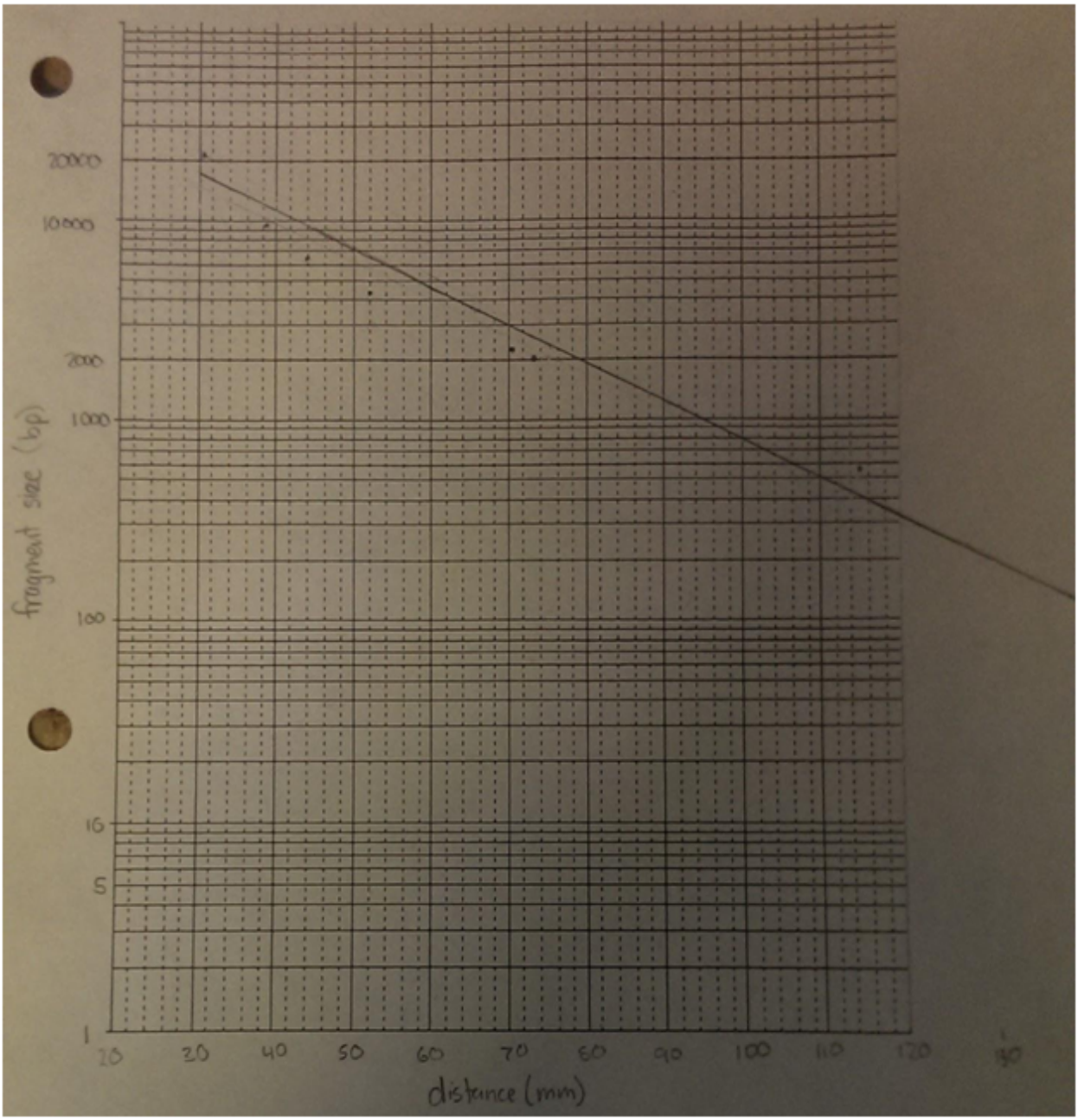
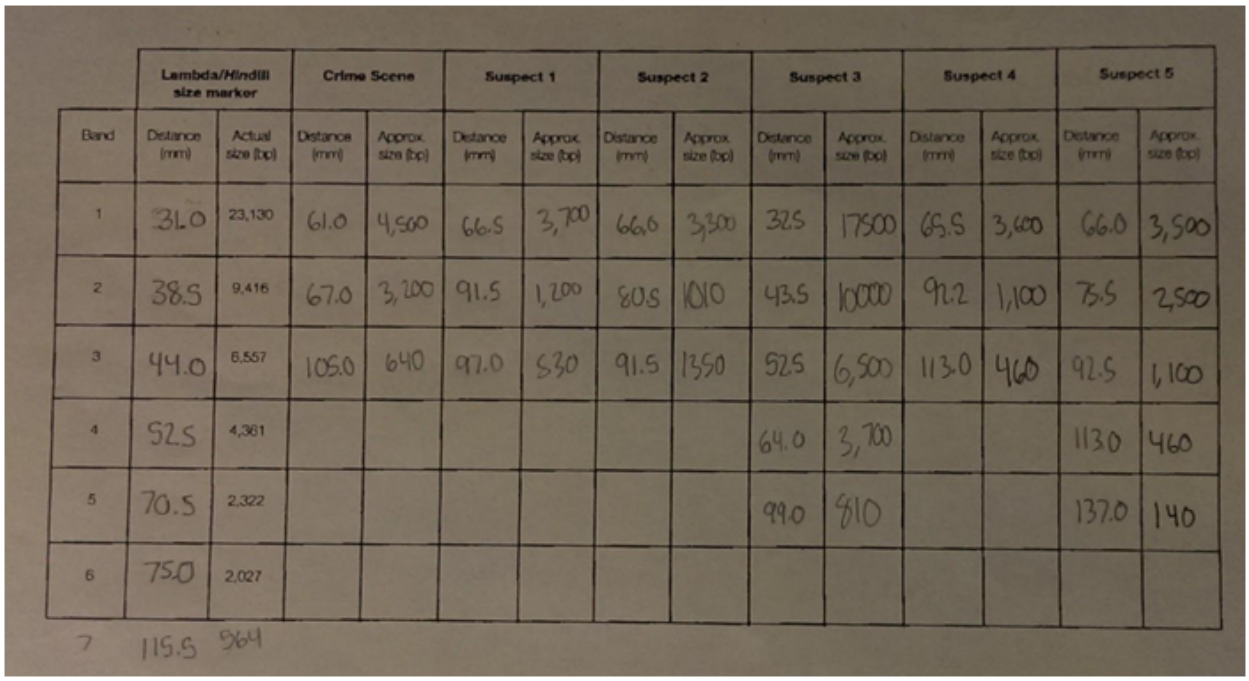
Stephen Clark
Block B

Restriction Enzyme Lab

Part 1: Quantitative Analysis of DNA Fragment Sizes





	Lambda/HindIII size marker		Crime Scene		Suspect 1		Suspect 2		Suspect 3		Suspect 4		Suspect 5	
Band	Distance (mm)	Actual size (bp)	Distance (mm)	Approx. size (bp)	Distance (mm)	Approx. size (bp)	Distance (mm)	Approx. size (bp)	Distance (mm)	Approx. size (bp)	Distance (mm)	Approx. size (bp)	Distance (mm)	Approx. size (bp)
1	31.0	23,130	61.0	4,500	66.5	3,700	66.0	3,300	32.5	17500	65.5	3,600	66.0	3,500
2	38.5	9,416	67.0	3,200	91.5	1,200	80.5	1010	43.5	10000	92.2	1,100	73.5	2,500
3	44.0	6,557	105.0	640	97.0	530	91.5	1350	52.5	6,500	113.0	460	92.5	1,100
4	52.5	4,361							64.0	3,700			113.0	460
5	70.5	2,322							99.0	810			137.0	140
6	75.0	2,027												
7	115.5	964												

Part 2: Analysis Questions

1. The purpose of the Restriction Enzyme Lab is to become familiar with restriction enzymes, become comfortable using lab equipment such as micropipettes, and perform an electrophoresis while attempting to match suspects to crime scene DNA.
2. A restriction Enzyme is an enzyme that is able to cut DNA. Each enzyme has a varying recognition sequence, the base-pair sequence that the enzyme will cut.The Restriction Enzyme can cut the DNA with blunt or sticky ends. Blunt ends are where the DNA has just been cut and all bases have a pair that they are Hydrogen bonded to. Sticky ends are cuts made that leave the two, now separated ends of DNA, with unpaired bases, allowing them to Hydrogen bond, therefore making them “sticky”. Restriction Enzymes are found in bacteria and are believed to be a defence against invading viral DNA because the Restriction Enzymes will cut sequences known to be in viral DNA, leaving the host’s DNA uncut.
3. make water bath
4. First, restriction enzymes and DNA samples, generated through PCR, were taken from a stock and placed in vials. Also, a water bath was set to 37°C and allowed to warm. From each DNA vial, 10µL were transferred to a vial that was immediately labeled. After all 5 suspects and the crime scene DNA had been transferred to a reaction vial, from the enzyme vial, 10µL enzyme was added to six (6) different reaction vials. During this transfer process, micropipettes were used at the 10µL setting and tips were changed with every change of reagent that touched the tip. Following the combination of DNA and enzymes, the tubes were mixed and then added to a centrifuge, with care taken toward

balancing the centrifuge. Next, the pre-prepared water bath was used to incubate the DNA reaction vials. Reaction vials were placed in foam stands and floated on the surface of the water bath. While incubating, prepare and agarose gel. Heat a container of agarose and pour into your casting plate. Once filled to about a 1cm height, insert the comb to make the wells in the agarose gel. Allow the agarose gel to cool. Now, add 5μL loading dye to the reaction vials remembering to switch pipette tips every time the tip touches a different solution. Mix the reaction vials. Place a solid agarose gel in a platform box and fill with electrophoresis buffer to cover the agarose. Using a micropipette add 10μL of HindIII lambda digest to a well in the agarose. Then, using a micropipette, add 20μL of each solution from the six (6) reaction vials to the wells in the agarose. Take note of what solution is in each well. Place the lid on the platform box and connect to a power supply. Set power supply to 60V and run the gel for about one (1) hour. Once complete. Safely turn off and disconnect the power supply from the platform box and take a picture of the agarose gel under a UV light. This picture can now be used for analysis.

5. From our gel, no suspect can be conclusively verified as being present at the crime scene. This is due to what is believed to be an incomplete digest of suspect three (3). To obtain conclusive results, Another electrophoresis would need to be run on all of the suspects. This stated, suspects 1,2,4, and 5 can probably be ruled out as suspects at the crime scene because their DNA does not closely resemble that found at the crime scene.
6. There are three (3) restriction enzyme recognition sites in suspect 2's DNA (if in the form of a plasmid) because there are three (3) clearly visible bands that formed during electrophoresis. To have three segments cutting from a circle, one must cut three (3) times. This is why there are probably three (3) restriction enzyme recognition sites in suspect 2's DNA.
7. Shorter bands of DNA in terms of base-pair length would appear fainter because the loading fluorescent dye must bind to the DNA. The shorter the DNA molecule, the less change of the loading fluorescent dye attaching to the shorter chains of DNA. This effect is what causes the bands farther down the lane in the gel to be faint and sometimes almost undetectable.
8. A standard curve allows for the comparison of distance down the agarose gel to be compared to the fragment size using a standard. This was done in the Restriction Enzyme Lab to find the length (in base-pairs) of the different strands of DNA in the agarose gel. The standard curve was generated by plotting the distance from the well different bands of the HindIII lambda digest were from the well vs the know base-pair lengths of those bands. Then a best fit line was applied to the graph to allow for comparison of other DNA bands lengths from the wells to the base-pair length of that DNA.