(b) If the RNA polymerase fails to recognize the promoter region, then the process of transcription will not take place since the RNA polymerase cannot transcribe DNA unless it is bound to it. The promoter region acts as the initial binding site.

8.

Replication	DNA is copied by unzipping and uncoiling. DNA nucleotides are inserted into the sequence using base pair rules using DNA polymerase. DNA molecule is rezipped and coiled, producing two identical strands.
Transcription	The leading strand of DNA is copied at a start code. DNA is uncoiled and unzipped at this location. RNA nucleotides are inserted using base pair rules, except uracil nucleotides are substituted in place of thymine nucleotides. Once a stop code is reached, the single RNA strand is released into the cytoplasm.

9. (a) The A and P sites are found in a ribosome that is translating an mRNA sequence into protein. The A (acceptor) site is where tRNA molecules bring in the appropriate amino acid. The P (peptide) site is where peptide bonds are formed between adjoining amino acids on a growing polypeptide chain.
   (b) A codon is a triplet of ribonucleotides on mRNA that encodes a single amino acid. An anticodon is a triplet of ribonucleotides on tRNA that recognizes and pairs with a codon on the mRNA.
   (c) The start codon signals to the ribosome to start synthesizing the polypeptide chain. The start codon is always AUG and codes for methionine. The stop codon signals to the ribosome to stop the process of translation. The stop codons are UGA, UAG, and UAA.
   (d) DNA is double-stranded, whereas RNA is single-stranded. The sugar group DNA is deoxyribose, whereas in RNA, it is ribose. The thymine bases of DNA are replaced with uracil in RNA.
10. D UGA CGA GGU
11. An error in the third base of a codon in mRNA may not necessarily result in an error during the process of translation because more than one codon encodes a particular amino acid. The codons differ by the third nucleotide. For example, proline can be encoded by the codons CCU, CCC, CCA, and CCG. If a mistake is made in the third nucleotide of the codon, it is negligible. It does not matter what the third nucleotide is—the two first nucleotides, CC, will always code for proline. The possibility of flexibility in the third nucleotide of a codon is termed the wobble hypothesis.

20.3 DNA AND BIOTECHNOLOGY

Web Activity: Simulation—Electrophoresis

(Page 677)

1. Lane AA and lane Aa each have eight proteins of MWs 200 kDa, 125 kDa, 80 kDa, 57 kDa, 50 kDa, 45 kDa, 30 kDa, and 25 kDa. Lane aa has seven proteins of MWs 200 kDa, 125 kDa, 80 kDa, 57 kDa, 50 kDa, 30 kDa, and 25 kDa. The standard lane has four proteins of MWs 200 kDa, 66 kDa, 45 kDa, and 22 kDa. Note that students' estimates of the MWs of the samples will vary but should be similar to these.
2. Large, high molecular weight proteins are always found nearer the top of the gel.
3. The MW of the protein missing from the homozygous recessive extract is 45 kDa.
4. There are eight proteins in the extract from the homozygous dominant individual.
5. There are seven proteins in the extract from the homozygous recessive individual.
6. It appears that one protein is related to the differences in shell colour.
7. The protein profiles for the AA and AA individuals are identical.
8. Yes, it is reasonable to conclude that the 45 kDa protein is involved in shell colour.
9. The absence of the 45 kDa protein correlates with the *a* allele. The presence of this same protein correlates with the *A* allele.

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10. The production of the 45 kDa protein appears to be under the control of the *A* allele.
11. Specific alleles code for particular proteins. If that allele is not present, then the protein will not be produced.

Web Activity: Canadian Achievers—Researchers in Human Genetic Disorders

(Page 678)
Students' views will vary but should indicate that they have considered the impact of genetic diseases on those who inherit them, on their families, and on society in general. They should also note that, for each of the conditions mentioned in the activity, more than one scientist or group of scientists is researching the condition and that the findings of one often further the work of another. Furthermore, the work of scientists in the lab can have direct consequences on the diagnosis and treatment of individuals with genetic diseases.

Mini Investigation: Examining the Human Genome

(Page 679)
(a) Chromosome 1 contains the most genes.
(b) The Y chromosome contains the fewest genes.
(c) The human genome is estimated to have over 34 300 genes. Students should touch each chromosome and find the estimated number of genes. To calculate the size of the human genome (number of genes), students should sum these values:

Chromosome pair	Number of genes (minimum number estimated)
1	3000
2	2500
3	1900
4	1600
5	1700
6	1900
7	1800
8	1400
9	1400
10	1400
11	2000
12	1600
13	800
14	1200
15	1200
16	1300
17	1600
18	600
19	1700
20	900
21	400
22	800
X	1400
Y	200
Total	**34 300**

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Practice

(Page 681)

1. (a) The location of the cuts created by *Sma*I is between the C and the G in the highlighted sequences:
   5′–AATTCGCCC GGGATATTACGGATTATGCATTATCCGCCC GGGATATTTTAGCA–3′
   3′–TTAAGCGGG CCCTATAATGCCTAATACGTAATAGGCGGG CCCTATAAAATCGA–5′
   (b) Three fragments will be produced if *Sma*I digests this sequence.
   (c) *Sma*I produces blunt ends, which are defined as fragment ends that are fully base paired.
2. A cleavage produced by *Hin*dIII would yield sticky ends because the enzyme cuts between the two A's of the sequence (AAGCTT), which is palindromic.
   Refer to the diagram below:
   5′–AAGCCTT–3′
   3′–TTCGGAA–5′
3. Restriction endonucleases are considered molecular tools because they are biological molecules that allow biotechnologists to cut stands of DNA at specific sequences, the way a pair of scissors would cut a piece of paper. Restriction enzymes allow for the manipulation of DNA and are one of the fundamental instruments required by molecular biologists.
4. The palindromic sequences are highlighted below:
   GCGCTAAGGATAGCATTCGAATTCCCAATTAGGATCCTTTAAAGCTTATCC
   CGCGATTCCTATCGTAAGCTTAAGGGTTAATCCTAGGAAATTTCGAATAGG

Investigation 20.2: Restriction Enzyme Digestion of Bacteriophage DNA

(Pages 696–699)

Purpose

To examine how the patterns of DNA fragments compare when a piece of DNA is digested using different restriction endonucleases

Problem

How do the patterns of DNA fragments compare when a piece of DNA is digested using different restriction endonucleases?

Hypothesis

Restriction endonucleases cut DNA at specific recognition sites. Cleavage of a piece of DNA using particular endonucleases will therefore generate fragments of specific sizes.

Prediction

Using Figure 1, students should predict the following number of fragments: *Eco*RI and *Bam*HI, six fragments, *Hin*dIII, eight bands.

Design

In this investigation, bacteriophage lambda DNA will be digested using the restriction endonucleases *Eco*RI, *Hin*dIII, and *Bam*HI. The fragments produced will be separated using gel electrophoresis. Fragment sizes will be calculated from an analysis of the agarose gel. Bacteriophage lambda DNA is obtained from a virus that infects bacterial cells and is 48 514 base pairs in length. The manipulated variable is the restriction enzyme used, and the responding variable is the size of the resulting restriction fragment. The controlled variables include the DNA sample used, the gel used to separate the fragments, and the gel stain used.

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Materials

- safety goggles
- gloves
- 70 % ethanol solution (or 10 % bleach)
- four 1.5 mL Eppendorf tubes
- waterproof pen for labelling
- masking tape
- polystyrene cup
- freezer
- crushed ice
- 20 µL of 0.5 g/L lambda DNA
- 5 µL 10× restriction buffer
- 1.0–20 µL micropipette with tips
- 2 µL each of *Bam*HI, *Eco*RI, and *Hin*dIII restriction endonucleases
- microcentrifuge (optional)
- 37 °C water bath
- thermometer
- 1 g agarose
- paper boat
- electronic balance
- 500 mL Erlenmeyer flask
- 250 mL graduated cylinder
- microwave or hot plate
- flask tongs or oven mitts
- gel casting tray and gel electrophoresis box (bought from a scientific supply company or made using basic materials)
- 1 L 1× TBE buffer
- 5 µL loading dye
- power supply (45 V)
- plastic wrap
- 25–30 mL 0.025 % methylene blue, or enough to cover the gel in the staining tray
- light box or overhead projector
- acetate sheet

Procedure

Day 1: Restriction Enzyme Digestion

1. Safety goggles and gloves were worn. The bench was wiped with a 70 % ethanol solution (or 10% bleach).
2. Four 1.5 mL Eppendorf tubes were labelled "*Bam*HI," "*Eco*RI," "*Hin*dIII," and "control." The tubes were placed in a polystyrene cup containing crushed ice. Reagents as outlined in Table 1 were added to each tube.
3. A fresh tip was placed on the micropipette for each reagent. The 4 µL of DNA was added to each tube first, followed by the 10× reaction buffer and then the water. The enzyme was added last. All the contents were dispensed close to the bottom of the Eppendorf tubes, ensuring that the pipette tip was touching the side of the tubes. Everything was kept on ice at all times.
4. The Eppendorf tube tops were closed. The tubes were placed in the microcentrifuge. The microcentrifuge was closed and spun at maximum speed for approximately 3 s. If no microcentrifuge was available, the tubes were tapped on a soft pad or thick paper towel on the bench, pooling the contents to the bottom.

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5. The tubes were placed in a 37 °C water bath for at least 45 min. A thermometer was used to check the temperature of the water.
6. Once the digestion was complete, the tubes were placed in the labelled polystyrene cup, and the cup was placed in a freezer until the next class.

Day 2: Gel Electrophoresis

7. 0.96 g of agarose powder was measured in a paper boat on an electronic balance and transferred to a 500 mL Erlenmeyer flask.
8. Using a graduated cylinder, 125 mL of 1× TBE buffer was added and swirled to mix.
9. The flask was heated on a hot plate or in a microwave until the solution was completely clear. The flask was handled carefully, using tongs or oven mitts.
10. The gel casting tray was prepared. The plastic comb was inserted properly.
11. Once the flask with agarose solution was cool enough to handle with bare hands, the mixture was poured into the gel casting tray. The comb teeth were immersed in about 6 mm of agarose, and the gel covered about one-third of the height of the comb teeth. Bubbles were removed from the gel with a micropipette tip as soon as it was poured.
12. The agarose was allowed to set for at least 20 min. The gel was cloudy as it solidified.
13. Once the gel had set (this was tested by gently touching the lower righthand corner with a finger), the gel was flooded with 1× TBE running buffer. The comb was gently pulled out without ripping any of the wells.
14. The tray containing the gel was oriented in the gel electrophoresis box so that the wells made by the comb were at the end with the positive electrode.
15. The 1× TBE buffer was added to the gel electrophoresis box until the buffer was approximately 5 mm above the gel. The gel electrophoresis box was placed to the side.
16. 1 µL of loading dye was added to each of the Eppendorf tubes. These were microfuged for 3 s.
17. The full contents of one Eppendorf tube were micropipetted into a well on the gel. The micropipette was steadied over the well using both hands. This was repeated for each tube while recording the order in which the tubes were dispensed.
18. The gel box was closed and connected to the power supply. If a gel box that was handmade was used, the voltage was set to 45 V dc and turned on. It was electrophoresed for 12 h. Alternatively, if a stronger power supply or a store-bought electrophoresis unit was used, the unit was electrophoresed at 110 V for 2.5 h.
19. The power supply was unplugged, and the gel was carefully removed. The gel was wrapped in plastic wrap and placed in the refrigerator for a maximum of one day.

Day 3: Staining the Gel

20. The gel was unwrapped and placed in the staining tray.
21. The gel was flooded with 0.025 % methylene blue solution and left to sit for at least 20 to 25 min. The water was poured off and replaced with fresh water. This process was repeated three more times, keeping an eye on the intensity of the DNA bands. If destained for too long, the smaller fragments could be lost. If not destained for long enough, the whole gel remains blue and the fragments cannot be differentiated.
22. The destained gel was placed on a light box or on an overhead projector.
23. A blank acetate sheet or plastic wrap was placed over the gel. The pattern of bands was traced onto the wrap or sheet, being sure to draw a line where the bottom of each well started.

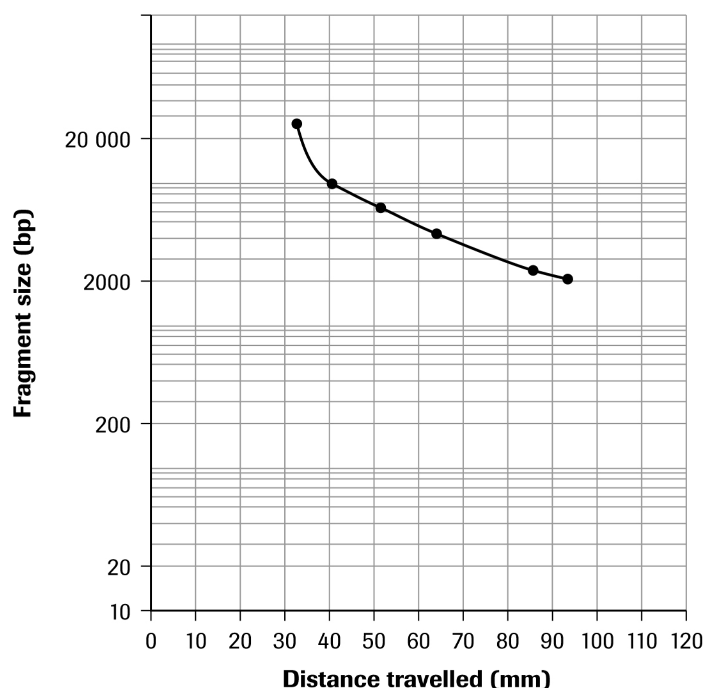
Evidence and Analysis

Answers to questions (a) to (d) will vary depending on experimental results. Students should present their graph of distance travelled versus fragment size for the *Hin*dIII digestion and a completed Table 2 in their lab reports. Sample data are given below:

Table 2 Distance Travelled by Each Band From the Well Origin

*Hin*dIII		*Eco*RI			*Bam*HI		
Actual fragment size	**Distance travelled (mm)**	**Actual fragment size**	**Distance traveled (mm)**	**Calculated fragment size**	**Actual fragment size**	**Distance traveled (mm)**	**Calculated fragment size**
27 491		21 226			16 841		
23 130	34	7421	37	13 000	7233	35	17 500
9416	40	5804	42	8500	6768	40	10 000
6557	52	4973	46	7500	6527	57	5250
4361	63	4878	49	7000	5626	62	4750
2322	86	3528	52	6000	5505	71	3750
2027	93						

Sample semilogarithmic graph of *Hin*dIII digestion; fragment size (bp) versus distance travelled (mm).



Calculated fragment sizes are as follows. Use Figure 1 on page 697 of the Student Book. Sample calculation for *Eco*RI. Total length of bacteriophage DNA is 48 500 bp. *Eco*RI cuts at 21 226 bp, 26 104 bp, 31 747 bp, 39 168 bp, and 44 972 bp. Therefore, fragment sizes are 21 226 bp (21 226 – 0), 4878 bp (26 104 – 21 226), 5643 bp (31 747 – 26 104), 7421 bp (39 168 – 31 747), 5804 bp (44 972 – 39 168), and 3528 (48 500 – 44 972).

Actual Fragment Size of Bacteriophage DNA Restriction Enzyme Digest

*Hin*dIII (bp)	*Eco*RI (bp)	*Bam*HI (bp)	Control (bp)
27 491	21 226	16 841	48 500
23 140	7421	7233	
9416	5804	6768	
6557	4973	6527	
4361	4878	5626	
2322	3528	5505	
2027			

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Students can calculate the percentage error using the following formula (actual fragment size – calculated fragment size)/actual fragment size × 100 %. The answers will vary depending on experimental results. Please note that some bands will travel as a doublet and not be resolved by the gel. For example, fragments 4878 and 4973 in the *Eco*RI digest will appear as one band.

Evaluation

(e) Each tube contained the bacteriophage DNA and a restriction enzyme. Hence, the *Bam*HI tube illustrates the banding pattern that will be produced with digestion with *Bam*HI, the *Hin*dIII tube illustrates the banding pattern that will be produced with digestion with *Hin*dIII, and the *Eco*RI tube illustrates the banding pattern that will be produced with digestion with *Eco*RI. The three tubes produce three different banding patterns, illustrating that each restriction enzyme has different recognition sites that it recognizes and cleaves. The control illustrates that the digestion of the DNA is due to the presence of the restriction enzymes and not because of any other factor.

(f) Smaller bands migrate faster than larger bands since they have an easier time navigating through the molecular sieve of the gel.

(g) To resolve bands that migrate close together, a higher percentage agarose gel can be used.

(h) The running buffer contains electrolytes; hence, it can carry the current that is being used to separate the different-sized fragments.

(i) The gel is partially made of running buffer so that the circuit is completed between the anode and the cathode.

(j) Bubbles appear because of the electrolysis of water in the running buffer. Hydrogen and oxygen gas are being produced.

(k) Loading dye is added to the samples before they are loaded into the wells. The loading dye contains glycerol, which is a heavy molecule, resulting in the sinking of the DNA samples into their respective wells. Also, the dye contains small molecules that migrate faster than the dye. These coloured molecules are used as visual markers to track the progression of the DNA through the gel. Once the small molecules reach the end of the gel, it is wise to turn off the energy source since the DNA follows and can be electrophoresed right off the gel.

(l) Larger fragments are stained darker than smaller fragments since larger fragments contain more DNA and therefore will absorb more dye.

(m) The following errors may occur and can be corrected as indicated. If the bands in the lanes are not separated, the gel should be run for a longer time to allow the bands to migrate further through the gel. If the bands on the gel after staining are faint, the gel can be left in the staining solution for a longer time to ensure that all the bands, including the smaller ones, are picked up by the dye. If there are no cuts in any lane, resulting in all the bands looking like the control lane, this indicates a problem with the restriction enzyme digest itself. Ensure that the temperature was maintained at 37 °C throughout the restriction digest for optimum restriction enzyme activity. If the bands are skewed throughout the gel, this indicates that the gel contains inconsistencies in its matrix due to varying concentrations. The agarose would not have been completely melted and/or mixed uniformly with the buffer before it was allowed to set.

Web Activity: Case Study—Transformation of Eukaryotes

(Page 685)

Answers will vary. Sample answers for plant and animal transformation technologies are given below:

The most common method for transforming plants is through use of *Agrobacterium tumefaciens*. *Agrobacterium* is a naturally occurring soil bacteria that can transfer its DNA into the genome of a number of plant species. *Agrobacterium* has a plasmid into which foreign DNA

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can be introduced in the lab (i.e., used as a transformation vector). In designing transformation vectors, scientists include the gene or genes of interest, a marker gene to distinguish cells that have been transformed, and sequences that control the expression of the gene(s). Transformation occurs by incubating a culture of *Agrobacterium* containing the transformation vector with sterile tissue (cells or explants) of the plant to be transformed. The transformed cells or explants are identified by a selection protocol that relies on the marker gene. For example, if a gene for antibiotic resistance is used as a marker, the tissue is exposed to the antibiotic. Any surviving cells/tissue will contain the *Agrobacterium* plasmid and, therefore, the gene of interest. The cells or explants are then cultured in a manner that regenerates whole plants. The plants are then analyzed for the presence of the gene (e.g., DNA is extracted, and a Southern blot analysis is carried out to confirm the presence of the gene sequence in the plant genome).

One method for producing transgenic animal cells is to introduce the DNA using microinjection. The DNA segment to be transferred usually contains the gene of interest and sequences needed for its correct expression. Cells of the organism to be transformed are then isolated. In animals, these may be stem cells or, more commonly, fertilized eggs. Then, under a microscope, a single cell is held in place using gentle suction through a blunt capillary tube. A very fine-tipped pipette is then inserted into the cytoplasm or nucleus of the cell using a micromanipulator. The DNA is then injected. The cell/fertilized egg is then allowed to develop. If the technique is successful, the animal's genome will contain the foreign DNA sequence and be able to pass it to the next generation. Even when the DNA is successfully integrated into the host's genome, the gene is not always expressed appropriately or at all.

Section 20.3 Questions

(Page 686)

1. Restriction endonuclease: a group of molecules that are able to cleave DNA into fragments at a specific location within the DNA strand.
   Methylase: an enzyme that adds a methyl group to one of the nucleotides found in a restriction endonuclease recognition site, altering its chemical composition.
2. (a) The role of restriction endonucleases in the bacterial cell is to act as the cell's immune system. The endonucleases digest any foreign DNA, or DNA that is unmethylated, by identifying and cutting at the endonuclease's recognition site along the DNA fragment. This protects the bacterial cell's genetic material from threats, such as bacteriophages.
   (b) In a laboratory setting, restriction endonucleases are used to cleave DNA fragments at specific sequences with the intent of manipulating the DNA, be it for creating recombinant DNA or for simply using the smaller fragments for another purpose.
3. Blunt ends are fragment ends of a DNA molecule that are fully base paired at the end, whereas sticky ends possess short, single-stranded overhangs.
4. Recognition site: a specific DNA sequence with double-stranded DNA, usually palindromic and consisting of four to eight nucleotides, that a restriction endonuclease recognizes and cleaves. An example of this is the recognition site that *Hin*dIII recognizes and cuts between the two A's as shown below:
   5′–AAGCTT–3′
   3′–TTCGAA–5′
   The site is palindromic because both strands have the same sequence when read 5′ to 3′ as shown below:
   5′–AAGCTT–3′ 5′–AAGCTT–3′
5. A two-base-pair recognition site is too short to be useful in genetic engineering because it would occur too frequently and likely result in too much cleaving of the targeted DNA fragment. A 14-base-pair fragment would occur too infrequently to be useful for molecular biologists. The following calculations are applied to a 75 000 bp sequence:

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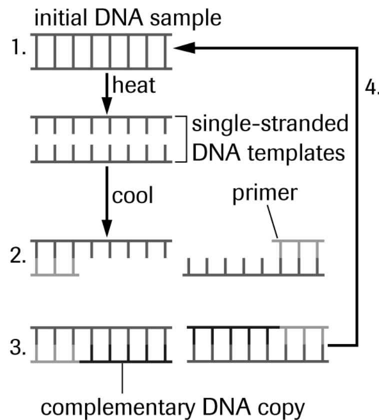
Two-base-pair recognition site:
Frequency of cuts: 4 × 4 = 16 nucleotides
Expected number of cuts: 75 000/16 = 4687.5 (4687 cuts)
Fourteen-base-pair recognition site:
Frequency of cuts: 4 × 4 × 4 × 4 × 4 × 4 × 4 × 4 × 4 × 4 × 4 × 4 × 4 × 4
= 268 435 456 nucleotides
Expected number of cuts: 75 000/268 435 456 = 0.0002 (0 cuts)

6. Students' diagrams should include all the features included in the figure below. For simplicity, the two original single strands are not shown after step 3.



7. The initial years were spent working on methods to speed up the process of sequencing.
8. Isolate the gene responsible for producing human growth hormone. Using a restriction enzyme, insert the gene into a piece of bacterial DNA. After allowing the bacteria to multiply, the sample can be stimulated to produce the hormone that can be purified and sold.
9. Canadian regulations regarding GMOs include proper food labelling and submission of all product information, including test data, to Health Canada. These guidelines appear to be adequate as Health Canada performs additional research on the product to ensure the safety of public health. As well, through proper labelling, people can make informed decisions about the foods they consume. Having the food companies also advertise the potential risks of the food, much like tobacco companies are required to do, may alleviate the worry many people have about GMOs. The guidelines that are currently in place give the public a sense of security about the food they eat while forcing food manufacturers to prove that their product is not dangerous.
10. Electrophoresis is a technique used to separate and sometimes purify macromolecules—especially proteins and nucleic acids—that differ in size, charge, or conformation.

    A Southern blot is a method in molecular biology of enhancing the result of an agarose gel electrophoresis by marking specific DNA sequences.

    The Northern blot is a technique used in molecular biology research to study gene expression. RNA, rather than DNA, is the substance being analyzed by electrophoresis and detection with a hybridization probe.
11. PCR identifies individuals by matching a sequence of their DNA with a sample obtained at a crime scene. It is good for society to have a record of individuals who have been convicted of a serious crime. This allows law enforcement to determine more quickly the identity of a suspect following another crime. Some students may argue against it, saying that it opens the question of guilt to false accusation and errors made in the sampling of DNA at the crime scene.

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