LABORATORY

Human Genetic Traits

15

OVERVIEW

All people are recognizably human, but no one is exactly like anyone else, not even an identical twin. The basis for the similarity and the reasons for the diversity that coexist in all species have puzzled and intrigued people for thousands of years. Recently, experiments with a wide variety of plants, animals, and microorganisms have yielded detailed knowledge of how traits are passed from one generation to the next, how genetic information is decoded and expressed during development, and how genetic variability can account for gradual evolution.

STUDENT PREPARATION

To prepare for this laboratory, read the text pages indicated by your instructor. Familiarizing yourself in advance with the information and procedures covered in this laboratory will give you a better understanding of the material and improve your efficiency. Work through Exercise A before coming to the laboratory.

EXERCISE A Human Cytogenetics

Many human hereditary defects caused by chromosomal abnormalities may be identified by examining human chromosomes from cells that have been arrested in metaphase of mitosis—a stage when chromosomes are very short and compact. Leukocytes (white blood cells) or fetal cells obtained by amniocentesis or chorionic villus sampling are often used for diagnosis.

The cells are cultured (to increase their number), treated with a chemical that disrupts the mitotic spindle apparatus, and placed in a hypotonic salt solution to swell their nuclei. The mixture is then centrifuged (to increase the concentration of cells) and transferred to a glass slide. As a drop of the cell

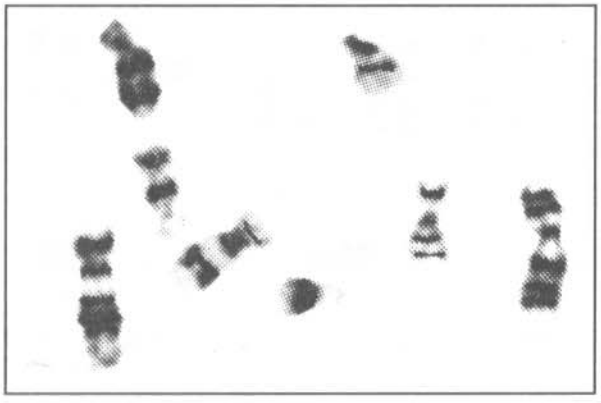


Figure 15A-1 *G-banded chromosomes. This pattern is produced by using Giemsa stain. Bands do not represent genes, but they do serve as markers for locating genes and gene families.*

suspension hits the slide, the nuclei break open and the chromosomes spread apart; usually chromosomes from a single cell remain in an identifiable group. The cells are then stained, sometimes using special procedures that result in banded chromosomes (Figure 15A-1).

The "metaphase spread" produced by a single cell is then photographed. The photograph can be cut apart and homologous chromosomes can be arranged in pairs according to size, location of the centromeres, and length of the chromosome arms. Chromosome pairs are arranged in a specific order and labeled. The result is called a **karyotype.**

Objectives

- ☐ Match and order pairs of human chromosomes to make a karyotype.

Procedure

1. Figure 15A-2 is a diagrammatic representation of G-banded human chromosomes (only one chromosome from each homologous pair is represented). Figure 15A-3b (page 15-20) is a metaphase preparation of banded chromosomes. Cut out these chromosomes and match them to the chromosomes shown in Figure 15A-2. Match homologous chromosomes by size, length of arms, and location of the centromere. Place the homologous pairs together above corresponding numbers in Figure 15A-3a (page 15-3). A sample karyotype, Figure 15A-4, will also assist you in matching chromosome pairs by size and banding.
2. Note that the *X* chromosome has a single thick band on its upper end and four bands on its lower end. The *Y* chromosome is very small and has a single thick band on the tips of its arms at one end.
3. Once you have matched the chromosomes, tape them onto the blank karyotype sheet (Figure 15A-3a).

   a. Are these chromosomes from a male or a female? _____

Bring the completed karyotype with you to the laboratory.

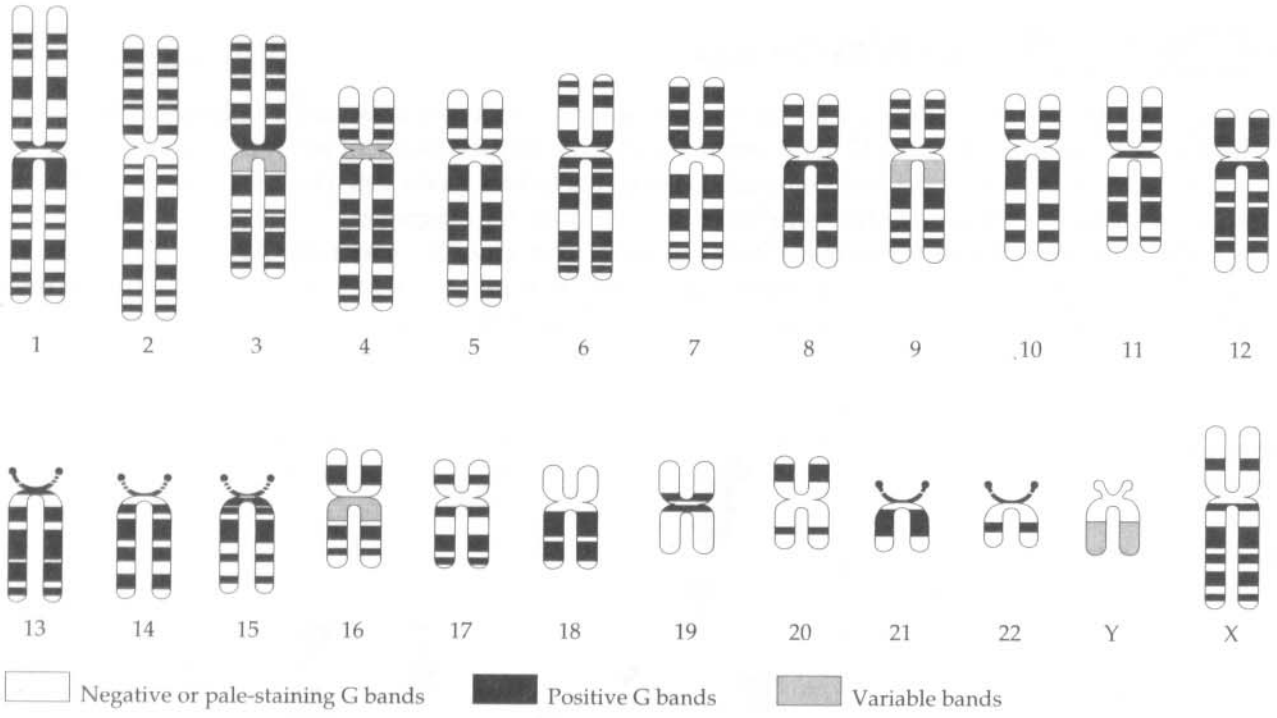


Figure 15A-2 *Diagram of G-banded human chromosomes.*

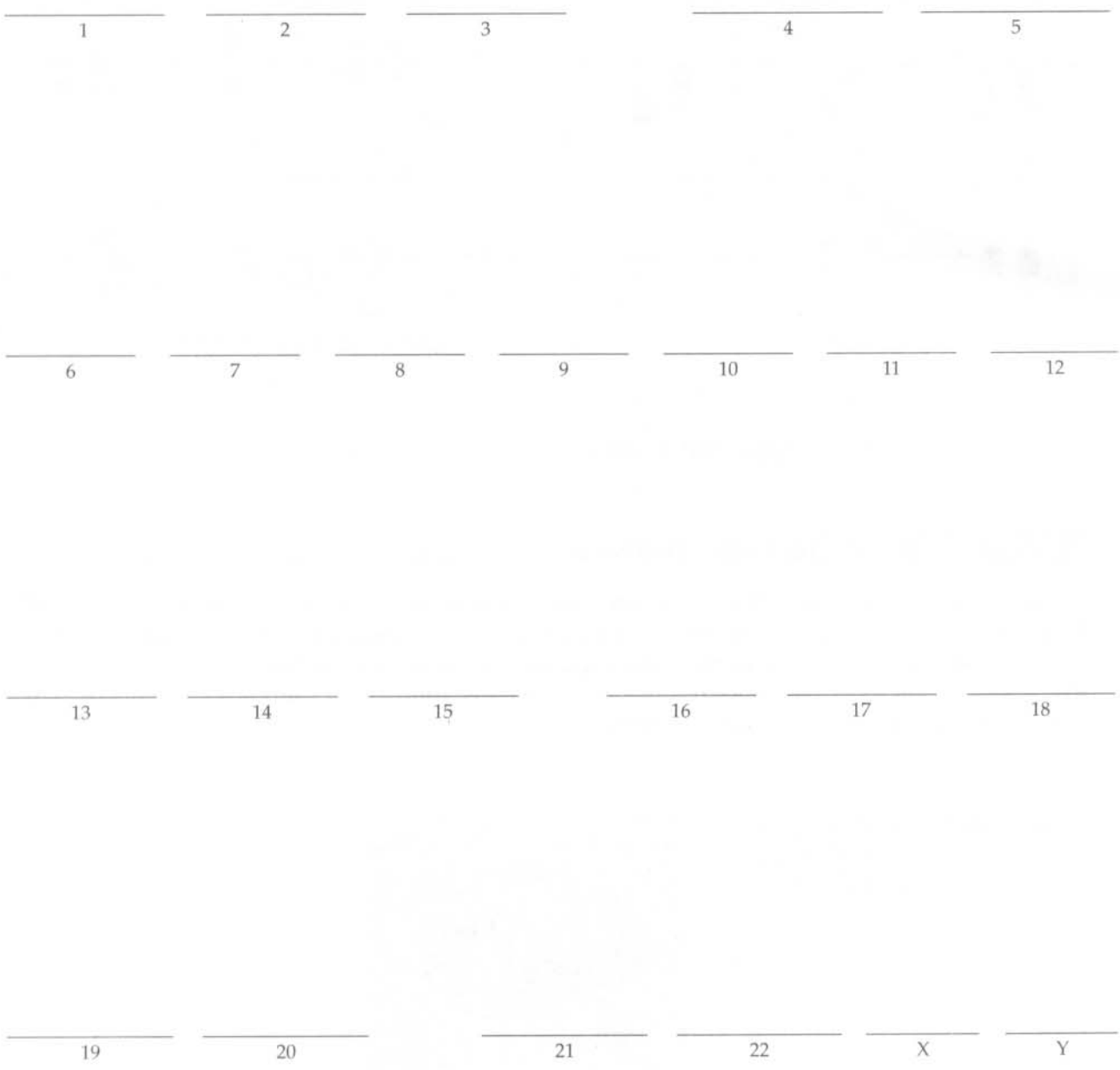


Figure 15A-3a *Match homologous chromosome pairs from Figure 15A-3b (page 15-20) and attach them in appropriate spaces above. Use the karyotype (Figure 15A-4) to help you match the pairs by size and banding patterns.*

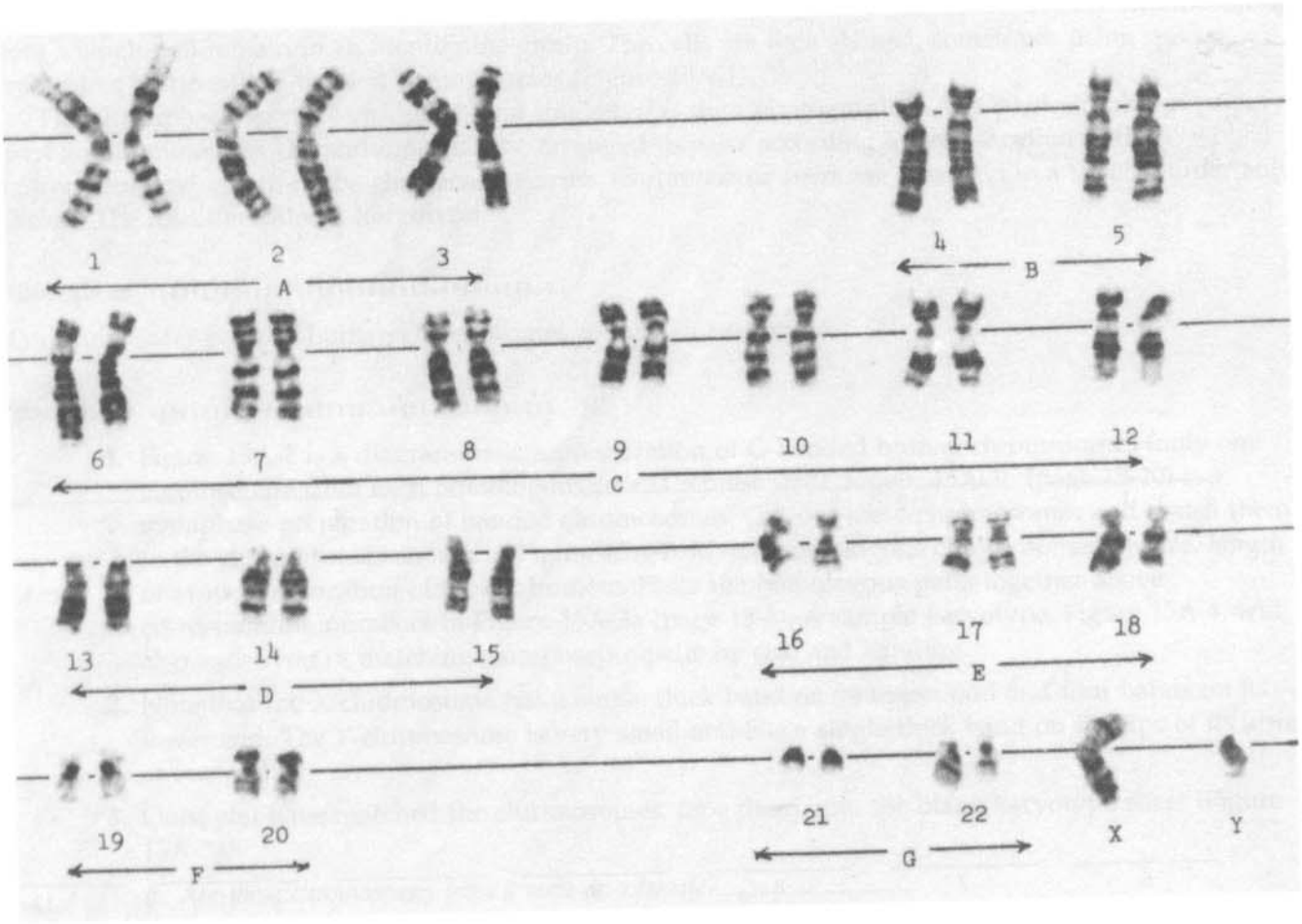
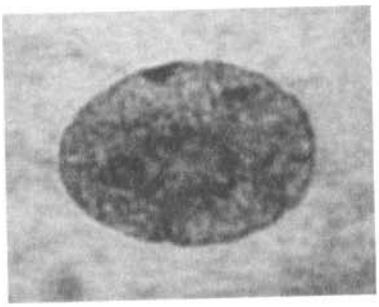


Figure 15A-4 *Karyotype of G-banded human chromosomes.*

EXERCISE B X and Y Chromosomes

An individual's sex chromosomes can be identified more simply than by karyotyping. Cells scraped from the inside of the mouth can be stained with a dye specific for DNA. If the individual is female, a darkly staining **Barr body,** or sex-chromatin body, appears near the nuclear membrane (Figure 15B-1). This structure is one of the *X* chromosomes that has condensed; only one *X* chromosome remains active in females. No such body is found in male cells.

Figure 15B-1 *Part of a cell from the squamous epithelium of a female, showing nucleus with Barr body (top).*



a. How many X chromosomes are found in male cells? _____

Both males and females ultimately have just one active *X* chromosome, which provides the correct amount of *X* chromosome products required for the normal functioning of cells in both males and females. In cases where more than two *X* chromosomes are present (see Exercise D, Part 1), the number of Barr bodies in a cell is $N - 1$, where N is the number of chromosomes in the cell.

While Barr-body stains reveal the number of *X* chromosomes present, they do not reveal anything about the presence or number of *Y* chromosomes. Instead, a special stain, acridine orange, is used to stain the *Y* chromosome, which then stands out as a very bright fluorescent structure in the cell.

Objectives

- ☐ Describe how, without karyotyping, an individual's sex chromosomes composition can be determined.

Procedure

Examine the two slides on demonstration. Both slides show cheek scrapings, one from a female and the other from a male. In the space provided below, draw what you see on these slides. Determine which slide shows tissue from a female and which shows tissue from a male.

EXERCISE C Mendelian Inheritance in Humans

PART I Monohybrid Crosses in Humans

Several human traits may be used to demonstrate Mendel's law of segregation. The traits you will study are controlled by a single gene that can occur in two forms (alleles). One allele is dominant, the other is recessive. A person's phenotype is determined by the combination of alleles present. An individual can be homozygous dominant, heterozygous, or homozygous recessive.

Objectives

- ☐ Describe the genetic basis for several human traits evident in the classroom population.
- ☐ Determine the extent to which individuals are different from one another based on the presence or absence of selected genetic traits.

Procedure

For each of the following 11 traits, determine your phenotype and enter it in Table 15C-1. Based on your phenotype, record your possible genotypes for each of the traits. Remember that if you have a recessive characteristic you must have both recessive alleles, but if you have a dominant characteristic you may be either homozygous dominant or heterozygous. If you have a dominant phenotype, you may have no way of knowing whether you carry a recessive allele. In this case, use a dash (—) to represent the unknown second gene.

With the help of your laboratory instructor, compile the data for your section. Calculate the percentages for each trait.

1. **Dimpled chin.** A cleft in the chin is a dominant trait. (*D*, allele for dimpling; *d*, allele for absence of a dimple.)
2. **Free ear lobe.** In most people the ear lobes hang free (dominant allele, *E*), but in a person with two recessive alleles (*e*), the ear lobes are attached directly to the head. Use a mirror or the opinion of your classmates to determine your phenotype.

3. **Widow's peak.** The action of the dominant allele (*W*) results in a hairline that forms a distinct point, known as a widow's peak, in the center of the forehead. The recessive allele (*w*) produces a continuous hairline. Omit this tabulation if a gene for baldness has had some effect on your hairline.
4. **Ability to taste PTC.** Some persons detect a distinct bitter taste in small concentrations of the chemical phenylthiocarbamide (PTC), while others do not taste it. A dominant allele *T* confers the ability to taste this chemical; those who are homozygous for the recessive allele *t* are nontasters. Place a PTC paper strip on your tongue and allow it to remain there for about 10 seconds. If you are a taster you will know it. If you have any doubt about your ability to taste the substance, you are a nontaster.
5. **Interlocking fingers.** When the fingers are interlocked, some people will almost invariably place the left thumb on top of the right (dominant allele *F*), whereas others will place the right over the left (recessive allele *f*).
6. **Bent little finger.** A dominant allele *B* causes the last joint of the little finger to bend inward toward the fourth finger (*b* is the recessive allele for a straight finger). Lay both hands flat on the table, relax your muscles, and note whether you have a bent or a straight little finger.
7. **Hitchhiker's thumb.** This characteristic, more precisely called distal hyperextensibility of the thumb, can be determined by bending the distal joint of the thumb back as far as possible. While there tends to be some degree of variation, certain individuals can bend it back until there is almost a 90-degree angle between the two joints. This characteristic is an effect of a recessive allele *h* (dominant allele, *H*).
8. **Long palmar muscle.** A person homozygous for a recessive allele *l* has a long palmar muscle that can be detected by examination of the tendons running over the inside of the wrists. Clench your first tightly and flex your hand. Now feel the tendons. If there are three, you have the long palmar muscle. If there are only two tendons (the large middle one will be missing) you do not have this muscle. Examine both wrists—if you find this trait in one or both wrists you have two recessive alleles. If not, you have the dominant allele *L*.
9. **Pigmented irises.** When a person is homozygous for the recessive allele *p*, there is no pigment in the front part of the eyes and a blue layer at the back of the iris shows through, resulting in blue eyes. A dominant allele of this gene, *P*, causes pigment to be deposited in the front layer of the iris, thus masking it blue to varying degrees. Other genes determine the exact nature and density of this pigment, thus there are brown, hazel, violet, green, and other eye colors. Here, you are concerned only with the presence or absence of such pigment.
10. **Mid-digital hair.** Some people have hair on the second (middle) joint of one or more of the fingers, while others do not. The complete absence of hair on this joint for all fingers is due to a recessive allele *m* and the presence of hair is due to a dominant allele *M*. There seem to be a number of alleles determining whether hair will grow on one, two, three, or four fingers. This hair may be very fine, so you should use a hand lens to look carefully on all fingers before deciding whether this hair is present on any one of your fingers, indicating the presence of dominant allele *M*.
11. **Second (index) finger shorter than the fourth.** This is a characteristic that appears to be sex-influenced. Use the symbol S^S for a shorter second finger and the symbol S^L for a longer second finger. Tabulate your results by sex, since the frequency should vary by sex.

    a. *For the traits observed, did you find that the dominant alleles were expressed most often in your laboratory section?* _____

    b. *For which traits was this not true?* _____

    _____

    c. *How do you explain your results?* _____

    _____

Table 15C-1 Genetic Traits

Characteristic	Your Phenotype	Your Possible Genotypes	Data for Your Laboratory Section	
			Number of Each Phenotype	Percentage
1. Dimpled chin (*D*)				
Nondimpled chin (*d*)				
2. Free ear lobes (*E*)				
Attached ear lobes (*e*)				
3. Widow's peak (*W*)				
No widow's peak (*w*)				
4. Taster of PTC (*T*)				
Nontaster (*t*)				
5. Left thumb on top (*F*)				
Right thumb on top (*f*)				
6. Bent little finger (*B*)				
Finger not bent (*b*)				
7. Hitchhiker's thumb (*h*)				
Normal thumb (*H*)				
8. Long palmar muscle (*l*)				
Two tendons only (*L*)				
9. Pigmented iris (*P*)				
Unpigmented iris (*p*)				
10. Mid-digital hair (*M*)				
No mid-digital hair (*m*)				
11. Shorter second finger (S^S)			♂	♂
Longer second finger (S^L)			♀	♀

PART 2 How Individual Is Each Individual?

Procedure

1. Pick a member of your class to serve as an "individual."
2. Everyone in the class should stand up.
3. Have the "individual" call out his or her phenotype for each of the traits studied. As each phenotype is called out, all those who do not have that phenotype should sit down.

   a. *How many characteristics must be considered before the "individual" stands out as a unique individual?* _____

   b. *In the United States, a great mixing of genotypes has taken place through immigration and intermarriage. In a country where there has been little immigration, would you expect an individual to stand out sooner or later than occurred in your class?* _____

4. Instead of considering the inheritance of only one of the traits just studied, consider the inheritance of two of these traits. All of these traits are unlinked, thus Mendel's principle of independent assortment applies.

   c. What is the phenotype of a person with the genotype EETT? _____

   d. What alleles would be present in gametes produced by this individual?

   _____

   e. What is the phenotype of a person with the genotype eett? _____

   f. What alleles would be present in gametes produced by this individual?

   _____

   *g. If the two homozygous individuals above (*EETT *and* eett*) produce offspring, what would be the expected genotypes and phenotypes of their offspring?*

   Genotypes _____ *Phenotypes* _____

   *h. Assume that two individuals heterozygous for both of these traits (*EeTt*) marry and produce offspring. What would be the expected genotypes and phenotypes of their offspring?*

   Genotypes _____ *Phenotypes* _____

✓ EXERCISE D Chromosomal Abnormalities—Nondisjunction and Translocation

Mitosis and meiosis are usually very exact processes that result in the correct distribution of chromosomes to the daughter cells. However, mistakes occasionally result in an abnormal number of chromosomes or pieces of chromosomes in the daughter cells. Usually, zygotes with abnormal chromosomal compositions are spontaneously aborted. However, if the combinations are not lethal at an early stage in development, the phenotype and viability of the resulting individual may be seriously affected.

Recall that during meiosis, homologous chromosomes synapse during prophase I and then separate from each other during anaphase I (Figure 15D-1a). Sometimes a pair of chromosomes may adhere so tightly that they do not pull apart during anaphase I. This will result in one of the daughter cells receiving duplicate chromosomes and the other receiving none of that type of chromosome (see Figure 15D-1b). This failure of chromosomes to separate is called **nondisjunction.** Nondisjunction may also occur during the second meiotic division if the chromatids of a dyad chromosome do not separate from each other (Figure 15D-1c).

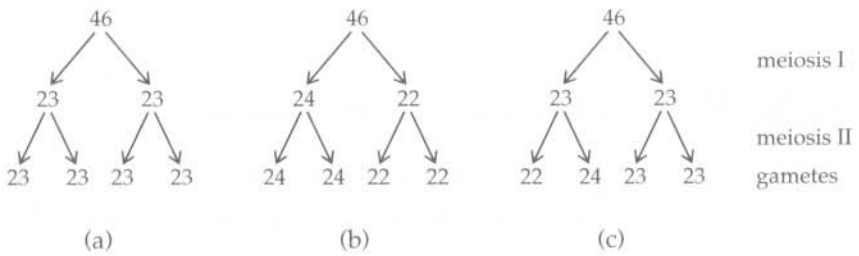


Figure 15D-1 *Meiosis in humans (*n = 23*). (a) Normal meiosis. (b) Nondisjunction at meiosis I. (c) Nondisjunction at meiosis II. Numbers indicate the number of chromosomes present in the cell after each division.*

Other chromosomal abnormalities are caused by chromosome breakage and redistribution of chromosome parts. Actual breakage of the chromosome can be caused by a variety of factors, including ionizing radiation and certain drugs and chemicals. The transfer of a portion of one chromosome to another, usually nonhomologous, chromosome is called a **translocation.** The abnormal chromosome may then be passed on from parent to offspring. Some cases of Down syndrome are caused by a translocation in which a large portion of chromosome 21 becomes attached to chromosome 15.

Objectives

- ☐ Define and distinguish between nondisjunction and translocation.
- ☐ Explain the chromosomal basis of Klinefelter's, Turner's, triple-*X*, Jacob's, and Down syndromes.

✔ PART I Nondisjunction: Sex Chromosomes

Normal meiosis in a human female results in the production of egg cells with 22 autosomes and one *X* chromosome. Normal meiosis in a human male results in the production of two types of sperm: one half with 22 autosomes and one *X* chromosome and one half with 22 autosomes and one *Y* chromosome.

a. *If an X-containing egg is fertilized by an X-containing sperm, an XX zygote will be formed. Will this individual be male or female?* _____

b. *How many Barr bodies (sex-chromatin bodies) will the zygote have (see Exercise B)?* _____

c. *If an X-containing egg is fertilized by a Y-containing sperm, an XY zygote will be formed. Will it be male or female?* _____

d. *How many Barr bodies will it have?* _____

If nondisjunction of the *X* chromosomes occurs during meiosis in a human female, some of the eggs will contain two *X* chromosomes and others will contain no *X* chromosome. In a male, nondisjunction can result in four kinds of sperm: nondisjunction during meiosis I will result in sperm with both the *X* and *Y* chromosomes and sperm with no sex chromosome, designated *0*. Nondisjunction during meiosis II may result in sperm with *XX* and sperm with no sex chromosome (*0*) or in sperm with *YY* and sperm with *0*. Gametes produced by nondisjunction usually fertilize, or are fertilized by, normal gametes.

Procedure

Several syndromes resulting from nondisjunction in humans are listed below.

Triple-*X* syndrome The individual will develop into a normal-appearing female, but may be sterile. She may also be mentally retarded.

Klinefelter's syndrome The individual will develop as a male. During early development he appears normal, but abnormalities become apparent at puberty. Testes do not fully develop. The person is usually taller than average, his muscular development may be somewhat feminine, breast development may occur, and his voice may be higher-pitched than normal. Although the individual may develop a "female" appearance, fluorescent cell staining always reveals the presence of a *Y* chromosome.

Turner's syndrome The individual will be female. She appears normal during early development, but at puberty does not menstruate, breasts do not develop, and no eggs are produced by the ovaries.

Table 15D-1 Nondisjunction in a Human Female

Abnormal Gamete (egg)	Normal Gamete (sperm)	Zygote (genotype)	Expected Sex	Number of Barr Bodies	Name of Syndrome
XX	*X*				Triple-*X* syndrome
XX	*Y*				Klinefelter's syndrome
0	*X*				Turner's syndrome
0	*Y*				Nonviable, not seen

Table 15D-2 Nondisjunction in a Human Male

Abnormal Gamete (egg)	Normal Gamete (sperm)	Zygote (genotype)	Expected Sex	Number of Barr Bodies	Name of Syndrome
X	*XX*				Triple-*X* syndrome
X	*XY*				Klinefelter's syndrome
X	*0*				Turner's syndrome
X	*YY*				Jacob's syndrome

Jacob's syndrome The individual appears to be sexually normal.

Note that all of these syndromes, except Jacob's syndrome, may result from nondisjunction in either the male or the female parent.

Based on the information given in this and previous exercises, complete Tables 15D-1 and 15D-2.

✓ PART 2 Nondisjunction: Autosomes

Autosomal nondisjunction involves chromosomes other than the sex chromosomes. The incidence of Down syndrome caused by autosomal nondisjunction of chromosome 21 increases with the age of the mother (although evidence suggests that the father may sometimes be responsible for providing the extra chromosome).

a. *What explanations might be proposed for the relationship between increasing maternal age and the increasing frequency of Down syndrome?* _____

_____

Down syndrome—trisomy 21 Three number 21 chromosomes are present. Individuals are characterized by a fold of the upper eyelid, short stature, broad hands, stubby feet, a wide, rounded face, a large tongue, and mental retardation.

Trisomy 18 This syndrome involves an extra chromosome 18. Individuals are characterized by a misshapen skull, eye problems, overlapping fingers, heart defects, feeding problems, and severe mental and developmental retardation.

Procedure

Work in pairs. Obtain a blank karyotype form and a copy of a photograph of human chromosomes. The chromosomes may be banded or simply stained with Feulgen stain. Carefully cut out the individual chromosomes. Using as a guide the sample karyotype that you prepared in Exercise A, arrange each homologous pair of chromosomes on the blank karyotype form. (Remember that the comparison of bands is helpful.) Do not fasten the chromosomes until they have been checked by your instructor. Keep all scraps until you have identified each chromosome. Now tape the chromosomes to the karyotype form. Identify the karyotype you have made. The possible choices are normal female; trisomy 21 (Down syndrome) male; trisomy 21 (Down syndrome) female; trisomy 18 male or female.

✓ EXERCISE E Constructing a Human Pedigree

Genetic analysis of the inheritance pattern for a specific human trait often requires collecting information about a family's genetic history and using it to construct a pedigree chart, which traces the occurrence of particular characteristics through several generations, or "lines." Such pedigrees enable genetic counselors

to derive facts about a couple's genetic makeup and thereby accurately calculate the chances that their offspring will inherit detrimental genes.

The pedigree chart provides a systematic and convenient method for recording data. One set of symbols and arrangements commonly used in pedigree construction is given in Table 15E-1. Individuals are designated by specific symbols, ○ for female and □ for male. Phenotypes are represented by shading the symbols. Familial relationships must also be indicated on pedigree charts: marriages, offspring, siblings, and identical or fraternal twins. For easy reference, symbols are often numbered, and birth or death dates are recorded beside the symbols.

Objectives

- ☐ Construct a human pedigree from case history information.

Procedure

1. Read the true case history that follows. Analyze and complete the pedigree chart in Figure 15E-1 using the symbols given in Table 15E-1.

Case History 147*

Jane M. requested genetic counseling because she and her husband were planning to begin a family and were concerned because Jane's brother, Brian, was moderately retarded.

The family history indicated that Brian was the third of four children. The pregnancy was uncomplicated, but delivery required the use of forceps. Shortly after his birth, the family noticed that Brian was fussy and was slow to crawl and sit. After a neurological evaluation, Brian's parents were told that he had brain damage, probably due to the forceps delivery. Brian had attended special schools and now, at the age of 22, lived at home and had no major physical problems.

Jane's and Brian's mother, Mrs. S., indicated that her daughter, Carol, had a 6-year-old daughter who required a special education program. Another daughter, Nancy, had a son, 4 years old, considered to be developmentally delayed. Brian's mother denied that any of her three daughters, Jane, Carol, or Nancy, were mentally retarded or had any learning disability, but it was noted during counseling that Brian's mother, as well as Jane and Carol, appeared to have below-average intelligence.

After genetic screening tests, Brian was diagnosed as having a condition known as "fragile *X*" syndrome. This *X*-linked recessive condition can be identified by karyotype studies of cells cultured in media deficient in specific nutrients. Under these conditions, the *X* chromosome breaks very close to the end of its long arm and releases a fragment. Males who carry the fragile *X* chromosome are usually severely retarded. If the carrier of the fragile *X* chromosome is female, it is likely that, in the heterozygous condition, the presence of a normal *X* chromosome will prevent severe mental retardation. Protection by the normal *X* chromosome is not complete, however, for heterozygous female carriers usually exhibit mild forms of mental retardation.

After further screening of family members, it was explained to the family that Brian's mother carried the fragile *X* chromosome, as did Jane and Carol, but Nancy's karyotype did not reveal the fragile *X* chromosome. Women who carry the fragile *X* are at 50 percent risk of having affected sons and 50 percent risk of having girls who are "carriers." Nancy would not be at risk for having affected children and fragile *X* was not the explanation for her child's developmental delay.

Further investigation revealed additional information about the family background as noted in the partial pedigree (Figure 15E-1).

2. Examine Figure 15E-1. On a separate sheet of paper, write a paragraph describing the family history prior to that for Mrs. S.

**Case history information was provided by Dr. Nina Caris, Department of Biology, Texas A & M University.*

Table 15E-1 Symbols Used in Pedigree Analysis

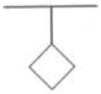
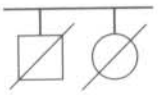
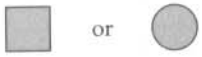
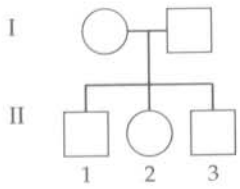
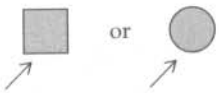
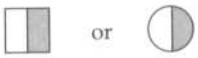
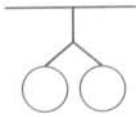
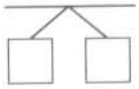
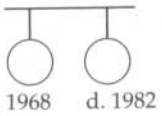
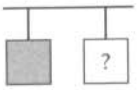
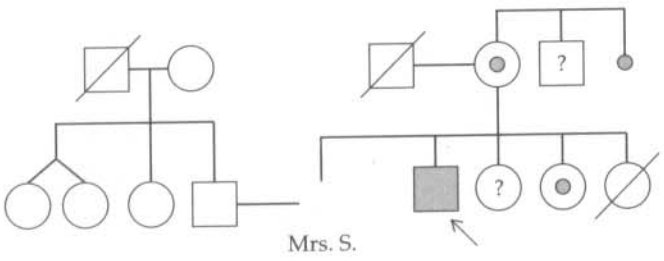
	Male		Offspring of unknown sex
	Female		Aborted or stillborn offspring
	Mating between individuals		Deceased offspring
	Mating between close relatives		Affected individual
	Parents (top row) and their offspring (bottom row) listed in birth order. Roman numerals indicate generations; arabic numbers indicate birth order within a generation		Propositus (male) or proposita (female). First case in family that was identified.
			Heterozygotes
	Identical (monozygotic) twins		X-linked carrier
	Nonidentical (fraternal) twins		Date of death
			Questionable whether individual had trait

Figure 15E-1 *Partial pedigree for the fragile X syndrome in Mrs. S.'s generation and in the generation preceding hers. Complete the chart by drawing the symbol for Mrs. S. in the position indicated, then add symbols (see Table 15E-1) for the family members described in the case study.*



3. Complete the pedigree (Figure 15E-1) for Mrs. S., her children, and grandchildren according to the information given in the family history.

   a. *How would you counsel Jane? And, if Carol sought genetic counseling about having further children, how would you counsel her? (Write your answer on a separate sheet.)*

   b. *If Carol's daughter marries and decides to have children, what would you tell her if she sought your counsel? (Write your answer on a separate sheet of paper.)*

   c. *What would you infer about the genotype of Mrs. S.'s grandparents?* _____

   d. *What might have been the genotype of Mrs. S.'s uncle?* _____

EXERCISE F Forensic Science: DNA Fingerprinting

In human DNA, there are many long sequences of base pairs that are similar in all individuals. Some of these sequences code for proteins, but others do not. (In fact, because most of the human genome does *not* code for protein, a large amount of variation can occur without consequence.) Mutations in coding regions may randomly eliminate restriction sites (places where restriction enzymes cut DNA) or form new ones. Thus, if the same restriction enzyme is used to cut the DNA from two individuals, the resulting fragments from corresponding allelic regions may be of different lengths. These different-length pieces are called **RFLPs** (pronounced "riflips"), **restriction fragment length polymorphisms** (Figure 15F-1a).

Restriction fragments of different lengths (RFLPs) can be separated on a gel by electrophoresis to produce a **DNA fingerprint;** the pattern of bands on the gel will be different for each individual (except for identical twins). The steps involved in analyzing DNA fingerprints, used to identify or match individuals (Figure 15F-2), can be summarized as follows:

1. Once RFLPs are separated on a gel, the gel is treated with an alkaline solution to denature the DNA. The two strands of the DNA separate, forming single strands.
2. A sheet of nitrocellulose paper or a nylon membrane is blotted onto the gel to pick up the single-stranded DNA fragments.
3. Artificially synthesized, radioactive, single-stranded, DNA fragments called **probes,** complementary in sequence to portions of specific RFLPs, are applied to the blotting sheet. They base-pair only with complementary sequences. Excess probe is rinsed off.
4. The blotting sheet is used to expose X-ray film. Since only radioactive materials will expose the film, only those sequences that have base-paired with the radioactive probes will show up on the film. The resulting pattern is called a DNA fingerprint.

Since mutations in DNA are cumulative over time, more closely related individuals and groups can be recognized by their genetic fingerprints: similar banding patterns of RFLPs. Thus, DNA fingerprinting can be used not only to identify genetic material from the same individual, but also to establish paternity and to discern evolutionary relationships.

For added precision, especially when forensic evidence is needed, RFLP analysis is accompanied by another type of DNA fingerprinting that analyzes the **variable number of tandem repeats (VNTRs)** present in genomic DNA. Within the human genome, short nucleotide sequences of 5 to 10 base pairs may be repeated over and over again (in tandem, and often head to tail) to form longer sequences of 20 to 200 base pairs. Often these tandem nucleotide sequences occur in front of or behind single-copy genes that code for proteins.

The sequence of base pairs in tandem repeats shows little variation from individual to individual, but the *number* of repeats can vary greatly, leading to different fingerprint patterns among individuals. Differences in length among arrays of tandem repeats result from unequal crossing-over during meiosis. VNTRs protect genes from damage by allowing for some "slippage" in front of and behind a gene being "cut out" of one chromosome and "pasted into" or recombined with another during crossing-over. As a consequence, the lengths of tandem repeat arrays located close to a particular allele on maternal and paternal homologous chromosomes belonging to the same individual also may differ (Figure 15F-3).

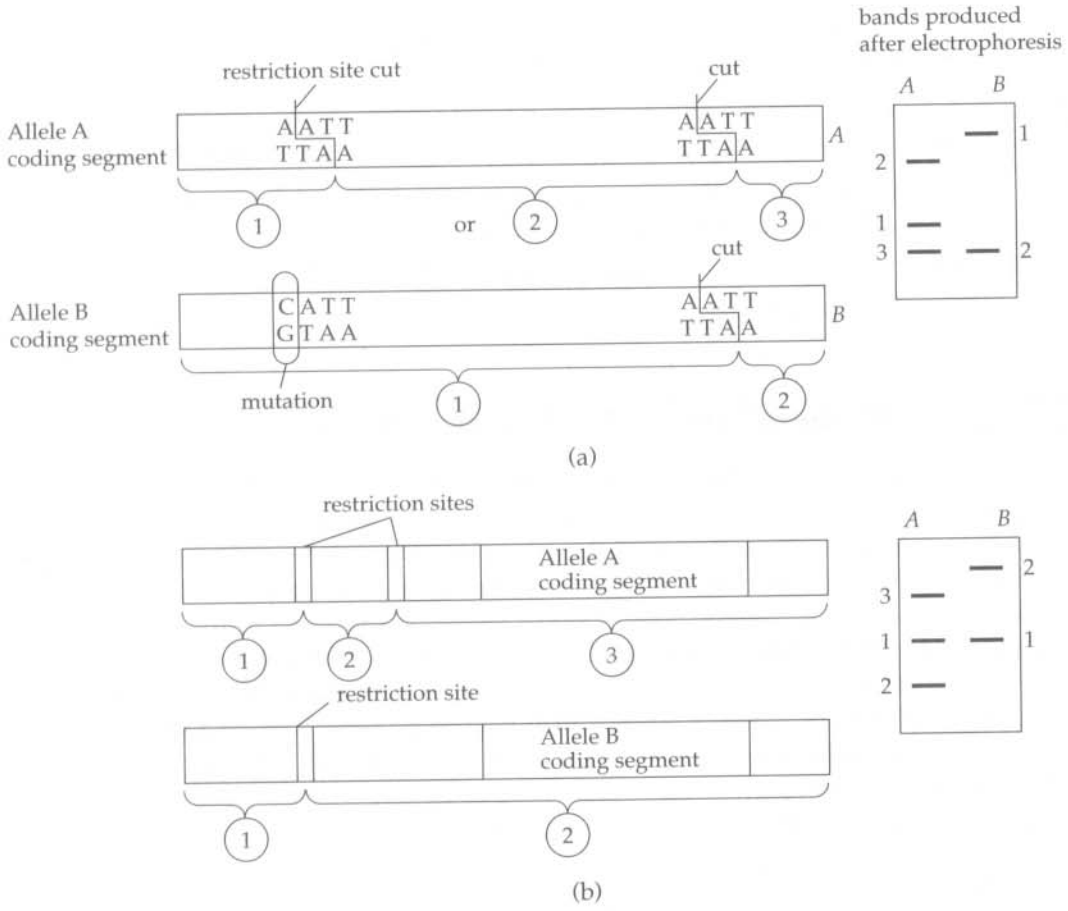


Figure 15F-1 *DNA fingerprinting using RFLP sequences. Mutations in DNA can result in eliminating or forming new restriction sites where restriction endonucleases can cut the DNA to form fragments of varying lengths (RFLPs).*

(a) Elimination of a restriction site within the coding sequence of an allele due to a base-pair change (converting allele A *to allele* B*) results in only two fragments being formed during enzymatic digestion, rather than three. Since the fragments vary in length, a different fingerprint is obtained for alleles* A *and* B*.*

(b) If restriction sites in a DNA sequence preceding an allele are affected, rather than the allele itself, differences can also be detected from DNA fingerprints. Often, a unique fingerprint pattern results from a mutation in a noncoding sequence found in front of a DNA sequence (allele) suspected of causing a specific genetic disorder. If this pattern is consistently found among those with the genetic disorder (pattern B *in this case), this fingerprint pattern can be used for diagnostic purposes even though identification of the gene is not conclusive and its specific location is not known.*

To obtain a DNA fingerprint using VNTR regions, the DNA is cut with restriction enzymes that do *not* cut within the tandem repeat area. Instead, they cut in front of and behind the repeat sequence. The polymerase chain reaction (PCR) is used to augment the number of copies of the isolated repeat areas. (For accuracy, several different repeat areas are analyzed by cutting with different restriction enzymes and are then amplified using PCR.) The PCR reaction uses short DNA primers to synthesize many copies of the VNTR regions to be used for electrophoresis and fingerprinting (Figure 15F-3). PCR makes it possible to obtain the required amount of DNA from even the smallest traces of blood, semen, and other tissues and cells of an individual.

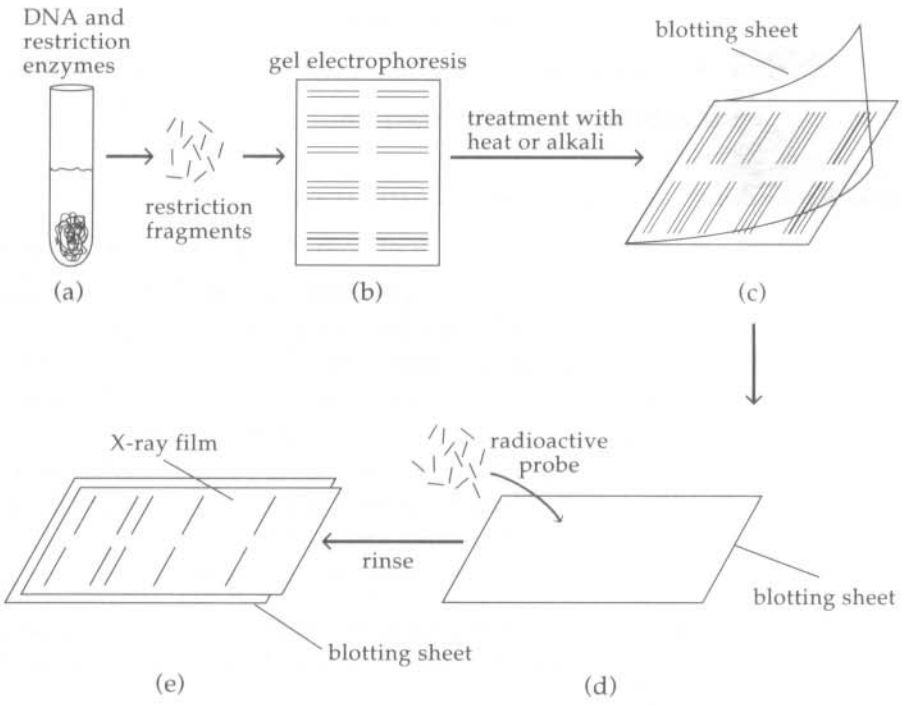


Figure 15F-2 *DNA fingerprinting. (a) DNA is broken into fragments by restriction endonucleases. (b) The fragments are separated by gel electrophoresis, then the gel is treated by heat or an alkaline solution to separate the strands. (c) A sheet of nylon or nitrocellulose film is used to "blot" the gel and pick up DNA. (d) When radioactive DNA probes are added to the sheet, they base-pair only with complementary strands in various regions, producing a DNA fingerprint. (The sheet is then rinsed to wash away any probes that do not hybridize so that these pieces will not interfere with the pattern.) (e) The blotting sheet is allowed to expose X-ray film. The radioactive probes will identify the RFLPs of interest—only these will be visible on the film.*

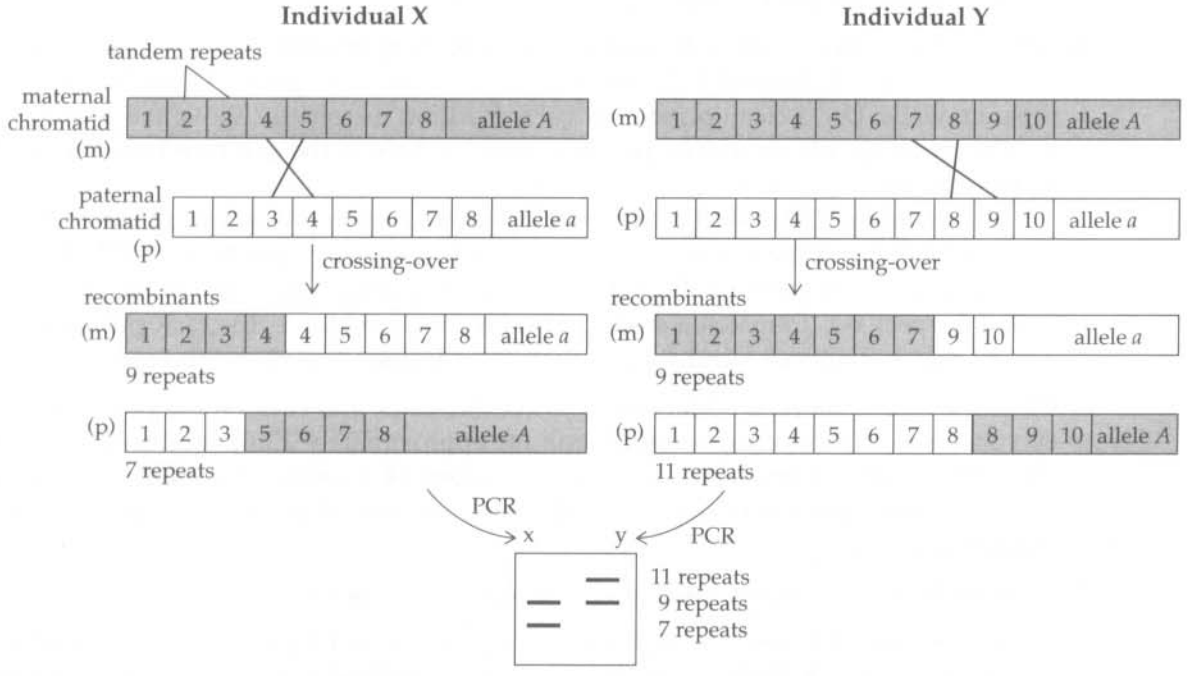


Figure 15F-3 *VNTR differences in homologous chromosomes. The number of tandemly repeated sequences of DNA associated with particular genes on a chromosome differs widely among individuals. These differences are generated by unequal crossing-over, so that even maternal and paternal homologues of the same individual, as well as different individuals, have different numbers of sequences associated with the same allele.*

Objectives

- ☐ Describe how restriction enzymes are used in DNA fingerprinting.
- ☐ Interpret results from a DNA fingerprint.

Procedure*

A crime has been committed. A brief scenario of the crime is given below.

> *A woman jogging through a mid-city park at dusk was accosted by a man who dragged her into a stand of trees and bludgeoned her with a baseball bat. The man fled when interrupted by a fellow jogger who heard the woman's screams. The jogger who witnessed the crime saw the man flee from the scene but could not identify the suspect for certain because it was fairly dark at that time of evening. In the police lineup, the witness tentatively identified a suspect, but the man standing next to him also looked a lot like the alleged attacker. Fortunately, the victim had pulled some hair from the attacker's head. DNA was extracted from the papillae of the hairs and a DNA fingerprint was developed to compare the DNA from the evidence (hairs), and the two suspects, X and Y. (Electrophoresis of PCR-amplified VNTRs was used to create the needed forensic evidence.)*

1. Insert a gel comb into an electrophoresis gel tray and cast an agarose gel as described in Laboratory 14, Exercise D. (Add 2 drops of Carolina Blu stain to 50 ml of agarose, if instructed to do so.)
2. When the gel has solidified (about 10 minutes), place it in the gel box so that the comb is at the negative (black) end.
3. Fill the gel box with TBE (tris-borate EDTA) buffer until the buffer covers the gel. (Add Carolina Blu stain to the buffer if instructed to do so.)
4. Gently remove the gel comb and check for bubbles.
5. Add DNA samples to the wells using a microcapillary pipette. Load the contents of each tube—Suspect X-1, Suspect X-2, Evidence 1, Evidence 2, Suspect Y-1, and Suspect Y-2—into separate wells. Use a clean pipette for each. Position the pipette above the well to load. *Do not put the pipette tip into the well* or you may punch a hole in the gel! (See Laboratory 14, Exercise D, for details.)

   Note: During PCR, two different primers were used to produce multiple copies of the suspects' DNA and the evidence DNA. Primer 1 was used to produce the DNA of Suspect X-1, Evidence 1, and Suspect Y-1. Primer 2 was used to produce the DNA of Suspect X-2, Evidence 2, and Suspect Y-2. To match a suspect to crime scene evidence, the PCR products from Primer 1 must match Evidence 1 and the products from Primer 2 must match Evidence 2.
6. Close the electrophoresis apparatus, connect the leads, and turn on the power supply to 80 volts. Allow the electrophoresis to run for approximately 2 hours or until the bromophenol blue band nears the end of the gel (see Laboratory 14, Exercise D, for details). If Carolina Blu stain has been added to the agarose and buffer, you will also see faint blue bands in the "fingerprint" pattern.
7. Turn off the power supply, disconnect the leads, and open the gel box.
8. Remove the gel and stain it using the procedure described by your instructor (staining procedures vary with the preparation of agarose), using either methylene blue or Carolina Blu Final Stain.
9. Analyze the gel by placing it on a light box or transilluminator. You may wish to use plastic wrap or transparency film to make "copies" of the gel and its bands for analysis. (See Laboratory 14, Exercise D, for details.)

**This exercise is adapted from the Carolina Biological Supply Kit 21-121-, PCR Forensic Simulation Kit.*

a. Which "suspect" DNA matches the "evidence" DNA? Who committed the crime?

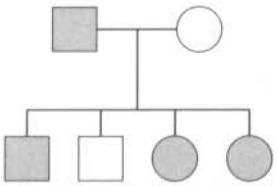
_____

b. If identical twins had been included among the suspects, what would you have observed in the DNA fingerprint pattern? _____

Laboratory Review Questions and Problems

Use a separate sheet of paper to answer the following questions. Show all of your work. Indicate all genotypes and phenotypes, where appropriate. If more than one type of offspring is produced, indicate the proportion of each type.

1. A man with attached ear lobes marries a woman who is heterozygous for free ear lobes. What types of offspring and what proportion of each type would be expected from this mating?
2. Polydactyly is an inherited human trait in which the affected individual has extra fingers or toes. This defect results from the dominance of the defective gene (*P*) over the normal allele (*p*).
   a. If a man with polydactyly (assume that he is heterozygous) marries a normal woman, what are the possible genotypes of their children?
   b. What are the phenotypes of these genotypes?
   c. If two individuals heterozygous for this trait marry, what are the possible genotypes of their children?
   d. What are the phenotypes of these genotypes?
3. Formation of Barr bodies takes place when a zygote has already divided to form a multicellular embryo. Because of this, the body of a female can be a "mosaic of sex-linked phenotypes." Explain.
4. Color blindness is an *X*-linked recessive trait. The phenotypes within a family pedigree are shown below. Determine the genotypes of each individual.



5. A color-blind man marries a woman whose father was color-blind but whose mother had normal vision. Would it be possible for the couple to have a color-blind son? A color-blind daughter? What is the probability of each of these events happening?
6. The inheritance of human blood types is dependent on multiple alleles. The ABO blood system, characterized by three alleles, is commonly used to determine the suitability of donors and recipients for blood transfusions.

   Normally, the body does not store antibodies for proteins it has never encountered. The ABO blood system, however, is an exception to this rule. In this system, antibodies to other blood types occur naturally whenever the antigen (A or B) is not present. An individual with

type A blood possesses type A antigen and type B antibody; an individual with type B blood possesses type B antigen and type A antibody; type O blood contains neither the A nor the B antigen, but both antibodies; type AB blood contains both antigens and neither antibody. As a result, if, for instance, a type A person receives type B blood, the B antigen will be agglutinated by the B antibodies present in the type A blood. Antibodies in the donor's blood are generally of little consequence because they are so diluted in the recipient's blood.

The general rule in transfusions is never to allow an individual to receive an antigen that does not occur in his or her blood. Since type O blood contains neither antigen A nor antigen B, this blood type is considered as the "universal donor" and may be transfused to any blood type. To study the inheritance of the ABO alleles, the symbols *I* and *i* with superscripts are used, since more than two alleles must be considered (capital and lowercase letters will not suffice). I^A, I^B, and *i* represent alleles of antigens A and B and the allele for blood type O (which produces no antigens), respectively.

An individual homozygous for type A blood (I^AI^A) will produce I^A gametes; a heterozygote (I^Ai) will produce I^A and *i* gametes. (Remember that in this case the genotype cannot be determined from the phenotype.) A similar situation holds for type B individuals. Individuals with type AB blood will produce both I^A and I^B gametes. Only *i* gametes will be produced by an individual with type O blood. Remember that each parent contributes only one allele to its offspring, and if the parent produces more than one type of gamete, each allele has the same probability of being passed on to the offspring.

a. What types of gametes will a woman with type AB blood produce?

b. What types of gametes will a man with type O blood produce?

c. If these two people were to marry and have children, what would be the possible genotypes of their children's blood?

d. Which blood types could their children have?

e. Which blood types could not be inherited by the children of these parents?

7. The pattern of inheritance of blood groups has some practical application in medico-legal cases involving disputed parentage.

a. If a child has type A blood, could both parents be of type O? Why?

b. If the mother is type A and the father is type B, would blood tests help to determine whether a particular child belonged to them? Why?

c. If a woman has type O blood and her child has type A blood, could a man with type B blood possibly be the father of the child? Why?

d. If both husband and wife have type AB blood, what possible blood types could their offspring have?

8. A child of blood type O has a mother whose blood type is also O. Which of these men could be the father of this child: a man of blood type A, one of blood type B, or one of blood type O? Recall that blood tests reveal only the phenotype of the individual, not the genotype, so an individual with type A blood may carry the alleles for types A and O. (Refer to question 6.) Based on the results of blood typing alone, can you rule out any of the three men as possible fathers? Why or why not?

Conclusive evidence can be gained by DNA fingerprinting, using a sample of DNA from each of the three men and from the child and the mother. The child's DNA fingerprint should contain bands that are also present in the mother's fingerprint. If additional bands are present, they must be represented in the father's fingerprint. The following results are obtained after a restriction enzyme digest, followed by autoradiography.

Key to bands:

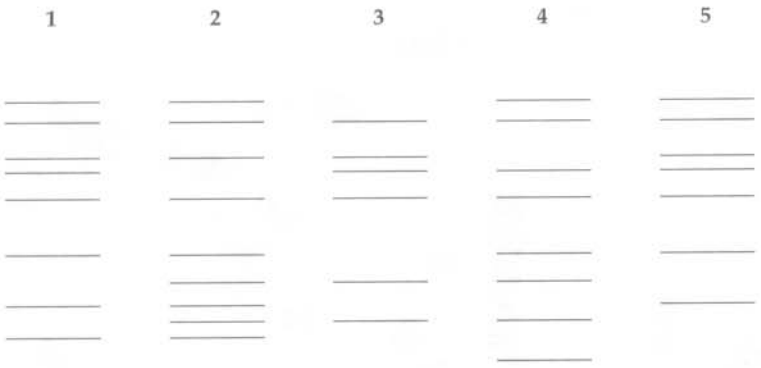
1 Mother

2 Child

3 Possible father 1, blood type A

4 Possible father 2, blood type B

5 Possible father 3, blood type O



Which of the three men is the father of the child? How do you know?

9. List the steps involved in DNA fingerprinting. Why would there be an advantage to using more than one probe when developing a fingerprint?

10. A cemetery next to a river has been flooded after a spring of continuous rain. Many of the coffins have ruptured and bones are scattered about. Members of three families are buried in the cemetery. The families would like to reclaim the bones of their ancestors and place them in a proper burial place. As a forensic scientist, you have been asked to assist. How might your proceed to determine to which families the bones belong?

Figure 15A-3b *Cut out chromosomes and use Figures 15A-2 and 15A-4 to help you match homologous chromosome pairs. Arrange these pairs above the appropriate positions in Figure 15A-3a.*