

Genetic Analysis

Karyotyping, Pedigree and Gel Electrophoresis

Vocabulary

- Karyotype
- Autosome
- Sex chromosome
- Nondisjunction
- Monosomy
- Trisomy
- Pedigree
- Carrier
- Restriction enzyme
- Restriction site
- Restriction fragment
- Sticky ends
- Recombination
- Gel electrophoresis

Essential Question

- How can scientists determine genetic conditions before a child is born?

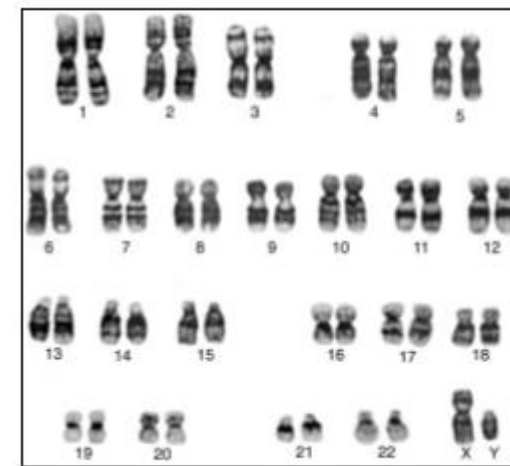
Karyotype

- Karyotype – a picture of the paired homologous chromosomes, taken during Prophase (sometimes Metaphase) of Mitosis, arranged from largest chromosome to smallest.
- Purpose: Allows for the analysis of chromosomes, to show abnormalities.

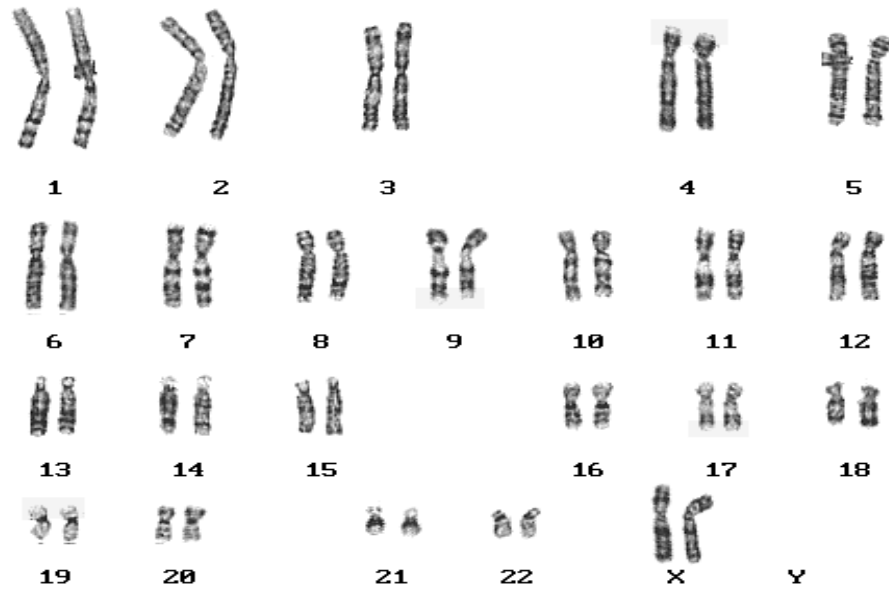


Metaphase spread

Chromosomes arranged
in homologous pairs and
ordered from largest to
smallest

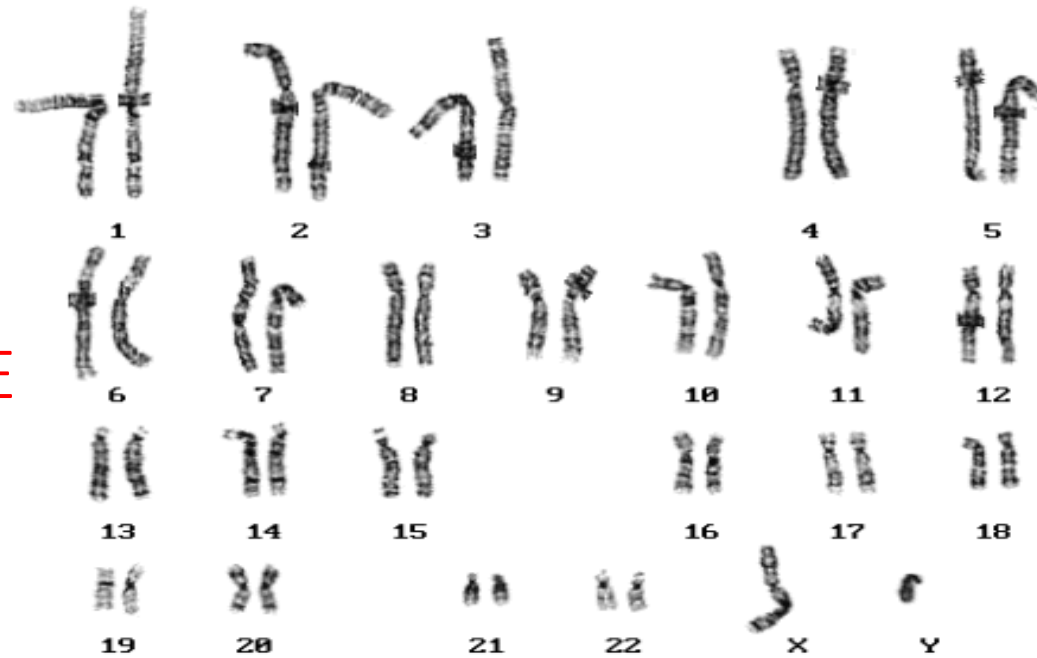


Karyotype



Normal Female

NORMAL MALE



Karyotype vocabulary

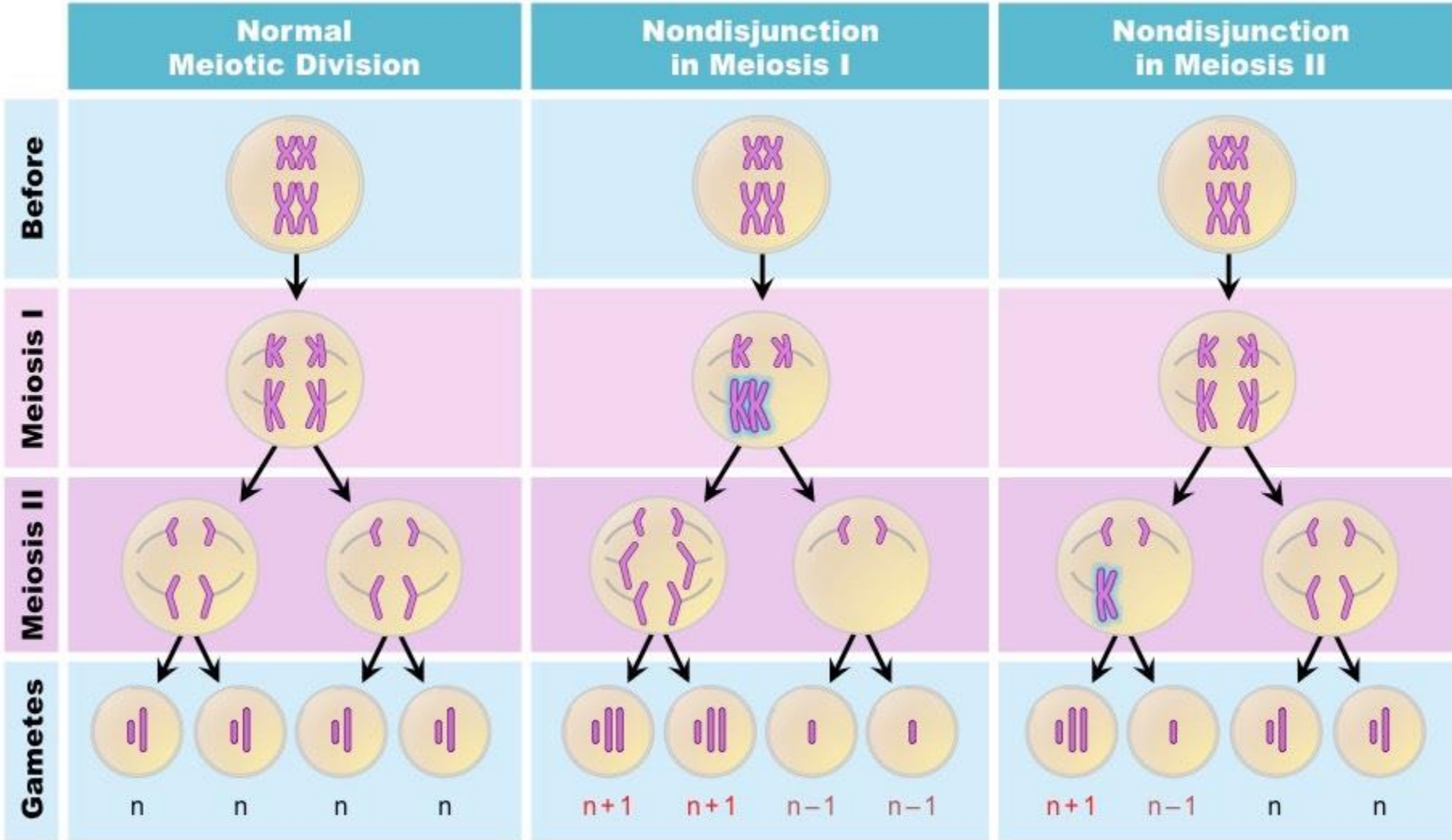
- Nondisjunction – a failure to separate chromosomes or chromatids in any anaphase stage.
- Use your science to break down the word...

Junction – to be together

Disjunction – to come apart

Nondisjunction – failure to come apart, and stays together

Nondisjunction Examples

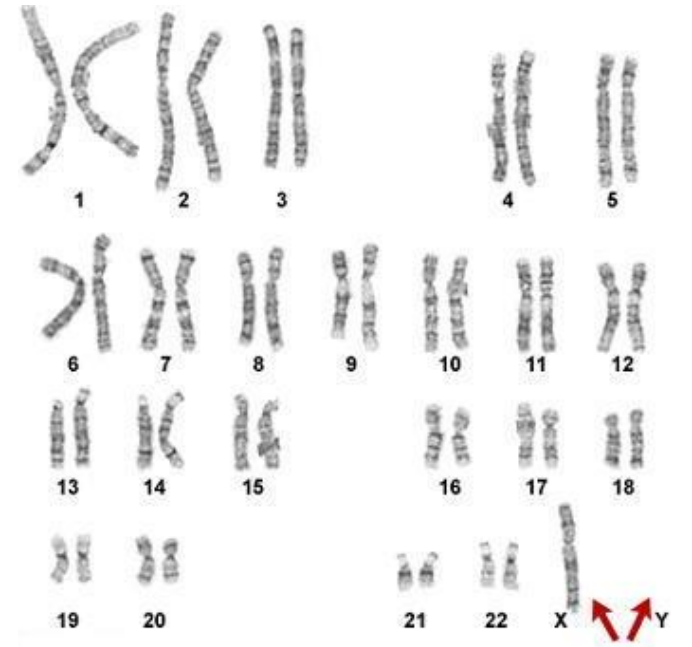


Karyotype vocabulary

- Nondisjunction mutations create gametes with too few or too many chromosomes.
 - When those gametes fertilize normal gametes, the diploid numbers are off. In humans they do not equal 46 chromosomes.
- **Monosomy** – A cell with too few chromosomes. One of the homologous pairs is a single chromosome ($2n = 45$ in humans)
- **Trisomy** – A cell with too many chromosomes. One of the homologous pairs has 3 total chromosomes ($2n = 47$ in humans)

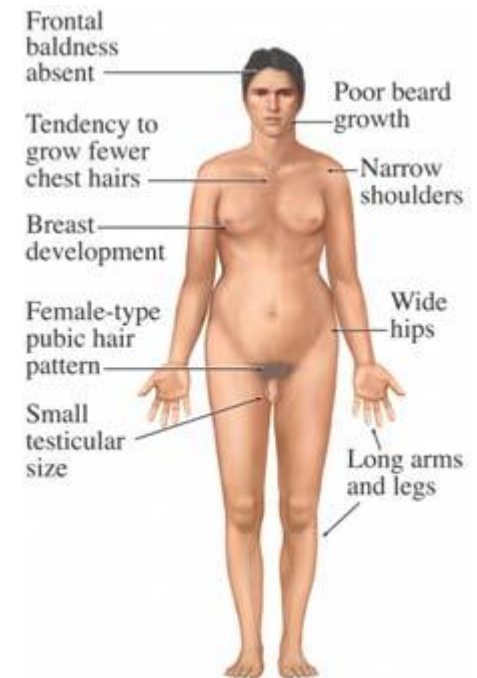
Turner's Syndrome

- Missing a X chromosome on 23rd chromosome
- Causes underdeveloped ovaries, short stature, webbed, and only found in women.
- Bull neck and broad chest. Individuals are sterile and lack expected secondary sexual characteristics.
- Mentally handicapped typically not evident.



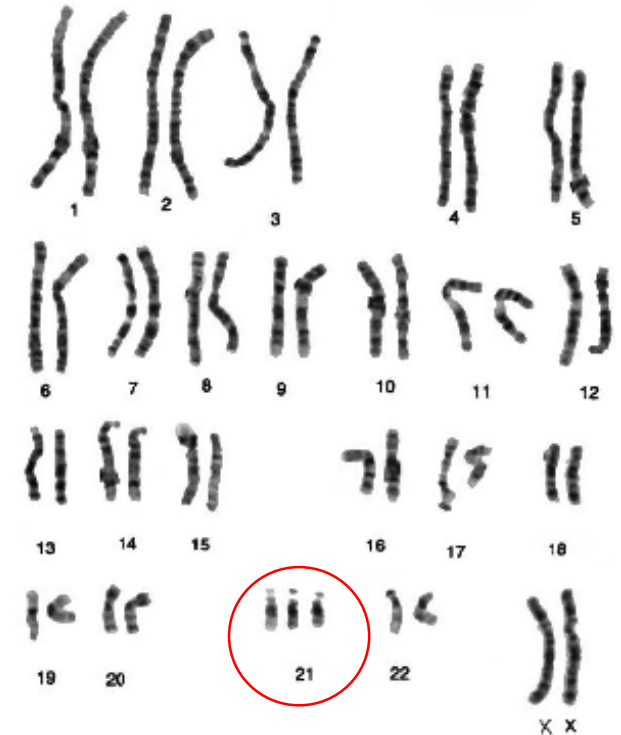
Klinefelter's Syndrome

- Caused by nondisjunction of the X chromosome on 23rd chromosome (XXY, XXYY)
- Males with some development of breast tissue
- Individuals have little body hair, typically tall, and have small testes.
- Infertility results from absent sperm.
- Mental handicapped may or may not be present.



Down Syndrome

- Caused by non-disjunction of the 21st chromosome.
- The individual has a trisomy 21.
- Some form of mental retardation is usually present



Practice reading a Karyotype

- Use the pages from Doctor's Karyotype Activity and the Disorder chart to identify the condition and sex of the individual.

Disorders Chart

NAME OF DISORDER	CHROMOSOME NOTATION	SYMPTOMS
Turner Syndrome 1:5,000 births	45, X #23 Missing Nondisjunction	90 - 98% do not survive to birth no menstruation no breast development no hips broad shoulders and neck
Co-Down Syndrome 1:16,000 births	46, XY or XX #5 Deletion of lower arm	moon-shaped face heart disease mentally retarded enlarged larynx normal lifespan
Acro-Rhomboid Tumor Syndrome 1:5,000,000	46, XY or XX #11 Deletion of upper arm	mentally retarded growth retardation widened tumors on kidneys short lifespan
Fifteen Q Deletion Syndrome 1:500,000 births	46, XY or XX #15 Deletion of lower arm	mentally retarded downward face no thumbs heart disease short lifespan
Phelan-Will Syndrome 1:500,000 births	46, XY - 97% XX - 3% #18 Deletion of lower arm	small low-set head mentally retarded respiratory problems abnormally short lifespan
Eighteen Q Deletion 1:16,000,000 births	46, XY or XX #18 Deletion of lower arm	mentally retarded heart disease abnormal hands and feet large ears large eyes normal lifespan
Co-Eye Syndrome 1:1,000,000 births	46, XY or XX #22 Deletion of bottom arm	Fused fingers and toes mentally retarded small jaw heart problems normal lifespan

Doctor's Karyotype Activity

You are a doctor and you are given these 12 baby karyotypes to look. You have five things to tell each parent about their baby.

Baby 7

Baby 8

Baby 9

1. Sex of baby -
2. Normal baby or abnormal baby -
3. Chromosome # with mistake -
4. Name of the disorder -
5. Three symptoms of disorder -

1. Sex of baby -
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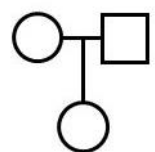
1. Sex of baby -
2. Normal baby or abnormal baby -
3. Chromosome # with mistake -
4. Name of the disorder -
5. Three symptoms of disorder -


Pedigree


- A Pedigree is a graphical representation of genetic crosses covering multiple generations.

● ● ● | Symbols Used in Pedigree Charts

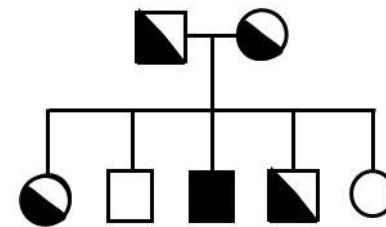
□ male ○ female

 Vertical lines connect children to their parents

 A solid square or circle indicates that the person has a certain trait

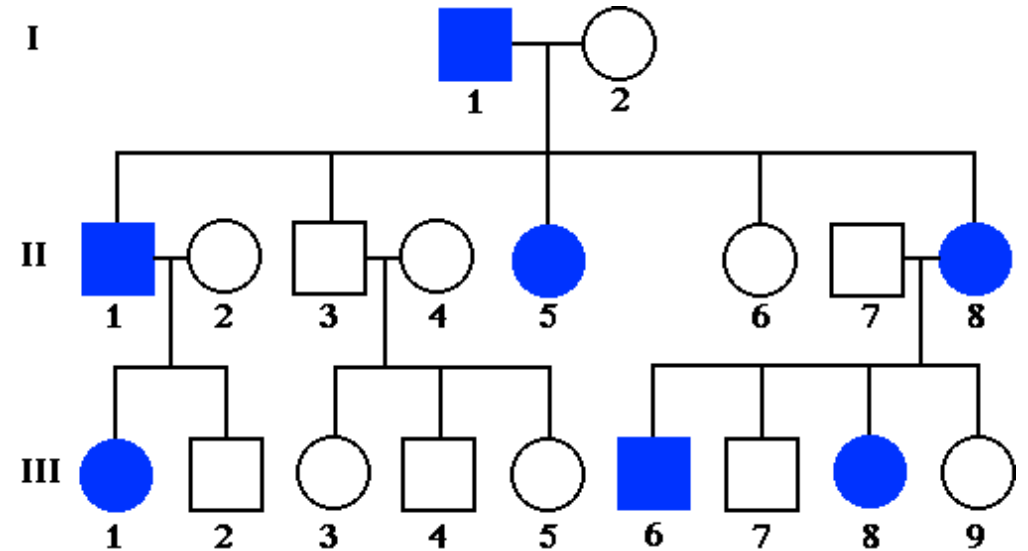
 A half-filled square or circle indicates that the person is a **carrier** of the trait.

A marriage with five children, two daughters and three sons.



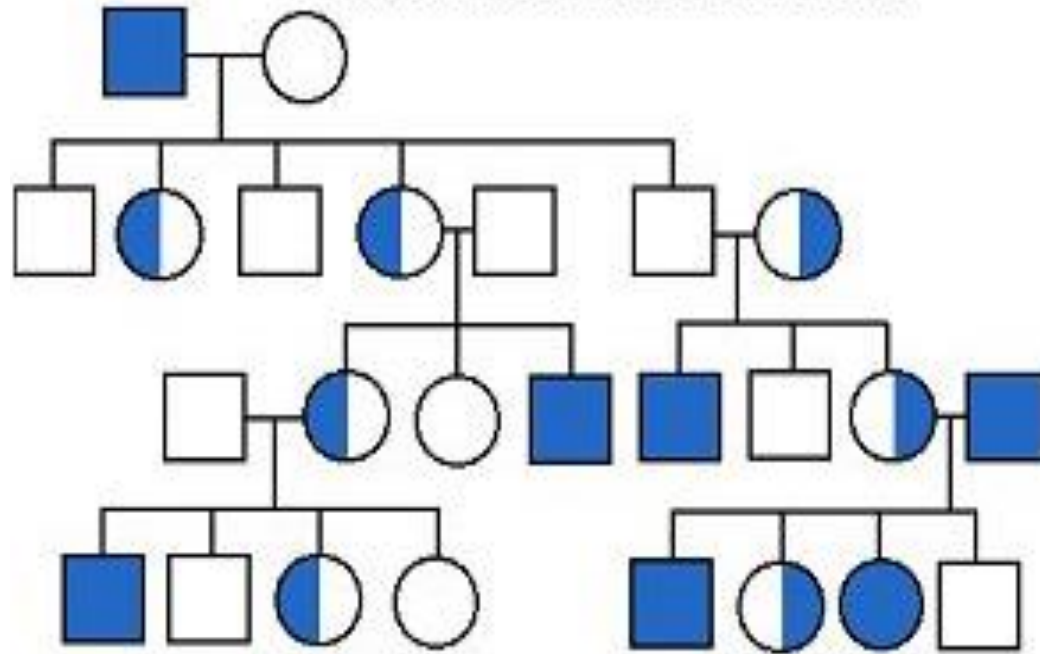
Oldest child ↔ Youngest child

How to read a pedigree...



- How many generations are shown? **3**
- How many affected people are there? **7**
- How many affected people are female? **4**
- How many affected people are male? **3**

Pedigree showing sex-linked trait



- All carriers are female. Most affected are male.

Bozeman Genetic Analysis video

- Watch the following video...

<http://www.bozemanscience.com/molecule-biology/>

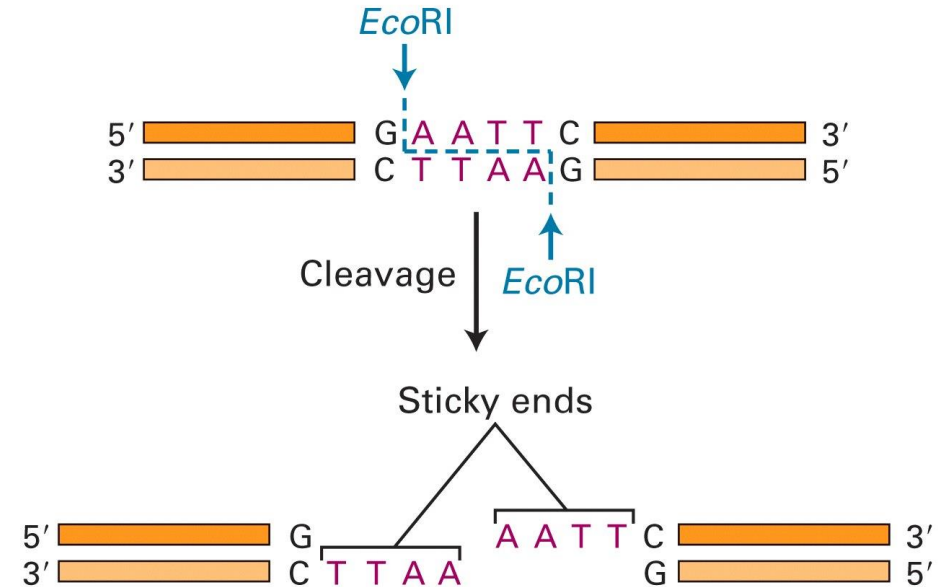
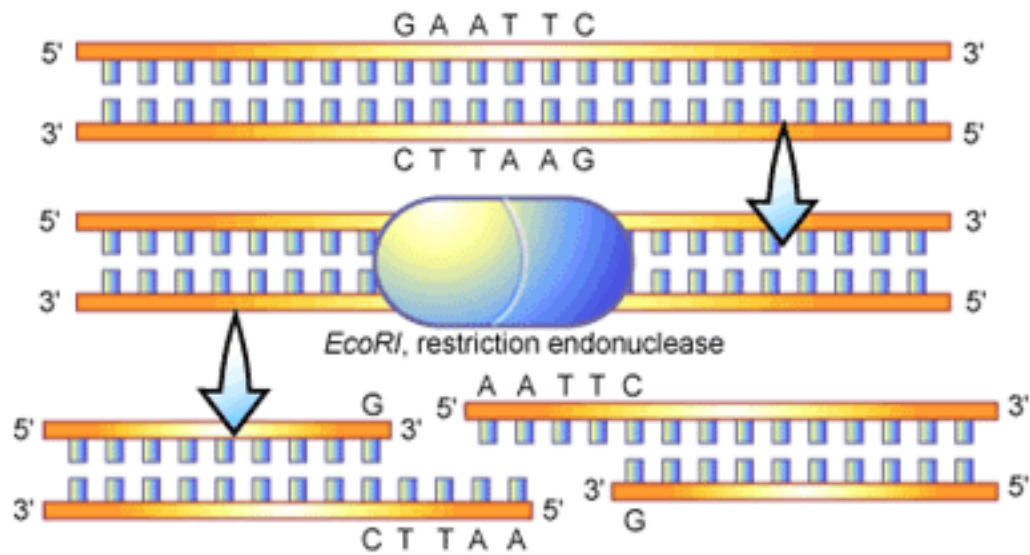
Gel Electrophoresis

- Technique used to sort and compare DNA from different sources (individuals)
- Restriction enzymes must be used to cut the DNA into small pieces called restriction fragments.
- Restriction enzymes can only work on VERY specific sequences of DNA called restriction sites.

Restriction Enzymes

- Restriction enzymes, like all enzymes, are very specific.
- Most restriction enzymes you will see are based off of prokaryotic enzymes (EcoR1, BamH1, Hind3, etc)
- Each enzyme cuts a different sequence of nitrogenous bases in DNA.
- Think of restriction enzymes like a pair of scissors.

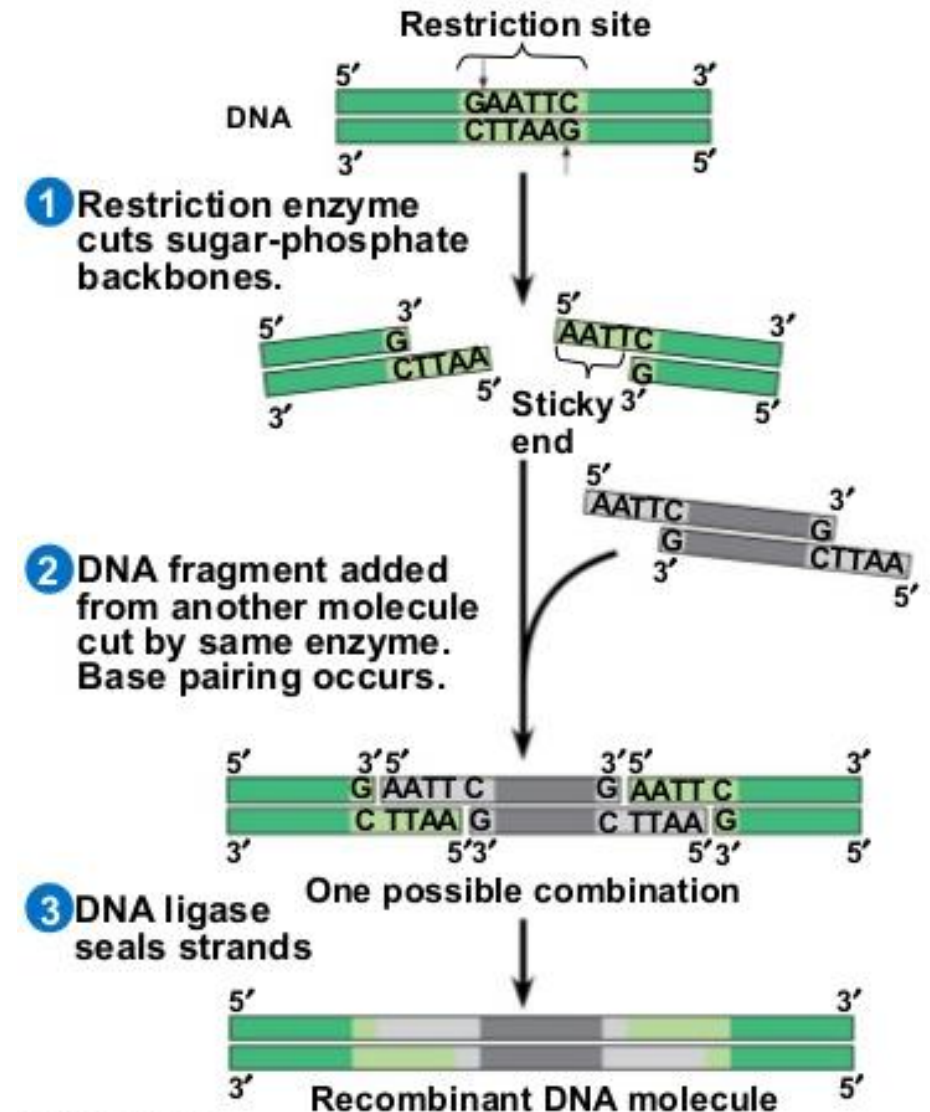
EcoRI example



- Many restriction enzymes leave “sticky ends” when they cut.
- These “sticky ends” want to pair back up following base pairing rules.

Recombinant DNA

- If the same restriction enzyme is used on different DNA pieces, all cuts will make the same “sticky ends” and the pieces can be connected.
- Using this method scientists can merge the DNA of different organisms.

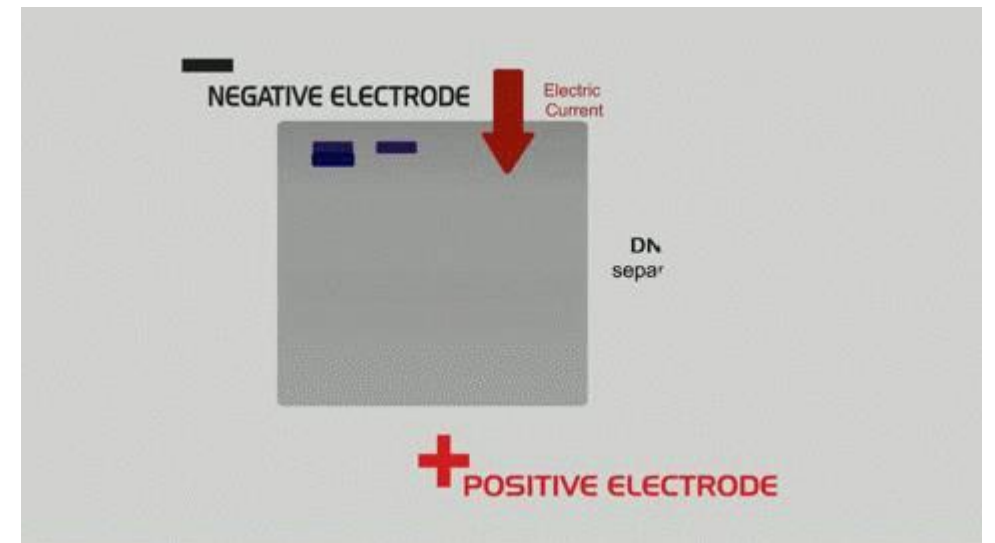


Or...organize the fragments by length!

- Gel electrophoresis uses the fact that DNA is a negatively charged molecule.
- If the fragments are pushed/pulled from a negative end of agar gel, to the positive end, then they can be separated by size.
- Small pieces of DNA will travel faster/further to the positive end, than larger pieces of DNA (which get stuck/move slow).

Gel plate creation

- It is harder for the large DNA pieces to move through the agar protein matrix (think of this as a set of monkey bars on a playground)
- Small pieces can move very quickly through the agar gel matrix (monkey bars) and get to the positive end faster.
- This sorts the DNA pieces cut by restriction enzymes by length over time



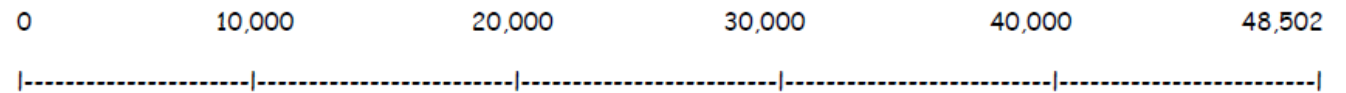
Draw a Gel Electrophoresis Plate

Practice work...

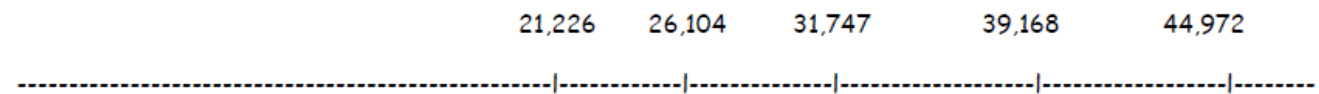
- Step 1 – calculate the length of the first fragment using EcoRI.

Restriction maps of the linear λ genome

Lambda (λ)



EcoRI Sites



Fragment 1

Subtract the final number (21,226 bps) from the initial number (in this case 0 bps) and you get...

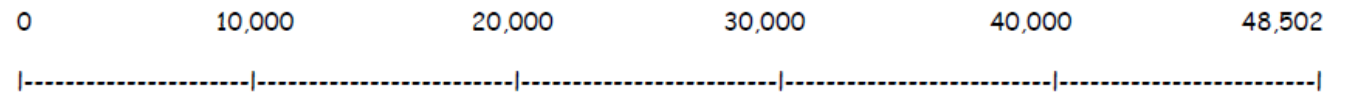
$$21,226 - 0 = 21,226$$

Draw a Gel Electrophoresis Plate

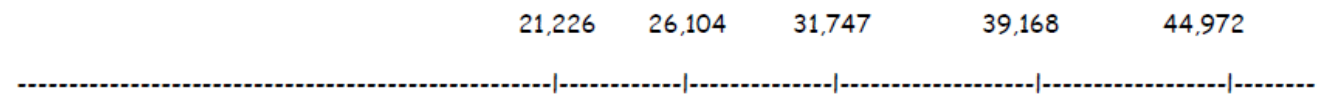
- Step 2 – calculate the length of the next fragment using EcoR1.

Restriction maps of the linear λ genome

Lambda (λ)



EcoRI Sites



Fragment 2

Subtract the final number (26,104) from the initial number (21,226) and you get...

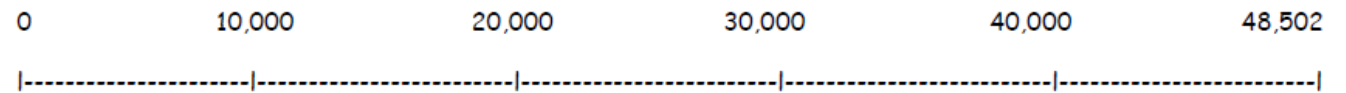
$$26,104 - 21,226 = 4,878 \text{ bps}$$

Draw a Gel Electrophoresis Plate

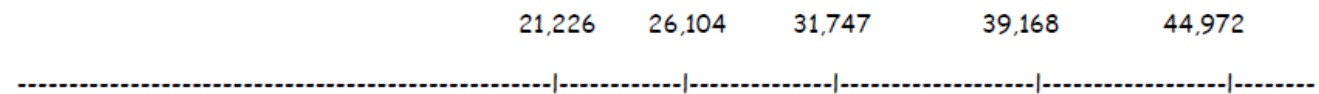
- Step 3 and beyond – repeat steps for each fragment

Restriction maps of the linear λ genome

Lambda (λ)



*Eco*RI Sites



Fragment 3

Subtract the final number (31,747) from the initial number (26,104) and you get...

$$31,747 - 26,104 = 5,643 \text{ bps}$$

Record in the table on next page

- Put the fragments in order from largest to smallest in the table on the next page for each restriction enzyme.

DNA restriction fragment size chart

Directions:

List your DNA fragments in the following chart

List each fragment, from largest to smallest.

<i>EcoRI</i>	<i>HindIII</i>	<i>BamI</i>
21,226		
7,421		
5,804		
5,643		
4,878		
3,530		

Draw a line representing the length

- Draw lines for the fragment lengths at the appropriate position bases on the marker lengths.
- Congratulations, you just made an electrophoresis plate.

