or unacceptable with respect to hypothesis, evaluate, the results of the Chi-square observations. The 5 percentage points (0-05) on the table are usually chosen as an arbitrary standard for determining the significance or goodness of fit.

Worked Out Example:

Let us take the example of ratio of phenotype and genotype of experiments conducted by Mendel. By applying X^2, results show that the observed frequencies are in agreement with the predicted ratios. The data of Mendel's actual experiments are given in the following table. The variations in the expected and predicted ratio are due to experimental error alone.

Observed, Os	Expected, E	$(O-E)^2$ Z	$\frac{(O-E)^2}{E}$
315	312.75	5.06	0.016
101	104.25	10.56	0.101
1.08	104.25	14.00	0.135
32	34.75	7.56	0.218
Total 556	556		$0.470 = \chi^2$

Mendel observed in his experiment, the ratio as 9 : 3 : 3 : 1 in dihybrid cross in the F_2generation while the ratio in monohybrid cross in F_2 generation was 1: 2 : 1. He found 315 rounds, yellow seeds, 101 rounds, green seeds, 108 wrinkle, yellow seeds and 32 wrinkle, green seeds.

So the expected numbers of each phenotype are 556 (9/16) = 312.75 round, yellow seeds; 556(3/16) = 104.25 round. Green seeds; 556(3/16) = 104.25 wrinkle, yellow seeds and 556(1/16) = 34.75 wrinkle, green seeds. Chi-square will show whether the difference between the actual and predicted ratio is due to experimental error or not.

The Chi-square value calculated is 0.470, which is got by applying by the following formula:

$X^2 = \sum$ = (observed value – expected value)^2/expected value

Degree of freedom is required for the calculation of X^2, the number of independent constraints determined the number of degree of freedom (or d.f.). The degree of freedom is a measure of the number of independent variables present in a given experiment.

It is stated that the chances of error affect only one independent variable. In Mendels experiments stated above the variables are only 4 so the degree of freedom is 4 -1 = 3. The number of any three phenotypic classes is determined, the number of the fourth class is fixed. Table value of X^2 for 3 degree of freedom at 5% level is 7.82, a chi-square value is 0.47 which is lower than the table value hence it is correct. In other words, it may be said that a probability at 5% level of significance is 7.82 which is more/greater hence the hypothesis is correct. If it is less than 5%, then rejected.

Solve the Problems:

1. Genetic theory states that children having one parent of blood type A and the other of blood type B will always be of one of the three types A, AB, B and that the proportion of the three types will on an average be as 1: 2 :1. A sample of 300 children was collected— 30% were found to be type A, 45% — type AB and the remainder —type B.

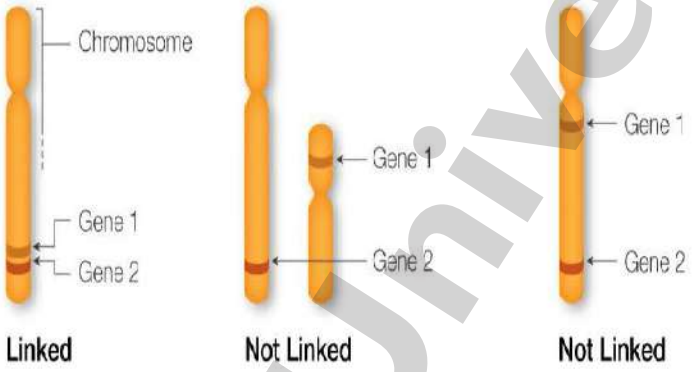
2. The proportion of the bean in four groups namely A, B, C and D should be 9 : 3 : 3 :1. A farmer sowed 1600 beans. The results of his experiment show that in four groups the data is 882,313,287 and 118. Does the experimental result support the theory that they are in the ratio of 9 : 3 : 3 :1?

Additional Details:

Practical 9: Determination of linkage and cross over analysis through two point test cross

https://www.youtube.com/watch?v=qCrulK8PPAg

Definition: Linked genes sit close together on a chromosome, making them likely to be inherited together. Genes on separate chromosomes are never linked but not all genes on a chromosome are linked. Genes that are farther away from each other are more likely to be separated during a process called homologous recombination.



The discovery of linkage:

Example 1:

In the early 1900s, William Bateson and R. C. Punnett were studying inheritance in the sweet pea. They studied two genes: one affecting flower color (*P*, purple, and *p*, red) and the other affecting the shape of pollen grains (*L*, long, and *l*, round). They crossed pure lines $P/P \cdot L/L$ (purple, long) × $p/p \cdot l/l$ (red, round), and selfed the F_1 $P/p \cdot L/l$ heterozygotes to obtain an F_2. The table below shows the proportions of each phenotype in the F_2 plants.

Number of Progeny in F2		
Phenotype/Genotype	Observed	Expected
Purple long(P-L	4831	3911
Purple round (P-ll)	390	1303
Red long (pp-L)	393	1303
Red round (pp-ll)	1138	435

The F_2 phenotypes deviated strikingly from the expected 9:3:3:1 ratio. What is going on? This does not appear to be explainable as a modified Mendelian ratio. Note that two phenotypic classes are larger than expected: the purple, long phenotype and the red, round phenotype. As a possible explanation for this, Bateson and Punnett proposed that the F_1 had actually produced more $P \cdot L$ and $p \cdot l$ gametes than would be produced by Mendelian independent assortment. Because these genotypes were the gametic types in the original pure lines, the researchers thought that physical **coupling** between the dominant alleles *P* and *L* and between the recessive

alleles *p* and *l* might have prevented their independent assortment in the F2. This phenomenon is called linkage. The more recovery of the parental type indicates linkage.

Problems to be solved:

1. In fruitfly brown body (B) colour is dominant over black body (b) and winged (N) is dominant over (n) wingless condition. The F1 heterozygote was crossed with a double recessive parent. In F2 the progeny count was shown in table, interpret the result?

Brown winged	800
Brown wingless	0
Black winged	0
Black wingless	800

2. The progeny count in F2 generation is what is the genetic distance between wing and colour gene?

Brown winged	85
Brown wingless	75
Black winged	728
Black wingless	712

3. Drosophila genes linked to X-chromosome, the eye colour white and a body colour gene yellow, the wild type eye colour is red and wild type body colour is brown. What is the recombination frequency?

White, brown males	4484
Red, yellow males	4413
Red, brown males	76
White, yellow males	53

Problem 4: Explain the linkage ratios from above three examples?

Additional Details:

Practical 10: Determination of linkage and cross over analysis through three point test cross

https://www.youtube.com/watch?v=8z1L-wxTQPk

Example 1: A standard problem in genetics is to determine the order of three loci known to be linked on one pair of the autosomes. Solution of the problem requires (1) a determination of the relative order of loci, and (2) the map distances between loci.

A cross is made between homozygous wild-type female *Drosophila* (**$a^+a^+b^+b^+$ c^+c^+**) and triple-mutant males (**aa bb cc**) (the order here is arbitrary). The F_1 (**a^+a b^+b c^+c**) females are test crossed back to the triple-mutant males and the F_2 phenotypic ratios are as follows:

Progeny Type	Number
a^+ b^+ c^+ female	321
a b c male	308
a+ b c+	18
a b+ c	15
a+ b c	66
a b+c+	59
a+b+c	102
a b c+	112
Total	1001

Progeny Type	Number
c^+ a^+ b^+ female	321
c a b male	308
c+ a b+	18
c a+ b	15
c+ a b	66
c a+ b+	59
c+ a+ b	102
c a b+	112
Total	1001

---

b to c = (102+112 + 18 +15)/1001X100 = 24.67cM

a to b = (66+59 + 18+ 15)/1001X100 = 15.78cM

b _____a _____ c

----- 24.67cM----------

_____

----15.78cM-------a

Co-efficient of Coincidence (CC) = Obtained DCO/ expected DCO = 33/38.2= 0.846=84.6%

Interference I = 1- CC = 0.154= 15.4 %

Expected Double cross over (DCO) progeny = frequency of Single cross over 1 X frequency of Single cross over 2 X total progeny = 0.2467X.1578X1001=

Calculate Map Distance:

Problems to be solved: 3 point test cross (triple recessive parent for all the 3 genes or traits)

sc_____ ec_____ cv

sc_____ec_____ cv

From the below mentioned test cross data construct a linkage map, recombination percentage

Progeny Phenotype	Progeny Genotype	Number
Echinus Crossveinless	sc ec cv/sc ec cv	4
Wild type	+ + +/ sc ec cv	1
Scute	sc + +/sc ec cv	997
Echinus Crossveinless	+ ec cv/sc ec cv	1002
Scute Echinus	sc ec +/sc ec cv	681
Crossveinless	+ + cv/ sc ec cv	716
Scute Crossveinless	sc + cv/sc ec cv	8808
Echinus	+ ec +/sc ec cv	8576
Total		20,785

Practical 11: Study of different parts of Compound Microscope

https://www.youtube.com/watch?v=ArVJO6AaY3c

Introduction

A good microscope is an essential tool for any microbiology laboratory. There are many kinds of microscopes but the type most useful in diagnostic work is the compound microscope.

Principle

- A general biological microscope mainly consists of an objective lens, ocular lens, lens tube, stage, and reflector. An object placed on the stage is magnified through the objective lens. When the target is focused, a magnified image can be observed through the ocular lens. Microscope is designed to emit light onto or through objects and magnify the transmitted or reflected light with the objective and ocular lenses.

- A microscope is an optical instrument used to view small objects by enlarging them with two convex lenses. Optical microscopes, used for research, illuminate samples with visible or ultraviolet light. Depending on its structure, a biological microscope is categorized as an upright or inverted with a magnification ranging from 10x to 1500x.
- Different types of microscopes are used based on the desired level of magnification. Magnifying glasses or loupes are used for quick inspection with a low magnification; binocular microscopes are used to observe from 10x to 50x, and upright/inverted microscopes are used to observe from 50x to 1500x.

Care and Handling of the Microscope

- Always use both hands to carry the microscope, one holding the arm, other under the base.
- Before each use, examine the microscope carefully and report any unusual condition or damage.
- Keep the oculars, objectives, and condenser lens clean. Use dry lens paper only.
- At the end of each laboratory period in which the microscope is used, remove the slide from the stage, wipe away the oil on the oil-immersion objective, and place the low-power objective in vertical position.
- Replace the dust cover, if available, and return the microscope to its box.

Basic Parts of Microscope

1. **Eye Piece Lens**

2. **Tube**

3. **Arm**

4. **Base**

5. **Coarse focus**

6. **Fine focus**

7. **Illuminator**

8. **Stage**

9. **Object lens**

10. **Rack Stop**

11. **Condenser lens**

12. **Diaphragm or Irish**

PRINCIPLES OF MICROSCOPY, RESOLVING POWER AND NUMERICAL APERTURE AND USE

Using of Microscope

1. To carry the microscope grasp the microscopes arm with one hand. Place your other hand under the base.
2. Place the microscope on a table with the arm toward you.
3. Turn the coarse adjustment knob to raise the body tube.
4. Revolve the nosepiece until the low-power objective lens clicks into place.
5. Adjust the diaphragm. While looking through the eyepiece, also adjust the mirror until you see a bright white circle of light.
6. Place a slide on the stage. Center the specimen over the opening on the stage. Use the stage clips to hold the slide in place.
7. Look at the stage from the side. Carefully turn the coarse adjustment knob to lower the body tube until the low power objective almost touches the slide.
8. Looking through the eyepiece, VERY SLOWLY the coarse adjustment knob until the specimen comes into focus.
9. To switch to the high power objective lens, look at the microscope from the side. CAREFULLY revolve the nosepiece until the high-power objective lens clicks into place. Make sure the lens does not hit the slide.
10. Looking through the eyepiece, turn the fine adjustment knob until the specimen comes into focus.

Draw a neat diagram of Compound Microscope with labelling:

Problems to be solved:

1. Function of Simple Microscope:

2. Function of Compound Microscope:

3. Function of Electron Microscope:

4. Function of Fluorescent Microscope:

Practical 12: Preparation of Mitotic slides and observation of different stages

https://www.youtube.com/watch?v=5-ur7bWqlDQ

Aim of the Experiment

To study and demonstrate mitosis by preparing a mount of onion root tip cells.

Experiment Theory: In mitosis, the nucleus of the Eukaryotic cells divides into two, subsequently resulting in the splitting of the parent cells into two daughter cells. Hence every cell division involves two chief stages:

- Cytokinesis – Cytoplasm division
- Karyokinesis – Nucleus division

Stages Of Mitosis

The various stages of mitosis are:

Prophase

Metaphase

Anaphase

Telophase

Materials Required

- Compound microscope
- Acetocarmine stain
- Water
- Burner
- N/10 Hydrochloric acid
- Filter paper
- Coverslip
- Aceto alcohol (Glacial acetic acid and Ethanol in the ratio 1:3)
- Glass Slide
- Onion root peel
- Forceps
- Blade
- Watch glass
- Needle
- Vial

Write down the Procedure:

Observations:

Conclusion:

Precaution:

Inference:

Additional Details:

Practical 13: Preparation of Meiotic slides and observation of different stages

https://www.youtube.com/watch?v=hJttpMnSqiU

Materials:

- Onion flower
- Aceto-carmine stain
- Glass slides
- Cover slips
- blotting paper

Write down the Procedure:

Phases

Interphase

Before under going in meiosis-I, each cell will remain in an interphase, during which the genetic materials are duplicated due to active DNA replication.

Prophase-I

In the first meiotic division, production in the chromosome number occurs without separation of chromatids. Prophase is the longest phase and has 5 stages.

Leptotene

Chromosomes appear as long threadlike structure interwoven together. Chromosomes display a beaded appearance and are called chromomeres. Ends of chromosomes are drawn toward nuclear membrane near the centriole. In some plants, chromosomes may form synthetic knots.

Zygotene

The homologous chromosomes pair with one another, gene by gene, over the entire length of the chromosomes. The pairing of the homologous chromosomes is called synapsis. Each pair of homologous chromosomes is known as bivalent.

Pachytene

Each paired chromosomes become shorter and thicker than in earlier substages and splits into 2 sister chromatids except at the region of the centromere. As a result of the longitudinal division of each homologous chromosome into chromatids, there are 4 group of chromatids in the nucleus parallel to each other, called tetrads.

Diplotene

During the diplotene stage, chiasmata appear to move towards the ends of the synapsed chromosomes in the process of terminalization. Repulsion of homologous chiasmata are very clear in pachytene because of the increased condensation of the chromosomes.

Diakinesis

The chromosomes begin to coil, and so become shorter and thicker. Terminalization is completed. The nucleolus detaches from the nucleolar organizer and disappears completely. The nuclei envelope starts to degenerate and spindle formation is well underway.

Metaphase-I

The bivalents orient themselves at random on the equatorial plate. The centromere of each chromosome of a terminalized tetrad is directed toward the opposite poles. The chromosomal microtubular spindle fibers remain attached, with the centromeres and homologous chromosomes ready to separate.

Anaphase-I

It is characterized by the separation of whole chromosomes of each homologous pair (tetrad), so that each pole of the dividing cell receives either a paternal or maternal longitudinally double chromosome of each tetrad. This ensures a change in chromosome number from diploid to monoploid or haploid in the resultant reorganized daughter nuclei.

Telophase-I

The chromosomes may persist for a time in the condensed state, the nucleolus and nuclear

membrane may be reconstituted, and cytokinesis may also occur to produce 2 haploid cells.

Metaphase-II
Metaphase-II is of very short duration. The chromosomes rearrange in the equatorial plate. The centromere lies in the equator, while the arms are directed toward the poles. The centromeres divide and separate into 2 daughter chromosomes.

Anaphase-II
Daughter chromosomes start migrating toward the opposite poles and the movement is brought about by the action of spindle fibers.

Telophase-II
The chromosomes uncoil after reaching the opposite poles and become less distinct. The nuclear membrane and nucleolus reappear, resulting in the formation of 4 daughter nuclei, which are haploid.

Cytokinesis
This separates each nucleus from the others. The cell wall is formed and 4 haploid cells are produced.
To study and demonstrate mitosis by preparing a mount of onion root tip cells.

Observations:

Conclusion:

Precaution:

Inference:

Additional Details:

Practical 14: Calculation of Mitotic Index

https://www.youtube.com/watch?v=GJFqkcgOib4

How to Calculate Mitotic Index?

$$\text{mitotic index} = \frac{\text{number of cells in mitosis}}{\text{total number of cells}} \times 100$$

$$= \frac{\text{no.of cells in prophase + metaphase + anaphase + telophase}}{1000}$$

$$= \frac{30+20+20+10+20}{1000} \times 100$$

$$= \boxed{10\%}$$

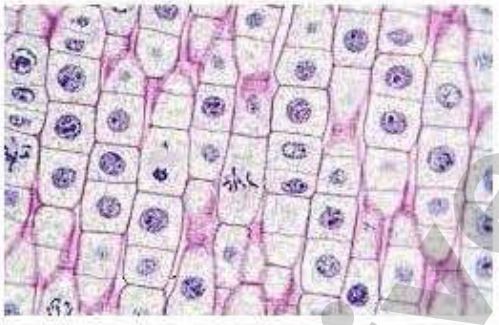
Problems to be solved:

Data 1: Calculate Mitotic Index

Stage	*Number of cells*
Interphase	462
Prophase	23
Metaphase	24
Anaphase	4
Telophase	16

Calculation =

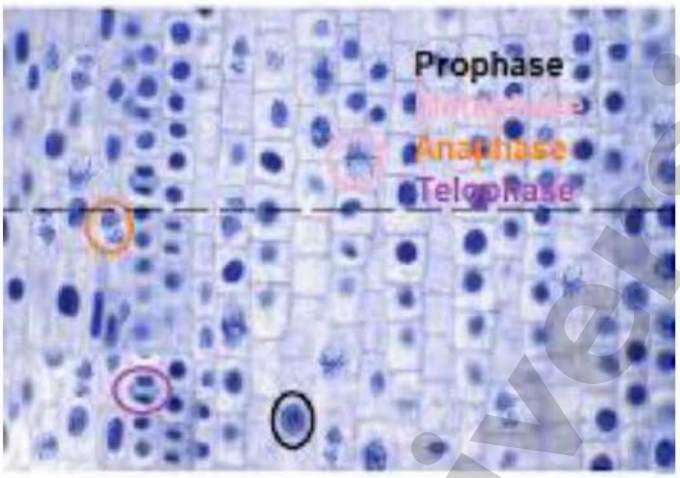
Data 2: Calculate Mitotic Index



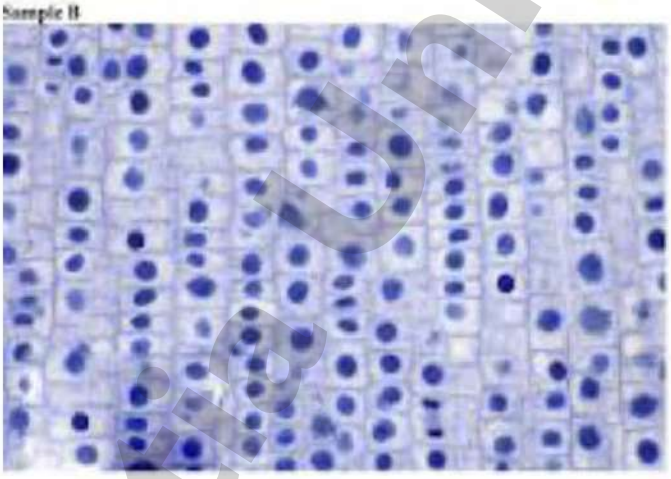
Calculation =

Data 3: Calculate Mitotic Index

Sample A



Sample B



Calculation =

Precaution:

Application:

Additional Details:

Practical 15: Study on sex linked inheritance in Drosophila

https://www.youtube.com/watch?v=H1HaR47Dqfw

Sex-linked traits are genetic characteristics determined by genes located on sex chromosomes. Genes are segments of DNA found on chromosomes that carry information for protein production and that are responsible for the inheritance of specific traits. Genes exist in alternative forms called alleles. One allele for a trait is inherited from each parent. Like traits originating from genes on autosomes (non-sex chromosomes), sex-linked traits are passed from parents to offspring through sexual reproduction. Sex-linked diseases are passed down through families through one of the X or Y chromosomes. X and Y are sex chromosomes.

Dominant inheritance occurs when an abnormal gene from one parent causes disease even though the matching gene from the other parent is normal. The abnormal gene dominates.

Recessive inheritance is when both matching genes must be abnormal to cause disease. If only one gene in the pair is abnormal, the disease does not occur, or is mild. Someone who has one abnormal gene (but no symptoms) is called a carrier. A carrier can pass this abnormal gene to his or her children.

The term "**sex-linked**" usually refers to **X-linked** traits.

Directions: Answer the questions below about sex linked traits.

1. X-linked (also called sex-linked traits) are inherited on the _____ chromosome.
2. Which gender (male or female) is most likely to have X-linked traits? _____
3. How many X chromosomes do males have? _____
4. How many X chromosomes do females have? _____
5. Why does a single X chromosome that carriers the allele for red-green colorblindness cause males
   to be color blind but doesn't cause females to be color blind?
6. What is a "carrier" for an X-linked trait?

Problems to be solved

1. In fruit flies, the gene for white eyes is sex-linked recessive. (R) is red and (r) is white. Cross a white-eyed female with a normal red-eyed male.
   a. What percent of the males will have red eyes? White eyes?
   b. What percent of the females will have red eyes? White eyes?

c. What **total percent** of the offspring will be white-eyed?

d. What **percent** of the offspring will be carriers of the white eye trait?

2. Using the same information as for question #1, cross a heterozygous red-eyed female with a red-eyed male.

a. What are the genotypes of each parent?

b. What **fraction** of the children will have red eyes?

c. What **fraction** of the children will have white eyes?

d. What **fraction** of the female children will carry the white eyed trait?

3. In humans, hemophilia is a sex-linked recessive trait. If a female who is a carrier for hemophilia marries a male with normal blood clotting, answer the following questions.

a. What fraction of the female children will have hemophilia?

b. What fraction of the female children will be carriers?

c. What fraction of the male children will have normal blood clotting?

d. What fraction of the male children will be carriers?

e. What fraction of the male children will have hemophilia?

a.

Practical 16: Study of DNA and RNA Models to know the 3D structure of Gene

https://youtu.be/7Hk9jct2ozY

DNA molecules are composed primarily of four chemicals: adenine (A), tyrosine (T), cytosine (C), and guanine (G). These four chemicals spell out the "words" in DNA that make up genes. DNA sequencing reveals the order of A, T, C, and G in a strand of DNA and has led to a deluge of information about the 1D sequence of DNA (*i.e.* the order of the letters). Technologies that have been recently developed now allow scientists to use sequencing to go beyond the 1D sequence and see the 3D structure of DNA as it actually exists within a nucleus. Research into the 3D structure of DNA has not only led to a new understanding of how genes organize themselves in 3D space, but also to the development of new medical applications like non-invasive prenatal testing for genetic diseases.

Project: Make your known DNA and RNA model and take a picture and paste

Additional Details:

Reference:

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Additional Sheet:

Additional Sheet:

Additional Sheet: