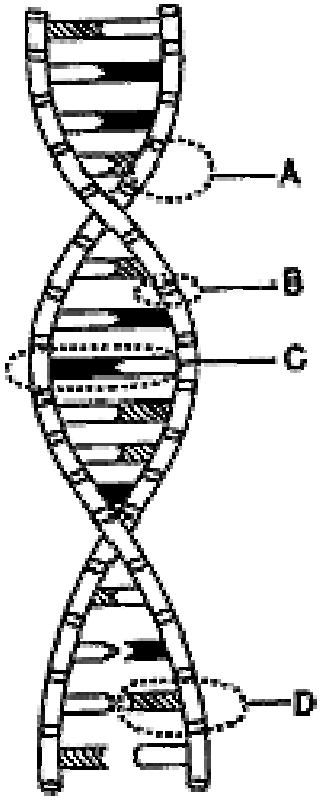
DESIGNER GENES: PRACTICE- BIOTECHNOLOGY

DNA COMPOSITION PROBLEMS



Using the diagram, answer the following

1. What is A?

2. What is B?

3. What is C?

4. What is D?

Remember:

For the nitrogen bases, the amount of **A**denine in DNA will equal the amount of **T**hymine and the amount of **G**uanine equals the amount of **C**ytosine for the molecule to be double stranded because A bonds to T and G bonds to C.

If A does not equal T and G does not equal C, then the DNA is single stranded.

Answer the following questions

5. If 22% of bases in DNA are A, what per cent is expected to be G?

6. If A= 29, T=21, G=32, and C=18 %, what is nature of DNA?
   single strand or double strand

7. If it takes 15 minutes, using PCR, to make a copy of the molecule, how many copies will be present after 2 hours?

8. If a DNA is labeled with a heavy isotope of nitrogen, and allowed to replicate in the presence of only light nitrogen, what fraction of the molecules will be of H/L composition after three rounds of duplication?

9. Determine the total length of DNA in one adult human.

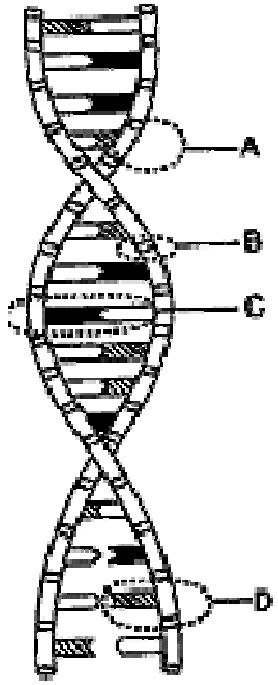
   The total length of DNA present in one adult human is calculated by the multiplication of
   (length of 1 bp)(number of bp per cell)(number of cells in the body)
   $(0.34 \times 10^{-9}\ m)(6 \times 10^{9})(10^{13})$ = **?**

Scientists usually describe the length of DNA using a unit called kb or kbp. One kb is 1000 base pairs, the base pair being the basic repeating nucleotide unit of the DNA chain. Each base pair has a length of 0.33 nm

10. The length of human chromosome number 1 DNA is 200,000 kb. What is its length in nm? in microns?

DESIGNER GENES: PRACTICE- BIOTECHNOLOGY

DNA COMPOSITION PROBLEMS



Using the diagram, answer the following

1. What is A? **sugar deoxyribose**

2. What is B? **phosphate**

3. What is C? **nitrogen base pair** A-T or G-C

4. What is D? **nucleotide (sugar deoxyribose, phosphate, and nitrogen base – A, T, G, or C**

Remember:

For the nitrogen bases, the amount of **A**denine in DNA will equal the amount of **T**hymine and the amount of **G**uanine equals the amount of **C**ytosine for the molecule to be double stranded because A bonds to T and G bonds to C.

If A does not equal T and G does not equal C, then the DNA is single stranded.

Answer the following questions

5. If 22% of bases in DNA are A, what per cent is expected to be G? ***28%***

6. If A= 29, T=21, G=32, and C=18 %, what is nature of DNA? ***single stranded***

7. If it takes 15 minutes, using PCR, to make a copy of the DNA molecule, how many copies will be present after 2 hours? **256**

8. If a DNA is labeled with a heavy isotope of nitrogen, and allowed to replicate in the presence of only light nitrogen, what fraction of the molecules will be of H/L composition after three rounds of duplication? ***¼ or 2 of the 8***

9. Determine the total length of DNA in one adult human.

   The total length of DNA present in one adult human is calculated by the multiplication of

   (length of 1 bp)(number of bp per cell)(number of cells in the body)

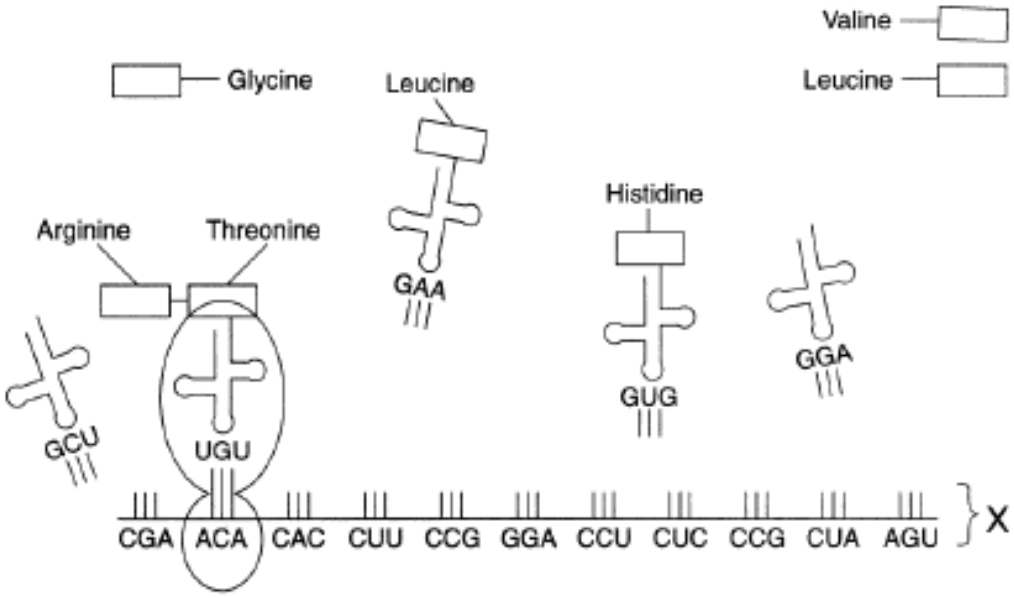
   $(0.34 \times 10^{-9}\ m)(6 \times 10^{9})(10^{13})$ **$= 2.0 \times 10^{13}$ meters**

Scientists usually describe the length of DNA using a unit called kb or kbp. One kb is 1000 base pairs, the base pair being the basic repeating nucleotide unit of the DNA chain. Each base pair has a length of 0.33 nm

10. The length of human chromosome number 1 DNA is 200,000 kb. What is its length in nm? in microns? , **67,000 nm 67 microns**

DESIGNER GENES: PRACTICE- BIOTECHNOLOGY

TRANSLATION
PROBLEMS



1. What is X in the above diagram?

2. What are the symbols coming to attach to X?

3. What do the rectangles represent?

4. Which end of molecule X is CGA ï the $3^{\hat{0}}$or the $5^{\hat{0}}$?

5. List the sequence of DNA which produced molecule X ï be sure to label the ends.

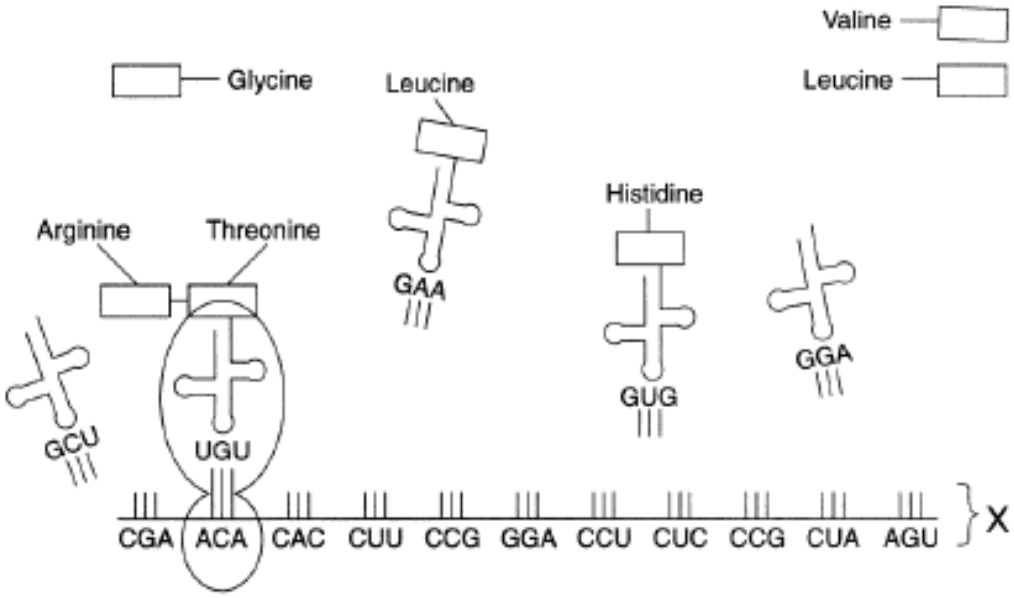
A gene will begin with a start codon and end with a stop codon, so you look for **AUG the start codon**, and then begin marking off groups of 3 until you reach a **stop codon – UAA, UAG, OR UGA** Remember that AUG is a start codon and it also codes for an amino acid.

AC G C A U G C C A U G C U U C A C G U A G

6. In the above section of M-RNA, how many codon follow the Start Codon before you reach the Stop Codon? How many amino acids will be formed by this section of m-RNA?

DESIGNER GENES: PRACTICE- BIOTECHNOLOGY

TRANSLATION PROBLEMS



1. What is X in the above diagram? **m-RNA**

2. What are the symbols coming to attach to X? **t-RNA**

3. What do the rectangles represent? **amino acids**

4. Which end of molecule X is CGA ï the 3$^{\hat{0}}$or the 5$^{\hat{0}}$? **the 5$^{\hat{}}$**

5. List the sequence of DNA which produced molecule X ï be sure to label the ends.
   3$^{\hat{}}$ GCT TGT GTG GAA GGC CCT GGA GAG GGC GAT TCA 5$^{\hat{}}$

A gene will begin with a start codon and end with a stop codon, so you look for **AUG the start codon**, and then begin marking off groups of 3 until you reach a **stop codon – UAA, UAG, OR UGA** Remember that AUG is the start codon and it also codes for an amino acid.

AC G C A U G C C A U G C U U C A C G U A G

6. In the above section of M-RNA, how many codon follow the Start Codon before you reach the Stop Codon? How many amino acids will be formed by this section of m-RNA?

   This message would make 5 amino acids; begin at AUG, and count successive groups of 3. UAG is stop codon and does *not* code for any amino acid.

DESIGNER GENES: PRACTICE- BIOTECHNOLOGY

MUTATION PROBLEMS

Mutations will change one or more bases in DNA and hence in RNA. Some mutations will replace one amino acid, some will produce no change. Adding or removing a base will totally alter the message.

Point mutations: one base is replaced by another base.

Depending upon location of this change,

a) either one or no amino acids will be changed,

b) a start or stop signal may be altered, resulting in no protein or a longer than normal protein

c) intron splice site may be altered, yielding larger, usually non-functional proteins

Point mutations in the coding region of DNA can have one of three consequences:

1) No effect if the mutated codon still codes for the same amino acid
2) It can result in the protein having one different amino acid. The effect on protein function can vary from no effect to devastating diseases such as cycstic fibrosis.
3) It can truncate the protein if the mutation results in a formation of a stop codon

PROBLEMS :

ORIGNAL SEQUENCE

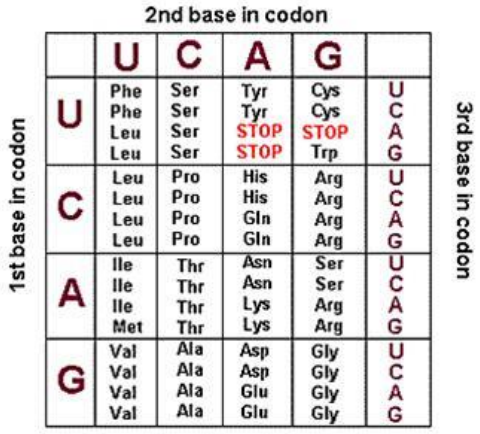
· **UGUAC AUG UAU ACG UCU CAA UGA UCCA**
Met Tyr Ser Thr Gln STOP

POINT MUTATIONS

· **UGUAC AUG UAU ACG UCU CA<u>G</u> UGA UCCA**
Met Tyr Ser Thr Gln STOP

· **UGUAC AUG UAU ACG <u>C</u>CU CAA UGA UCCA**
Met Tyr Ser Pro Gln STOP

· **UGUAC AUG UA<u>A</u> ACG UCU CAA UGA UCCA**
Met STOP



1. Why does the amino sequence begin at AUG and UGUAC are not translated?

2. What happens to the amino acid sequence when **CAA** is replaced by **CA<u>G</u>**?

3. What happens when **UCU** is replace by **<u>C</u>CU**?

4. What happens when **UAU** is replaced by **UA<u>A</u>**?

DESIGNER GENES: PRACTICE- BIOTECHNOLOGY

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3) It can truncate the protein if the mutation results in a formation of a stop codon

PROBLEMS :

ORIGNAL SEQUENCE

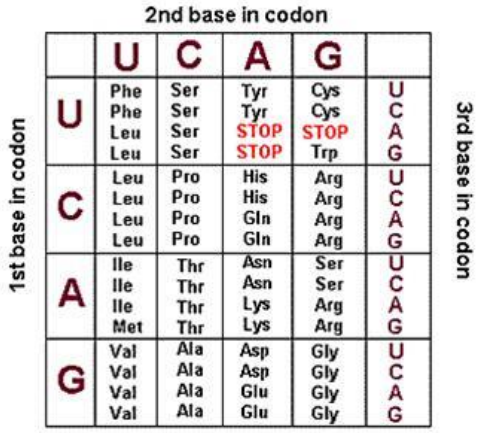
· UGUAC AUG UAU ACG UCU CAA UGA UCCA
Met Tyr Ser Thr Gln STOP

POINT MUTATIONS

· UGUAC AUG UAU ACG UCU CA**G** UGA UCCA
Met Tyr Ser Thr Gln STOP

· UGUAC AUG UAU ACG **C**CU CAA UGA UCCA
Met Tyr Ser **Pro** Gln STOP

· UGUAC AUG UA**A** ACG UCU CAA UGA UCCA
Met **STOP**



1. Why does the amino sequence begin at AUG and UGUAC are not translated?
   It is the start codon for the new amino acid sequence and codes for the amino acid Met UGUAC are not part of this coding sequence
2. What happens to the amino acid sequence when **CAA** is replaced by **CA**G?
   The amino acid is not changed because CAA and CAU both code for Gln
3. What happens when **UCU** is replace by **C**CU?
   The amino acid sequence is changed from Thr to Pro
4. What happens when **UAU** is replaced by **UA**A?
   Try is replaced by UAA which is a stop code so the sequence of amino acid stop and four of the normal amino acids are not attached

DESIGNER GENES: PRACTICE- BIOTECHNOLOGY

MUTATION PROBLEMS

Frameshift mutations: one or more bases is added or deleted, making the wrong, and usually garbage codons after the mutation

Original Sequence

· UGUAC AUG UAU ACG UCU CAA UGA UCCA
Met Tyr Ser Thr Gln STOP

Deletions A

· UGUAC AUG UAU CGU CUC AAU GAU CCA
Met Tyr Arg Leu Asn Asp Pro

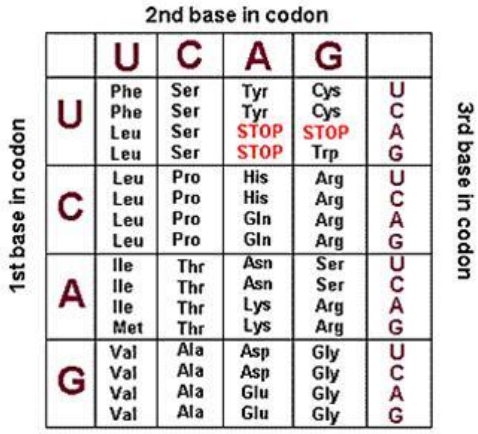
ACG

· UGUAC AUG UAU UCU CAA UGA UCCA
Met Tyr Thr Gln STOP

Insertion

A

· UGUAC AUG UAU ACG AUC UCA AUG AUC
Met Tyr Ser Ile Ser Met Ile



1. What happens to the amino acid sequence when an the base **A** is deleted from the original sequence ?

2. What happens to the amino acid sequence when the base sequence **ACG** is deleted from the original sequence?

3. What happens to the amino acid sequence when the base **A** is inserted into the original sequence?

Human Genetic Disorders caused by Mutations

4. In sickle cell anemia, G**A**G is replaced by G**T**G. What will be the base sequence of the mRNA codon and what amino acid will replace the normal Glutamic Acid in the protein sequence?

DESIGNER GENES: PRACTICE- BIOTECHNOLOGY

MUTATION PROBLEMS

Frameshift mutations: one or more bases is added or deleted, making the wrong, and usually garbage codons after the mutation

Original Sequence

· UGUAC AUG UAU ACG UCU CAA UGA UCCA
Met Tyr Ser Thr Gln STOP

Deletions A

· UGUAC AUG UAU CGU CUC AAU GAU CCA
Met Tyr Arg Leu Asn Asp Pro

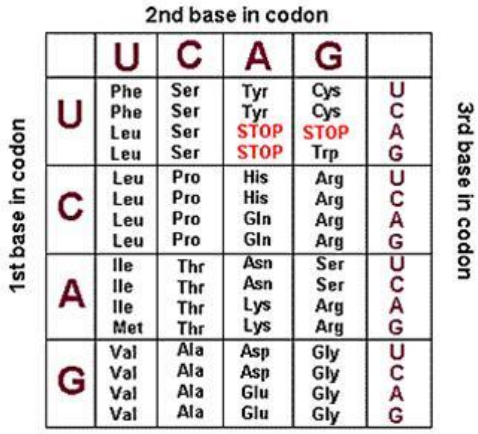
ACG

· UGUAC AUG UAU UCU CAA UGA UCCA
Met Tyr Thr Gln STOP

Insertion

A

· UGUAC AUG UAU ACG AUC UCA AUG AUC
Met Tyr Ser Ile Ser Met Ile



1. What happens to the amino acid sequence when an the base **A** is **deleted** from the original sequence ? **all of the codons are changed from this point on and all of the amino acids are changed**
2. What happens to the amino acid sequence when the base sequence **ACG** is deleted from the original sequence?
   one of the amino acids (Ser) is missing from the amino acid sequence because its codon is missing
3. What happens to the amino acid sequence when the base **A** is **inserted** into the original sequence? **all of the codons are changed from this point on and all of the amino acids are changed**

Human Genetic Disorders caused by Mutations

4. In sickle cell anemia, GAG is replaced by GTG. What will be the base sequence of the mRNA codon and what amino acid will replace the normal Glutamic Acid in the protein sequence? **valine substitutes for glutamic acid which causes the hemoglobin molecule to collapse resulting in a sickle cell red blood cell Gene therapy is being studied in an attempt to correct the problem**

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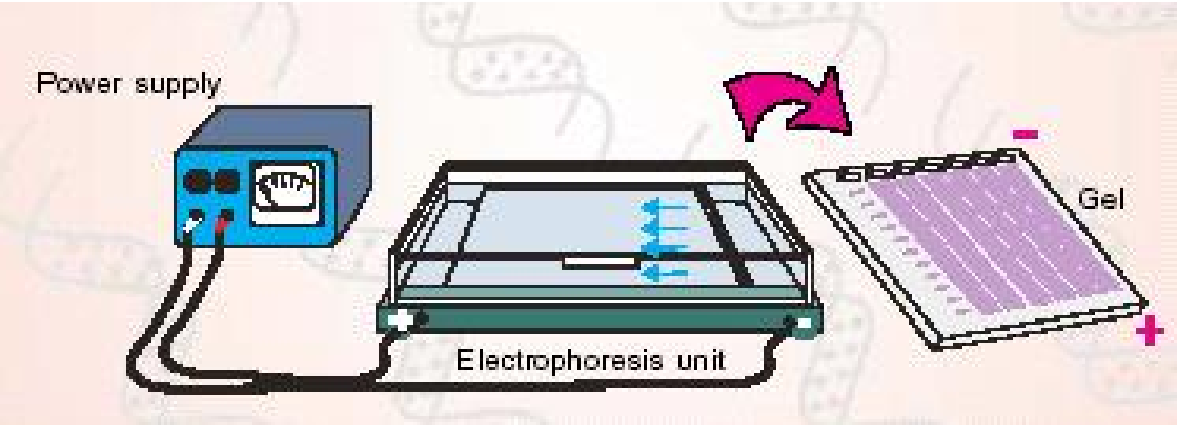
ELECTROPHORESIS



What is the function of each of the following in gel electrophoresis of DNA?

1. Agarose gel

2. Electric current:

3. "Wells" in the gel:

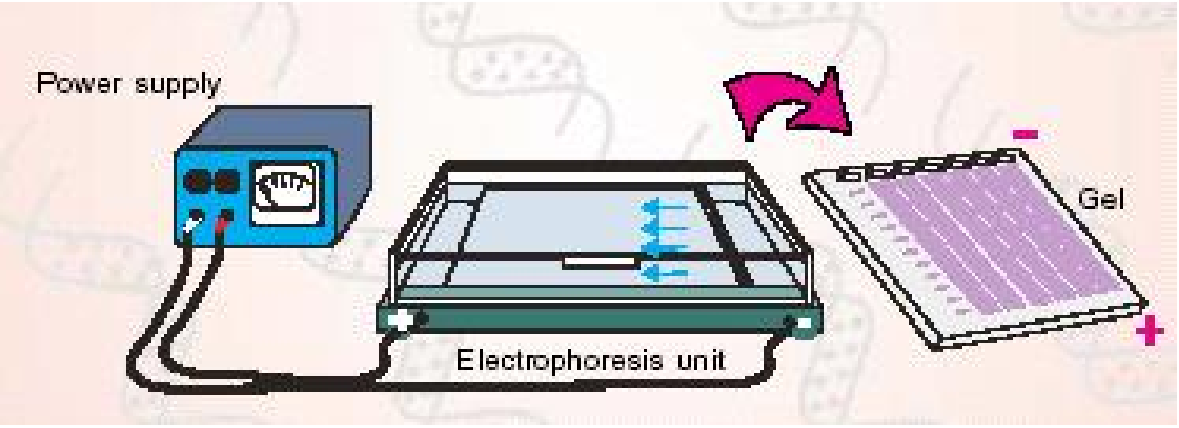
4. Toward which pole (positive or negative) does DNA migrate when electric current is run through the gel? Why do the DNA molecules move toward this pole?

5. What would happen to the DNA fragments if you forgot to turn the current off?

6. Describe how different sized DNA fragments are separated by the gel matrix.

DESIGNER GENES: PRACTICE- BIOTECHNOLOGY

ELECTROPHORESIS



What is the function of each of the following in gel electrophoresis of DNA?

1. Agarose gel:
   The agarose gel provides a matrix with pores to allow molecules to travel through and be sorted by size

2. Electric current:
   The electric current is the force that causes the negatively charged DNA molecules to more toward the positive pole.

3. "Wells" in the gel:
   The wells are the "starting gates" for the DNA molecules to be loaded into before starting the "race"

4. Toward which pole (positive or negative) does DNA migrate when electric current is run through the gel? Why do the DNA molecules move toward this pole?
   positive pole The DNA molecules are negatively charged (opposite charges attract one another

5. What would happen to the DNA fragments if you forgot to turn the current off?
   The DNA fragments would keep on running through the gel until they ran off the end

6. Describe how different sized DNA fragments are separated by the gel matrix.
   Longer DNA fragments take longer to work their way through the pores of the gel matrix, they don't travel as far through the gel as the shorter fragments in the same amount of time

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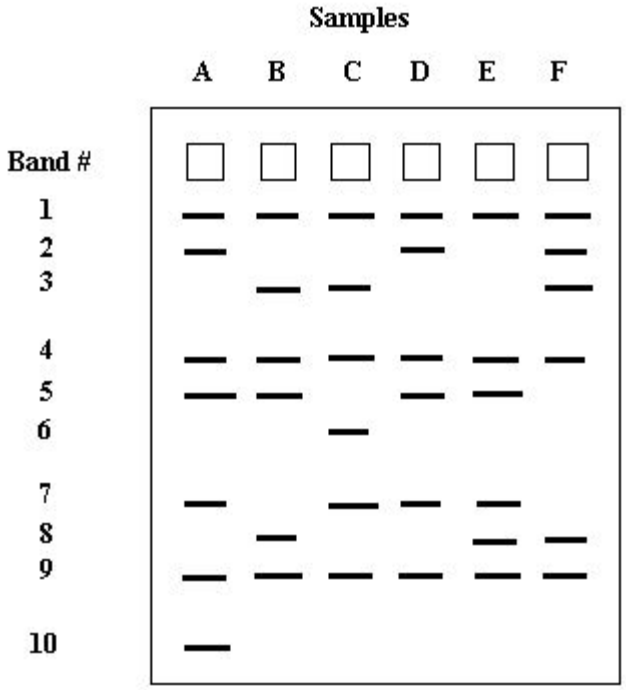

Examine the diagram of the gel and answer the following questions.

1. What do the bands in the drawing of the gel represent?

2. Which band(s) traveled fastest?

3. Which band(s) traveled slowest ?

4. On the above drawing, which is the positive and negative ends of the gel.

5. Are there any bands which are unique to only one individual? If so, which one?

6. How many bands are shared in common by **all** of the individuals?

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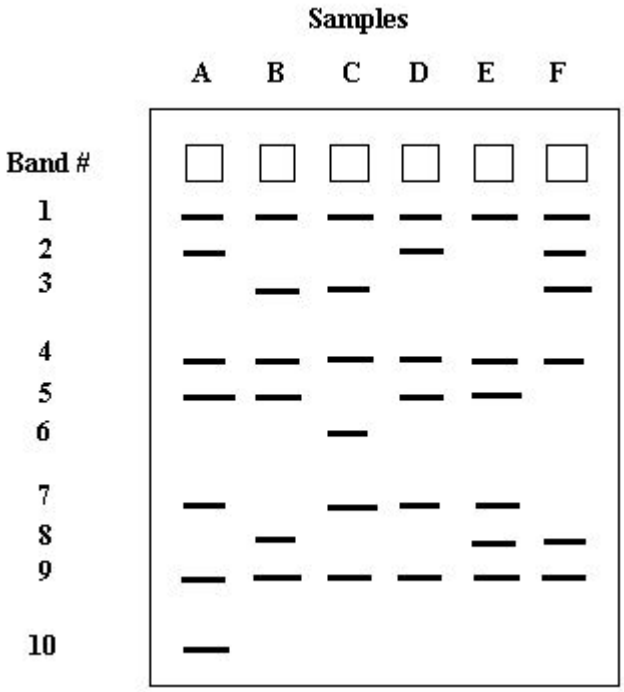


Examine the diagram of the gel and answer the following questions.

1. What do the bands in the drawing of the gel represent?
   DNA fragments which are the same size

2. Which band(s) traveled fastest?
   The bands farthest from the wells (containing the shortest DNA fragments) traveled the fastest--Bands #10

3. Which band(s) traveled slowest?
   The bands nearest the wells (containing the longest DNA fragments) traveled the slowest --Band #1

4. On the above drawing, which is the positive and negative ends of the gel.
   The positive pole is located farthest from the wells and the negative pole is located closest to the wells

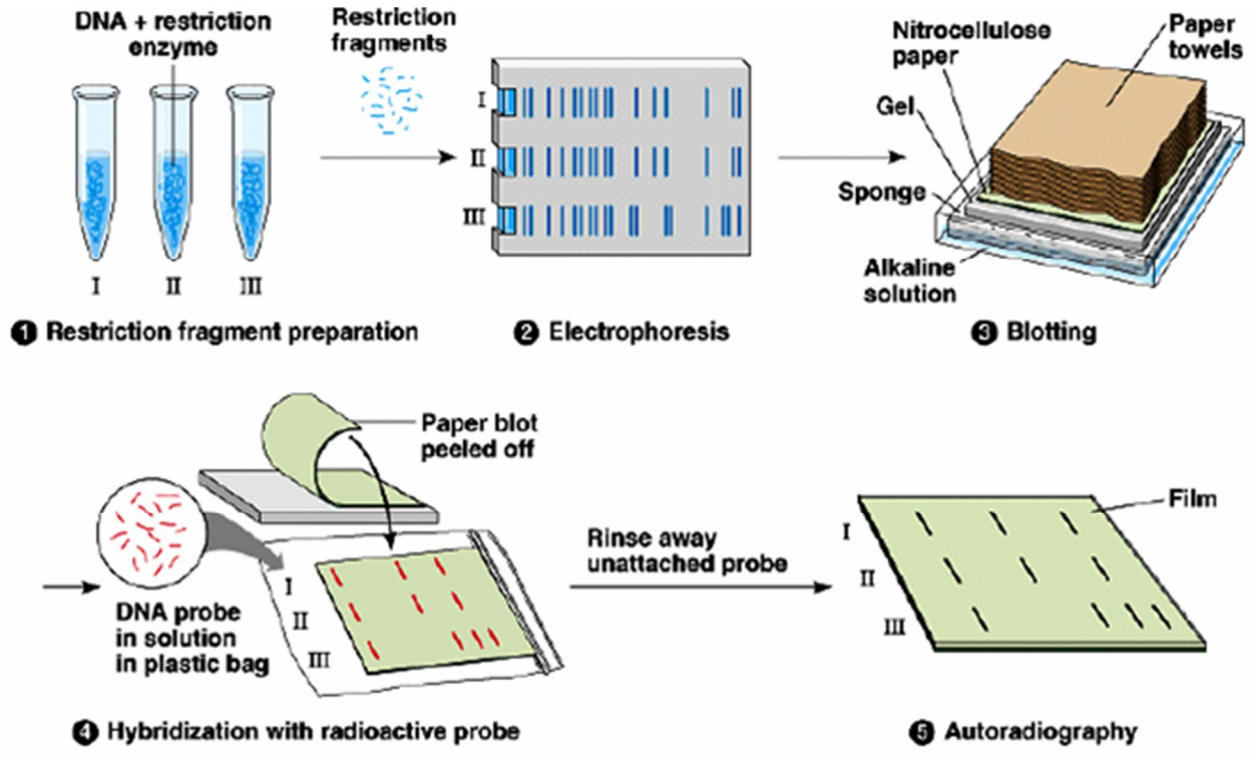
5. Are there any bands which are unique to only one individual? If so, which one?
   Yes --Bands #6 (sample C) and #10 (sample A).

6. How many bands are shared in common by **all** of the individuals?
   2 (Bands #4 and #9)

DESIGNER GENES: PRACTICE- BIOTECHNOLOGY

DNA ANALYSIS - Blotting Techniques

Type of Blot	Molecules separated by electrophoresis	Probe
Southern	ssDNA	cDNA or RNA
Northern	denatured RNA	RNA or cDNA
Western	Protein	Antibodies



Match the following techniques with its use.
Some answers may be used more than once; others not at all.
Each use will have only one answer.

A. Southern Blotting
B. Northern Blotting
C. Western Blotting
D. Eastern Blotting
E. none of these

1. Transferring electrophoresed RNA to filters
2. Determining if particular binding protein was inserted into the membrane
3. Transferring electrophoresed DNA to filters
4 Transferring hybrid DNA to an enucleated egg.

DESIGNER GENES: PRACTICE- BIOTECHNOLOGY

DNA ANALYSIS - Blotting Techniques

Type of Blot	Molecules separated by electrophoresis	Probe
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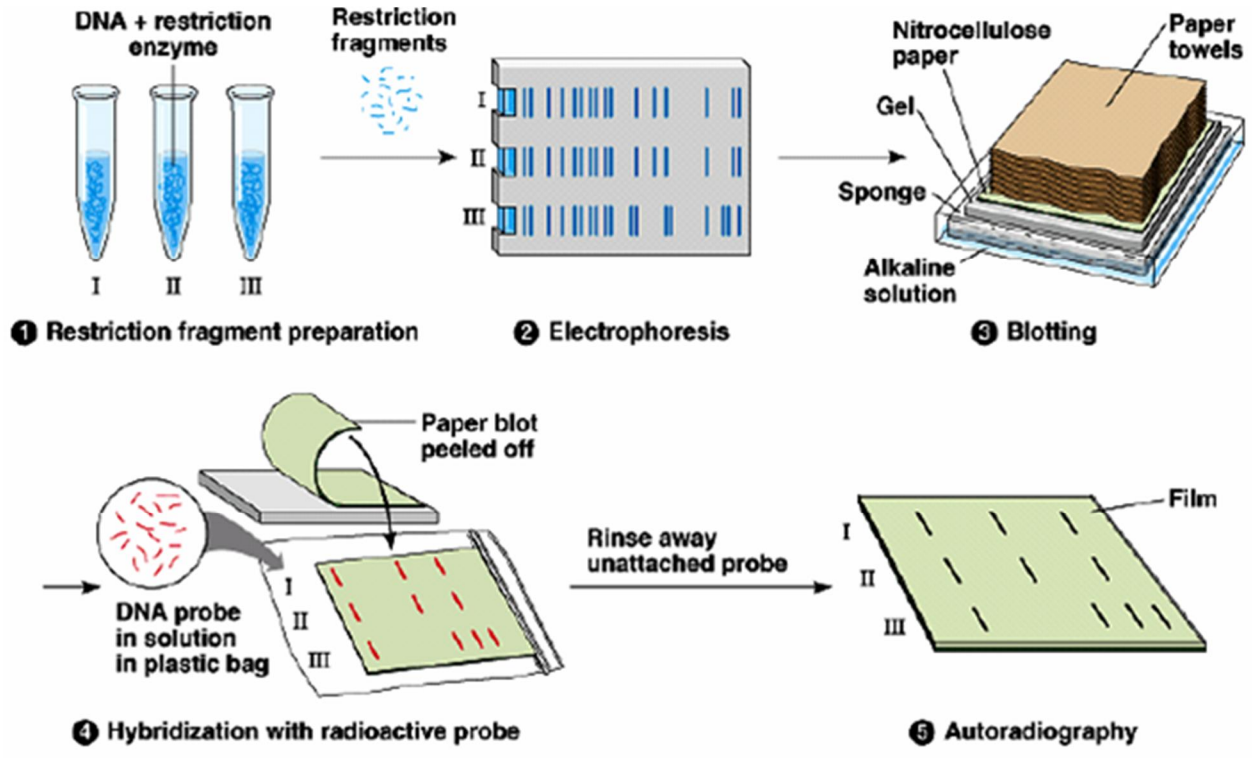

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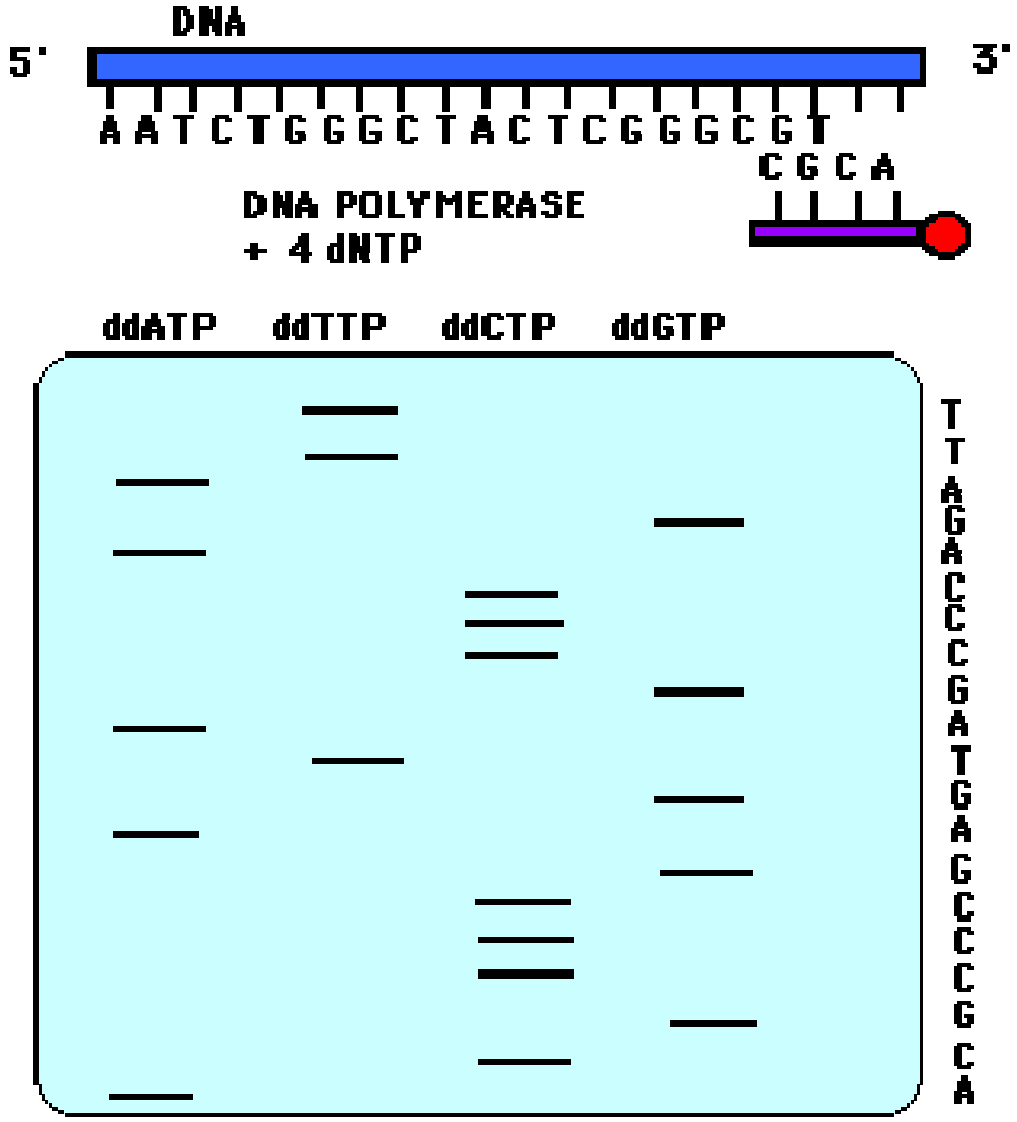
Each use will have only one answer.

A. Southern Blotting
B. Northern Blotting
C. Western Blotting
D. Eastern Blotting
E. none of these

1. Transferring electrophoresed RNA to filters **B**
2. Determining if particular binding protein was inserted into the membrane **C**
3 Transferring electrophoresed DNA to filters **A**
4. Transferring hybrid DNA to an enucleated egg **E**

DESIGNER GENES: PRACTICE- BIOTECHNOLOGY

DNA SEQUENCING

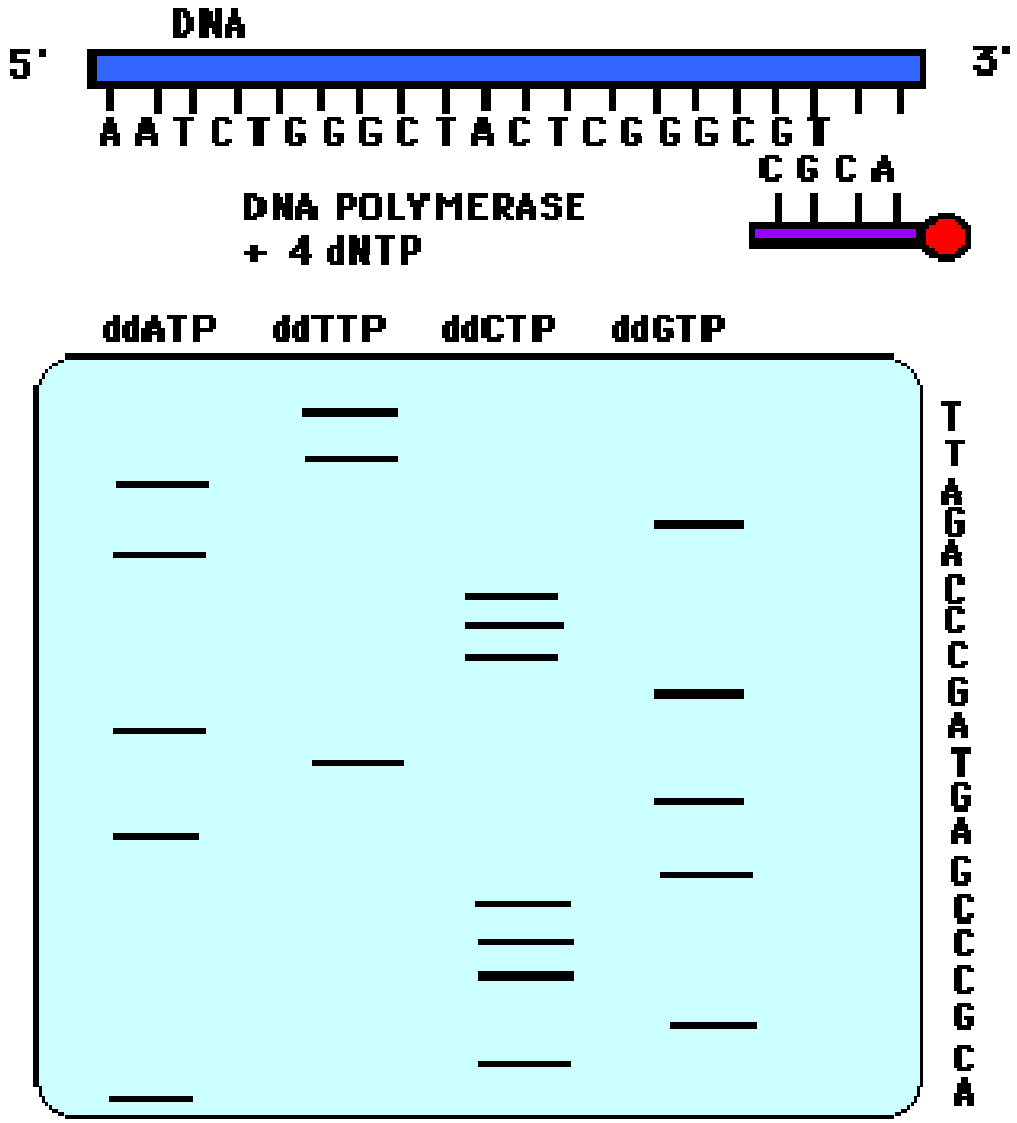


The gel was produced by the Sanger method of DNA sequencing.

Using the information provided, determine the complete sequence of the DNA from the gel.

DESIGNER GENES: PRACTICE- BIOTECHNOLOGY

DNA SEQUENCING



The gel was produced by the Sanger method of DNA sequencing.

Using the information provided, determine the complete sequence of the DNA from the gel.

Read rows from bottom to top

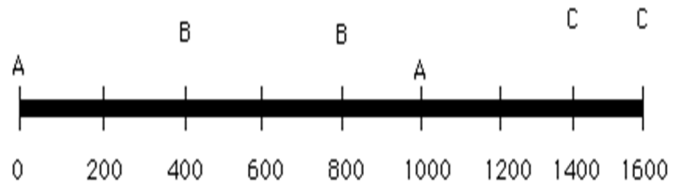
The sequence of the strand of DNA complementary to the sequenced strand is 5' to 3'
ACGCCCGAGTAGCCCAGATT

DESIGNER GENES: PRACTICE- BIOTECHNOLOGY

RESTRICTION MAPPING

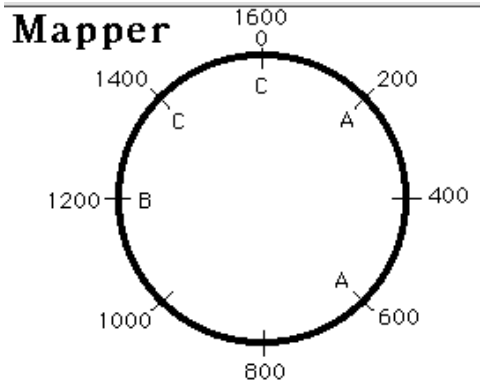
- Estimating fragment sizes from RE map
- There are both linear and circular maps
- When cutting a **linear DNA**, there will get **one more** fragment than you have RE sites. When cutting **circular DNA**, there will the **same number** of fragments as there are RE sites.

Given the map below, find the sizes generated with the RE combinations:



Restriction Enzyme	Fragment Sizes
A	
B	
C	
A + B	
A + C	
B + C	

A circular map:



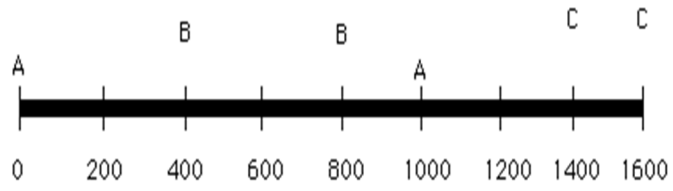
Restriction Enzyme	Fragment Sizes
A	
B	
C	
A + B	
A + C	
B + C	

DESIGNER GENES: PRACTICE- BIOTECHNOLOGY

RESTRICTION MAPPING

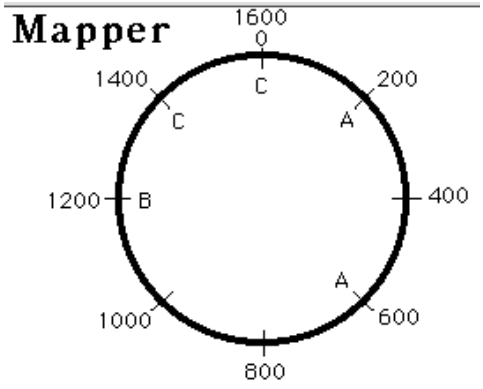
- Estimating fragment sizes from RE map
- There are both linear and circular maps
- When cutting a linear DNA, there will get one more fragment than you have RE sites. When cutting circular DNA, there will the same number of fragments as there are RE sites.

Given the map below, find the sizes generated with the RE combinations:



Restriction Enzyme	Fragment Sizes
A	600, 1000
B	400, 800
C	200, 1400
A + B	200, 400, 600
A + C	200, 400, 1000
B + C	200, 400, 600

A circular map:



Restriction Enzyme	Fragment Sizes
A	400, 1200
B	1600
C	200, 1400
A + B	400, 600
A + C	200, 400, 800
B + C	200, 1200

DESIGNER GENES: PRACTICE- BIOTECHNOLOGY

RESTRICTION MAPPING

- Two portions of the DNA sample are individually digested with different restriction enzymes
- A third portion of the DNA sample is double-digested with both restriction enzymes at the same time
- The total length of the fragments in each digestion will be equal
- Because the length of each individual DNA fragment depends upon the positions of its restriction sites, each restriction site can be mapped according to the lengths of the fragments
- **The restriction map is** the final drawing of the DNA segment that shows the positions of the restriction sites

DNA	Sizes of Fragments (bp)
uncut DNA	10,000
DNA cut with EcoRI	8000, 2000
DNA cut with BamHI	5000, 5000
DNA cut with EcoRI + BamHI	5000, 3000, 2000

Construct a restriction map of a linear fragment of DNA, using the following data.
Your map should indicate the relative positions of the restriction sites along with distances from the ends of the molecule to the restriction sites and between restriction sites

DESIGNER GENES: PRACTICE- BIOTECHNOLOGY

RESTRICTION MAPPING

- Two portions of the DNA sample are individually digested with different restriction enzymes
- A third portion of the DNA sample is double-digested with both restriction enzymes at the same time
- The total length of the fragments in each digestion will be equal
- Because the length of each individual DNA fragment depends upon the positions of its restriction sites, each restriction site can be mapped according to the lengths of the fragments
- **The restriction map is** the final drawing of the DNA segment that shows the positions of the restriction sites

DNA	Sizes of Fragments (bp)
uncut DNA	10,000
DNA cut with EcoRI	8000, 2000
DNA cut with BamHI	5000, 5000
DNA cut with EcoRI + BamHI	5000, 3000, 2000

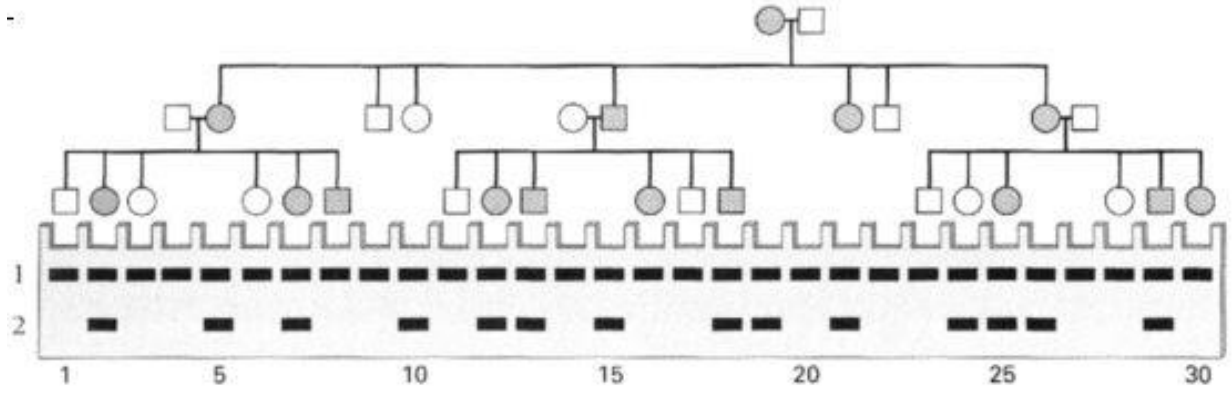
Construct a restriction map of a linear fragment of DNA, using the following data.
Your map should indicate the relative positions of the restriction sites along with distances from the ends of the molecule to the restriction sites

DESIGNER GENES: PRACTICE- BIOTECHNOLOGY

RFLP – Restriction Fragment Length Polymorphism

The pedigree below is for Huntington disease, an important, rare human genetic disorder. Below the pedigree is a diagram illustrating the DNA bands observed for a RFLP -restriction fragment length polymorphism - identified in this family.

Each individual's DNA pattern is shown in the lane directly underneath that individual's pedigree symbol.



1. Is this disease dominant or recessive? Provide <u>specific</u> evidence from the pedigree.

2. Is this disease sex-linked or autosomal? Provide specific evidence from the pedigree.

3. Using HD and hd for the Huntington alleles and A_1 and A_2 for the RFLP alleles (bands 1 and 2 from the gel, respectively), give the genotypes of individuals 1, 13, 19 and 24.

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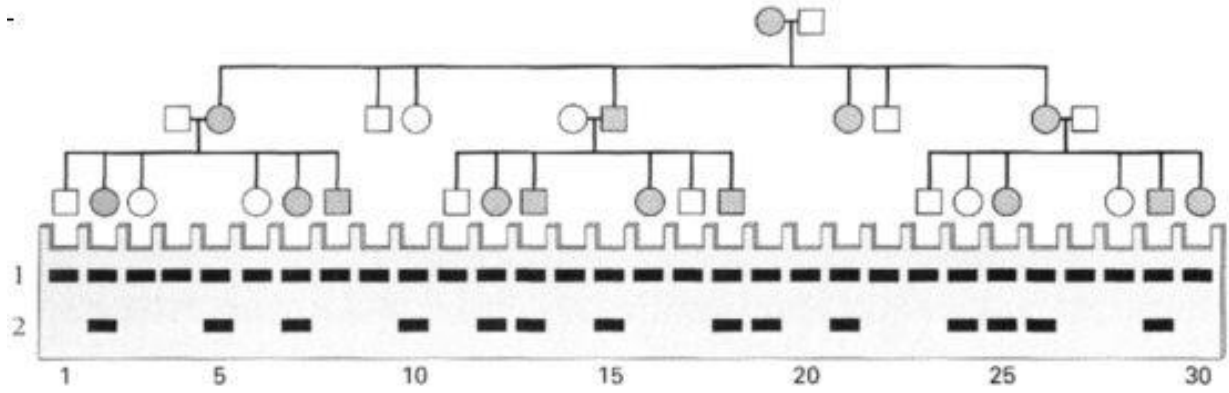

1 . Is this disease dominant or recessive? Provide <u>specific</u> evidence from the pedigree.

The disease is dominant because (1) Affected children always have one affected parent, and (2) If the disease is rare, the unrelated, unaffected spouses are likely to all be homozygous, yet their children show the trait.

2 . Is this disease sex-linked or autosomal? Provide specific evidence from the pedigree.
The disease is autosomal, because males 13 and 18 inherit it from their <u>father</u>.

3. Using HD and hd for the Huntington alleles and A_1 and A_2 for the RFLP alleles (bands 1 and 2 from the gel, respectively), give the genotypes of individuals 1, 13, 19 and 24.

Anyone who does not have the disease is homozygous for the recessive normal allele, hd hd. Anyone who has only one band is homozygous for that band, while anyone with both bands is heterozygous.

#1 is hd hd A_1 A_1
;#13 is HD hd A_1 A_2
#19 is HD hd A_1 A_2
#24 is hd hd A_1 A_2