

## **Novel Technique using Naturally Competent ADP1 to Clone Variable *lux* Genes via Homologous Recombination Tested**

### **I. Introduction**

What makes some species of bacteria benign and some pathogenic? Many times, pathogenicity is caused by virulence factors or pathogenicity islands, which for the most part are genomic segments that are highly different from a benign close relative; the difference between virulent *EHEC* and benign *E. coli* is one of these regions. These genetic “hotspots” usually signal that the genes originated in some other organism, in the case of *EHEC*, a key virulence factor can be traced to *Shigella*. Indeed, many of the genes that confer unique and interesting qualities on bacteria are located at areas of high variability – areas that have very different sequences or are nonexistent in different species. By studying these regions, one could gain new insights into the mechanics and evolutionary significance of horizontal gene transfer and identify key genes and their products.

In the past, if one wished to study these interesting genes, one would have had to sequence the entire genome of the new species, which would have been both costly and time consuming. Recently, a process involving yeast and homologous recombination to “trap” these regions of unknown sequence was developed, but it is laborious and costly; this process is not used widely out of the lab that invented it. The “trapping” of these genes, however, allows for sequencing of only these areas of interest.

Phoebe Lostroh of Colorado College and her colleague at Grinnell College propose that a naturally competent bacterium that is also adept at homologous recombination, ADP1, could vastly simplify and economize the processes of “trapping” these regions of high variability for sequencing. In this technique, a linearized plasmid with regions homologous to the conserved

“hotspot” flanking regions is introduced into ADP1’s vicinity along with chromosomal DNA from the target organism. ADP1 would then take up the linearized plasmid and chromosomal DNA and “trap” the target genes via homologous recombination. This new technique in combination with microarrays<sup>1</sup> – efficient methods using robotics and computer analysis to compare the genomes of numerous species of microorganisms at a time and identify regions of variability – would produce a powerful method of studying these genetic “hotspots.”

During this lab, we began the long process of testing this novel technique. Since some regions of high variability are merely an absence of those genes in one of the species, we chose to test this technique by attempting to trap the *lux* genes. These genes are the perfect test because they differ enough between species of *Vibrio fischeri* to be deemed variable, yet, because each of these species produces light, we know these genes exist in each species; these candidates were identified through a microarray (data not shown). The large majority of the work reported here deals with the construction and isolation of the plasmid, pLux-Gap, which would be used to “trap” the *lux* genes.

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<sup>1</sup> According to Salyers and Whitt, a more common use of microarrays is to study the regulation of genes. If one wanted to identify, for instance, genes that enable organism M to survive at low iron levels, one could use two microarray chips, each with thousands of compartments. Each compartment would contain the ssDNA complement of mRNA from one gene, and every gene in the organism’s chromosome would be represented by a compartment. One would then grow organism M on two different media, one with abundant iron, and one with low iron. One would then separate out the mRNA from these two sets of cells, fluorescently tag the mRNA, and have a robot spread the mRNA all over the chip. The mRNA would then bind to its complement and thus identify which genes were active in each condition. In this example, one would look for genes that were expressed in low iron conditions but not in abundant iron conditions.

## II. Materials and Methods

### ISOLATION OF PLASMID DNA

All isolations of plasmid DNA were achieved using a Qiagen Miniprep kit. pUC-GAP 15 was isolated from *E. coli*, pBAC 348 from ADP1, and pLux-Gap from transformed *E. coli*.

### ENZYME DIGESTION

For each enzyme digestion, this general procedure was followed:

Reagents in Table 1 were added, in order, to 1.5mL microfuge tubes and mixed gently, but thoroughly with the flicking method. The tubes were then incubated at 37°C for ~3 hours.

**Table 1. Volumes of Reagents (uL) Needed for Each Type of Restriction Digest**

Reagent	Digest 1 <sup>1</sup>	Digest 2 <sup>1</sup>	Digest 3* <sup>2</sup>	Digest 4* <sup>2</sup>	Digest 5* <sup>2</sup>	Digest 6* <sup>2</sup>	Digest 7* <sup>2n</sup>	Digest 8* <sup>2n</sup>
ddH <sub>2</sub> O	34	29	18.5	18.5	18.5	18.5	18.5	18.5
Buffer 2	5	5	3	0	3	0	3	0
Buffer 3	0	0	0	3	0	3	0	3
10x BSA	5	5	3	3	3	3	3	3
pBAC DNA	5	0	5	5	0	0	0	0
pUC-Gap15 DNA	0	10	0	0	5	5	0	0
Experimental DNA	0	0	0	0	0	0	5	5
BamHI <sup>a</sup>	0.5	0.5	0	0	0	0	0	0
HindIII <sup>a</sup>	0.5	0.5	0.5	0	0.5	0	0.5	0
SalI <sup>a</sup>	0	0	0	0.5	0	0.5	0	0.5

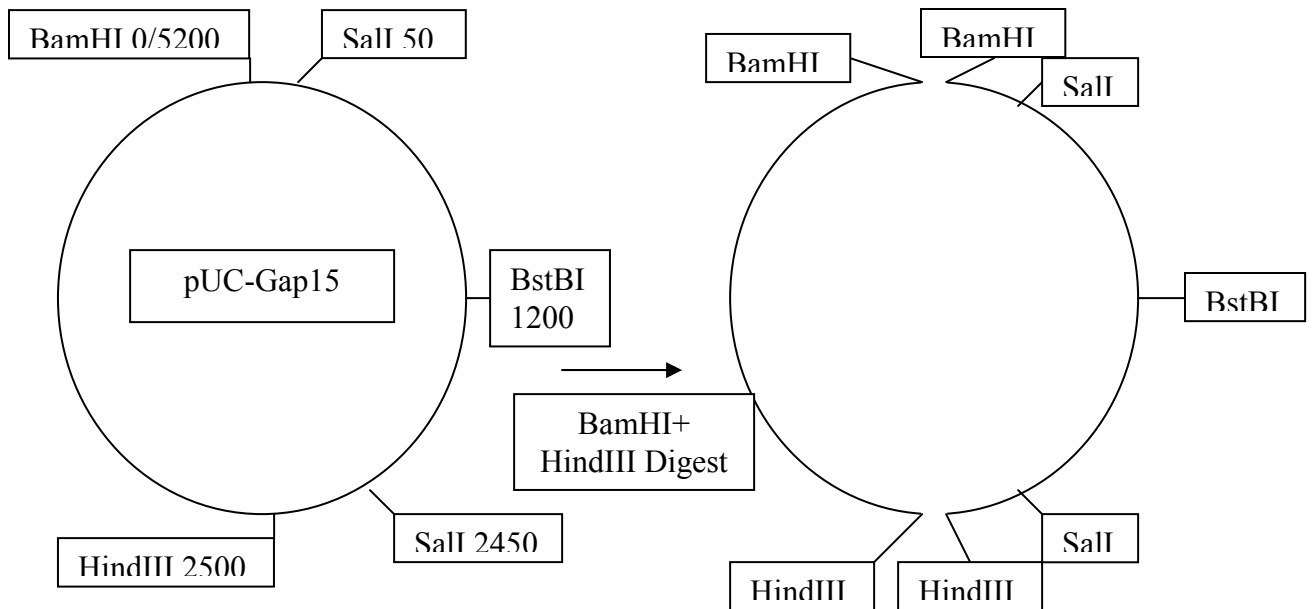
<sup>1</sup> used to construct pLux-Gap

\* for these digestions, a mother digestion solution, without DNA, was made with ten times the volume of the reagents listed above and then allocated evenly to each of the ten different DNA samples

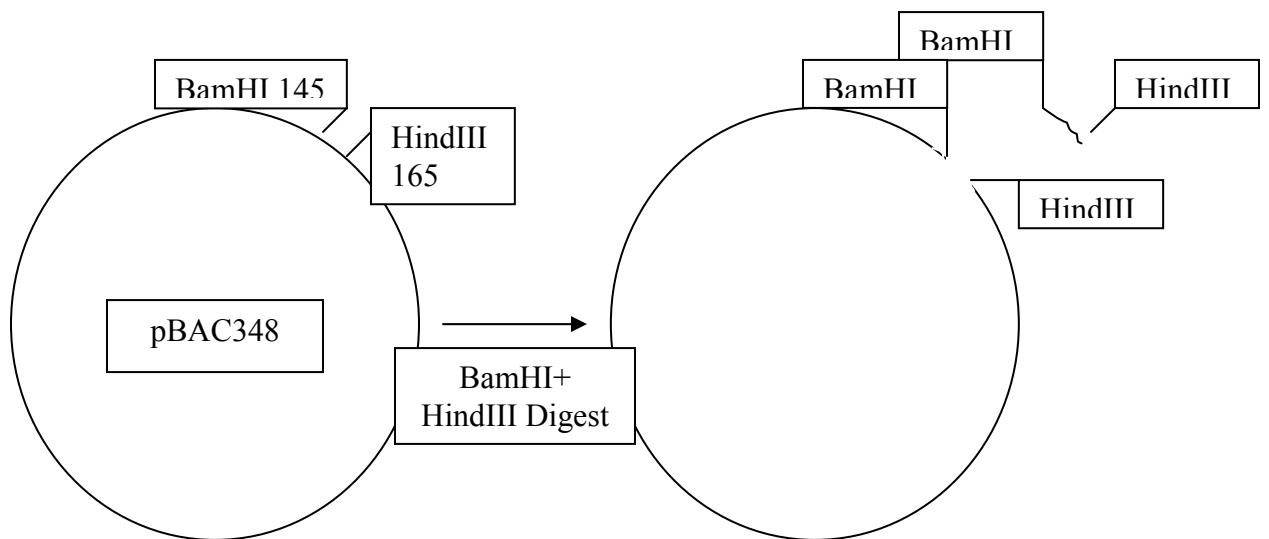
<sup>2</sup> made in preparation of running these DNA samples through an agarose electrophoresis gel

<sup>n</sup> Used for each of the eight DNA samples from colonies 11, 30, 14, 8, 41, 7, 5, 43

<sup>a</sup> added by PL or David



**Figure 1. Expected pUC-Gap15 BamHI/ HindIII double digest.** As shown in this diagrammatic representation, this double digest is expected to yield two linear DNA segments of 2450 BP and 2750 Bp respectively. The above rightmost segment will be ligated to most of pBAC348 (Figure 2) to create pLuxGap. The locations (in BP) of restriction enzyme sites are labeled.

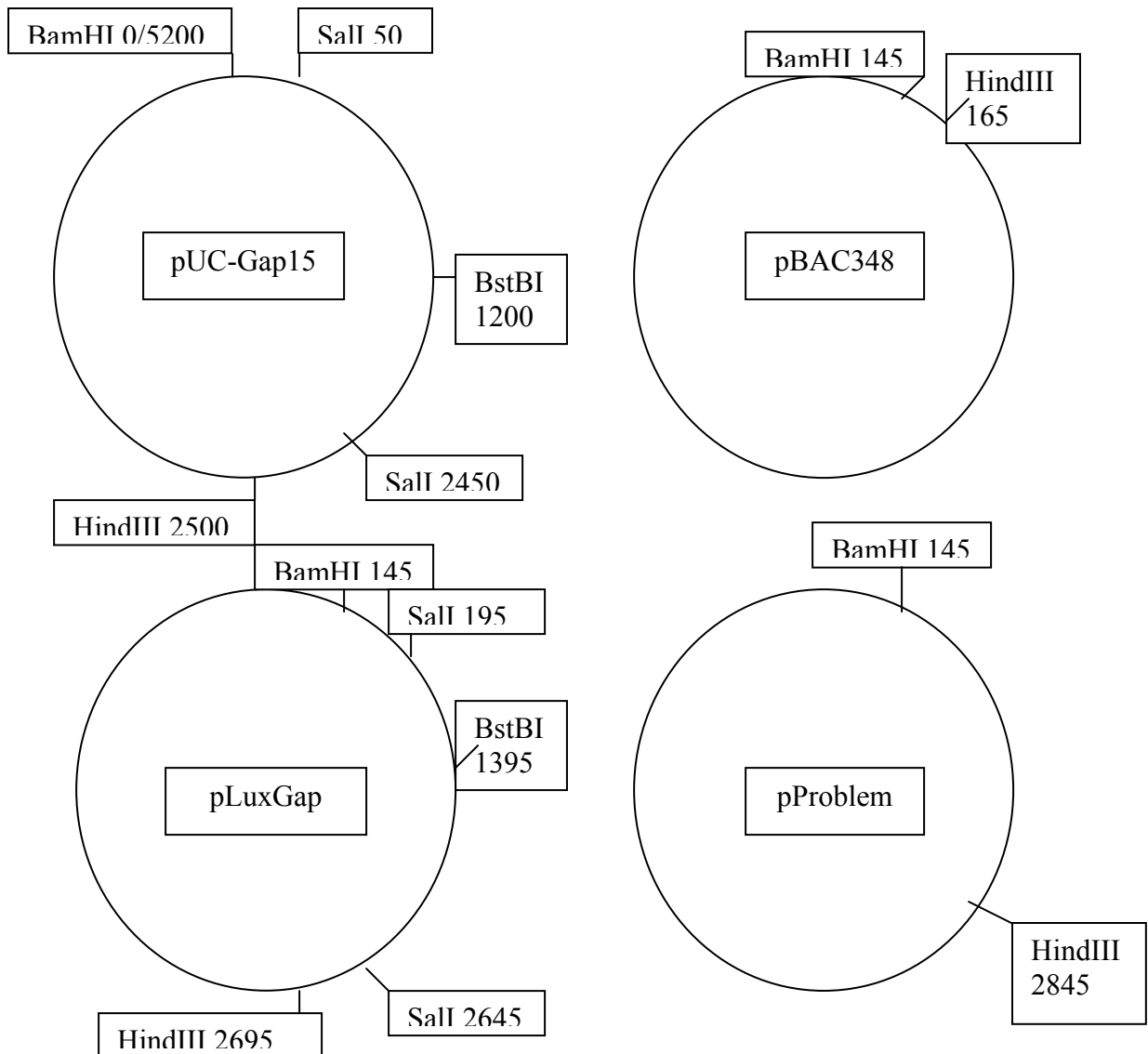


**Figure 2. Expected pBAC348 BamHI/ HindIII double digest.** As shown in this diagrammatic representation, this double digest is expected to yield two linear DNA segments of 20 BP and 6180 Bp respectively. The above right larger segment will be ligated to the BstBI site containing half of pUC-Gap15 to create pLuxGap. The locations (in BP) of restriction enzyme sites are labeled.

## LIGATION

Three separate ligations were performed during this lab following the BioLab's Quick Ligation Kit. Two were control ligations and one was experimental. First, the restriction enzymes in the plasmid DNA were heat inactivated on a heat block set at 70°C for 15 minutes. For the controls, 10uL pUC-Gap15 BamHI/ HindIII double digest was added to 80uL 1x buffer 2, and 10uL pBAC348 BamHI/ HindIII double digest was added to 80uL 1x buffer 2 in a 1.5mL microfuge tube. For the experimental, 10uL pUC-Gap15 and 10uL pUC-Gap15 BamHI/ HindIII double digest was added to 70uL 1x buffer 2 in a 1.5mL microfuge tube. The DNA solutions were then pelleted in a microfuge for 30 min at 14,000 rpm, the supernatant poured out, remaining liquid removed with a micropipette, and the microfuge tube refilled with 70% ethanol. The tubes were then spun in the microfuge for 5 min at 14,000 rpm, the supernatant poured out, liquid removed with micropipette, and tube filled with 100% ethanol. The tubes were then spun again in the microfuge for 5 min at 14,000 rpm, the supernatant poured out, liquid removed with micropipette, and tubes allowed to dry in the lab area.

The DNA pellet was then re-suspended in 10uL of ddH<sub>2</sub>O by repeatedly washing the side of the microfuge tube where the pellet dried. All the liquid was brought down to the bottom of the tube by briefly spinning in the microfuge. 10uL of ligation buffer was then added to each ligation, the tubes briefly spun, 1uL of ligase added to tubes, the tubes briefly spun again, and the tubes incubated on ice for roughly two hours.



**Figure 3. Possible Ligations in Experimental Tube.** Here, a schematic representation of the four possible ligations is shown; the locations (in BP) of restriction enzyme sites are labeled.

#### TRANSFORMATION

5uL of ligation and 50uL of artificially competent *E. coli* were added to each tube and mixed gently with a micropipette. The tubes were then incubated on ice for at least a half an hour. Afterwards, the cells were heat shocked at 37°C for 20 seconds, then iced for 2 minutes. Finally, 1mL of pre-warmed Luria Broth was added to each tube and incubated overnight at 37°C with shaking. After incubation, 100uL from each tube was plated on LB/amp plates. The remaining broth was pelleted in microfuge for 1 minute at 14,000 rpm, the supernatant dumped

out, the cells re-suspended with pipette, and the remaining solution plated on LB/amp plates. The plates were then incubated overnight at 37°C.

#### PATCH PLATING

Well isolated colonies from the transformation plates were picked with a sterile toothpick, and transferred to a spot designated on the target plate by an underlying grid with 50 spaces. An *E. coli* colony transformed with pUC-GAP15 HindIII/BamHI ligation was plated at position one of each plate and a colony transformed with pBAC348 HindIII/BamHI ligation was plated at position 50. Positions 2-49 were picked from colonies transformed with pUC-GAP15 and pBAC348 HindIII/BamHI ligation. Each colony was picked from the transformation plate, in order, first to an LB-amp positive control, then, with the same toothpick, to a succinate-amp negative control, and finally to an experimental succinate-amp plate with plated ADP1 (made competent by adding 10uL succinate stock to ADP1 and letting grow for 30 minutes prior).

#### AGAROSE GEL ELECTROPHORESIS

An ethidium bromide stained agarose gel was prepared using standard methods. Gel wells were loaded with 14uL sample DNA digestions or 10uL lambda-HindIII ladder (diluted in 1:4 with buffer EB from Qiagen miniprep kit and heated at 60°C for 3 minutes), and 1uL of sample buffer dye. Gels were then run for 90 minutes at 80 volts and photographed with a UV camera.

### III. Results

After artificially transforming *E. coli* with pUC-GAP15 HindIII/BamHI ligation (plate A), pUC-GAP15 and pBAC348 HindIII/BamHI ligation (plate B), and pBAC348 HindIII/BamHI ligation (plate C) and plating on Luria Broth/ Ampicillin plates, the respective colony counts found in Table 2 were observed. Plate A had the most colonies, followed by plate B, and plate C had no colonies.

**Table 2. Numbers of Transformed *E. coli* Colonies on LB/amp plates**

Plate*	Number of Colonies
Plate A <sup>1</sup> 100ul	16
Plate A <sup>1</sup> pellet	126
Plate B <sup>2</sup> 100ul	8
Plate B <sup>2</sup> pellet	73
Plate C <sup>3</sup> 100ul	0
Plate C <sup>3</sup> pellet	0

\*All plates were LB/Amp agar plates

<sup>1</sup> *E. coli* with pUC-GAP15 HindIII/BamHI ligation

<sup>2</sup> *E. coli* with pUC-GAP15 and pBAC348 HindIII/BamHI ligation

<sup>3</sup> *E. coli* with pBAC348 HindIII/BamHI ligation

We next observed that the *E. coli* colonies that we had patch plated at the positions shown in Table 3 seemed to have transformed the ADP1, thus allowing the ADP1 to acquire ampR and grow on the succinate ampicillin plate.<sup>2</sup> We then identified the eight *E. coli* colonies who's expelled DNA resulted in the transformation of the most robust ADP1 colonies on the succ/amp plate (determined roughly by the diameter of the ADP1 colonies). We ran agarose gel electrophoresis of these eight's isolated and digested plasmid DNA. The photograph of the ethidium- bromide-tagged DNA is shown in Figure 4.

**Colonies Who's Plasmid DNA Transformed ADP1<sup>1</sup>**

Colony Position # <sup>2</sup>	
5 <sup>3</sup>	30 <sup>3</sup>
7 <sup>3</sup>	32
8 <sup>3</sup>	37
11 <sup>3</sup>	38
12	40
13	41 <sup>3</sup>
14 <sup>3</sup>	43 <sup>3</sup>
23	46
24	

<sup>1</sup> transformation enabled ADP1 to survive on ampicillin (ampR genes were in the plasmids)

<sup>2</sup> Based on the circular numbered grid provided by PL

<sup>3</sup> candidates for pLux-Gap that were tested with electrophoresis

<sup>2</sup> Picked colonies 1-9 and 50 were from my group's plates, 9-34 from PL's plates, and 35-49 from GECC's plates.

**Table 4a. Approximate Sizes of DNA Segments Based on Agarose Gel Electrophoresis with a Sall Digest**

Colony #/Plasmid <sup>1</sup>	11	30	14 <sup>3</sup>	8	41	7	5	43 <sup>3</sup>	pBAC348 <sup>n</sup>	pUC-GAP15 <sup>n</sup>
Approximate	80.6	12.1	5.4	2.6	2.6	2.6	2.6	7.0	7.9	2.6
size	75.8	5.4	2.6	2.2	2.2		2.2	5.4		2.2
of	67.1	2.9	2.2					2.9		
DNA	2.6							2.6		
segment <sup>2</sup>	2.2							2.2		

<sup>1</sup> Candidates from Table 3 and control plasmids (pre-isolated)

<sup>2</sup> in kBP. Calculated from distance migrated (see calculations)

<sup>3</sup> possibly contain pLux-Gap

<sup>n</sup> control plasmids

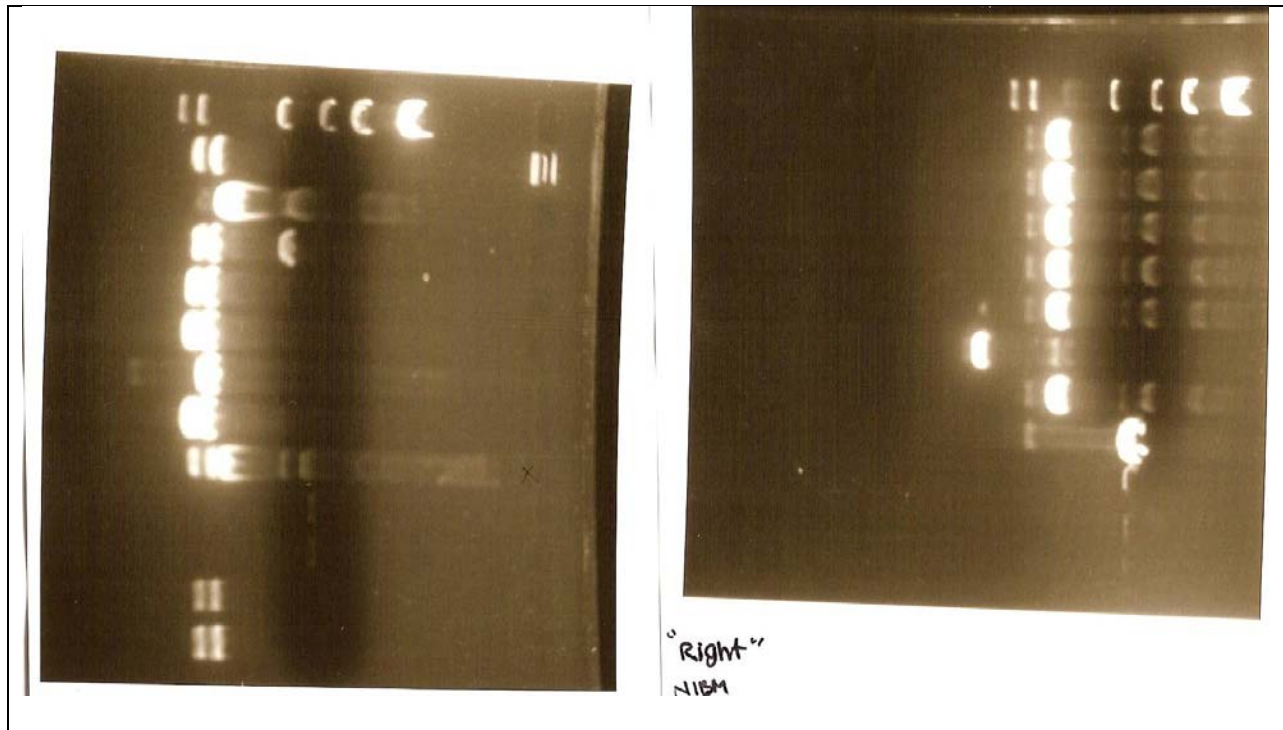
**Table 4b. Approximate Sizes of DNA Segments Based on Agarose Gel Electrophoresis with a HindIII Digest**

Colony #/Plasmid <sup>1</sup>	11	30	14	8	41	7	5	43	pBAC348 <sup>n</sup>	pUC-GAP15 <sup>n</sup>
Approximate	11.7	11.7	11.7	11.7	11.7	2.9	11.7	6.2	7.9	2.6
size	7.1	7.1	7.1	7.1	7.1	1.3	7.1	2.1		2.2
of	5.7	5.7	5.7	5.7	5.7		5.7			
DNA	2.8	2.8	2.8	2.8	2.8		2.8			
segment <sup>2</sup>	2.1	2.1	2.1	2.1	2.1		2.1			

<sup>1</sup> Candidates from Table 3 and control plasmids (pre-isolated)

<sup>2</sup> in kBP. Calculated from distance migrated (see calculations)

<sup>n</sup> control plasmids



**Figure 4. Agarose Gel Electrophoresis Results.** In both gels, the wells are located on the right-hand side. The wells on the rightmost side were cut off in the photography process. In both gels, the lambda-HindIII ladder appears as the topmost series of bands. The wells of the left-hand gel were loaded – in bottom to top order – with pUC-Gap15 SalI, pUC-Gap15 HindIII, pBAC348 SalI, pBAC348 HindIII, plasmid DNA digested with SalI from colony 43, then colony 5, colony 7, colony 41, colony 18, colony 14, colony 30, colony 11, then the ladder. The second gel was loaded – again in bottom to top order – with nothing in the first four wells, and then Hind III digested plasmid DNA from colony 43, then colony 5, colony 7, colony 41, colony 18, colony 14, colony 30, colony 11, then the ladder.

The approximate number of base pairs in each piece of DNA is shown in Table 4 (derived using a standard curve; see calculations section for details). The expected piece sizes of the plasmids, pUC-GAP15, pBAC348, pProblem, and pLux-Gap are shown in Table 5. As shown in Table 4a, the wells of colony number 43 and colony number 14 could possibly contain pLux-Gap,

**Table 5. Expected DNA Segment Sizes after Digestion**

	SalI	HindIII
pUC-GAP15	2.4/2.7 kBP	5.1kBP
pBAC348	6.2 kBP	6.2kBP
pProblem <sup>3</sup>	8.7 kBP <sup>n</sup>	8.7kBP
pLux-Gap <sup>1</sup>	2.4/6.2 kBP	~8.6 kBP <sup>2</sup>

<sup>1</sup> Desired plasmid

<sup>2</sup> True size unknown

along with other plasmids, if one looks at the Sall digest. The well of colony number 14 seems to also include pUC-Gap15 while that of colony number 43 seems to also contain pUC-GAP15, pProblem, and another piece of DNA of approximately 2.9 kBP.

<sup>3</sup> result of ligation of pBAC348 and the segment of pUC-GAP15 that does not contain the BstBI site  
<sup>n</sup> Plasmid remains circular after this digestion; abnormal migration through agarose gel during electrophoresis.

None of the DNA from the other colonies appears to contain pLux-Gap. According to the Sall digests, Colony 11 seems to contain pUC-Gap15 and some very large piece of DNA, probably pProblem. Colony 30 appears to contain a DNA fragment larger than any of the plasmids we worked with, as well as two smaller pieces, one that could be pBAC and one that is unidentified. Colony 8 contains pUC-Gap15, as do colonies 41 and 5. Colony 7 appears to only contain the larger portion of pUC-Gap15.

According to the HindIII digests, however, colonies 11, 30, 14, 8, 41, and 5 all seem to have the same plasmid DNA fingerprint. They all have two small fragments of 2.1 and 2.8 mBP that do not correspond to any expected HindIII digestion. Accounting for experimental error, it appears that all four expected plasmids could be in these wells. The fingerprint of colony number 14 does seem to contain a piece that could be around 8.6kBP (pLux-Gap) or 8.7kBP (pProblem). Colony 7 appears to contain to unexpected small DNA fragments. Colony 43 appears to contain pBAC and the smaller piece of pUC-Gap15 and lacks the expected pLux-Gap piece of approximately 8.6kBP.

#### IV. Discussion

During the transformation, the *E. coli* plated with HindIII/BamHI digested pUC-GAP15 (plate A) transformed at a higher frequency than those plated with HindIII/BamHI digested pUC-GAP15 and pBAC348 (plate B) and those plated with HindIII/BamHI digested pBAC348 (plate C). It would have been more desirable if plate B had the highest rate of transformation, because pLux-Gap could be ligated from only this experimental group; the more transformed colonies on this plate, the higher the probability that we would be able to isolate a pLux-Gap carrier. Plate B would also have been expected to have about twice as many transformed cells as the other plates because twice as much DNA was used to transform the cells on plate B.

Furthermore, of the *E. Coli* DNA that transformed ADP1 on the succinate amp plate, that of colonies 14 and 43 seem to possibly contain pLux-Gap mixed in with other DNA. Upon examination of the electrophoresis fingerprints of the Sall digest, it appears as if the DNA from these colonies 14 and 43 contains the right sized DNA pieces to make pLux-Gap and pUC-GAP15 or pLux-Gap, pUC-GAP15, and possibly pProblem or an unidentified large piece of DNA respectively.

Upon examination of the HindIII digest fingerprint, however, colony 43 does not contain the 8.7kBP fragment necessary for identification of pLux-Gap. Colony 14 has a band that could be 8.7kBP, but so do the two colonies on either side of it (indeed the fingerprints of these five are identical). It is possible that DNA from one of these wells – or many of them – spilled out during well preparation and contaminated the others. If well DNA containing pProblem meandered into other wells, it would leave a band at about 8.6kBP, which could easily be misinterpreted as the band necessary for pLux-Gap. Also, the plasmid DNA could have not been fully digested, which would account for the unexpected, abundant fragment sizes. This incomplete or incorrect

digestion is reinforced by the fact that the fingerprints of both our pUC-Gap15 controls are identical.

It is interesting to note the results of colony 11, with its apparently gigantic pieces of DNA; since not one of the plasmids we worked with amounted to anything close to 80kbp (the size of a small bacterial genome) these outstanding results could be due to circular pProblem not being able to migrate well through the gel, chromosomal DNA contamination, or some other type of contamination.

Due to the ambiguity of the electrophoresis results, more testing would be needed to determine conclusively whether or not pLux-Gap is present in one of these colonies. This extended testing could include a rerun electrophoresis of colonies 14 and 43. If the DNA from these colonies were run in the end wells and the ladder in the middle, DNA mixing would be minimized. If this rerun yielded pure pLux-Gap signature (only a 2.4 and a 6.2 kbp with a Sall digest and a single 8.7 kbp segment with a HindIII digest) then the lab could continue. If, however, it appeared that pLux-Gap were present but mixed in with other plasmids, it is possible that colony 14 or 43 is not a pure culture. In this case, a streak plate to isolate pure colonies, the testing of these colonies via ability to transform ADP1 and DNA fingerprinting would be necessary to attempt to isolate pLux-Gap. It is also possible that the *E. coli* from colonies 14 and 43 actually contains multiple plasmids. In this case, it would be impossible to isolate pLux-Gap without transforming another group of *E. coli* with the plasmid DNA from the multiple plasmid *E. coli*.

The prevalence of pUC-Gap15 in the colonies that allowed ADP1 to grow on amp plates is unexpected. pUC-Gap15 cannot replicate inside ADP1. It is possible that the extreme selective pressure applied to the ADP1 caused a process similar to one used to introduce not functional

genes to a genome to determine the function of these genes. In this process, a non-replicating plasmid with an incomplete (nonfunctional) copy of the gene in question and an ampR gene are introduced to the target organism and then the ampR is selected for. This selection insures that all those cells that survive have used homologous recombination to incorporate the plasmid sequence (along with the ampR and the nonfunctional gene) into its chromosome. The nonfunctional gene replaces the functional gene. It may be possible, due to the mastery of ADP1 rec proteins, that a region of homologous sequence (maybe just a few bp) was found both pUC-Gap15 and the ADP1 chromosome, and the rec proteins sliced in the ampR gene to the chromosome. This is merely a tentative hypothesis, however, and would require much additional testing.

If we were to continue the test of this novel technique of cloning genetic “hotspots,” we would first need to conclusively identify pLux-Gap. Once identified, pLux-Gap could be introduced along with *V. fischeri* on a succinate/amp plate inoculated with ADP1. Since the *V. fischeri* cannot survive with succinate as their only source of carbon, they would die and spew their chromosomal DNA all over the ADP1. Then, hopefully, some of the ADP1 would take up both pLux-Gap (linearized through a BstBI digest) and chromosomal *V. fischeri* DNA (containing the *lux* genes) and would use homologous recombination to create a closed plasmid with trapped *lux* genes. It is not certain whether or not the ADP1 will express the *lux* genes and glow once they have trapped these genes. Therefore, one final transformation of good old battered *E. coli* could finish the job. It is known that *E. coli* express the *lux* genes; therefore, if the *lux* genes have been successfully “trapped” by ADP1, the *E. coli* transformed with this newly constructed plasmid could be identified by their emission of photons. If the continuation described above works, *lux* genes from many different species can be trapped in this manner,

then all can be sequenced and the differences scrutinized. Also, the successful completion of this test would show that this technique could be feasibly used to study the variable regions of microorganisms in a more economic and less labor intensive way.

The applications of this novel technique could impact many areas of research. First, it could revolutionize the study of pathogenicity. If one could use this technique to find out what differs between pathogenic and nonpathogenic strains of bacteria, one could gain insight into the very nature of pathogenicity. On a more medical level, sequencing all these virulence factors could lead to countless novel treatments of disease, and maybe even some medications that halt pathogenicity without killing bacteria and selecting for resistant strains. If this second application comes to pass, this technique could help catapult us to Amabile-Cuevas' "post anti-biotic era."

Similarly, if one examined two strains with different phenotypes or different interesting metabolic processes, this technique could be used to quickly and efficiently isolate those interesting genes and line them up for sequencing. Finally, evolution scientists, such as Doolittle, could use this technique to examine the nature of horizontal gene transfer between species. If researchers could identify and catalogue these genetic "hotspots," people such as Doolittle could use this catalogue to create a more accurate and complex phylogenetic tree. The interwoven, three dimensional tree of Doolittle's dreams could become a reality.

Like any scientific inquiry, this lab also raises new questions. Why do *E. coli* become competent when you "beat them up?" does this mean that any cell has the potential to become competent? Could this idea somehow explain the success of naked DNA vaccines? Why are some bacteria better at homologous recombination than others? Can pUC-Gap15 be maintained somehow in ADP1? If the *lux* genes are present in all species of *V. fischeri* and all seem to function in the same way – by effecting the emission of photons – why are they variable? What

significance does this variability in the *lux* genes have? Are different *lux* genes required for establishment of symbiosis with different hosts? What purpose do the *lux* genes serve in non-symbiotic *V. fischeri*, such as those found at Woods Hole on the Cape?

If I learned one thing from Allen and Baker that made reading their book worth while, it is to always look for new questions, always continue the (now hackneyed) “process of inquiry.”

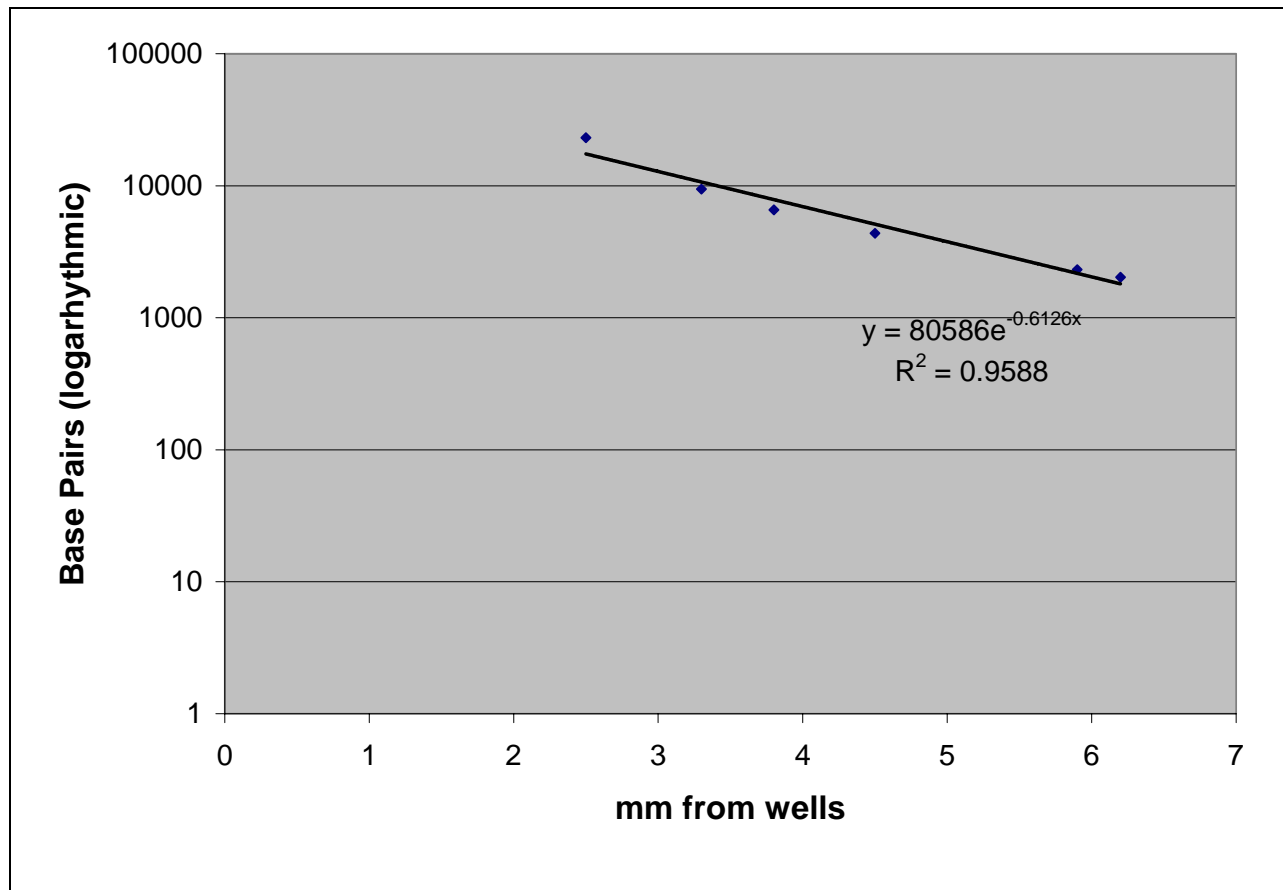
## V. Calculations

Colony # <sup>2</sup>	11 Sall	30 Sall	14 Sall	8 Sall	41 Sall	7 Sall	5 Sall	43 Sall	
	0	3.1	4.4	5.6	5.6	5.6	5.6	4	
m	0.1	4.4	5.6	5.9	5.9		5.9	4.4	
m	0.3	5.4	5.9					5.4	
	5.6							5.6	
	5.9							5.9	
Plasmid or ladder <sup>3</sup>	pBAC 348 Sall	pBAC348 HindIII	pUC-Gap15 Sall	pUC-GAP15 HindIII	ladder <sup>n</sup>				
	3.8	3.8	5.6	5.6	2.5				
m			5.9	5.9	3.3				
m					3.8				
					4.5				
					5.9				
					6.2				

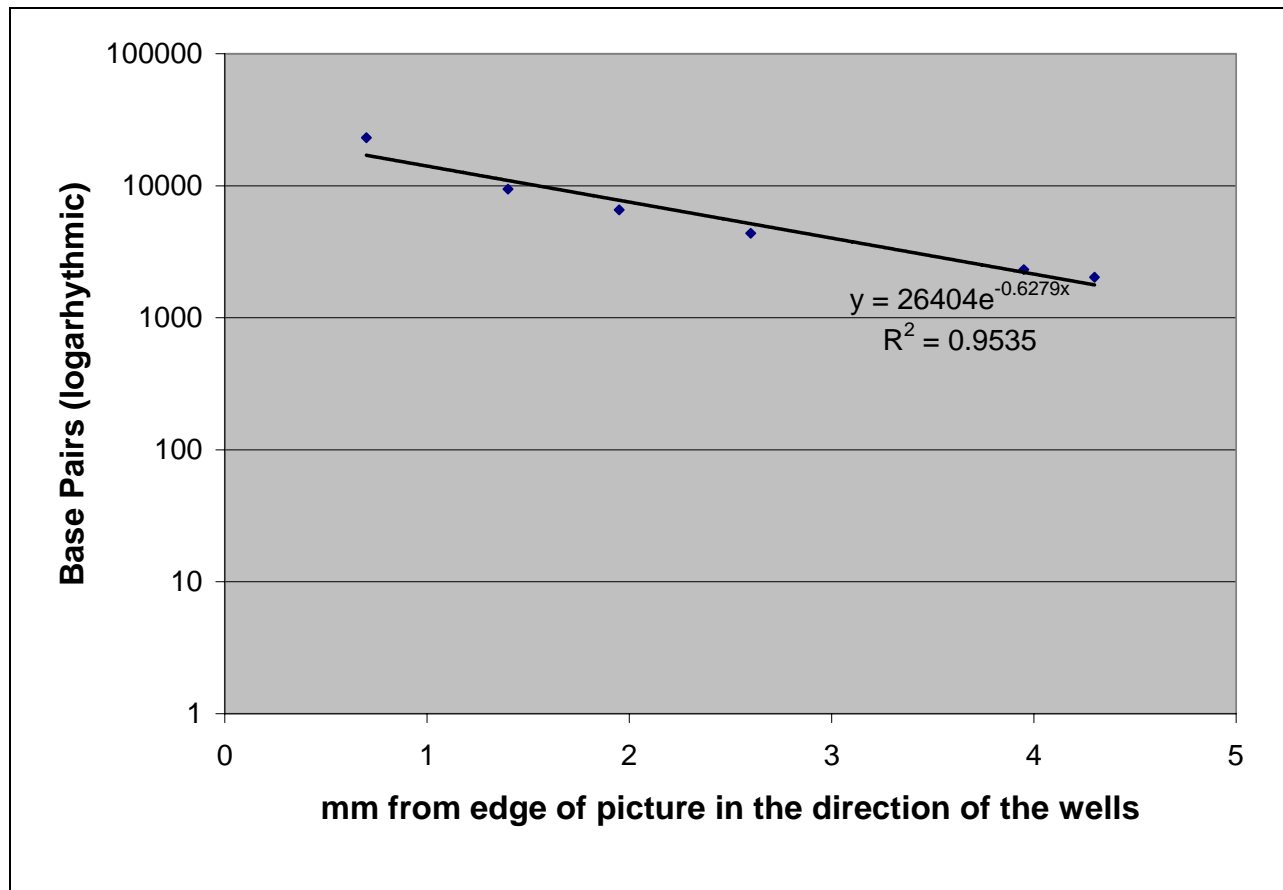
<sup>1</sup> Distance traveled from wells; measurement with a ruler of a photocopy of the gel photo  
<sup>2</sup> Colonies from Table 3 that transformed ADP1 on succ/amp  
<sup>3</sup> Controls and standards  
<sup>n</sup> Lambda DNA HindIII digest

Colony # <sup>2</sup> or ladder <sup>3</sup>	11	30	14	8	41	7	5	43	ladder <sup>n</sup>
	1.3	1.3	1.3	1.3	1.3	3.5	1.3	2.3	0.7
m	2.1	2.1	2.1	2.1	2.1	4.8	2.1	4	1.4
m	2.45	2.45	2.45	2.45	2.45		2.45		1.95
	3.6	3.6	3.6	3.6	3.6		3.6		2.6
	4	4	4	4	4		4		3.95
									4.3

<sup>1</sup> Distance traveled from wells; measurement with a ruler of a photocopy of the gel photo  
<sup>2</sup> Colonies from Table 3 that transformed ADP1 on succ/amp  
<sup>3</sup> Controls and standards  
<sup>n</sup> Lambda DNA HindIII digest



**Figure 5. Standard curve for determining size of DNA fragments in gel 1.** This standard curve was created using the distance each band of ladder DNA migrated away from the wells in agarose gel 1 and the corresponding base pair number provided by the New England BioLabs Lambda HindIII digest direction sheet. Each point represents one band of ladder DNA, the distance in mm that that band traveled, and the size in base pairs of that band. The best fit line was created with Microsoft Excell add trend line function. The equation of this trend line was then used to estimate the size of each band of DNA in gel 1 based on the distance each of these bands migrated (Table 4a). Alternatively, one could draw a vertical line from the distance the band migrated to the best fit line, then draw a horizontal line from this point of intersection over to the Y-axis and read the intersecting Y coordinate (Base Pairs).



**Figure 6. Standard curve for determining size of DNA fragments in gel 2.** This standard curve was created using the distance each band of ladder DNA migrated away from the wells (measured from the edge of the picture because the wells were cut off) in agarose gel 2 and the corresponding base pair number provided by the New England BioLabs Lambda HindIII digest direction sheet. Each point represents one band of ladder DNA, the distance in mm that that band traveled, and the size in base pairs of that band. The best fit line was created with Microsoft Excell add trend line function. The equation of this trend line was then used to estimate the size of each band of DNA in gel 2 based on the distance each of these bands migrated (Table 4b). Alternatively, one could draw a vertical line from the distance the band migrated to the best fit line, then draw a horizontal line from this point of intersection over to the Y-axis and read the intersecting Y coordinate (Base Pairs).

**Calculating size of DNA bands in the agarose gel:**

*Agarose gel 1:*

$$80586 * 2.71828182845904^{(-0.6126 * (\text{distance, in mm, the band migrated}))}$$

*Agarose gel 2:*

$$26404 * 2.71828182845904^{(-0.6279 * (\text{distance, in mm, the band migrated}))}$$

These formulae were plugged into an excel spreadsheet with the numbers of Table 6 to generate the values located in Table 4. Only the first 15 digits of e were used due to the limitations of Excell. The first fifty digits of e were provided by corks cited reference 1.

## VI. Acknowledgements

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## VII. Works Cited

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### **VIII. Honor Code:**

I have neither given nor received any unauthorized assistance on this laboratory report, and I have not quoted anyone else's work without citation