DNA Fingerprinting

Restriction Enzymes/Gel Electrophoresis

Intro:

DNA Fingerprinting is one of the most powerful tools in biotechnology. By cutting DNA into smaller fragments with restriction enzymes and laying out these fragments in bands on a gel with the bands ordered by size, a "fingerprint" of the DNA is created. Since two different molecules of DNA will not have the exact same sequence of bases, the enzymes may cut them in different places, giving different size fragments. Since the banding pattern of the fingerprint is based on the size of the fragments, the fingerprint of two identical samples of DNA will match while that of two different samples of DNA will not match.

Restriction Enzymes: Restriction enzymes are chemicals that locate a specific sequence of base pairs along a molecule of DNA and cut the DNA within that sequence (there are other types of restriction enzymes that cut the DNA outside of the recognized sequence of base pairs, but they are not used in this type of work). The enzyme used in this activity is Alu1. It locates the base sequence AGCT and cuts the DNA between the G and the C. In the double strand of DNA, the cut looks like this:

AG CT
TC GA

Gel Elctrophoresis: Gel electrohporesis is a method of separating DNA, RNA or Proteins by size or charge. These molecules have an electrical charge. When they are place in a porous gel and an electrical charge is placed across the gel, the molecules will move through the gel towards the opposite charge. For instance, if negatively charged DNA is place in the well at the negatively charged end of the gel, it will migrate through the gel towards the positively charged end. The smaller the fragment, the faster it will move. When the electric current is turned off, the smaller pieces will line up further from the starting well than the larger pieces. The end result is distinct bands of similar sized DNA, ordered by size.

The steps that are modeled in this activity:

1. Locate the restriction sites on the DNA strips

2. Cut the DNA at the restriction sites
3. Run the gel to separate the DNA fragments and create the fingerprint
4. Stain the DNA fragments (alternative 2)

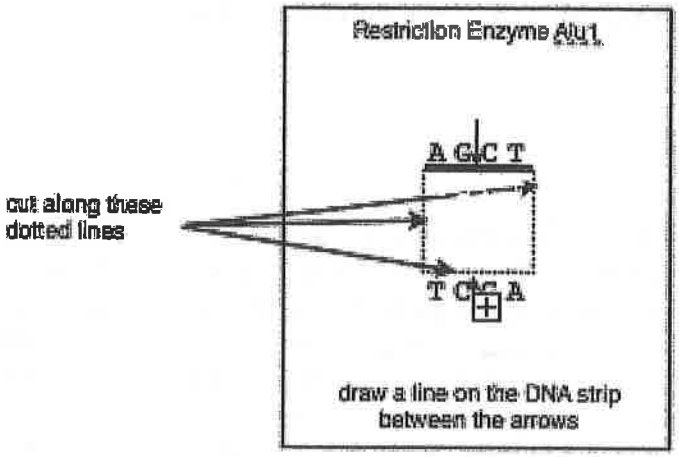
Teacher Prep for Activity

1. Print one set of this activity for each group.
2. Provide the following materials to each group:
   - Scissors
   - Tape

Student Prep for Activity

1. Cut out all of the pieces on page 7. The pieces to be cut include the 4 **DNA Strips** and the **Restriction Enzyme.**□

2. **Restriction Enzyme:** Cut out the flap in the restriction Enzyme. Cut *only* along the three dotted lines. Make sure you do not cut along the solid line at

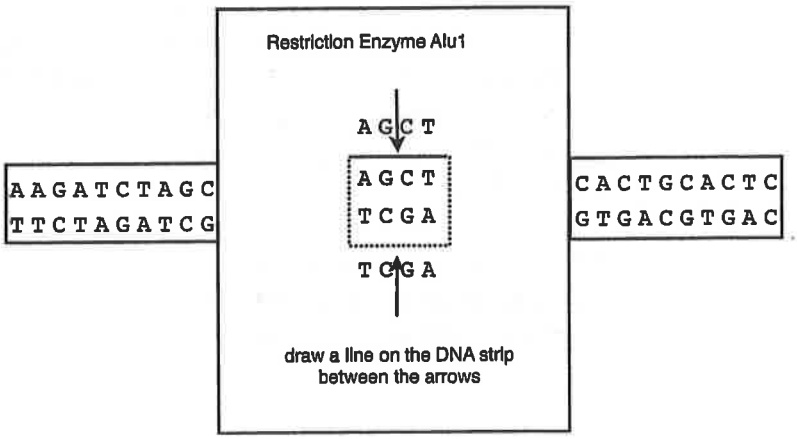


Perform the DNA Fingerprinting

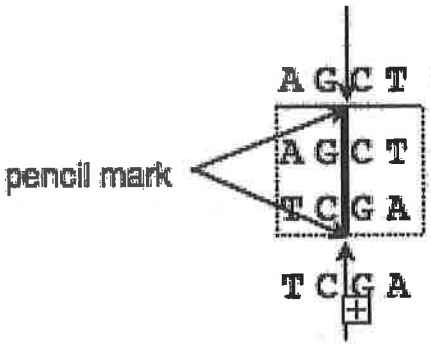
Locate the restriction sites on the DNA strips

1. The envelope with the plasmid inside Place the **Restriction Enzyme** over one of the **DNA strips** with the flap behind the **DNA Strip** and the base pairs showing through the opening in the **Restriction Enzyme.**

2. Slide the **Restriction Enzyme** along the **DNA Strip** until the base pairs match up. The AGCT on the **Restriction Enzyme** should be directly above an AGCT on the top line of the **DNA strip** and the TCGA on the bottom of the **Restriction Enzyme** should be directly below a TCGA on the bottom line of the **DNA strip**.



3. Draw a line on the **DNA Strip** between the two arrows on the **Restriction Enzyme**. Place the **plasmid DNA**, **Gene insert** and **Restriction Enzyme** on the table in front of you.



4. Continue sliding the **Restriction Enzyme** along the **DNA Strip**, drawing a line everyplace the base pairs match up.

Cut the DNA at the restriction sites

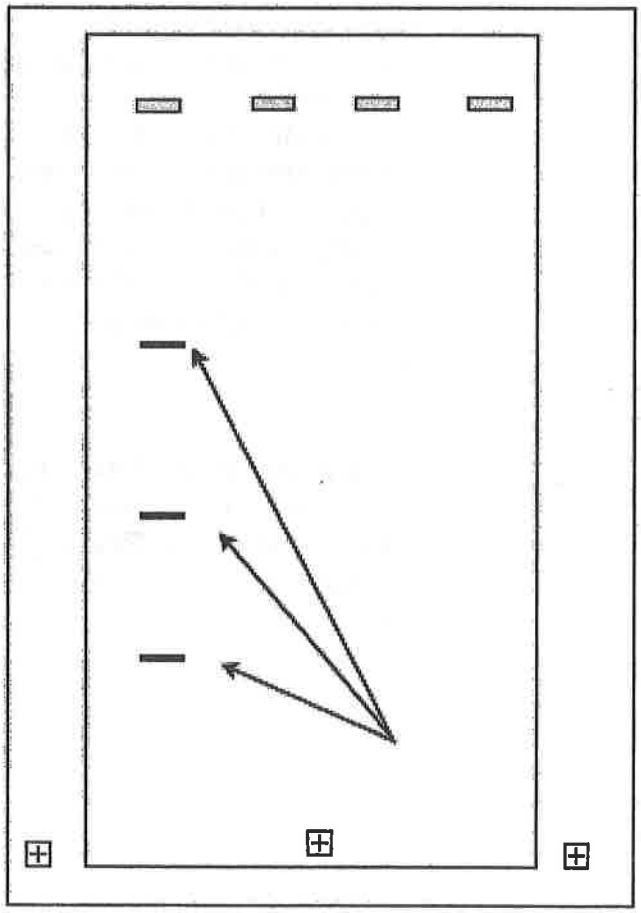
5. Cut the **DNA Strip** along each pencil mark you made. You should end up with a number of DNA fragments of different sizes.

6. Count the number of base pairs on each fragment of DNA and write the number on the back of the DNA fragment.

Run the Gel alternative 1 (on 8.5x11 sheet of paper)

7. On the handout on page 9 under well 1, make a mark that represents each piece of DNA from DNA Strip 1 - matching the DNA piece with the correct number of base pairs. Do this for all of the pieces from DNA strip 1.

8. Repeat the process for the other DNA Strips, placing the marks under the corresponding well number.



Run the gel alternative 2 (large scale) cont. from #6. ABOVE

9. Cut out all of the pieces on page 11. The pieces to be cut include the 3 **Number of Base Pairs** and the 4 **Loading Wells.**

10. Tape the 4 **Loading Wells** across a large space such as a blackboard or wall.

11. Tape the 3 **Number of Base Pairs** together and tape them down one side of the **Loading Wells.**

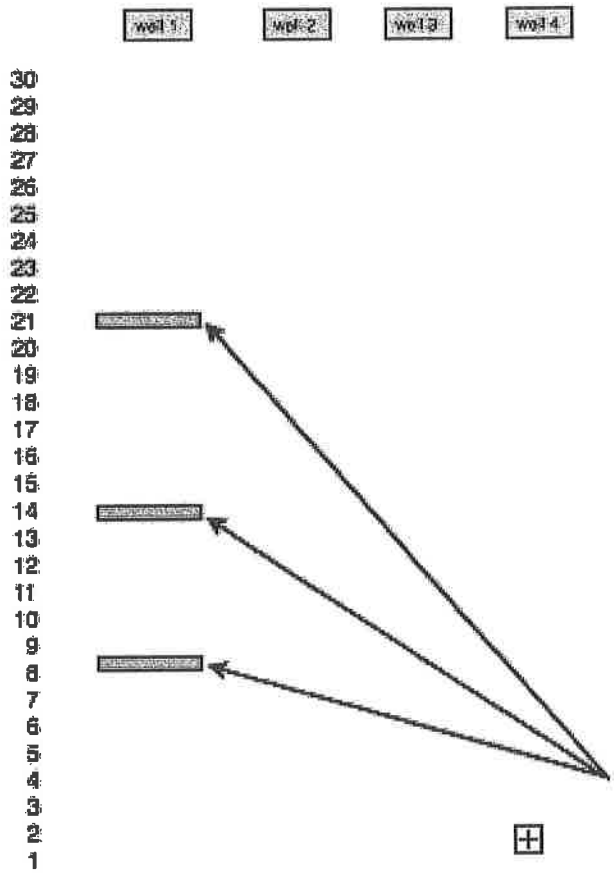
12. Under **Well 1**, tape each fragment of DNA from **DNA Strip 1** by the corresponding number of the **Number of base pairs**. Make sure to tape each DNA piece so that the base pairs do not show.

□□*In a real gel, the DNA can not be seen until it is stained. Even then, the base pairs are not known - only the size of the DNA strand.*
□

13. Repeat this process for the other three **DNA Strips**, placing their fragments under the corresponding **Wells**.

Stain the DNA Fragments□

14. Stain the DNA fragments by coloring in the DNA strips on your gel.



Extension

Have half the class perform this activity with the PCR Long Target DNA sequence and half with the PCR Short Target DNA Sequence. These two Target DNA sequences can then be used in the DNA Fingerprinting activity to demonstrate how the PCR can be used to differentiate slightly different fragments of DNA.

DNA Strips

DNA sample 1

AAGATCTAGCTCTTAGGGCTATGAGCTACATTGCTAGACCTAACCTCAAGCTTTACTTAGATAAGC
TTCTAGATCGAGAATCCCGATACTACTCGATGTAACGATCTGGATTGGAGTTCGAAATGAATCTATTCG

DNA sample 2

AAGATCTACCTCTTAGGGCTATGAGCTACATAACTAGACCTAAGCCCAAGCTTTACTTAGATAAGC
TTCTAGATGGAGAATCCCCGATACTACTCGATGTATTGATCTGGGTTCGAAATGAATCTATTCG

DNA sample 3

AAGATCTAGCTCTTAGGGCTATGAGCTACATAGCTAGACCTAAGCTCAAGCTTTACTTAGATAAGC
TTCTAGATCGAGAATCCCGATACTCGATGTATCGATCTGGATTCGAGTTCGAAATGAATCTATTCG

DNA sample 4

AAGATCTAGCTCTTAGGGCTATGCGCTACATAGCTAGACCTAAGCTCAAGCTTTACTTAGATAAGC
TTCTAGATCGAGAATCCCGATACGCGATGTATCGATCTGGATTCGAGTTCGAAATGAATCTATTCG

Criminal DNA

AAGATCTAGCTCTTAGGGCTATGAGCTACATAGCTAGACCTAAGCTCAAGCTTTACTTAGATAAGC
TTCTAGATCGAGAATCCCGATACTCGATGTATCGATCTGGATTCGAGTTCGAAATGAATCTATTCG

Restriction Enzyme Alu1

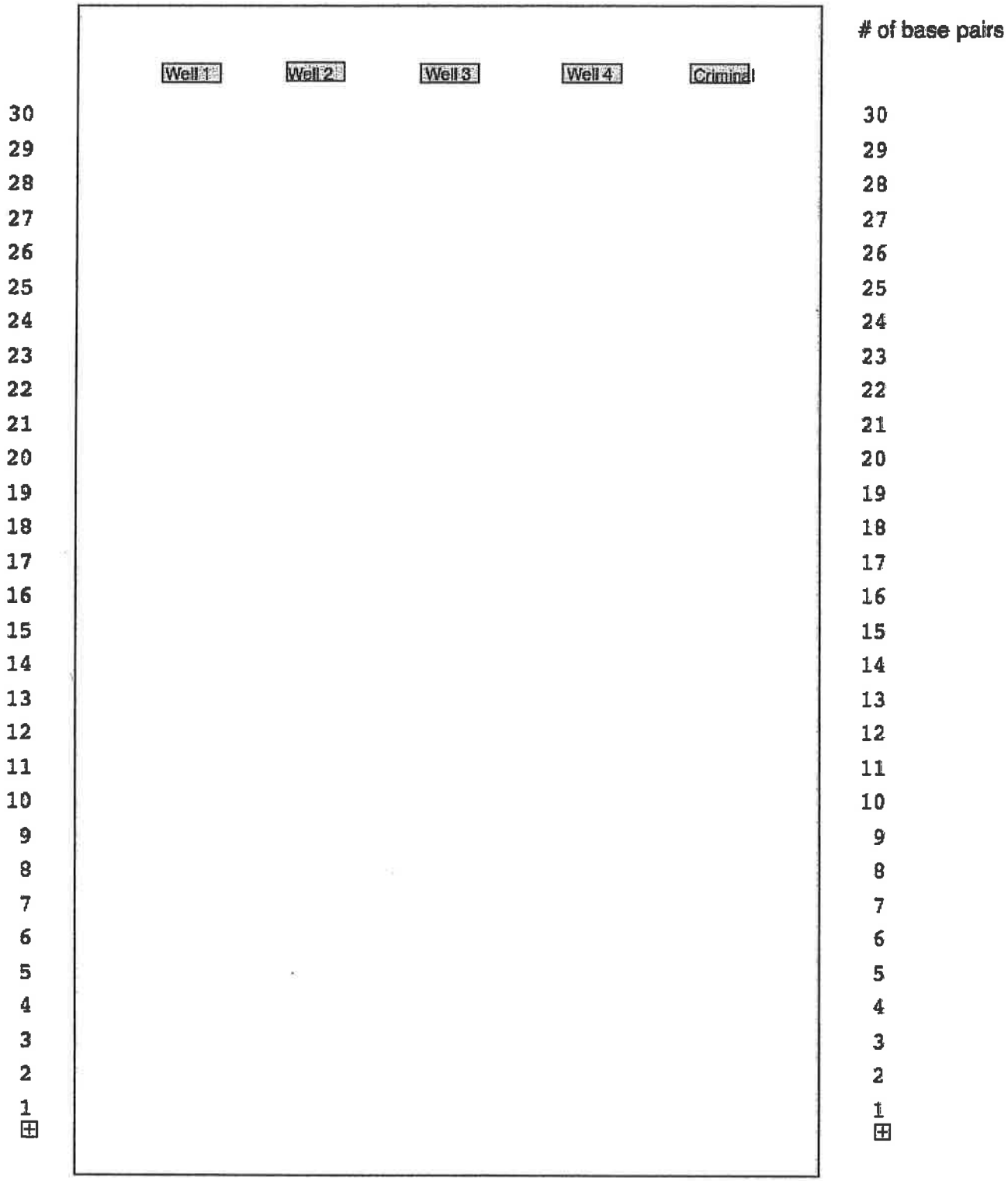
A G C T

T C G A

draw a line on the DNA strip between the arrows

Restriction Enzyme

Agarose Gel


Well 1
Well 2
Well 3
Well 4
Criminal
# of base pairs
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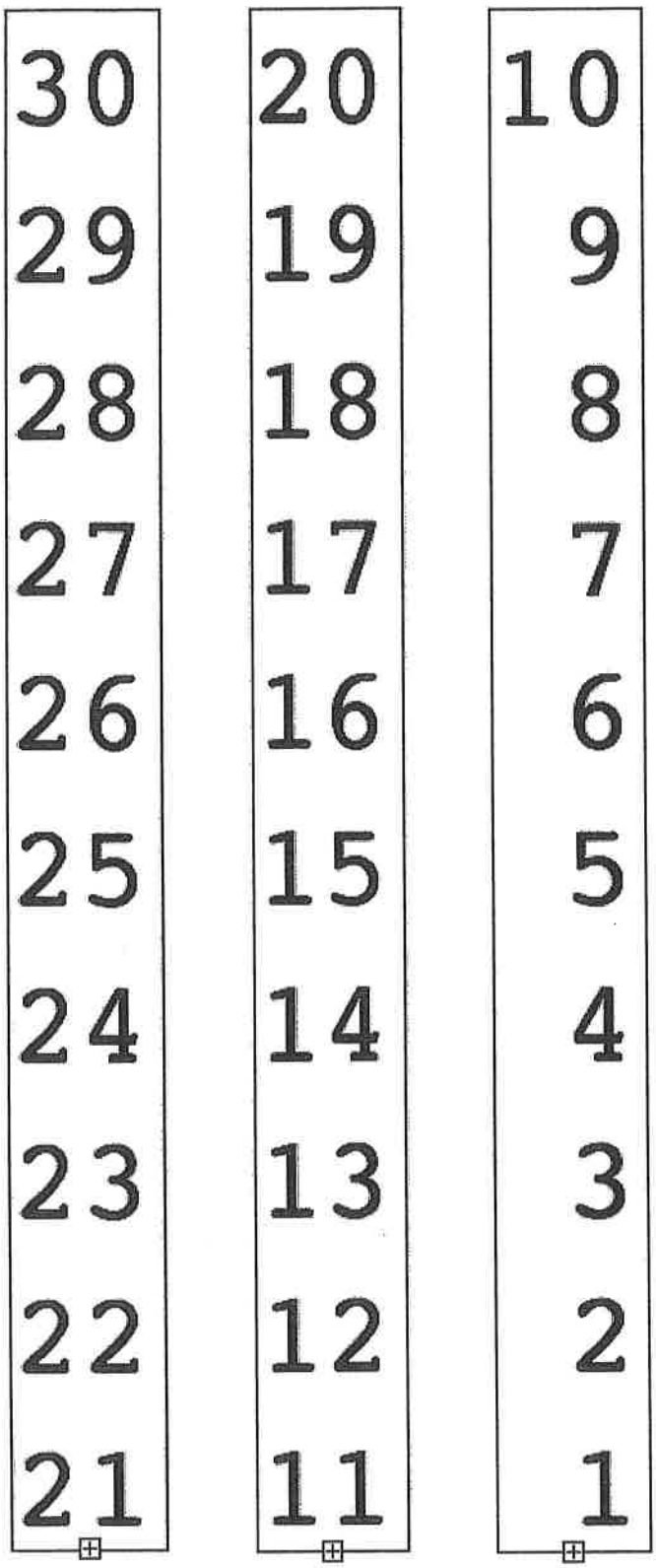
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4
3
2
1
Well 1
Well 2
Well 3
Well 4
Criminal DNA
Loading Wells
Number of Base Pairs