Quantitative Genetics

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Previously, we studied distinct and specific phenotypes (Qualitative traits) like tall stem and short stem pea plants. These traits have a small number of discrete phenotypes, or it means that they are showing discontinuous variation.

Now, we will study traits showing more variation (Quantitative traits), with a continuous range of phenotypes that cannot easily classify into distinct categories. Examples of these traits are human weight and height, meat and milk production in cattle and crop yield in plants. Continuous variations of a range of phenotypes are measured and described by quantitative measurement units such as distance, weight, volume, and etc...., so this genetic phenomenon is known as quantitative inheritance or genetics.

The multiple-gene hypothesis explaining the quantitative inheritance was based on experiments published by Nilsson-Ehle in 1909 , where he used grain color trait in his experiment by crossing the red color wheat with white color wheat. The first generation showed an intermediate color between the two parents. The second generation showed five categories of colors as below:

Red-coloured **White-colored grains**

P1: **AABB** X **aabb**

F1: **AaBb**

Intermediate color between red and white

P2: **AaBb** X **AaBb**

F2:

Additive alleles

4	3	2	1	0
1/16	4/16	6/16	4/16	1/16
AABB	2 AaBB 2 AABb	4 AaBb 1 AAbb 1 aaBB	2 Aabb 2 aaBb	aabb
4 dominant alleles	3 dominant alleles	Two dominant alleles	One dominant allele	No dominant allele

Red like the first parent ← A color more than the intermediate — Intermediate color — A color less than the intermediate → White like the second parent

Phenotypic ratio resmbling one of the parents = $\frac{1}{16} = \frac{1}{4^n}$

Number of genes= 2

Number of phenotypes= 2 n+1

Calculating number of gene pairs in quantitative inheritance

The first method: A table is used by which the frequency of both extremities of F2 that resemble the parent phenotype is calculated .

Phenotypic ratio resembling one parent	Number of gene pairs
$\frac{1}{4}$	1
$\frac{1}{16}$	2
$\frac{1}{64}$	3
$\frac{1}{256}$	4
$\frac{1}{1024}$	5

Example: Two varieties of wheat were crossed. The first having a grain weight of 30 gm and the other variety of 20 gm weight. F1 grain weight yield was of 25 gm and when the F1 plants were self-crossed, 4 of 254 plants in F2 generation had a grain weight of 30 gm. Determine the number of gene pairs controlling the trait.

Phenotypic ratio resmbling one of the parents = $\frac{4}{256} = \frac{1}{64} = \frac{1}{4^3} = \frac{1}{4^n}$

Number of genes= 3

The second method: Estimating number of genes by genetic differences in F2

Number of gene pairs can be found from the following equation

$$n = \frac{D^2}{8(\delta^2 P_{F2} - \delta^2 P_{F1})}$$

D: Difference between parents

n: Number of gene pairs

The contribution of each active allele can be found from

$$a = \frac{D}{2n}$$

a: **Contribution of each active allele**

Example: the following data represent the mean (average) and variance $ð^2$ of the spike length of two varieties of soft wheat, where the first filial generation resulted from the two parents and the second generation from self-cross of the first generation. Calculate a number of gene pairs controlling this trait.

Generations	Mean	variance
P1	17.146	3.560
P2	6.620	0.660
F1	12.10	2.30
F2	12.90	6.92

$$N = \frac{D^2}{8(\delta^2 P_{F2} - \delta^2 P_{F1})}$$

$$= \frac{(17.146-6.620)^2}{8(6.92-2.30)}$$

$$= \frac{(10.526)^2}{36.96}$$

$$= 3$$

Sex-linked

Inheritance

Sex-linked inheritance

Thomas H. Morgan demonstrated X-linked pattern of inheritance in Drosophila in 1910 when a white –eyed male appeared in a culture of wild type (red eyed) flies. The male was crossed with a wild type female. All of the offspring were wild type. When these F1 individuals were crossed with each other, their offspring fell into two categories: All the females were red but half the males were white-eyed. Ultimately, this was interpreted to mean that the white-eye locus was on the X-chromosome. In the figure below we denote the X-chromosome with white allele as X^w and X is chromosome with wild type allele. Y is the chromosome which does not have this locus.

Female, wild type **male, white eye**

P1: $X\,X$ $X^w\,Y$

G1: X $X^w,\ Y$

F1: $X\,X^w$ **+** $X\,Y$

Wild female **Wild male**

P2: $X\,X^w$ × $X\,Y$

G2: $X,\ X^w$ X,Y

F2: $X\,X$ **+** $X\,X^w$ **+** $X\,Y$ **+** $X^w\,Y$

Wild female **Wild female** **Wild male** **male, white eye**

The X-linked pattern has long been known as crisscross pattern of inheritance because the father passes a trait to his daughters, who pass it to their sons. This inheritance pattern is shown below in which a white-eyed female is crossed with a wild type male. Here the F1 males are white-eyed, the F1 females are wild type, and 50% of each sex in F2 generation is white-eyed.

	White eye, female		Wild type male
P1:	X^wX^w		$X\ Y$
G1:	X^w		$X\ ,\ Y$
F1:	$X\ X^w$	+	$X^w\ Y$
	Wild female		**White eye, male**

P2:	$X\ X^w$	×	$X^w\ Y$			
G2:	$X\ ,\ X^w$		X^w, Y			
F2:	X^wX^w	+	$X\ X^w$	+	$X\ Y$	+ $X^w\ Y$
	White eye, female		**Wild female**		**Wild male**	**male, white eye**

Examples of X-linked inheritance

Red-green color blindness in human (recessive, sex-linked trait):

	Normal (carrier) female		Affected male
P1:	X^cX		$X^c\,Y$
G1:	X , X^c		X^c , Y

F1:	$X^c\,X^c$	+	X^cX	+	$X^c\,Y$	+	$X\,Y$
	Affected female		Normal (carrier) female		Affected male		Normal male

	Normal (carrier) female		Affected male				
P1:	X^hX		$X^h Y$				
G1:	X , X^h		X^h , Y				
F1:	$X^h X^h$	+	X^hX	+	$X^h Y$	+	$X Y$
	Affected female		**Normal (carrier) female**		**Affected male**		**Normal male**

Inheritance pattern of barred plumage in chicken (Dominant, sex-linked)

Here the males are homogametic ZZ and the females are heterogametic ZW. The gene for barred plumage is Z-linked; barred plumage (Z^B) is dominant to non-barred plumage (Z^b).

	Barred male		Non-barred female
P1:	Z^BZ^B		Z^bW
G1:	Z^B		Z^b , W
F1:	Z^BZ^b	+	Z^BW
	Barred male		**Barred female**

P2:	Z^BZ^b	×	Z^BW				
G2:	Z^B, Z^b		Z^B,W				
F2:	Z^BZ^B	+	Z^BZ^b	+	Z^BW	+	Z^bW
	Barred male		**Barred male**		**Barred female**		**Non-barred female**

Q1: A normal chicken with non-barred plumage was mated with homozygous barred rooster. What are the resulting genotypes and phenotypes?

Q2: A man with normal vision married a normal woman whose father was affected with color blindness, what are the resulting genotypes and phenotypes?

Q3: A normal woman, whose father had hemophilia, marries a normal man. What are the resulting genotypes and phenotypes?

Q4: In chickens, the absence of barred feathers is due to a recessive allele. A barred rooster was mated with a nonbarred hen, and all the offspring were barred. These F1 chickens were intercrossed to produce F2 progeny, among which all the males were barred; half the females were barred and half were nonbarred. Are these results consistent with the hypothesis that the gene for barred feathers is located on one of the sex chromosomes?

Q5: In *Drosophila*, yellow body is due to an X-linked gene that is recessive to the gene for gray body.
a. A homozygous gray female is crossed with a yellow male. The F1 are intercrossed to produce F2. Give the genotypes and phenotypes, along with the expected proportions, of the F1 and F2 progeny.

b. A yellow female is crossed with a gray male. The F1 are intercrossed to produce the F2. Give the genotypes and phenotypes, along with the expected proportions, of the F1 and F2 progeny.

Q6: Chickens, like all birds, have ZZ-ZW sex determination. The bar-feathered phenotype in chickens results from a Z-linked allele that is dominant over the allele for nonbar feathers. A barred female is crossed with a nonbarred male. The F1 from this cross are intercrossed to produce the F2. What will the phenotypes and their proportions be in the F1 and F2 progeny?

Q7. In Drosophila, the wild-type eye color is red (X), and white eye color is recessive (X^w). If a white-eyed female Drosophila is crossed with a red-eyed male Drosophila, what will be the eye color of the F1 offspring?

Q8. In Drosophila, the wild-type eye color is red (X), and white eye color is recessive (X^w). In a cross between a heterozygous red-eyed female (XX^w) and a red-eyed male (XY), what will be the eye color of the F1 offspring?

Practical Genetics

Lab 12: Pedigree Analysis

A pedigree is a diagram of family relationships that uses symbols to represent people and lines to represent genetic relationships. Pedigrees are often used to determine the mode of inheritance of genetic diseases. The circles represent females and the squares represent males. Symbols that are filled in represent individuals who have the trait under the study and said to be affected. The open symbols represent those who do not have the trait. The direct horizontal line between one male and one female is called marriage line. Children are attached to a marriage line by vertical line. All the brothers and sisters from the same parents are connected by a horizontal line above their symbols and numbered according to birth order. Generations are numbered by Roman numerals.

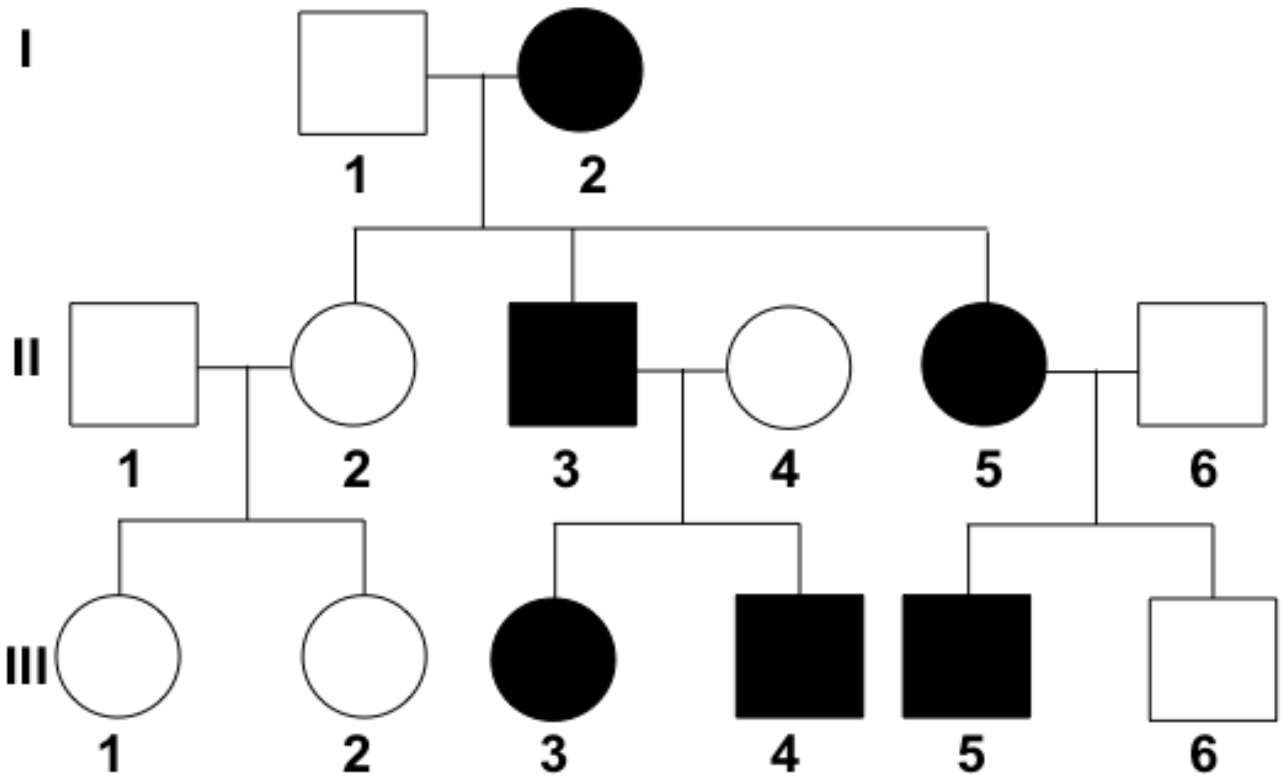


Figure 1: An example of pedigree

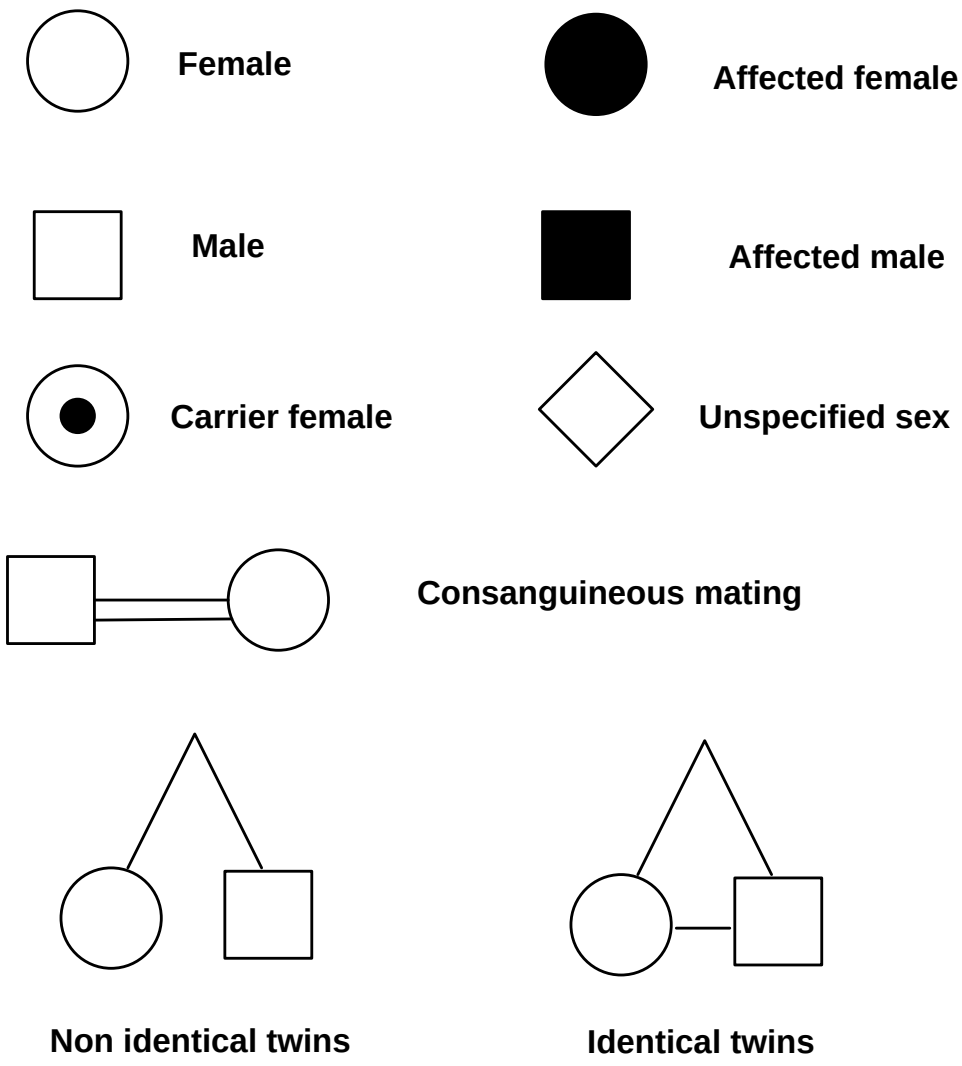


Figure 2: Symbols used in pedigrees

Autosomal recessive inheritance (e.g. albinism)

1. Trait often skips generations.
2. There should be almost equal number of affected males and females.
3. Traits often found in pedigrees with consanguineous matings.
4. If both parents are affected, all children should be affected.
5. In most cases of unaffected people mating with affected individuals, all children produced are unaffected. When at least on child is affected (indicating that unaffected parent is heterozygous), then approximately half the children should be affected.
6. Most affected individuals have unaffected parents. Figure 3.

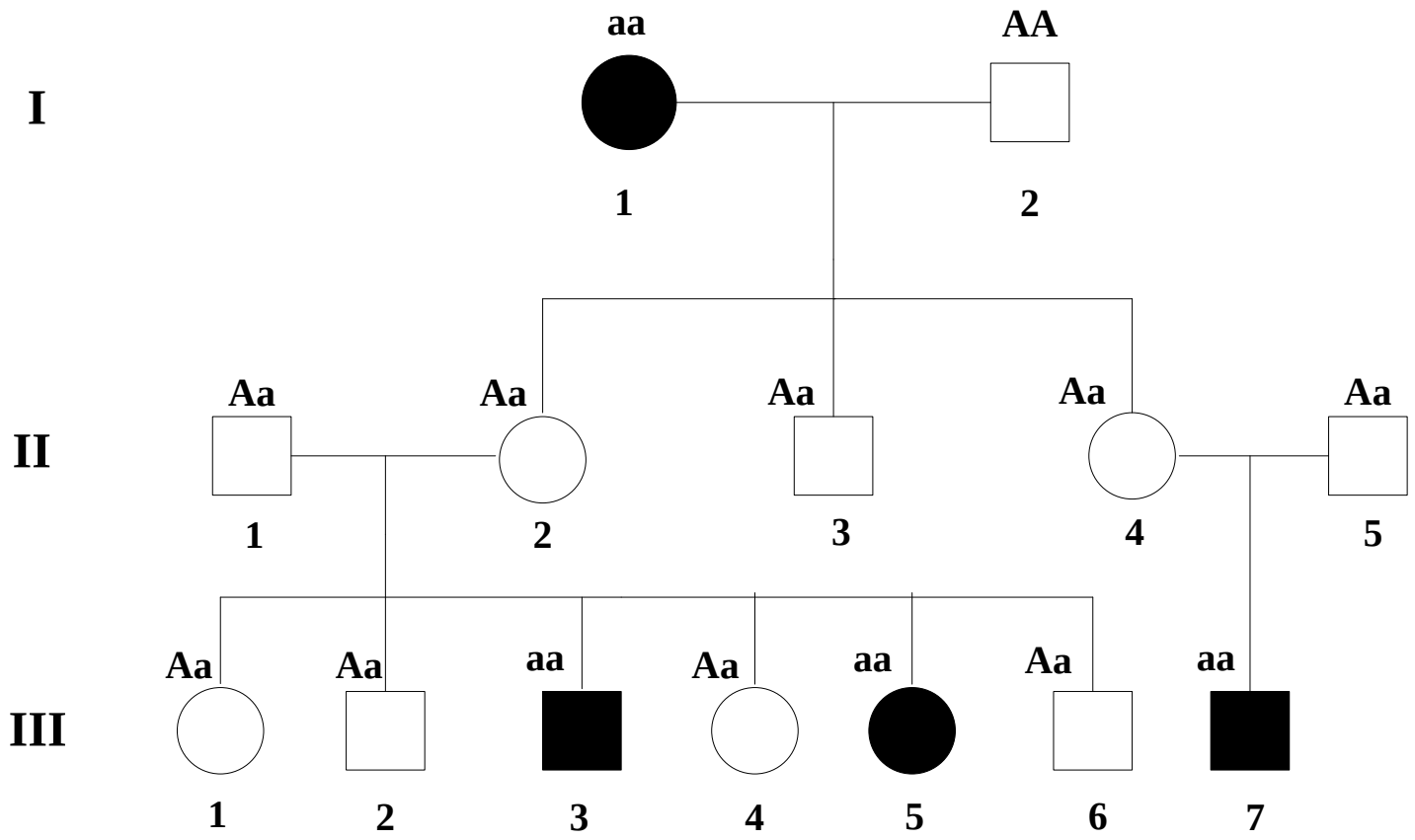


Figure 3: A pedigree of albinism, an autosomal recessive trait.

Autosomal dominant inheritance (e.g. PTC tasting)

1. Usually trait should not skip generations (unless penetrance is reduced).
2. All affected persons mating with unaffected persons should produce approximately 50% affected offspring (indicating also that the affected individual is heterozygous).
3. Distribution of the trait among sexes should be almost equal. Figure 4.

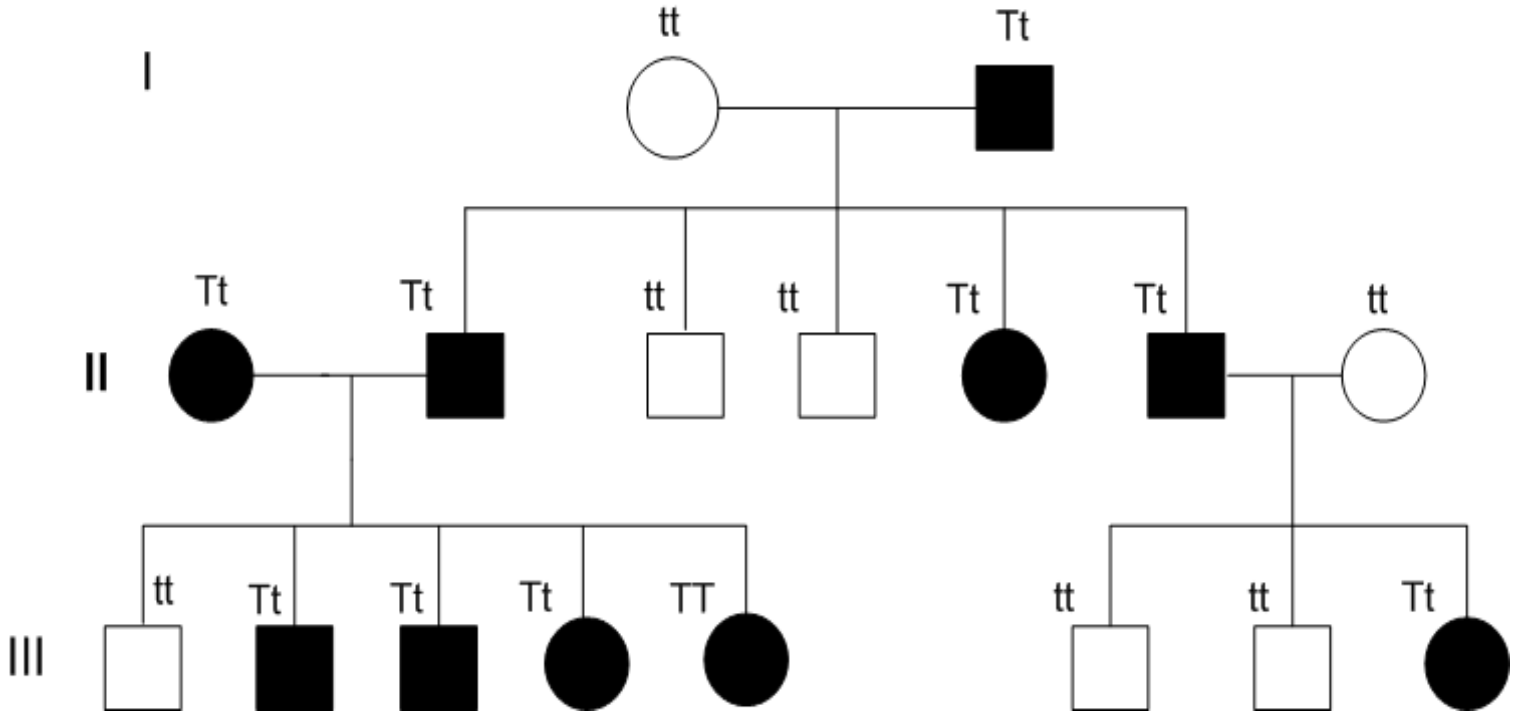


Figure 4: A pedigree of PTC tasting, an autosomal dominant trait.

Sex-linked recessive inheritance (Color blindness)

1. Most affected individuals are males.
2. Affected males result from mothers who are affected or who are known to be carriers (heterozygous) by having affected brothers, fathers or maternal uncles.
3. Affected females come from affected fathers and affected or carrier mothers.
4. The sons of affected females should be affected.
5. Approximately half the sons of carrier females should be affected. Figure 5.

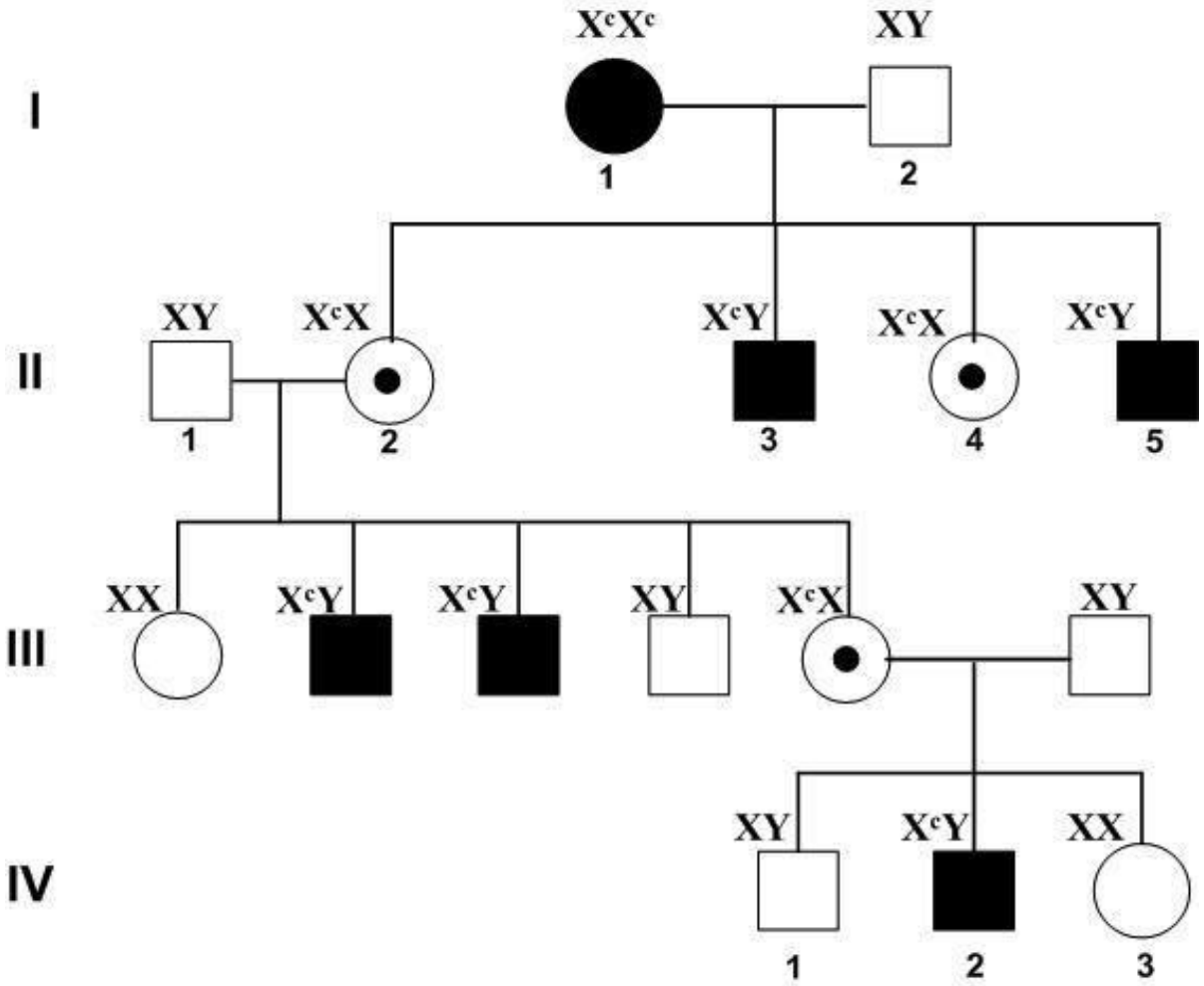


Figure 5: A pedigree of color blindness, a sex-linked recessive trait.

Sex-linked dominant inheritance

(vitamin-D- resistant rickets)

1. The trait does not skip generations.
2. Affected males must come from affected mothers.
3. Approximately half the children of an affected heterozygous female are affected.
4. Affected females come from affected mothers or fathers.
5. All the daughters, but none of the sons, of an affected father are affected. Figure 6.

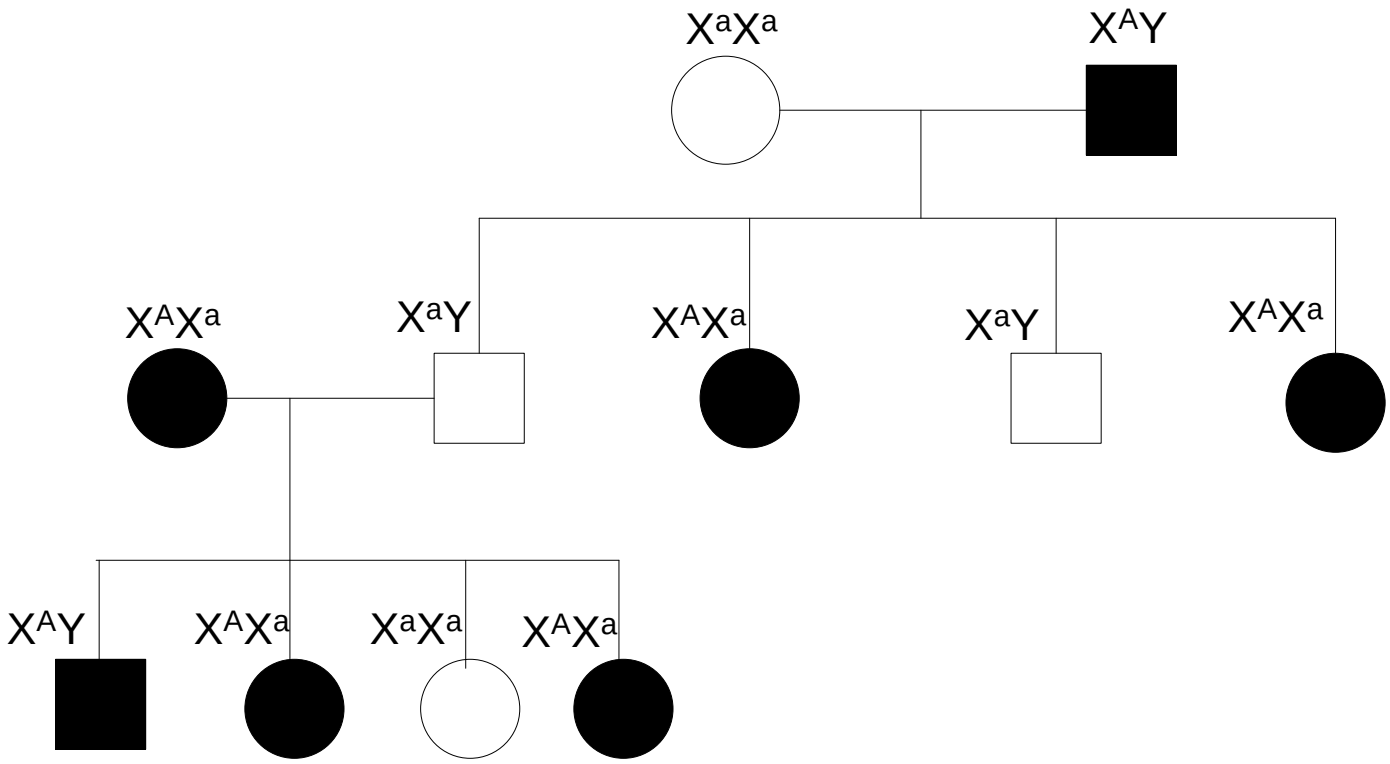


Figure 6: A pedigree of vitamin-D resistant rickets, a sex-linked dominant trait.

Codominance and Multiple Alleles

Codominance

The joint expression of both alleles in heterozygous state is called codominance such as MN blood groups and is characterized by an antigen called a glycoprotein found on the surface of red blood cells. In human population, two forms of this glycoprotein exist, designated M and N; an individual may exhibit either one or both of them and the two alleles designated L^M an L^N. Three combinations are possible:

Genotype	Phenotype (blood group)
$L^M L^M$	M
$L^M L^N$	MN
$L^N L^N$	N

P1: $L^M L^N \times L^M L^N$

G1: L^M, L^N L^M, L^N

F1: $L^M L^M + L^M L^N + L^M L^N + L^N L^N$

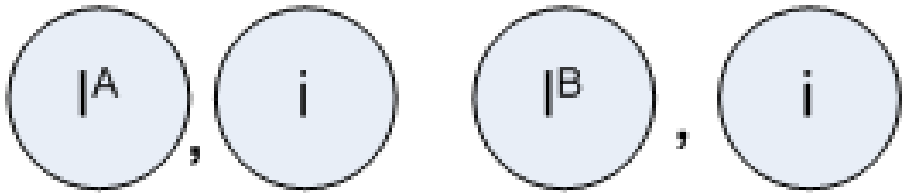
Multiple alleles

ABO blood groups in human

A gene may have more than two alleles. The gene for the ABO blood types has three alleles: I^A, I^B and i. Allele I^A give rise to blood type A by specifying an enzyme that add sugar A, I^B results in blood type B by specifying an enzyme that add sugar B. i does not produce a functional sugar-adding enzyme. Alleles I^A and I^B are codominant to each other and both are dominant to i: $I^A = I^B > i$. There are six possible genotypes as below:

Genotype	Phenotype (blood group)
$I^A I^A$, $I^A i$	A
$I^B I^B$, $I^B i$	B
$I^A I^B$	AB
ii	O

P1: $I^A i$ x $I^B i$

G1: 

F1: $I^A I^B$ + $I^A i$ + $I^B i$ + ii

AB A B O

Coat color in rabbits

The gene controlling coat color C has four alleles: c (albino) , C^h (himalayan), C^{ch} (chinchilla) and C (wild type). In homozygous condition each allele has a characteristic effect on the coat color. cc: white hairs over the entire body. C^hC^h black hair on the extremities but white hairs everywhere else. C^{ch} C^{ch}: white hairs with black tips on the body. CC: colored hairs over the entire body (agouti). The wild type allele is completely dominant over all the other alleles but the chinchilla is partially dominant over the himalayan and albino alleles. The himalayan allele is completely dominant over the albino allele. These dominant relations can be summarized as C> C^{ch} > C^h > c. The genotypes and phenotypes as follows:

Genotype	Phenotype
$C C^{ch}$, $C C^{h}$, Cc	wild type
$C^{ch} C^{ch}$	chinchilla
$C^{ch} C^{h}$	light chinchilla with black tips
C^{ch} c	light chinchilla
$C^{h} C^{h}$, C^{h} c	himalayan
cc	albino

P1: $C\ C^{ch}$ x $C^{h}\ c$

G1: C , C^{ch} C^{h} , c

F1: $C\ C^{h}$ + Cc + $C^{ch}\ C^{h}$ + $C^{ch}\ c$

Wild type | Wild type | Light chinchilla with black tips | Light chinchilla

Problems:

Q1: what are the resulting genotypes and phenotypes of mating a man with AB blood group with a woman with O blood group?

Q2: what are the resulting genotypes and phenotypes of mating a man with M blood group with a woman with N blood group?

Q3: Regarding coat color in rabbit, what are the resulting genotypes and phenotypes of mating the following? C^{ch} c × CC , Cc × C^{h}c , Cc × CC^{h} , Cc × C^{ch} C^{h}

Practical Genetics

Isolation of DNA

Lab 16: Purification of DNA from Living Cells

The fundamentals of DNA preparation are most easily understood by first considering the simplest type of DNA purification procedure, that where the entire DNA of a bacterial cell is required. The procedure for total DNA preparation from a culture of bacterial cells can be divided into four stages (Figure 1):

1 A culture of bacteria is grown and then **harvested**.

2 The cells are broken open to release their contents.

3 This **cell extract** is treated to remove all components except the DNA.

4 The resulting DNA solution is concentrated.

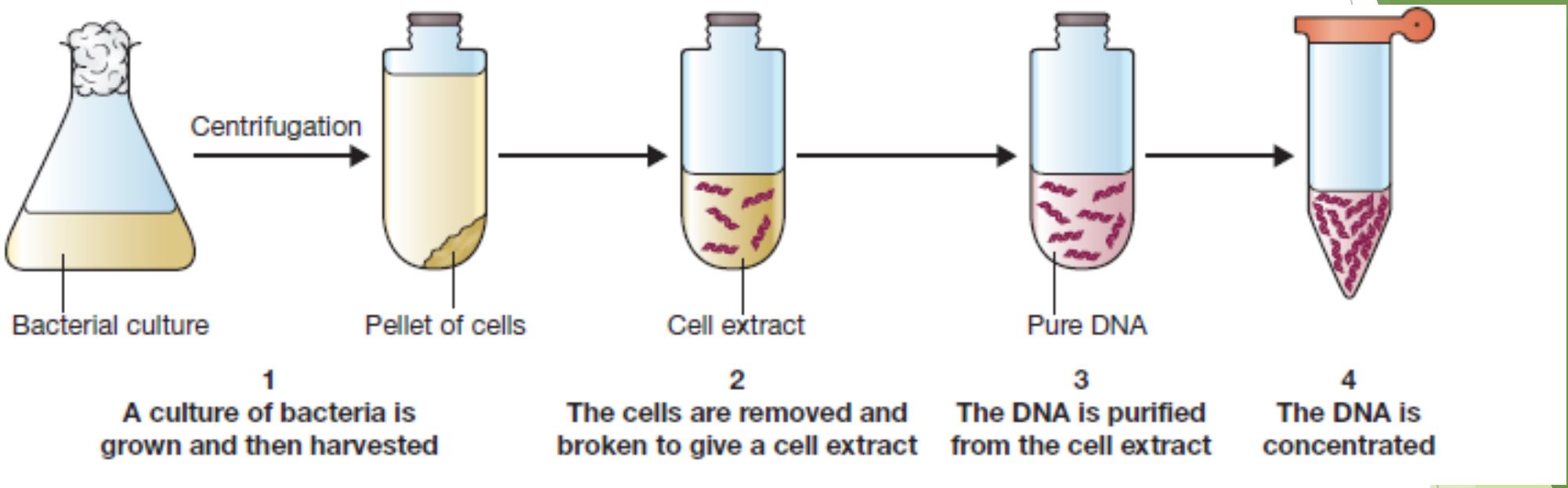


Figure 1: The basic steps in preparation of total cell DNA from a culture of bacteria

Growing and harvesting a bacterial culture

Most bacteria can be grown without too much difficulty in a liquid medium (**broth culture**). The culture medium must provide a balanced mixture of the essential nutrients at concentrations that will allow the bacteria to grow and divide efficiently. Luria-Bertani (LB) is a complex or **undefined medium**, meaning that the precise identity and quantity of its components are not known. This is because two of the ingredients, tryptone and yeast extract, are complicated mixtures of unknown chemical compounds. Tryptone supplies amino acids and small peptides, while yeast extract (a dried preparation of partially digested yeast cells) provides the nitrogen requirements, along with sugars and inorganic and organic nutrients.

In LB medium at 37°C, aerated by shaking at 150–250 rpm on a rotary platform, *E. coli* cells divide once every 20 min or so until the culture reaches a maximum density of about 2–3 × 10^9 cells/ml. The growth of the culture can be monitored by reading the optical density (OD) at 600 nm, at which wavelength 1 OD unit corresponds to about 0.8 × 10^9 cells/ml.

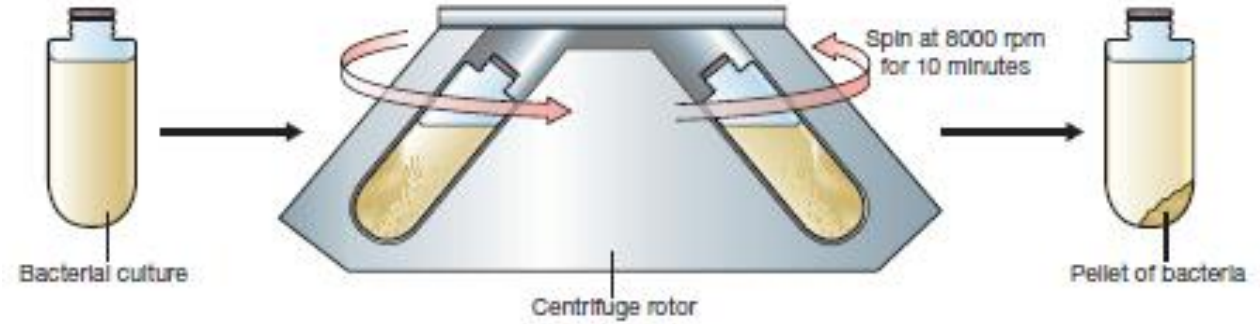


Figure 2: Harvesting bacteria by centrifugation

Preparation of a cell extract

The bacterial cell is enclosed in a cytoplasmic membrane and surrounded by a rigid cell wall. With some species, including *E. coli*, the cell wall may itself be enveloped by a second, outer membrane. All of these barriers have to be disrupted to release the cell components. By chemical methods, cell lysis is brought about by exposure to chemical agents that affect the integrity of the cell barriers. Chemical methods are most commonly used with bacterial cells when the object is DNA preparation.

Chemical lysis generally involves one agent attacking the cell wall and another disrupting the cell membrane. The chemicals that are used depend on the species of bacterium involved, but with *E. coli* and related organisms, weakening of the cell wall is usually brought about by **lysozyme**, ethylenediamine tetraacetate (EDTA), or a combination of both. Lysozyme is an enzyme that is present in egg white and in secretions such as tears and saliva, and which digests the polymeric compounds that give the cell wall its rigidity. EDTA removes magnesium ions that are essential for preserving the overall structure of the cell envelope, and also inhibits cellular enzymes that could degrade DNA.

Under some conditions, weakening the cell wall with lysozyme or EDTA is sufficient to cause bacterial cells to burst, but usually a detergent such as sodium dodecyl sulphate (SDS) is also added. Detergents aid the process of lysis by removing lipid molecules and thereby cause disruption of the cell membranes. Having lysed the cells, the final step in preparation of a cell extract is removal of insoluble cell debris. Components such as partially digested cell wall fractions can be pelleted by centrifugation, leaving the cell extract as a reasonably clear supernatant.

Purification of DNA from a cell extract

In addition to DNA, a bacterial cell extract contains significant quantities of protein and RNA. To remove contaminants organic extraction and enzyme digestion method is used. The standard way is to deproteinize a cell extract by adding a 1 : 1 mixture of phenol and chloroform. These organic solvents precipitate proteins but leave the nucleic acids (DNA and RNA) in aqueous solution. The result is that if the cell extract is mixed gently with the solvent, and the layers then separated by centrifugation, precipitated protein molecules are left as a white coagulated mass at the interface between the aqueous and organic layers. The aqueous solution of nucleic acids can then be removed with a pipette.

With some cell extracts the protein content is so great that a single phenol extraction is not sufficient to completely purify the nucleic acids. This problem could be solved by carrying out several phenol extractions one after the other, but this is undesirable as each mixing and centrifugation step results in a certain amount of breakage of the DNA molecules. The answer is to treat the cell extract with a **protease** such as proteinase K before phenol extraction. These enzymes break polypeptides down into smaller units, which are more easily removed by phenol.

Some RNA molecules, especially **messenger RNA (mRNA)**, are removed by phenol treatment, but most remain with the DNA in the aqueous layer. The only effective way to remove the RNA is with the enzyme **ribonuclease**, which rapidly degrades these molecules into ribonucleotide subunits.

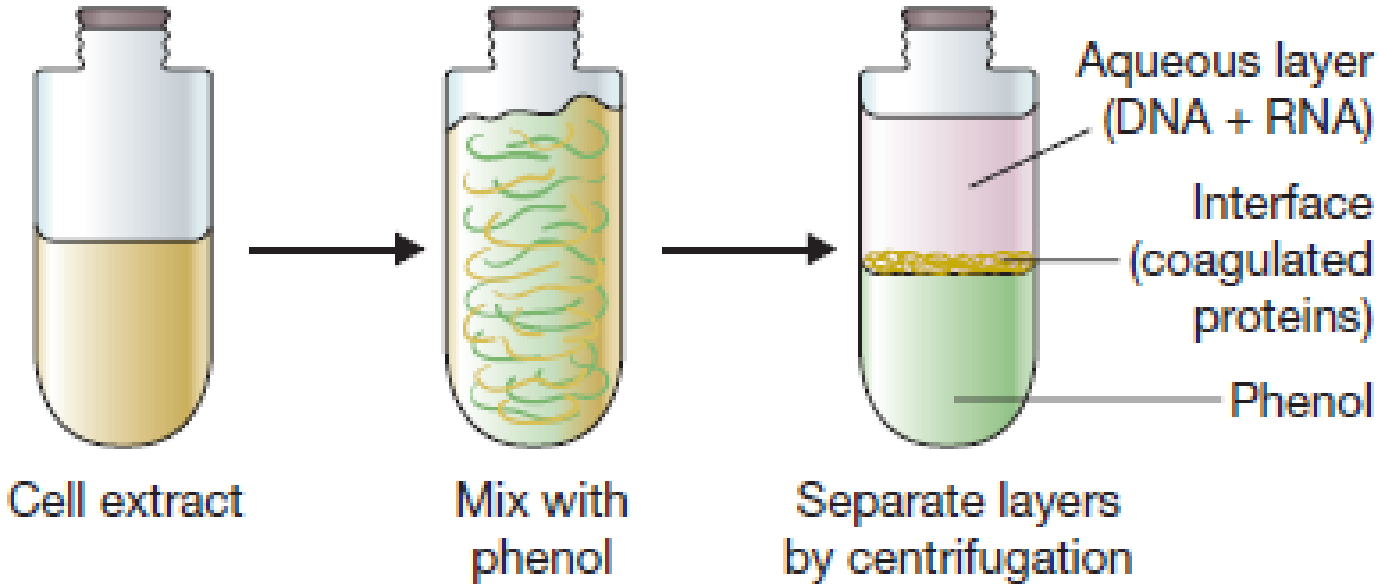


Figure 3: Removal of protein contaminants by phenol extraction

These treatments may not, however, be sufficient to give pure DNA if the cells also contain significant quantities of other biochemicals. Plant tissues are particularly difficult in this respect as they often contain large amounts of carbohydrates that are not removed by phenol extraction. Instead a different approach must be used. One method makes use of a detergent called cetyltrimethylammonium bromide (CTAB), which forms an insoluble complex with nucleic acids. When CTAB is added to a plant cell extract the nucleic acid–CTAB complex precipitates, leaving carbohydrate, protein, and other contaminants in the supernatant. The precipitate is then collected centrifugation and resuspended in 1 M sodium chloride, which causes the complex to break down. The nucleic acids can now be concentrated by ethanol precipitation and the RNA removed by ribonuclease treatment.

Concentration of DNA samples

Purification methods may give dilute solutions and it is therefore important to consider methods for increasing the DNA concentration. The most frequently used method of concentration is **ethanol precipitation**. In the presence of salt (strictly speaking, monovalent cations such as sodium ions (Na^+)), and at a temperature of −20°C or less, absolute ethanol efficiently precipitates polymeric nucleic acids.

A spectacular trick is to push a glass rod through the ethanol into the DNA solution. When the rod is removed, DNA molecules adhere and can be pulled out of the solution in the form of a long fiber (Figure 5). Alternatively, if ethanol is mixed with a dilute DNA solution, the precipitate can be collected by centrifugation (Figure 4), and then redissolved in an appropriate volume of water. Ribonucleotides produced by ribonuclease treatment are therefore lost at this stage.

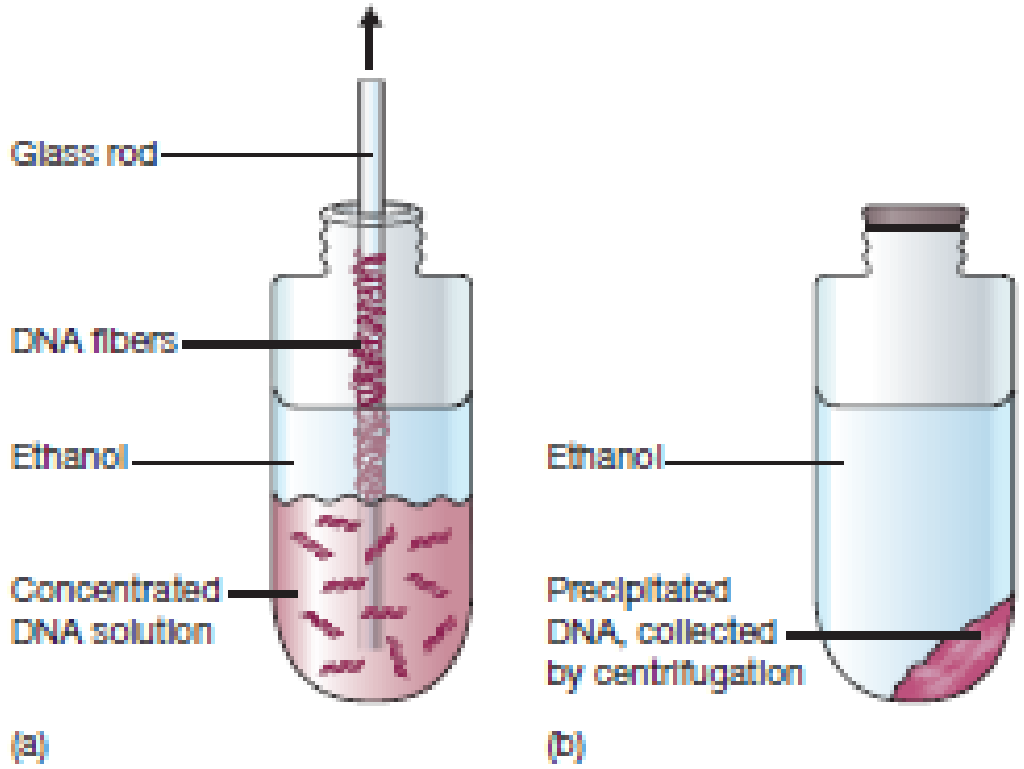


Figure 4: Collecting DNA by ethanol precipitation. (a) Absolute ethanol is layered on top of a concentrated solution of DNA. Fibers of DNA can be withdrawn with a glass rod. (b) For less concentrated solutions ethanol is added (at a ratio of 2.5 volumes of absolute ethanol to 1 volume of DNA solution) and precipitated DNA collected by centrifugation.

Measurement of DNA concentration

Fortunately DNA concentrations can be accurately measured by **ultraviolet (UV) absorbance spectrophotometry**. The amount of UV radiation absorbed by a solution of DNA is directly proportional to the amount of DNA in the sample. Usually absorbance is measured at 260 nm, at which wavelength an absorbance (A260) of 1.0 corresponds to 50 mg of double-stranded DNA per ml.

Ultraviolet absorbance can also be used to check the purity of a DNA preparation. With a pure sample of DNA, the ratio of the absorbances at 260 and 280 nm (A260/A280) is 1.8. Ratios of less than 1.8 indicate that the preparation is contaminated, either with protein or with phenol.

Procedure:

Preparation of Genomic DNA from Bacteria

Materials

- TE buffer (pH 8.0), 10mM Tris-HCl containing 1mM EDTA•Na2
- 10% sodium dodecyl sulfate (SDS)
- 20 mg/ml proteinase K (stored in small single-use aliquots at -20°C)
- 5 M NaCl
- CTAB/NaCl solution
- 24:1 chloroform/isoamyl alcohol
- 25:24:1 phenol/chloroform/isoamyl alcohol
- Isopropanol
- 70% ethanol

1. Inoculate a 5-ml liquid culture with the bacterial strain of interest.

2. Spin 1.5 ml of the culture in a microcentrifuge for 2 min, or until a compact pellet forms. Discard the supernatant.

3. Resuspend pellet in 567 µl TE buffer by repeated pipetting. Add 30 µl of 10% SDS and 3 µl of 20 mg/ml proteinase K to give a final concentration of 100 µg/ml proteinase K in 0.5% SDS. Mix thoroughly and incubate 1 hr at 37°C.

The solution should become viscous as the detergent lyses the bacterial cell walls. There should be no need to predigest the bacterial cell wall with lysozyme.

4. Add 100 µl of 5 M NaCl and mix thoroughly.

This step is very important since a CTAB–nucleic acid precipitate will form if salt concentration drops below about 0.5 M at room temperature . The aim here is to remove cell wall debris, denatured protein, and polysaccharides complexed to CTAB, while retaining the nucleic acids in solution.

5. Add 80 µl of CTAB/NaCl solution. Mix thoroughly and incubate 10 min at 65°C.

6. Add an approximately equal volume (0.7 to 0.8 ml) of chloroform/isoamyl alcohol,mix thoroughly, and spin 4 to 5 min in a microcentrifuge.

This extraction removes CTAB–protein/polysaccharide complexes. A white interface should be visible after centrifugation.

7. Remove aqueous, viscous supernatant to a fresh microcentrifuge tube, leaving theinterface behind. Add an equal volume of phenol/chloroform/isoamyl alcohol, extract thoroughly, and spin in a microcentrifuge for 5 min.

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8. Transfer the supernatant to a fresh tube. Add 0.6 vol isopropanol to precipitate the nucleic acids. Shake the tube back and forth until a stringy white DNA precipitate becomes clearly visible. The precipitate can be pelleted by spinning briefly at room temperature.

9. Wash the DNA with 70% ethanol to remove residual CTAB and respin 5 min at room temperature to repellet it. Carefully remove the supernatant and briefly dry the pellet in a lyophilizer.

10. Redissolve the pellet in 100 μl TE buffer.

References

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