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?

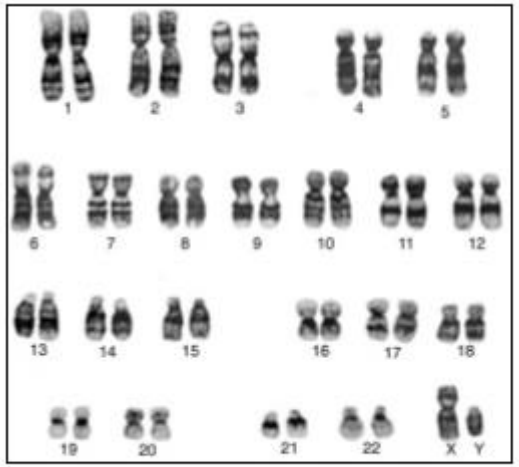
Karyotype

- Karyotype – a picture of the paired homologous chromosomes, taken during Prophase (sometimes Metaphase) of Mitosis, arranged from largest chromosome to smallest.

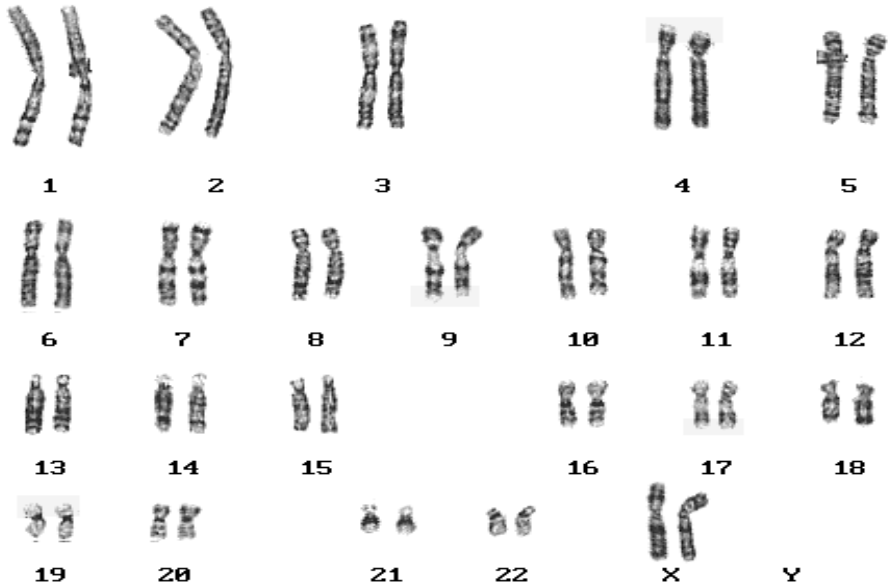
- Purpose: Allows for the analysis of chromosomes, to show abnormalities.

Metaphase spread

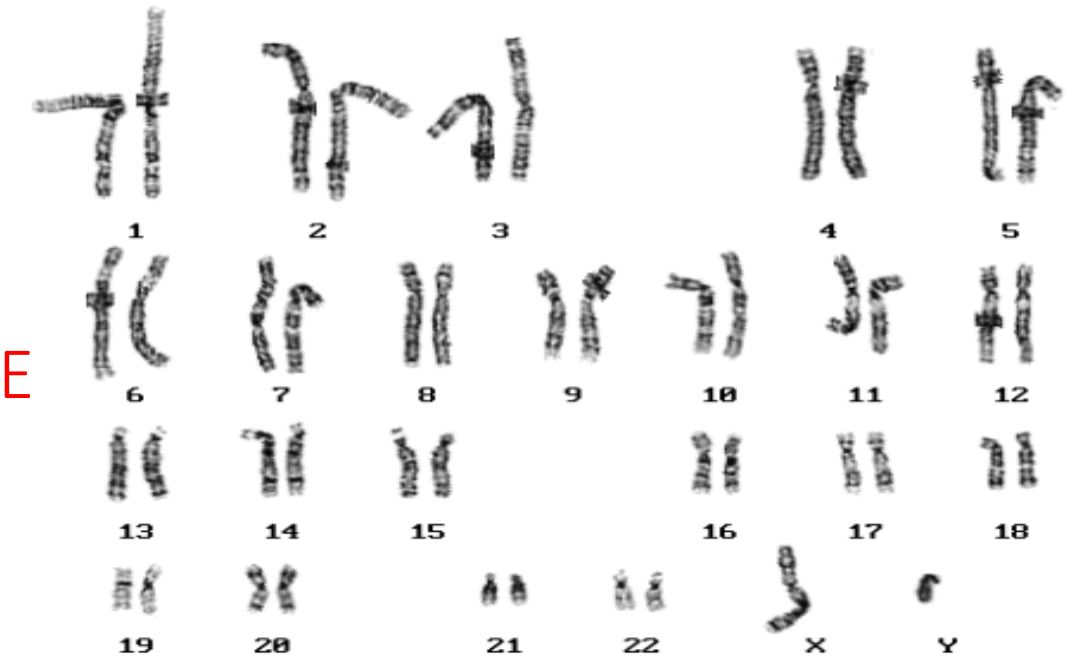
Chromosomes arranged in homologous pairs and ordered from largest to smallest



Karyotype



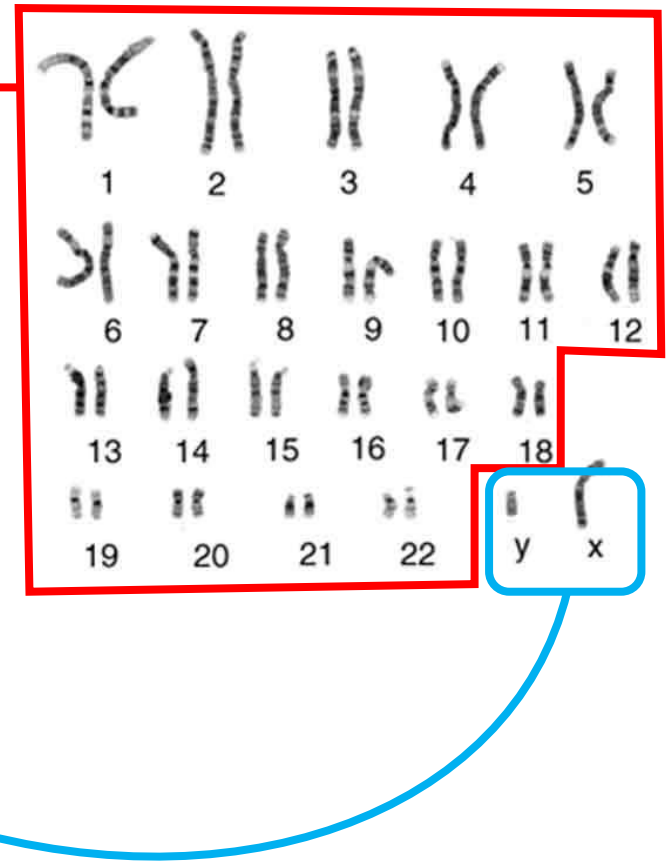
Normal Female



Normal Male

Karyotype vocabulary

- Autosomes – chromosome numbers 1 – 22 in humans
- Sex chromosomes – chromosome set 23 (X's and Y's)



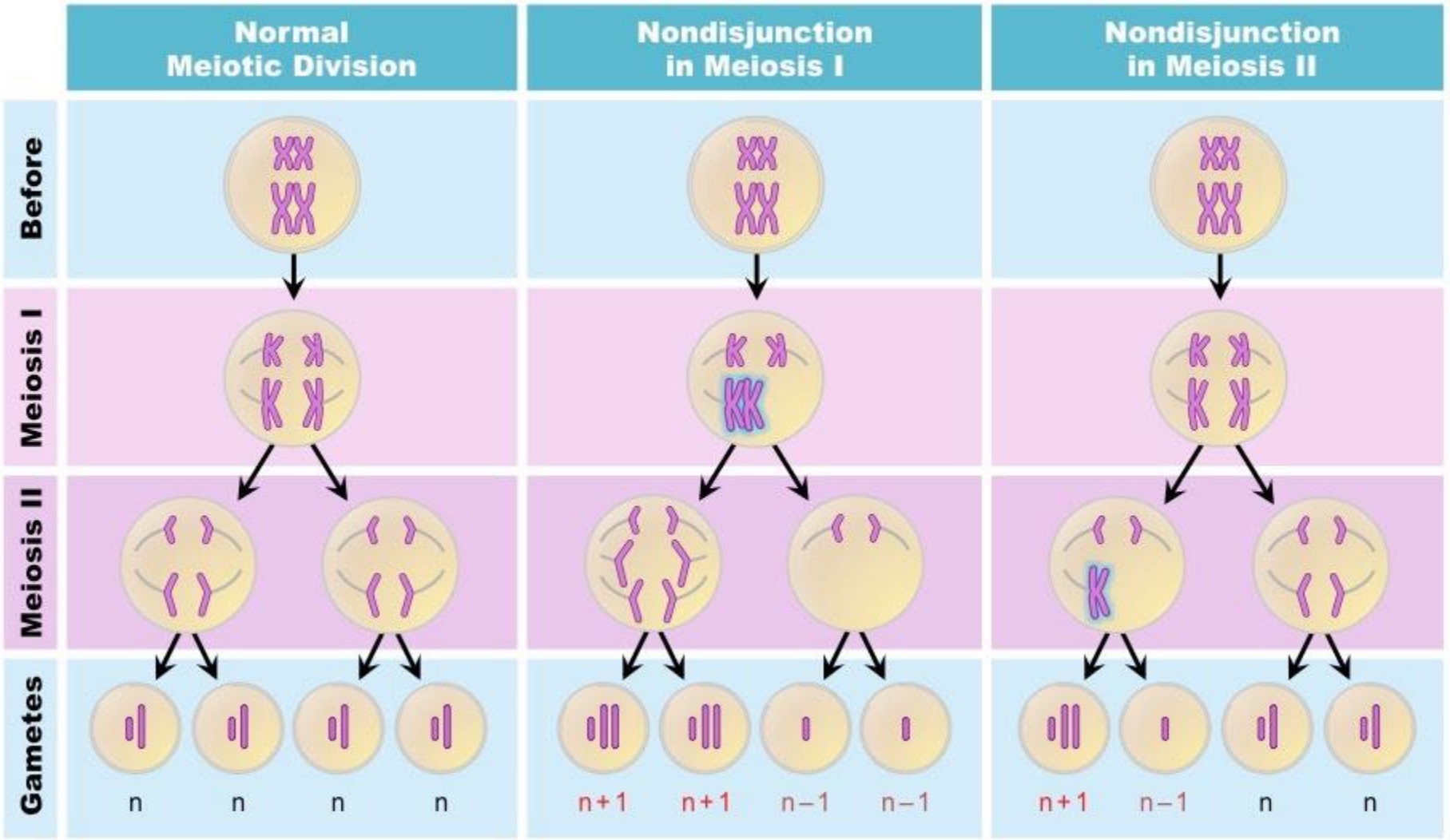
Karyotype vocabulary

- 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

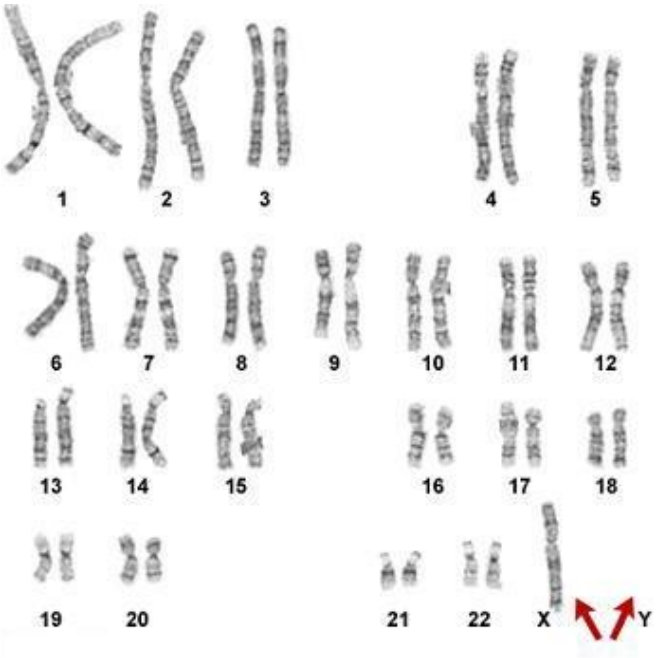
- Nondisjunction mutations create gametes with too few or too many chromosomes.
  - When those gametes fertilize normal gametes, the diploid numbers are off. In humans they do not equal 46 chromosomes.

- **Monosomy** – A cell with too few chromosomes. One of the homologous pairs is a single chromosome (2n = 45 in humans)

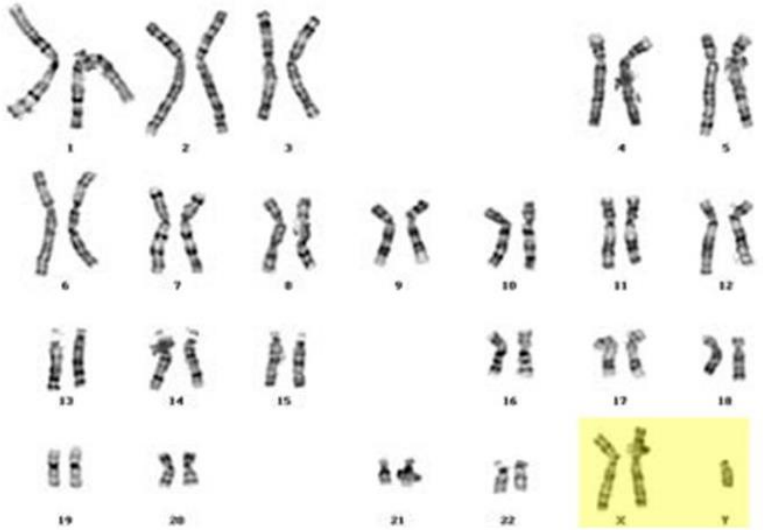
- **Trisomy** – A cell with too many chromosomes. One of the homologous pairs has 3 total chromosomes (2n = 47 in humans)

Turner's Syndrome

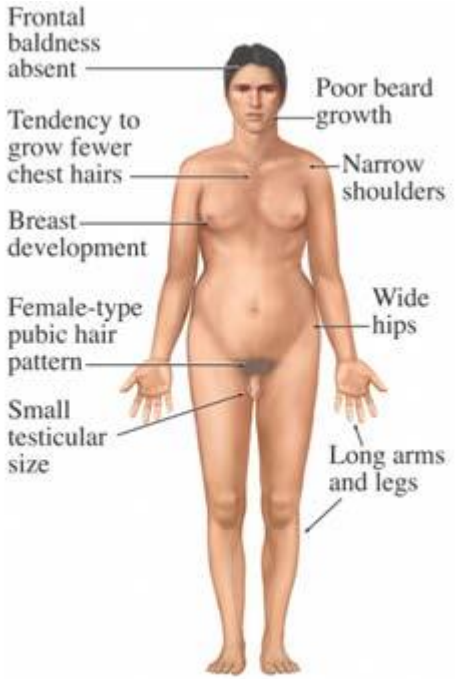
- Missing a X chromosome on 23rd chromosome
- Causes underdeveloped ovaries, short stature, webbed, and only found in women.
- Bull neck and broad chest. Individuals are sterile and lack expected secondary sexual characteristics.
- Mentally handicapped typically not evident.



Kleinfelter's Syndrome

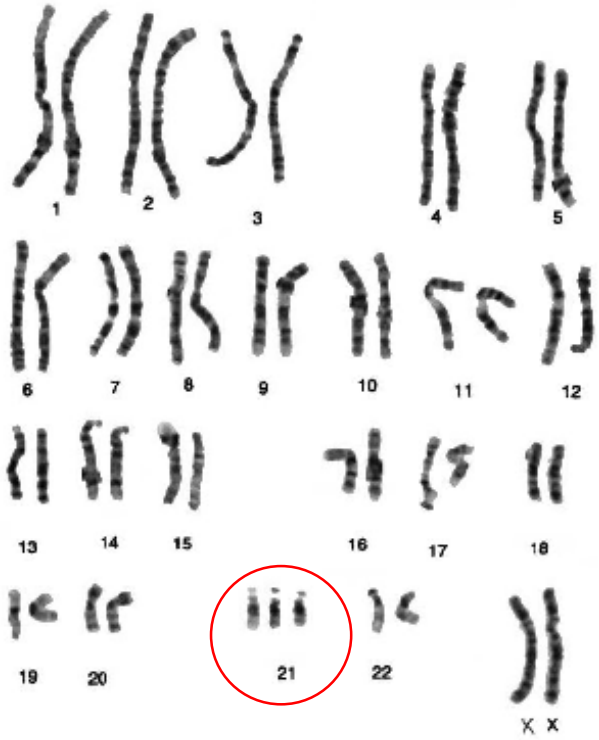


- Caused by nondisjunction of the X chromosome on 23rd chromosome (XXY, XXYY)
- Males with some development of breast tissue
- Individuals have little body hair, typically tall, and have small testes.
- Infertility results from absent sperm.
- Mental handicapped may or may not be present.



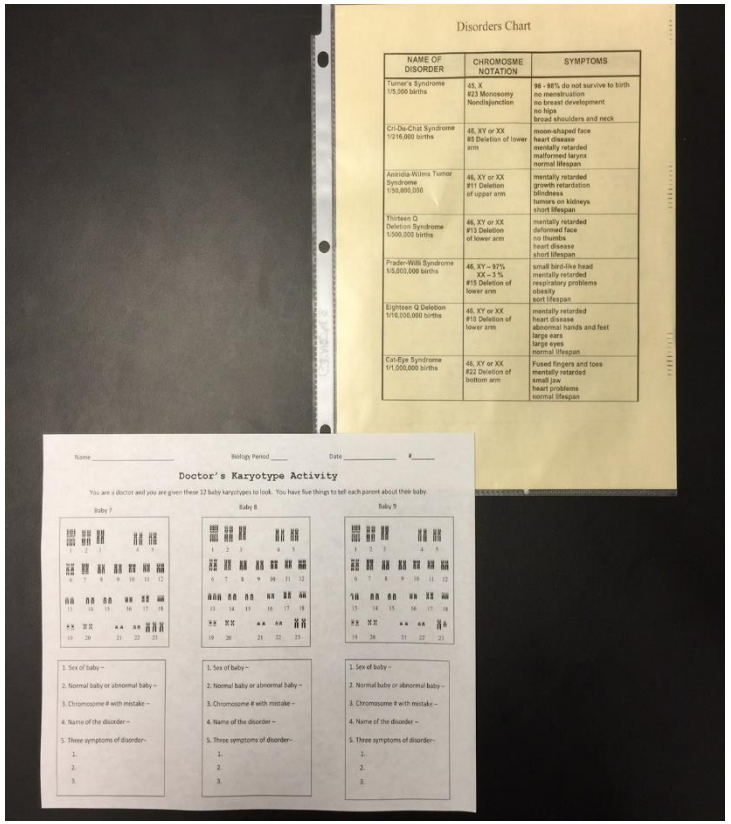
Down Syndrome

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



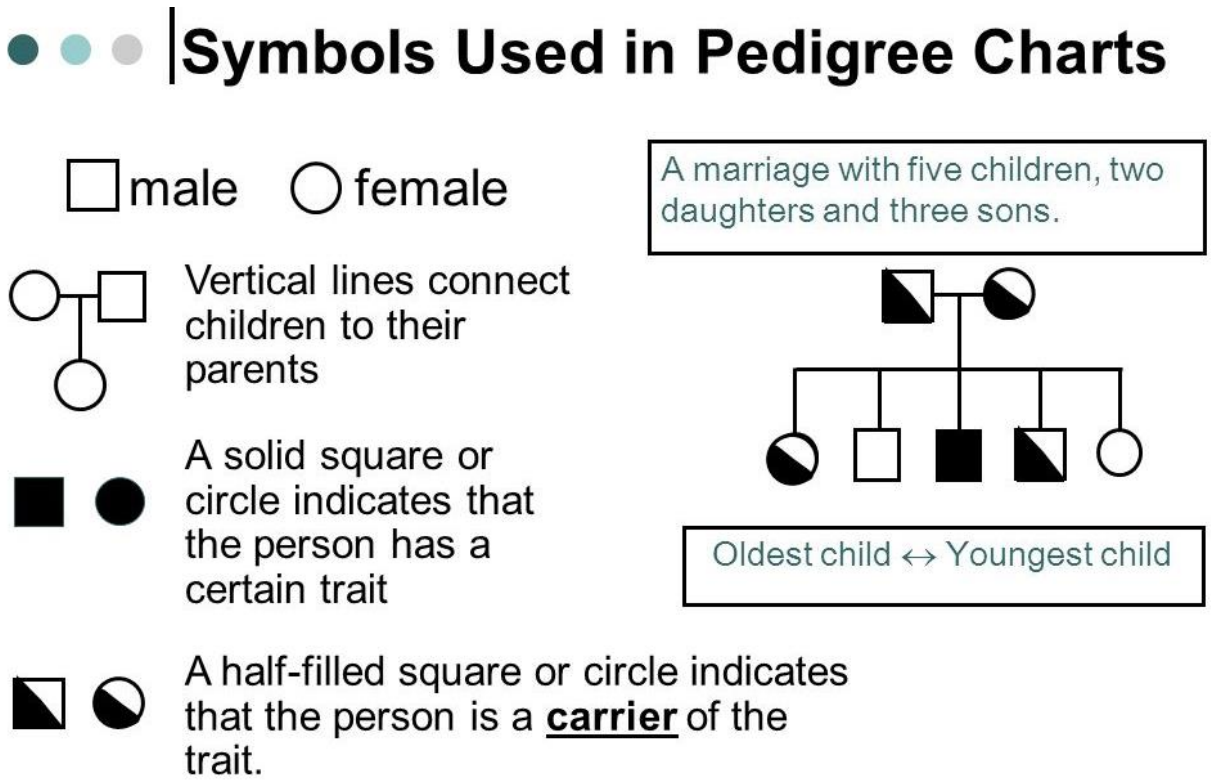
Practice reading a Karyotype

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

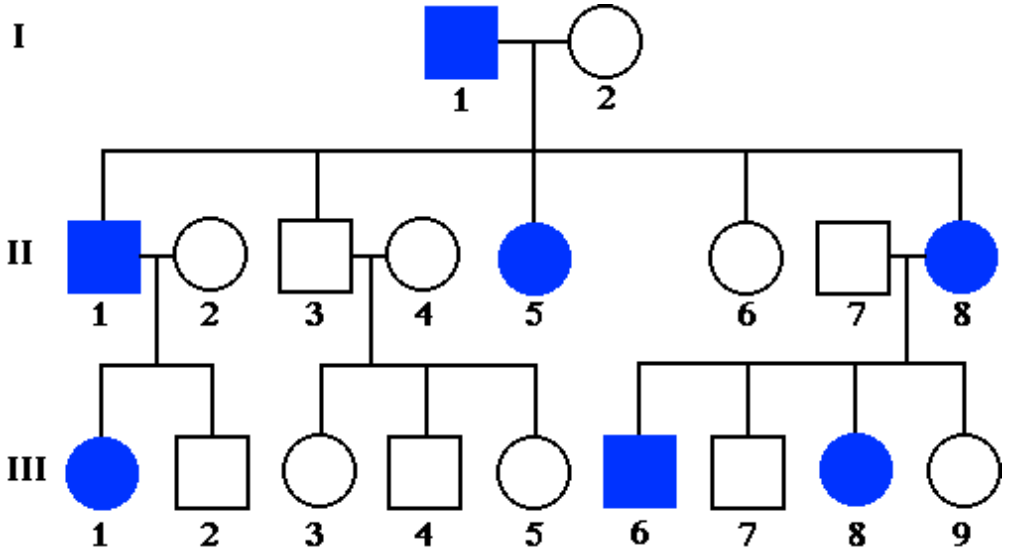


Pedigree

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

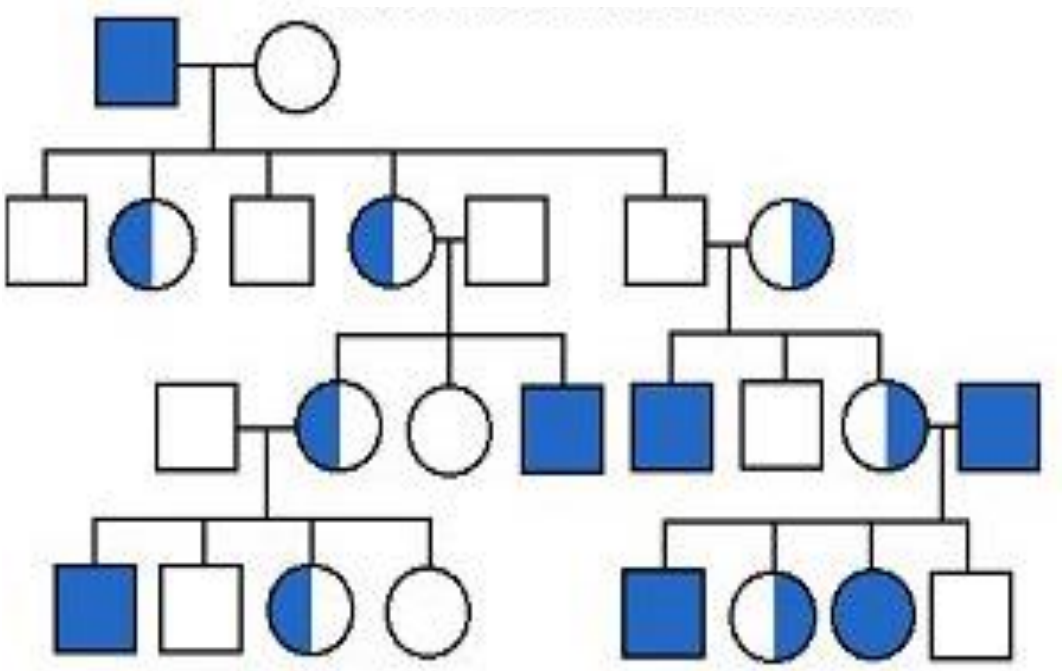


How to read a pedigree…



- 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)

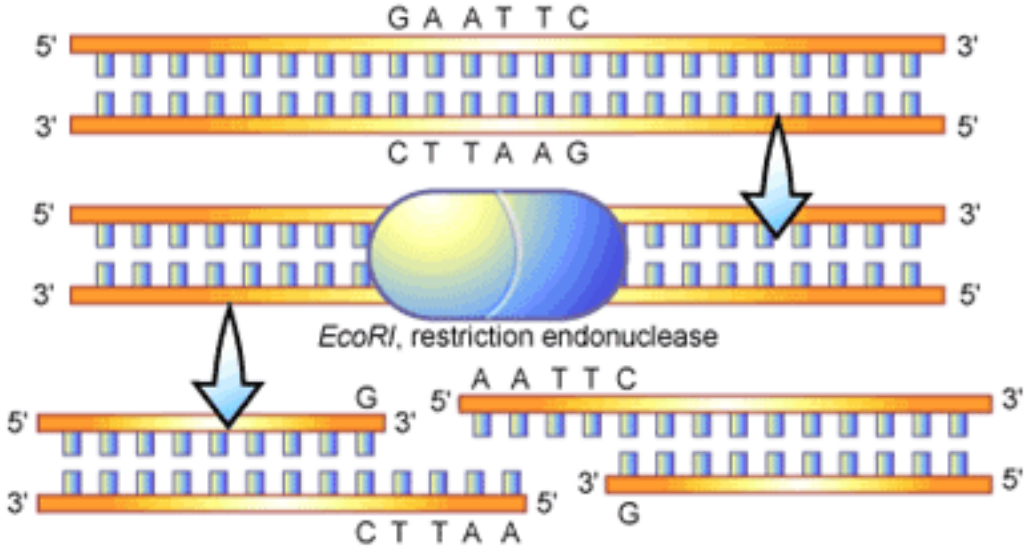
- Restriction enzymes must be used to cut the DNA into small pieces called restriction fragments.

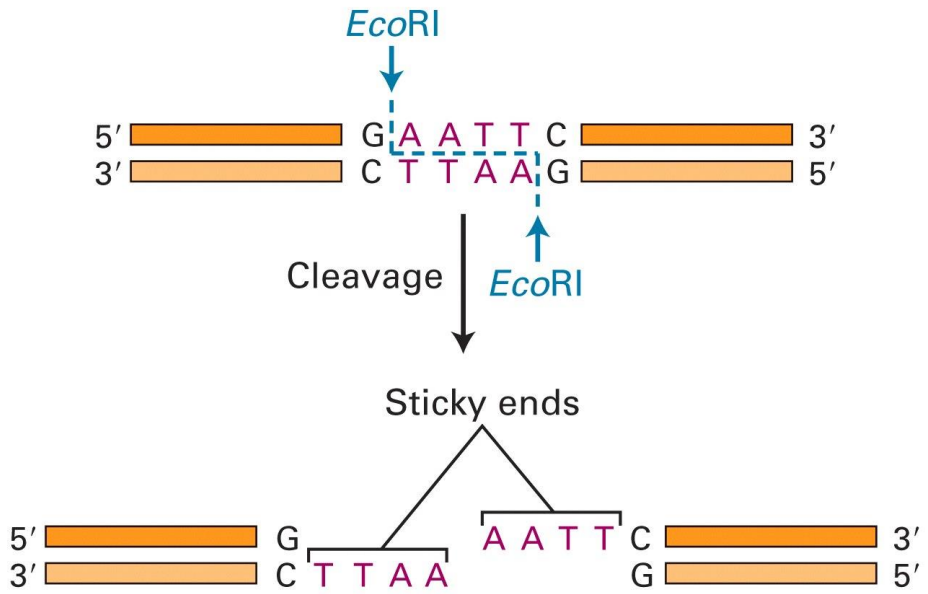
- Restriction enzymes can only work on VERY specific sequences of DNA called restriction sites.

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





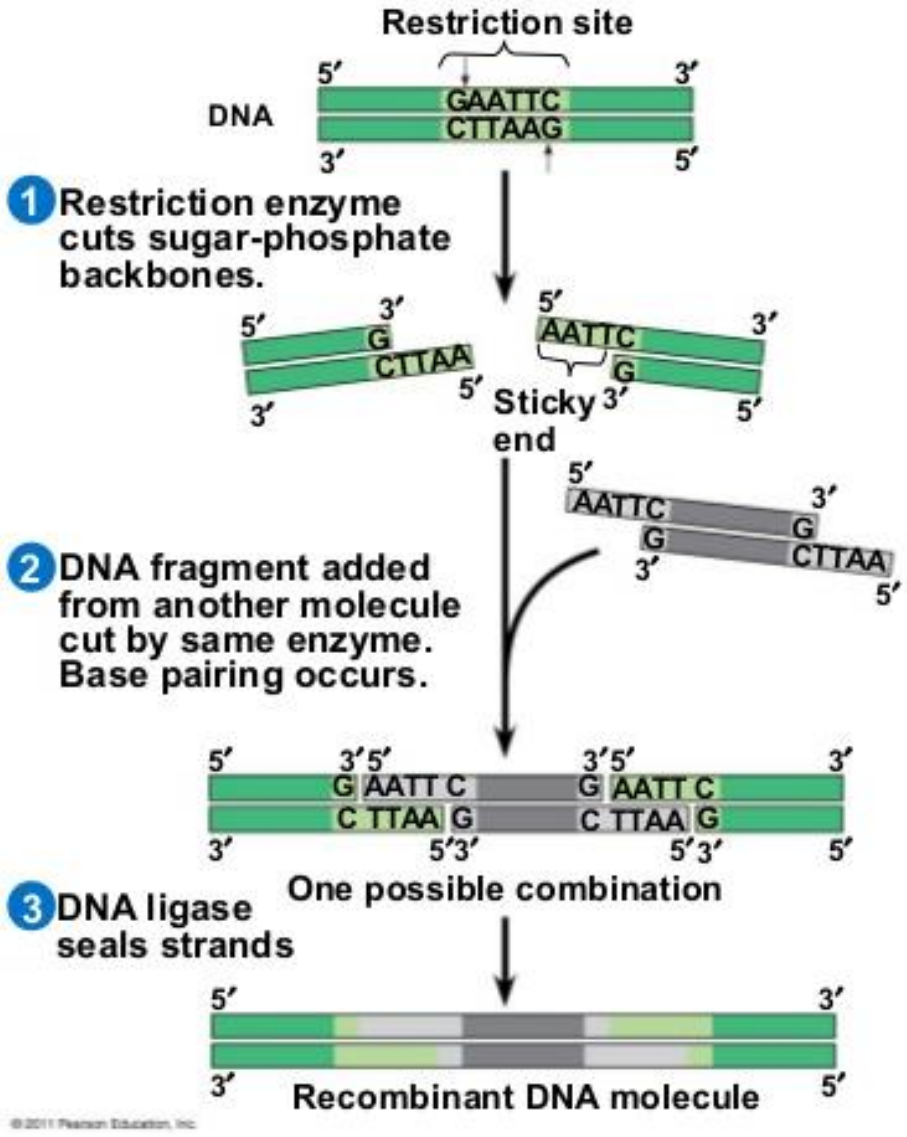
- Many restriction enzymes leave “sticky ends” when they cut.

- 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!

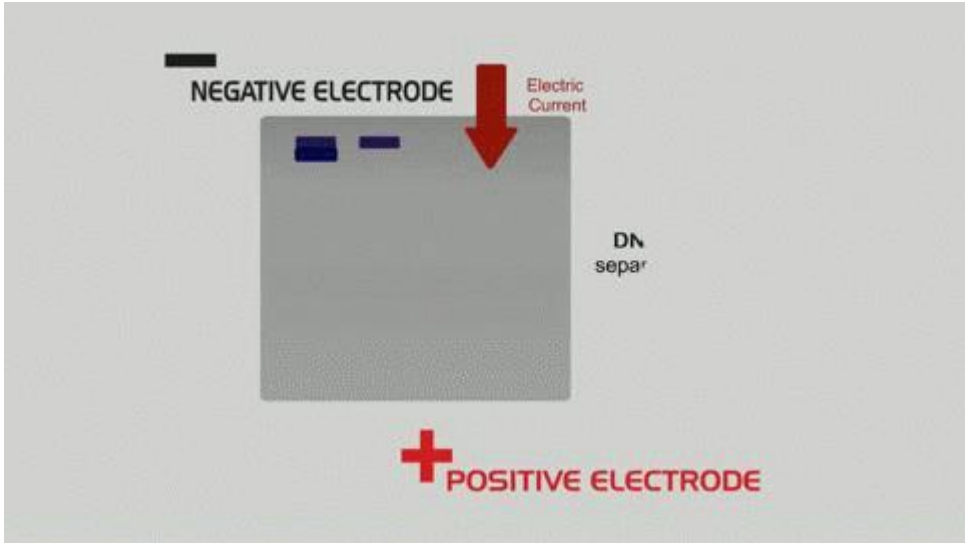
- Gel electrophoresis uses the fact that DNA is a negatively charged molecule.

- 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

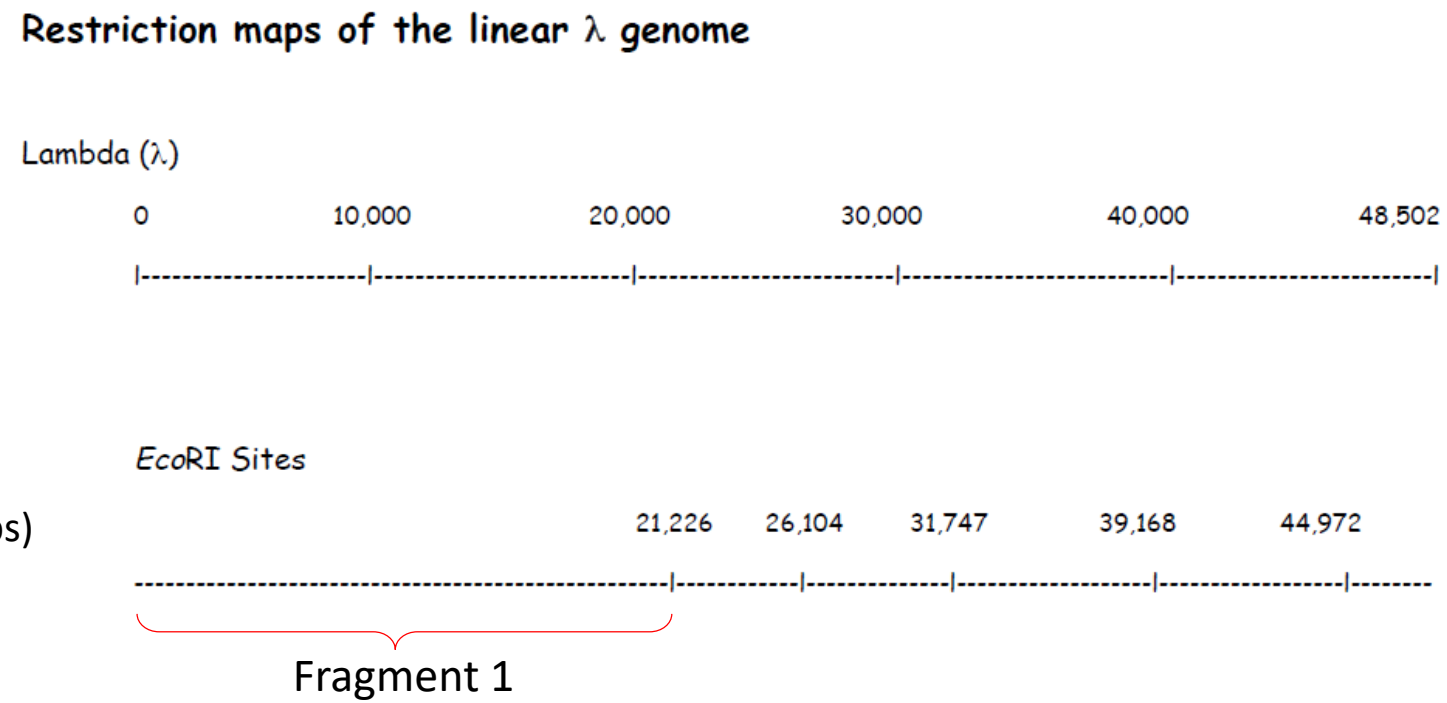
- It is harder for the large DNA pieces to move through the agar protein matrix (think of this as a set of monkey bars on a playground)
- Small pieces can move very quickly through the agar gel matrix (monkey bars) and get to the positive end faster.
- This sorts the DNA pieces cut by restriction enzymes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



Draw a Gel Electrophoresis Plate

Practice work…

- Step 1 – calculate the length of the first fragment using EcoR1.

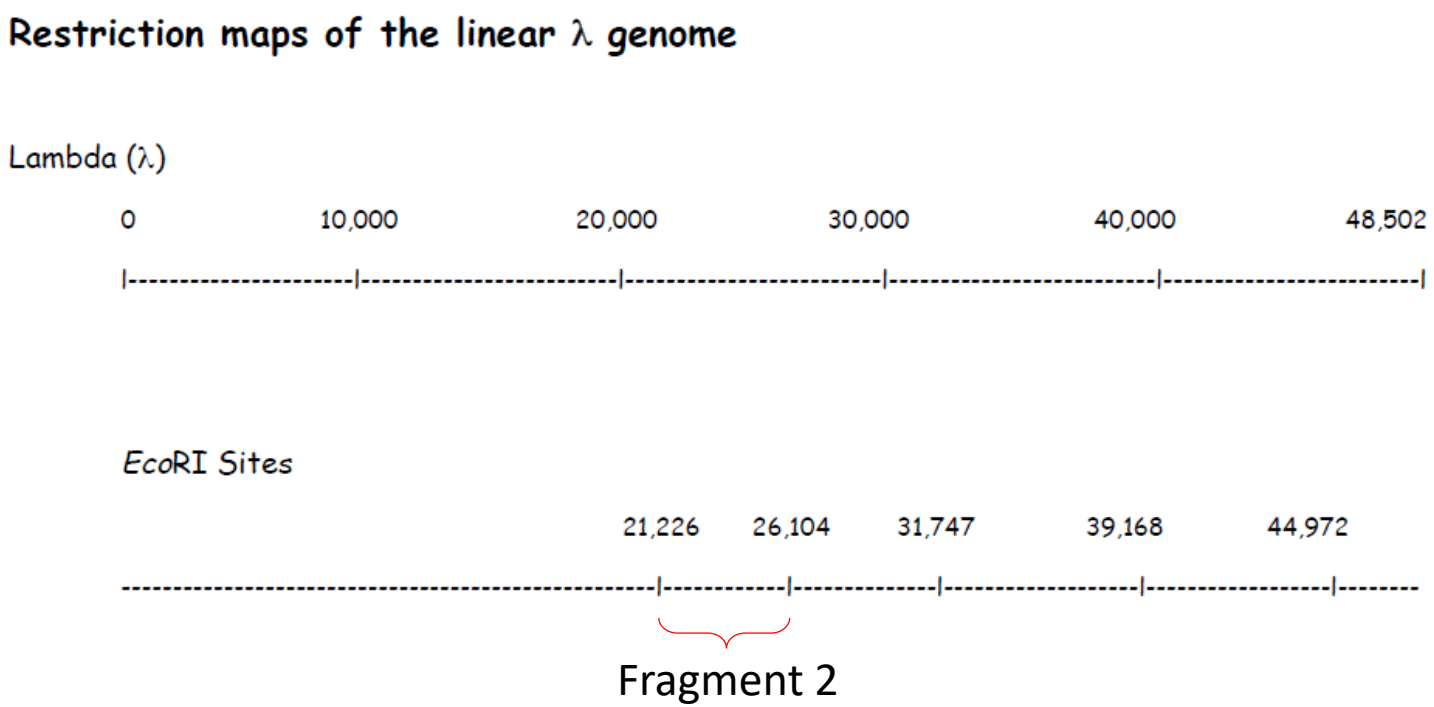


Subtract the final number (21,226 bps) from the initial number (in this case 0 bps) and you get…

21,226 – 0 = 21,226

Draw a Gel Electrophoresis Plate

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

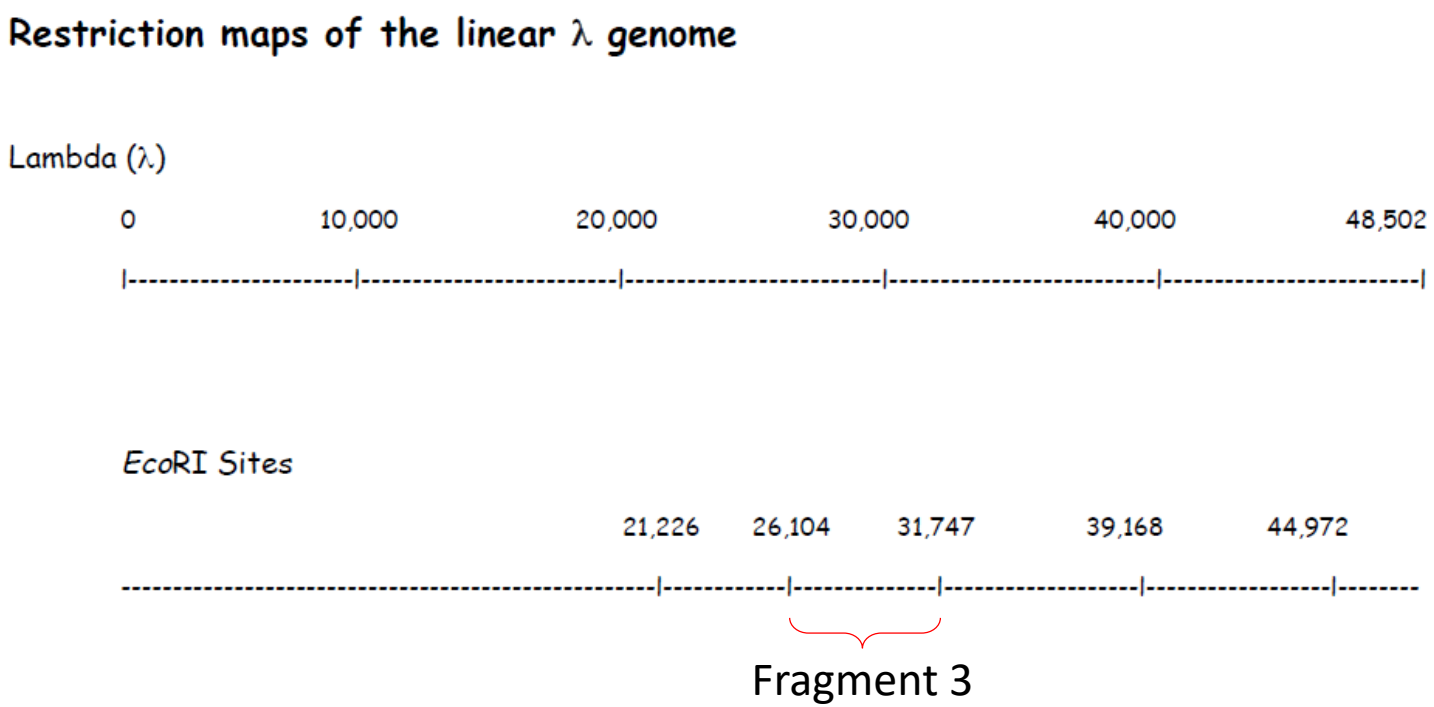


Subtract the final number (26,104) from the initial number (21,226) and you get…

26,104 – 21,226 = 4,878 bps

Draw a Gel Electrophoresis Plate

- Step 3 and beyond – repeat steps for each fragment



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

31,747 – 26,104 = 5,643 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 char

List each fragment, from largest to smallest.

EcoRI	*HindIII*	*BamH*
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.