Cloning of pUC18λ and Effects of Multiple Restriction Enzymes

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ABSTRACT

The need for large amounts of the same DNA is a common thing in the world of biology experiments. To do so one needs to clone the DNA, and that is what this experiment investigates. To begin a specific DNA, phage λ, was isolated as well as a specific vector, pUC18. These two DNAs were then cut with restriction enzyme EcoRI and then mixed with T4 DNA ligase to that the could be added together and the backbone of the DNA annealed. From there this new recombinant DNA was introduced to host cells, E. coli, and left to grow. The cells that took up the plasmid were then selected for by taking out the cells that didn't take up the plasmid by exposing them to ampicillin and X-gal. These cells were then lysed and the plasmid DNA separated from the chromosomal DNA. This DNA was then exposed to either just EcoRI or EcoRI and BamH1. The resulting segments were then run on a gel and the lengths compared. It was found that when pUC18λ was exposed to EcoRI and BamH1 resulted in more bands of shorter length when compared to when it was just exposed to EcoRI. From this data the hypothesis that if the plasmid pUC18λ is cut with more that one restriction enzyme then this would result in more, and shorter, segments of DNA was proven correct.

INTRODUCTION

Many biological experiments require large amounts of the same pieces of DNA. To do this one must first clone the original DNA. This DNA can then be sequenced and analyzed, and if cloned properly, without too much interference from unwanted DNA. This clone of DNA is called recombinant DNA cloning.

This method of DNA cloning include the insertion of the desired DNA segment into a vector, in this case the vector used is a plasmid and a phage. This is normally done by isolating the desired DNA and vector, then, the use restriction enzymes to create sticky ends, and then annealing the DNA with ligase. This DNA vector, with the desired segment is then reintroduced to the bacterial cells so that the bacteria can replicate, which also replicated the desired DNA. From there the cells are lysed, the DNA re-cut with restriction enzymes, and the desired DNA separated out.

Now onto the subject of the ingredients of this experiment. First let's have a look at restriction enzymes. Restriction enzymes are enzymes that are able to recognize a specific nucleotide sequence, and cut the DNA there. The nucleotide sequence is different for most restriction enzyme. When restriction enzymes cut the DNA they can create either blunt ends or sticky ends. Blunt ends have both strands of DNA being cut at the same point, making a flat end. Sticky ends have the strands cut at different locations so it created an overhanging strand that can then pair up with other nucleotides. This means that the sticky ends are good for adding different DNA together. In this experiment the restriction enzymes EcoR1 and BamH1 were used to create sticky ends.

Next the vector. For this experiment the plasmid pUC18 was used as the vector. This plasmid is fairly useful in the fact that it is small, about 2.7kb in length, and contains an origin of replication and some useful genes, ampicillin resistance and production of the enzyme ß-galactosidase. It also contains a site in which the restriction enzymes can cut, which is ultimately what is needed.

Another enzyme that is added to this mixture is ligases. For this experiment the ligase T4 DNA ligase was used. This ligase catalyzes the binding of two DNA strands by annealing the backbone of the DNA. T4 ligase is used in this experiment because it will catalyze the ligation of RNA and double-stranded DNA but will not do so with single-stranded DNA.

The other ingredients of this experiment are ethidium bromide, X-gal, and ampicillin. Ethidium bromide is used to stain the DNA in the agarose gel so that it can be seen under UV light. X-gal is a substrate of ß-galactosidase, that when cleaved can be oxidized and turned blue. Ampicillin antibiotic, and this experiment it is used to eliminate cells that have not taken up the plasmid, meaning they do not have the ampicillin resistance gene, and will die.

The purpose of this experiment was to investigate the methods of DNA cloning, and the effects of different restriction enzymes. We hypothesized that if the plasmid pUC18λ is cut with more that one restriction enzyme then this would result in more, and shorter, segments of DNA. To test this we followed the steps required to perform DNA cloning.

MATERIALS AND METHODS

All materials and methods from BIOL24200 Lab 4-8: Library Preparation 1-5 (online document) This lab experiment was spread over 5 weeks.

Week 1: This week was the first and we started with digestion and ligation of the desired DNA. To do this we mixed either phage λ DNA or pUC18 DNA with EcoRI to digest the DNA and then added DNA T4 ligase to each. These solutions were then frozen so that they could be used the following week.

Week 2: For this week the experiment consisted of electrophoresis, transformation of E. coli, and plating the cells. First the solutions were prepared for electrophoresis by mixing out DNA solutions with DNA sample buffer, and then small amounts were put into the wells in an agarose gel. The gel was then run at 90 volts until the bromophenol blue was near the end. Once it was done running it was stained with ethidium bromide and then photographed. For the transformation we added some of the ligation/DNA mix to a tube of competent E. coli cells. Then it went through heat shock so that the cells might take up the plasmid. Nutrient broth was then added to the cells, and they were then allowed to incubate for 45 minutes. When this time was up the mixture was added and spread onto 3 different X-gal ampicillin plates. These were then left to sit overnight, and once they started to show single colonies were then sealed and moved to the refrigerator to be used next week.

Week 3: Week three consisted of picking and growing the desired colonies. To begin the number of blue and white colonies on each plate was counted and recorded. Then a single blue colony was taken from the plate and was moved to a culture tube. Then another blue colony was taken from the plate and streaked on one third of a new plate, in the properly labeled section. These steps were then repeated twice with white colonies. Both the tubes and the plate were then left to incubate. The next day the plate was checked for single colonies, sealed, and placed in the refrigerator to be used next week.

Week 4: During week four's experiment we harvested colonies and performed miniprep. To begin we took the two culture tubes, pUC18 and pUC18λ, and poured the contents into microfuge tubes to then be centrifuged to form pellets of the cells. Solution one, which contained tris buffer, was then added to each tube and the pellet re-suspended. Then solution two, which contained SDS and NaOH, was added, and then left to react for one to two minutes. Then solution three, containing acetic acid and guanidinium chloride, was added, and the contents mixed. The tubes were then centrifuged again so that the chromosomal DNA would pellet at the bottom. Then two columns and collection tubes were set up and Equilibration Buffer added. Then the supernatant was slowly poured through the column, without dislodging the pellet, and then centrifuged. The flow through was discarded and HB buffer added, and, again, centrifuged. Then flow through discarded and wash buffer added, then centrifuged again. Flow through was discarded. The column was then placed in a microcentrifuge tube and warmed elution buffer was added, and then the tubes were centrifuged. The columns were then discarded and the tubes capped, labeled, and frozen for next week.

Week 5: Week five was the final week in which we performed digestion and electrophoresis. To begin six microcentrifuge tubes were obtained and labeled 1-6. Then EcoRI mixture was added to tubes 1-3, and EcoRI+BamH1 added to tubes 4-6. Then the DNAs were added as follows: Tube 1- phage λ, Tube 2- pUC18 miniprep, Tube 3- pUC18λ mini prep, Tube 4- phage λ, Tube 5- pUC18 miniprep, Tube 6- pUC18λ mini prep. Each was then briefly vortexed and centrifuged, and then incubated for some time. From there a small amount of electrophoresis sample buffer was added to each tube. Each sample was then loaded into the wells of an agarose gel. The gel was then run, stained with ethidium bromide, and photographed.

RESULTS

Table 1: Observations and notes. This is a table of observations and notes made each week while doing the experiment.

Week	Observations and note
1	- All solutions were clear
2	- Gel did not turn out clear - Plate 1 (pUC18): no colonies - Plate 2 (transformed cells): 1 white colony - Plate 3 (pUC18λ): 39 individual white colonies
3	Colonies over grew so the could not be used
4	-
5	- Gel was not clear

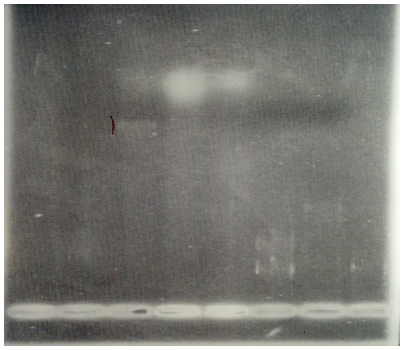
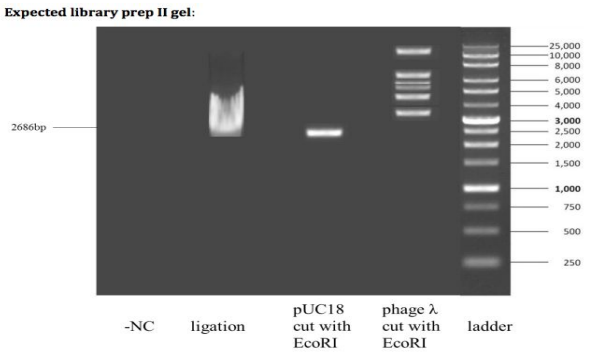
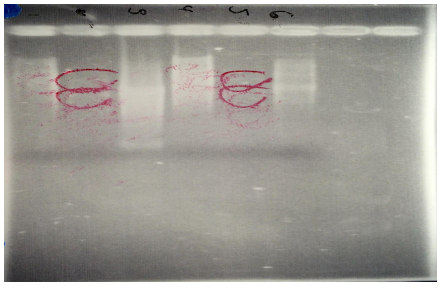
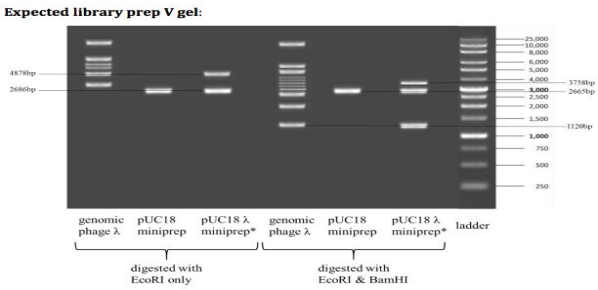
Table 2: Week Two Gels. This table contains the images of the gel made during week two of the experiment, as well as a list of what is in each lane of the gel.	
Personal Gel	Expected Gel
	
Lane 1: LE Lane 2: V-cut Lane 3: I-cut Lane 4: pUC Lane 5: pUC Lane 6: NC Lane 7: Ladder	Lane 1: NC Lane 2: Ligation Lane 3: pUC18 cut with EcoRI Lane 4: Phage λ cut with EcoRI Lane 5: Ladder

Table 3: Week Five Gels. This table contains the images of the gel made during week five of the experiment, as well as a list of what is in each lane of the gel.	
Personal Gel	Expected Gel
	
Lane 1: Phage λ Lane 2: pUC18 miniprep Lane 3: pUC18λ miniprep Lane 4: Phage λ Lane 5: pUC18 miniprep Lane 6: pUC18λ miniprep	Lane 1: Phage λ Lane 2: pUC18 miniprep Lane 3: pUC18λ miniprep Lane 4: Phage λ Lane 5: pUC18 miniprep Lane 6: pUC18λ miniprep Lane 7: Ladder

DISCUSSION

The purpose of the experiment was to transform E. coli cells to produce more of our desired DNA, and then compare the DNA when cut with different restriction enzymes. Looking at the above results proved our hypothesis that we started with, that if the plasmid pUC18λ is cut with more that one restriction enzyme then this would result in more, and shorter, segments of DNA.

First we look at table 2. Our personal gel did not turn out clear, so our observations and application of the information for our hypothesis came from the given expected gel. First we look at lane 3 and see that with only one enzyme the plasmid pUC18 results in only one band, meaning only one cut, and only one segment of DNA. Then we look at lane 4 and see that the phage λ has 5 places that it was cut.

Next we look at table 3. Again, our gel didn't turn out too well, so our observations had to come from the given expected gel. In the first two lanes of this gel we see similar results, as with the first gel, that is that pUC18 cut with just EcoRI results in one band, and phage λ cut with EcoRI results in 6 bands. Lane 3 is where it differs. In this lane pUC18λ was cut with just EcoRI and resulted in two bands. This would be our standard to test our hypothesis. Lane 4 shows that when phage λ is cut with EcoRI and BamH1, it results in eleven bands, while lane 5 shows that pUC18 cut with EcoRI and BamH1 results in, again, just one band. The big point of interest is in lane 6. This lane shows that pUC18λ cut with EcoRI and BamH1 results in three bands, which essentially proves our hypothesis.

CONCLUSION

We began this experiment to investigate the methods of DNA cloning, and the effects of different restriction enzymes. And after following the steps to isolate the DNA, insert it into a vector, insert into a cell, and harvesting it we observed our agarose gels to come to our conclusion. Our hypothesis was that if the plasmid pUC18λ is cut with more that one restriction enzyme then this would result in more, and shorter, segments of DNA. Through this experiment we found that our hypothesis was true. This was observed by the fact that pUC18λ cut with just EcoRI resulted in two bands, while when cut with EcoRI and BamH1 resulted in 3 bands.

Over this experiment there were several bumps in the road. Many times the experiment did not go as planned, such as cells overgrowing in the plate, or gels not coming out clear. If I were to change anything in this experiment it would be my starting knowledge. If I had know from the beginning what was the final goal, then there would be a better understand of each step.

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