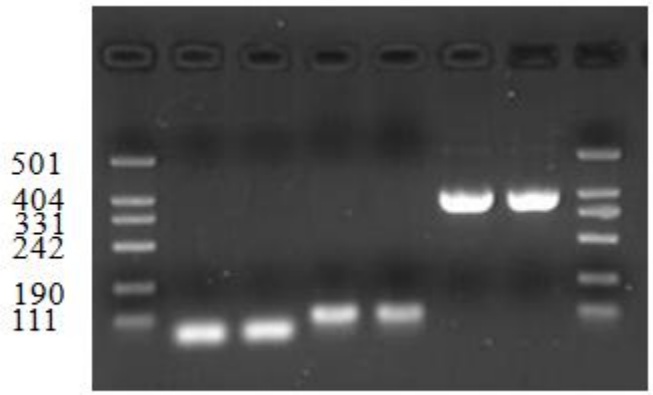
Name_____

Agarose Gel Interpretation

FS 362

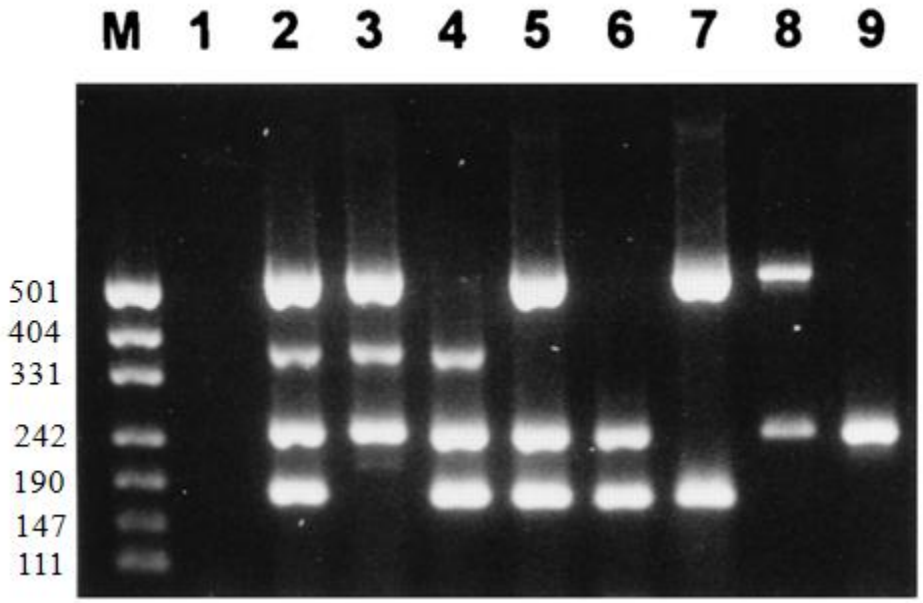
1. A PCR assay was designed to detect the *Salonella* spp. gene *invA.* The *invA* PCR product is 375 basepair. The lanes (wells) are as follow: 1) Marker/standard, 2) *E. coli,* 3) *Listeria monocytogenes*, 4) *Bacillus subtilis* 5) Negative control 6) Salmonella, 7) unknown, 8) Marker



1. What are the purposes of lanes 1 & 8
2. What is the role for the reactions in lanes 6 & 7
3. Interpret lane 8
4. Identify 3 ways to improve this assay

2. The PCR assay below is a multiplex PCR designed to differentiate among STEC *E. coli.* "Shiga toxigenic *Escherichia coli* (STEC) is an important cause of gastrointestinal disease in humans, particularly since such infections may result in life-threatening sequelae such as hemolytic-uremic syndrome (HUS. There is an increasing demand for improved diagnostic procedures for the detection of STEC in fecal samples and, in particular, in foods such as meat and dairy products. It has been recognized for a number of years that STEC strains causing human disease may belong to a very broad range of O serogroups. However, many of the STEC strains found in the gastrointestinal tracts of domestic animals (the principal source of human infections) may have a low degree of virulence in humans. These strains are less likely to produce putative accessory virulence factors such as intimin (encoded by *eaeA*) and the plasmid-encoded enterohemolysin (encoded by enterohemorrhagic *E. coli* (EHEC) *hlyA*). Within the human disease-associated strains, those producing Shiga toxin type 2 (Stx2, encoded by stx_2) appear to be more commonly responsible for serious complications such as HUS than those producing only Shiga toxin type 1 (Stx1, encoded by stx_1). We have developed multiplex PCR assays for the simultaneous detection of stx_1, stx_2, *eaeA*, and EHEC *hlyA.*"

Paton, A. W. et al. 1998. J. Clin. Microbiol. 36(2):598-602



stx_1 = 534 bp

stx_2 = 384 bp

eaeA = 255 bp

EHEC *hlyA* = 180 bp

Interpret each lane and state which isolates are most and least likely to cause disease.

Name_____

FS 362
Primer Design Group Project

Your goal is to design primers to amplify an approximate 600 base pair fragment of the *Salmonella* gene *invA.* InvA is essential for *Salmonella* spp. to enter epithelial cells. It is a member of a family of proteins involved in either flagellar biosynthesis or the secretion of virulence determinants by a number of plant and mammalian pathogens. This gene has been shown to be a suitable target for identification of *Salmonella* spp.

The rules for Primer Design:

1) Melting temperature should be between 58° and 60°C; melting T for forward and reverse primers should be the same

2) Avoid long "runs" of A's, T's, G's, or C's

3) Primers are always written 5' to 3', regardless whether it is the forward or reverse primer

4) Primers are between 18 and 24 bases long

5) Among the 5 bases on the 3' end of the primer, 3/5 must be a G or a C.

6) Avoid palindromes which may result in hairpin loops

7) DNA Polymerase recognizes the 3' free hydroxyl group.

Forward Primer:

5'_____3'

Reverse Primer:

5'_____3'

SOURCE: *Salmonella enterica* subsp. *enterica* serovar Saintpaul str. SARA29
LOCUS: *invA*
GENE: 2058 bp
MOLECULE: DNA

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   1 gtgctgcttt ctctacttaa cagtgctcgt ttacgacctg aattactgat tctggtacta
  61 atggtgatga tcatttctat gttcgtcatt ccattaccta cctatctggt tgatttcctg
 121 atcgcactga atatcgtact ggcgatattg gtgtttatgg ggtcgttcta cattgacaga
 181 atcctcagtt tttcaacgtt tcctgcggta ctgttaatta ccacgctctt tcgtctggca
 241 ttatcgatca gtaccagccg tcttatcttg attgaagccg atgccggtga aattatcgcc
 301 acgttcgggc aattcgttat tggcgatagc ctggcggtgg gttttgttgt cttctctatt
 361 gtcaccgtgg tccagtttat cgttattacc aaaggttcag aacgcgtcgc ggaagtcgcg
 421 gcccgatttt ctctggatgg tatgcccggt aaacagatga gtattgatgc cgatttgaag
 481 gccggtatta ttgatgcgga tgccgcgcgc gaacggcgaa gcgtactgga aagagaaagc
 541 cagctttacg gttcctttga cggtgcgatg aagtttatca aaggtgacgc tattgccggc
 601 atcattatca tctttgtgaa ctttattggc ggtatttcgg tggggatgac ccgccatggt
 661 atggatttgt cctccgccct gtctacttat accatgctga ccattggtga tggtcttgtc
 721 gcccagatcc ccgcattgtt gattgcgatt agtgccggtt ttatcgtgac tcgcgtaaat
 781 ggcgatagcg ataatatggg acggaatatc atgacgcagc tgttgaacaa cccatttgta
 841 ttggttgtta cggctatttt gaccatttca atgggaactc tgccgggatt cccgctgccg
 901 gtttttgtta ttttatcggt ggttttaagc gtactcttct attttaaatt ccgtgaagca
 961 aaacgtagtg ccgccaaacc taaaaccagc aaaggcgagc agccgctcag tattgaggaa
1021 aaagaagggt cgtcgttagg actgattggc gatctcgata aagtctctac agagaccgta
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1141 gagcgtctac gtagtcagtt ctttattgat tatggcgtgc gcctgccgga agtattgtta
1201 cgcgatggcg agggcctgga cgataacagc atcgtattgt tgattaatga gatccgtgtt
1261 gaacaattta cggtctattt tgatttgatg cgagtggtaa attattccga tgaagttgtt
1321 tcctttggca ttaatccaac aatccatcag caaggtagca gccagtattt ctgggtaacg
1381 catgaagagg gggagaaact ccgggagctt ggctatgtgt tgcggaacgc gcttgatgag
1441 ctttaccact gtctggcggt gacgctggcg cgcaacgtca atgaatattt cggtattcag
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1501 gaaacaaaac atatgctgga ccaactggaa gcgaaatttc ctgatttact taaagaagtg

1561 ctcagacatg ccacggtaca acgtatatct gaagttttgc agcgtttgtt aagcgaacgt

1621 gtttccgtgc gtaatatgaa attaattatg gaagcgctcg cattgtgggc gccaagagaa

1681 aaagatgtca ttaaccttgt ggagcatatt cgtggggcaa tggcgcgtta tatttgccat

1741 aaattcgcca atggcggtga attacgagca gtaatggtat ctgctgaagt tgaggatgtt

1801 attcgcaaag ggatccgtca gacctctggc agtaccttcc tcagccttga cccggaagcc

1861 tccgctaatt tgatggatct cattacactt aagttggatg atttattgat tgcacataaa

1921 gatcttgtcc tccttacgtc tgtcgatgtc cgtcgattta ttaagaaaat gattgaaggt

1981 cgttttccgg atctggaggt tttatctttc ggtgagatag cagatagcaa gtcagtgaat

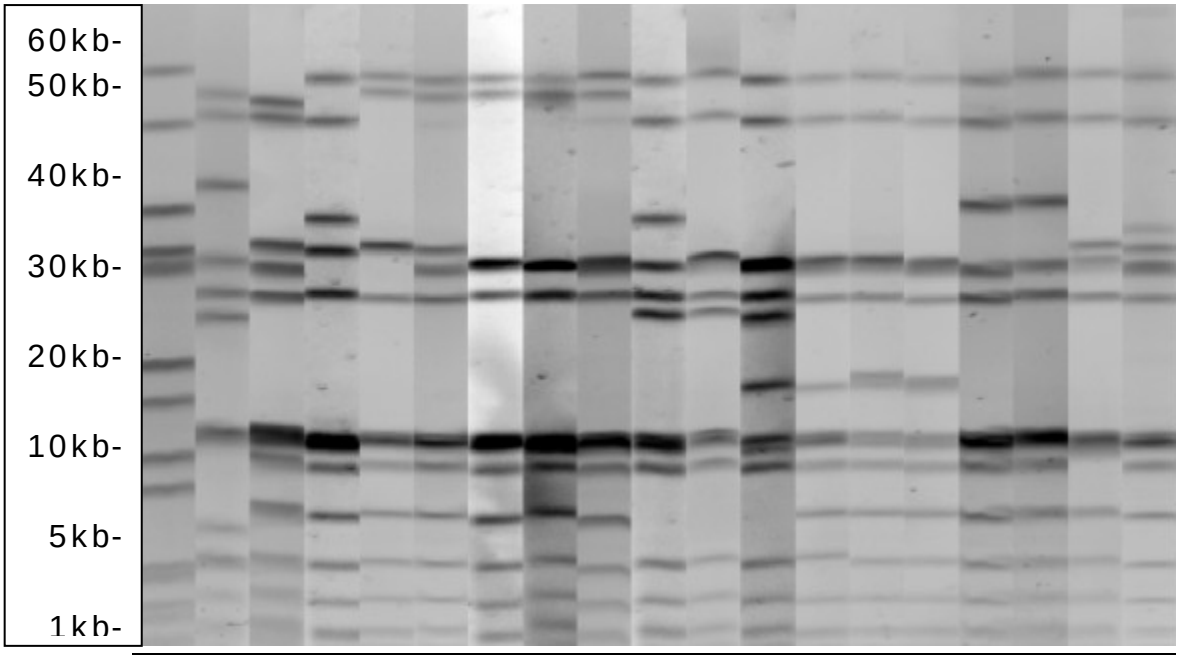
2041 gttataaaaa caatataa

Name:

PFGE Gel Activity

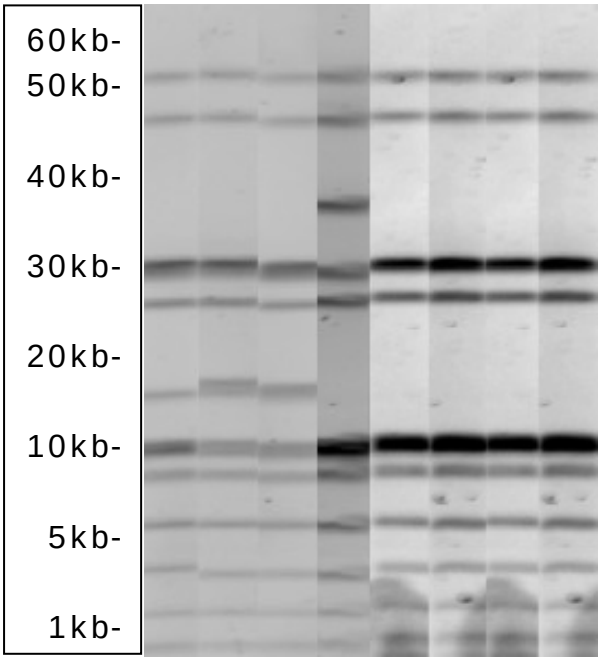
You and your group are currently working for PulseNet Canada, investigating an outbreak related to Tim Horton's custard-filled donuts and *Staphylococcus aureus*. From January to June 2011, there were 19 reported illnesses with 5 hospitalizations and 1 mortalities. No illnesses were reported from July to September. From October to December 2011, an additional 8 illnesses were recorded.

You and your team have been given the agarose gels resultant of separate PFGE runs from each month. Below is a summary of the merged gels from the first occurrence (6 months):



Question 1: How many different strains were present during the first 6 months of the outbreak?

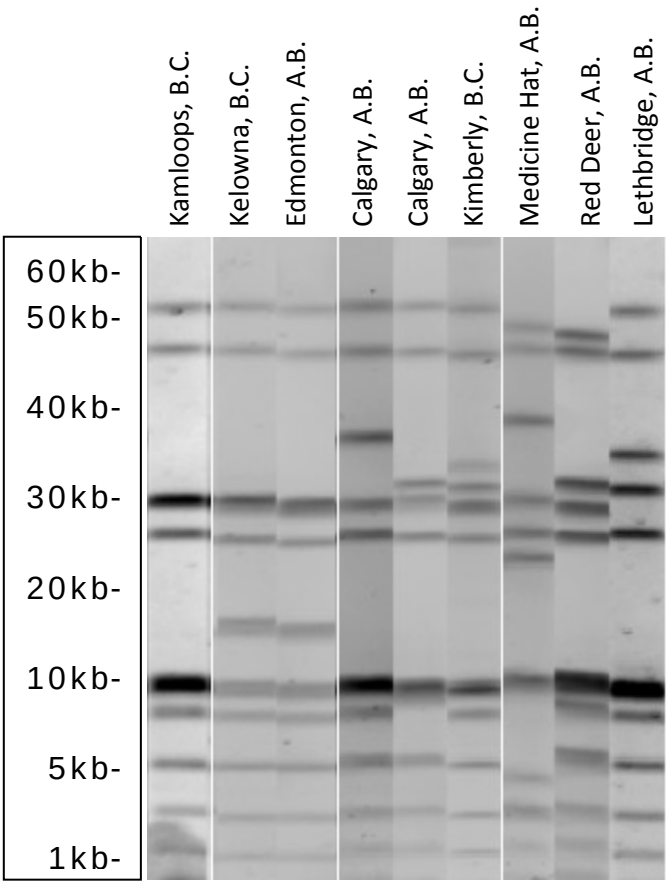
Following the interpretation of the first 6 months, you are provided with the data from the last three months (October to December).



Question 2: How many different strains were present during the last 3 months of the outbreak?

Question 3: Are there any similar strains from the first 6 months compared to the last 3 months?

Following the occurrences, samples were taken from all the Tim Horton's restaurants in the surrounding vicinity of living quarters for the reported cases and the victims.



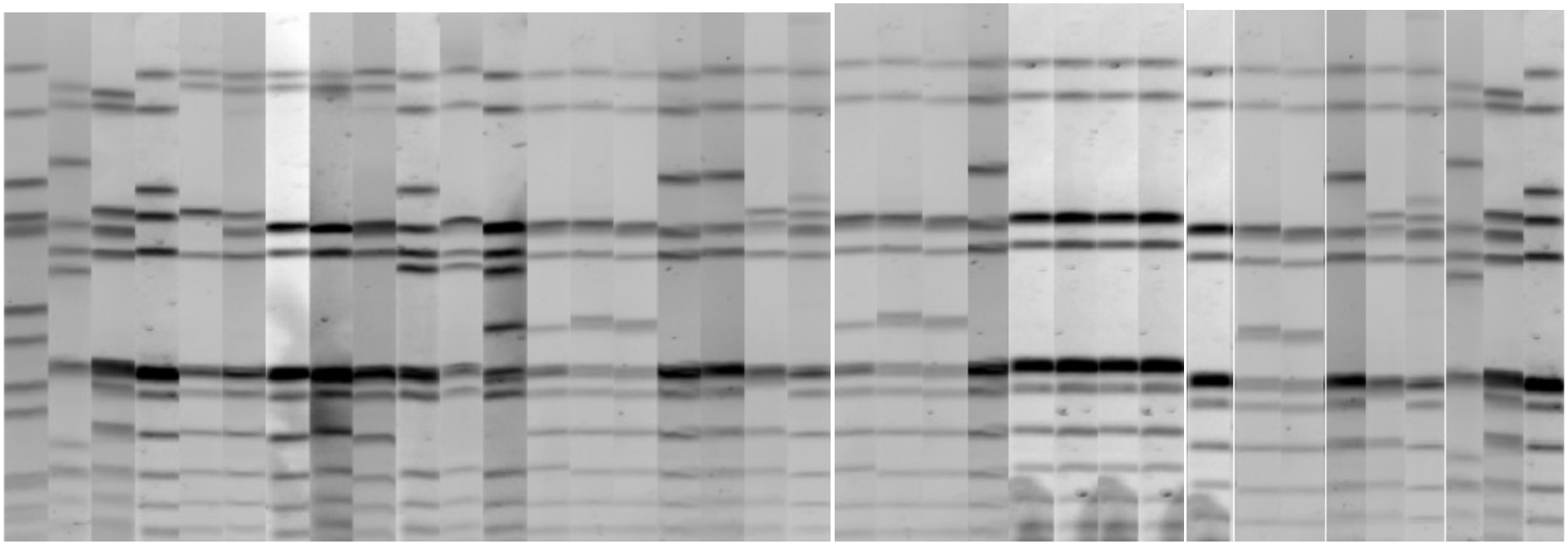
Question 4: How many outbreaks are related to each store?

Store	Outbreak Number
Kamloops, B.C.	
Kelowna, B.C.	
Edmonton, A.B.	
Calgary, A.B.	
Calgary, A.B.	
Kimberly, B.C.	
Medicine Hat, A.B.	
Red Deer, A.B.	
Lethbridge, A.B.	

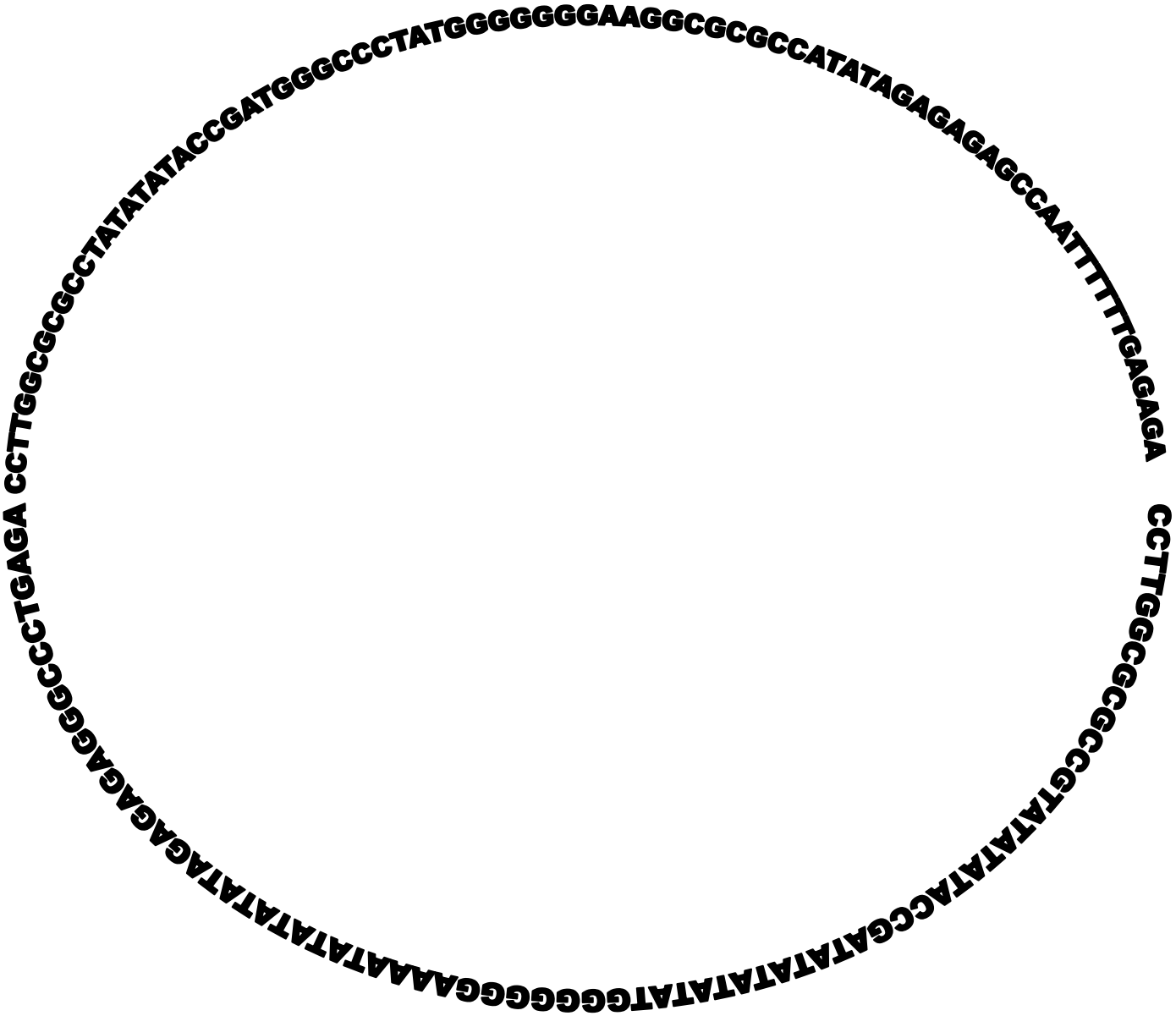
First 6 months

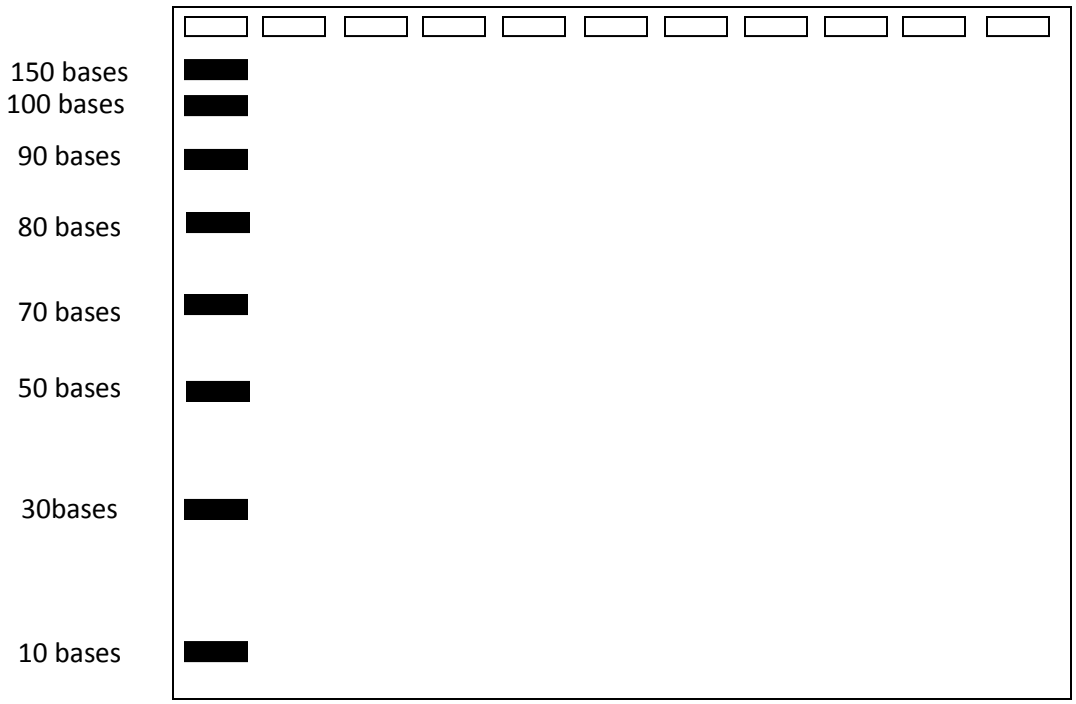
Last 3 months

Stores



Presented below is a sample bacterial chromosome, shortened for these purposes to 153 bases, forward strand only. Within the chromosome are restriction sites for ApaI and AscI. The recognition sequence for ApaI is 5' GGGCCC 3' and the recognition site for AscI is 5' GGCGCGCC 3'. Locate the restriction sites, mark where the RE's will cut and label as such. Calculate the segment size and place accordingly on the agarose gel on page 2. Remember, you only use one RE per experiment/investigation. You will have two lanes of samples on the next page.




150 bases
100 bases
90 bases
80 bases
70 bases
50 bases
30bases
10 bases