Bacterial Transformation of Antibiotic Resistant Recombinant DNA

By: Ashley K.
University of Massachusettes Lowell
Lab Section 801A
Lab Partners: S. K. and V. O.

Materials and Methods

DNA Digests:

We prepared two DNA digests from *DNA Science, a first Course, 2nd Ed., p447-448*. digested pAMP (5.5ul of 0.2ug/ul pAMP, 7.5ul 2x buffer, 2ul BamHI/HindIII) and digested pKAN (5.5ul of 0.2ug/ul pKAN, 7.5ul 2x buffer, 2ul BamHI/HindIII). The digests were mixed and incubated in a water bath at 80C for 20 minutes in preparation for gel electrophoresis.

Gel Electrophoresis:

We used the "Cast 0.8% Agarose Gel" protocol from *DNA Science, a first Course, 2nd Ed., p448-450*. to cast a gel for the DNA digests but added 5ul SybrSafe to stain the gel. We did not use control pAMP/pKAN and used 1X TBE running buffer. Lambda/HindIII DNA and the two digests were loaded into the gel and ran for 30 minutes at 125V.

Ligations:

We used the protocol adapted from NEB for DH5α cells from *New England Biolabs*. Two tubes each received 100ul of competent NEB DH5α cells and 10ul of ligation mix. Each tube was iced for 30 minutes, heat shocked at 42C for 30 seconds, and iced again for 5 minutes.

Bacterial Transformation:

We used the protocol adapted from NEB for DH5α cells from *New England Biolabs*. We added 200ul of super optimal broth with catabolite repression media (aka SOC media)

to each tube and shook at 250rpm at 37C for 60 minutes.

Bacterial Culture (plating bacteria):
We used the protocol adapted from NEB for DH5α cells from *New England Biolabs*. We used a LB agar plate, a LB +AMP agar plate, a LB +KAN agar plate, and a LB +AMP +KAN agar plate and spread for growth with 250ul of sample per plate. Plates then incubated at 37C.

Bacterial Culture (picking colonies):
We chose 2 colonies far apart of of the growth plates and added them using aseptic technique (bunsen burner) to two tubes with 5ml LB broth each. Tubes incubated at 37C for 7 days.

pAMP/pKAN Recombinants Plasmid Miniprep:
We used the “Perform Plasmid Miniprep” protocol from *DNA Science, a first Course, 2nd Ed., p484-486*. to prepare the samples for gel electrophoresis. However, we used 1-2mL LB broth to reconstitute the bacterial pellet and we used all of the DNA. The E.coli cells were resuspended, added between 2 tubes. One tube received 1,000uL M1 and the other tube received 1,000uL M2 and then both spun down and supernatant removed. Each tube then received 100uL of ice cold GTE solution, mixed, added 200uL SDS/NaOH solution and mixed. Tubes were left on ice for 5 minutes then received 150uL ice cold KOAc solution and mixed. Tubes incubated on ice for 5 miutes again and spun down. 400uL supernatant from M1 was added to a new M1 tube and 400uL

supernatant from M2 was added to a new M2 tube. Each of the new tubes received 400uL isopropanol, mixed, and spun down. The supernatant then removed and each tube adds 200uL of 10% ethanol, spun down, supernatant removed, and pellets left to dry. Once dry, each received 15uL of TE and the pellets resuspended.

Restriction Digests:
We prepared five DNA digests from *DNA Science, a first Course, 2nd Ed., p489-490.* To prepare the samples for electrophoresis. We only prepared five of the seven samples as seen in the book. Tubes were labled as M1+, M1-, M2+, M2-, and AK+. M1+ contained 5uL M1 sample from the miniprep, 2uL buffer, 2uL BamHI/HindIII, and 1uL water. M1 - contained 5uL M1 sample from miniprep, 2uL buffer, and 3uL water. M2+ contained 5uL M2 sample from miniprep, 2uL buffer, 2uL BamHI/HindIII, and 1uL water. M2- contained 5uL M2 sample from miniprep, 2uL buffer, and 3uL water. AK+ contained 5uL pAMP/pKAN, 2uL buffer, 2uL BamHI/HindIII, and 1uL water. Each tube was mixed then incubated at 37C for 30 minutes.

Gel Electrophoresis for Identification:
We used the “Cast 0.8% Agarose Gel” protocol and “Load Gel and Separate by Electrophoresis” from *DNA Science, a first Course, 2nd Ed., p490-492*. to cast a 0.8% agar gel for the restriction digests but added 5ul SybrSafe to stain the gel and used 1X TBE running buffer. We also only had five samples prepared to load plus a DNA marker (M1+, M2+, M1-, M2-, AK+, and lamda/HindIII DNA marker) and used a 37C incubator

rather than a 37C water bath. Each sample (M1+, M1-, M2+, M2-, AK+) received 1uL of loading dye. Samples in gel electrophoresis ran for 35 minutes at 120V.

Results

Figure 1, below, shows the experimental gel electrophoresis performed showed the digests to be complete. Digested pAMP bands can be seen in figure 1 in lane 3 at 3,755bp and 784bp. Digested pKAN bands can be seen in figure 1 in lane 2 at 2,332bp and 1,861bp. Lane 1 has a Lambda/HindIII DNA marker that was used to decipher lanes 2 and 3.

Figure 1: Gel Electrophoresis of Complete DNA Digest

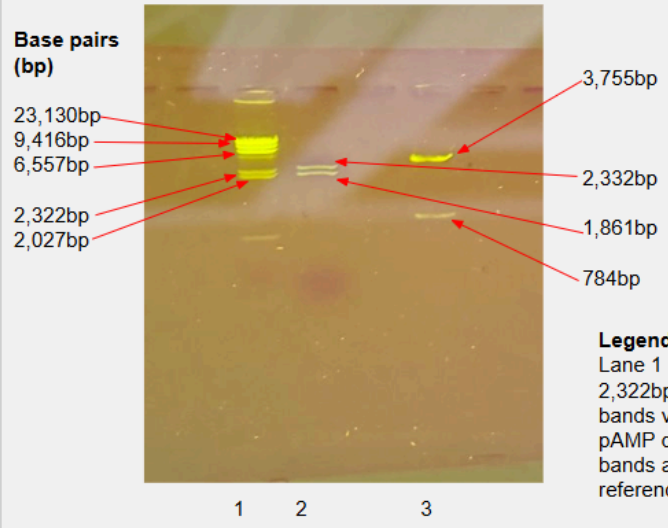

Figure 1: Lane 1 contains Lambda/HindIII DNA. Lane 2 contains pKAN digest. Lane 3 contains pAMP digest. The bands are estimated using the Lambda/HindIII DNA as reference.

Legend: Lane 1 contains 10uL Lambda/HindIII DNA marker. Lane 1 has viable bands at 23,130bp, 9,416bp, 6,557bp, 2,322bp, and 2,027bp. Lane 2 contains 10uL pKAN digest with bands visible at 2,332bp and 1,861bp. Lane 3 contains 10uL pAMP digest with bands visible at 3,755bp and 784bp. These bands are estimated using the Lambda/HindIII DNA marker as reference.

Figure 2, below, shows the growth observed on streak for growth plates in varied conditions.The streak for growth plates all resulted in growth. The LB plate had a lawn of growth that was irregular shape, creamy white color, with a wavy margin. Growth was

highest and more translucent on the inner portion and opaque edges with a rough surface. The LB+AMP plate growth was small and circular with a creamy white color. The growth was medium and transluecnt with a flat, glistening surface with a wavy margin. The LB+KAN plate was small and circular with a creamy white color. The growth was lowest and translucent with a flat, glistening surface with a wavy margin. The LB+AMP+KAN plate growth was small and circular with a creamy white color. The growth was medium and translucent with a flat, glistening surface with a wavy margin.

Figure 2: Growth of NEB 5-alpha Competent cells on agar

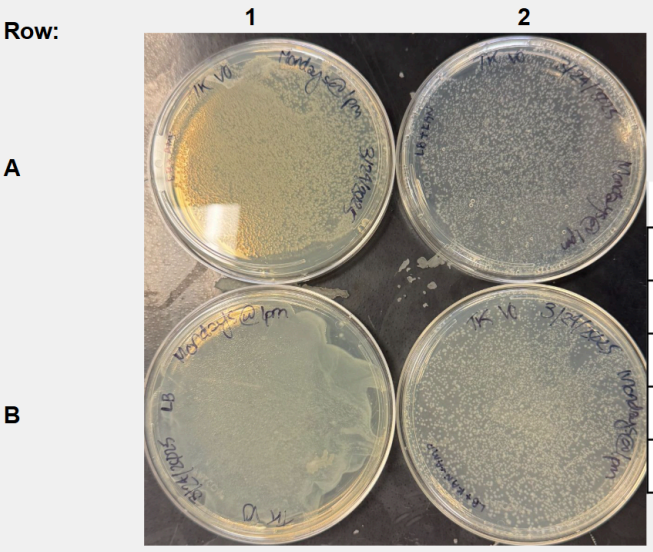

Figure 2: Row A1 depicts growth on the LB+AMP plate. Row A2 depicts growth on the LB+KAN plate. Row B1 depicts growth on the LB plate. Row B2 depicts growth on the LB+AMP+KAN plate. Table 2 reflects the growth rate seen on these plates in figure 2.

Table 2: Growth Rate of Figure 2

Plate:	Growth rate:
LB	Highest
LB+AMP	Medium
LB+KAN	Lowest
LB+AMP+KAN	Medium

Figure 3, below, shows the ideal gel electrophoresis with complete digests. Digested pAMP bands can be seen in figure 3 in lane 3 at 3755bp and 784bp. Digested pKAN bands can be seen in figure 3 in lane 2 at 2332bp and 1861bp. Lane 1 has a Lambda/HindIII DNA marker that was used to decipher lanes 2 and 3.

Figure 3: Ideal Gel Electrophoresis of Complete DNA Digest

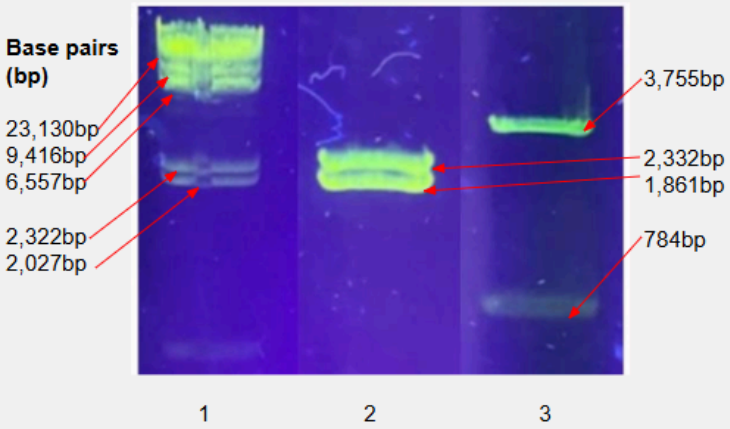

Figure 3: Lane 1 contains Lambda/HindIII DNA. Lane 2 contains pKAN digest. Lane 3 contains pAMP digest.

Legend: Lane 1 contains Lambda/HindIII DNA. Lane 2 contains pKAN digest with bands visible at 2332bp and 1861bp. Lane 3 contains pAMP digest with bands visible at 3755bp and 784bp. The bands are estimated using the Lambda/HindIII DNA as reference.

Figure 4, below, shows the experimental gel electrophoresis of isolated DNA. Lane 1 is the Lambda/HindIII DNA marker and has visible bands at 23,130bp, 9,416bp, 6,557bp, 2,322bp, and 2,027bp. Lane 2 contains M1- and has a visible band a 3,755bp, and nicked DNA above. Lane 3 contains M2- and has a visible band at 3,755bp with nicked DNA above. Lane 4 contains M1+ and has visible bands at 3,755bp, 2,322bp, 1,861bp, and RNA degradation. Lane 5 contains M2+ and has visible bands at 3,755bp, 2,322bp, 1,861bp, 784bp, and RNA degradation. Lane 6 contains AK+ and has visible bands at 3,755bp, 2,322bp, 1,861bp, and 784bp.

Figure 4: Gel Electrophoresis of Isolated DNA

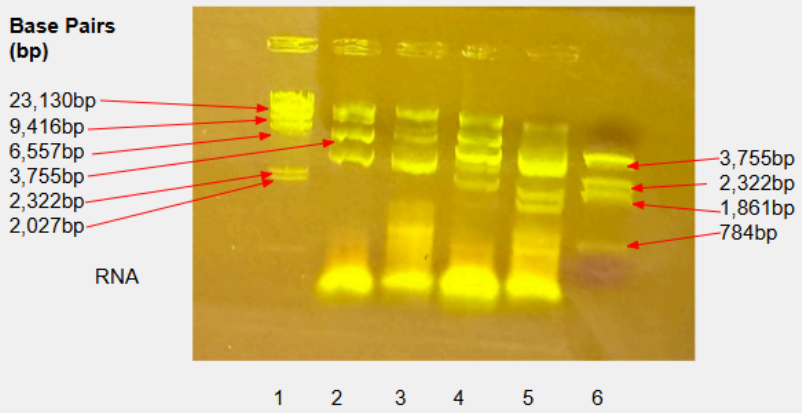

Figure 4: Lane 1 contains Lambda/HindIII DNA marker. Lane 2 contains M1-. Lane 3 contains M2-. Lane 4 contains M1+. Lane 5 contains M2+. Lane 6 contains AK+.

Legend: Lane 1 contains ~10uL Lambda/HindIII DNA marker. Lane 1 has visible bands at 23,130bp, 9,416bp, 6,557bp, 2,322bp, and 2,027bp. Lane 2 contains 10uL M1- and has a visible band a 3,755bp,and nicked DNA above. Lane 3 contains 10uL M2- and has a visible band at 3,755bp with nicked DNA above. Lane 4 contains 10uL M1+ and has visible bands at 3,755bp, 2,322bp, 1,861bp, and RNA degradation. Lane 5 contains 10uL M2+ and has visible bands at 3,755bp, 2,322bp, 1,861bp, 784bp, and RNA degradation. Lane 6 contains 10uL AK+ and has visible bands at 3,755bp, 2,322bp, 1,861bp, and 784bp.

Figure 5, below, shows the ideal gel electrophoresis of isolated DNA. Lane 1 contains Lambda/HindIII DNA marker and has visible bands at 23,130bp, 9,416bp, 6,557bp, 2,322bp, and 2,027bp. Lane 2 contains M1 - and has a visible band at 3,755bp with nicked DNA above. Lane 3 contains M2 - and has a visible band at 3,755bp with nicked DNA above. Lane 4 contains AK+ with visible bands at 3,755bp, 2,322bp, and 1,861bp. Lane 5 contains M1+ with visible bands at 3,755bp and 1,861bp. Lane 6 contains M2+ with visible bands at 3,755bp and 1,861bp.

Figure 5: Ideal Gel of Isolated DNA

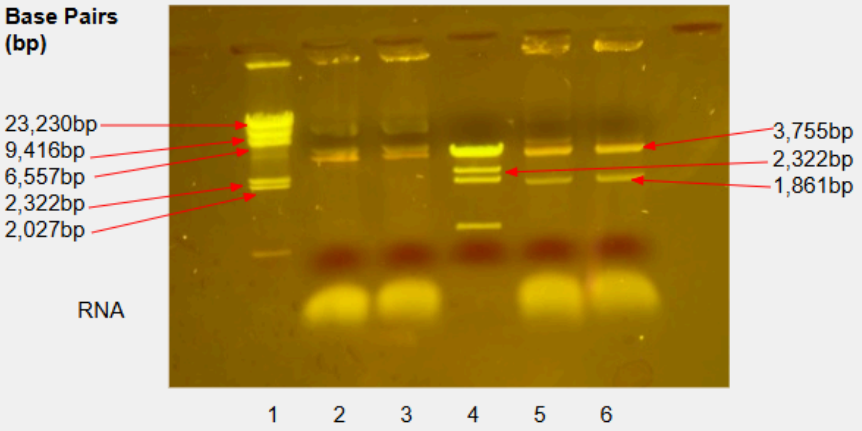

Figure 5: This figure represents an ideal gel. Lane 1 contains Lambda/HindIII DNA marker. Lane 2 contains M1 -. Lane 3 contains M2 -. Lane 4 contains AK+. Lane 5 contains M1+. Lane 6 contains M2+

Legend: Lane 1 contains Lambda/HindIII DNA marker and has visible bands at 23,130bp, 9,416bp, 6,557bp, 2,322bp, and 2,027bp. Lane 2 contains M1 - and has a visible band at 3,755bp with nicked DNA above. Lane 3 contains M2 - and has a visible band at 3,755bp with nicked DNA above. Lane 4 contains AK+ with visible bands at 3,755bp, 2,322bp, and 1,861bp. Lane 5 contains M1+ with visible bands at 3,755bp and 1,861bp. Lane 6 contains M2+ with visible bands at 3,755bp and 1,861bp.

Discussion

The goal of this study was to create a recombinant DNA that is pAMP/pKAN resistant and transform bacteria with the new DNA, creating pAMP/pKAN resistant bacteria. To verify the recombinant DNA, the plasmid DNA is purified, isolated, and analyzed. The gel electrophoresis performed on the digests resulted in a complete digest, giving the sign to perform ligation. The growth seen on the streak for growth bacterial culture was as expected. The growth on the LB plate had the highest growth, indicating that the bacteria was not killed off during the heat shock step. Growth on the LB+AMP plate was medium, as expected. This indicates that transformation was a success. The growth on the LB+KAN plate was the lowest, the growth was expected to be medium. However, the growth indicates that transformation was successful. The growth on the

LB+AMP+KAN was medium, the expected growth was lowest. The growth indicates that the ligation step was likely a success.

All steps to this point have yielded the results we had wanted, so we proceeded to purify and isolate the DNA. Figure 1, the experimental gel electrophoresis showing a complete DNA digest verifies that these digests are complete. Figure 1 shows bands that can be seen at 2332bp and 1861bp (pKAN) in lane 2 as well as 3755bp and 784bp (pAMP) in lane 3. This is what was expected to happen, as shown in Figure 3, the Ideal gel electrophoresis of complete DNA digest. Figure 2/Table 2 shows the growth of NEB 5-alpha Competent cells on agar plates in different conditions; the LB agar plate showed the highest growth, as expected. The LB+AMP plate showed medium growth, as expected. The LB+KAN plate showed the least growth, but still grew as expected. The LB+AMP+KAN plate showed medium growth, as expected.

Figure 4, the experimental gel electrophoresis of isolated DNA shows a band in lane 2 at 3,755bp with supercoiled and nicked DNA. In lane 3, a band can be seen at 3,755bp with supercoiled and nicked DNA. In lane 4, bands can be seen at 3,755bp, 2,322bp, 1,861bp, and has RNA degradation. Lane 5 has bands at 3,755bp, 2,322bp, 1,861bp, 784bp, and has RNA degradation. Lane 6 has bands at 3,755bp, 2,322bp, 1,861bp, and 784bp. The restriction digests completed on samples M1-, M1+, and M2- resulted in incomplete or partial digests. This was an unwanted result. If the digests had completed, proper base pairs may have resulted. The restriction digests completed on

samples AK+ and M2+ resulted in complete digests. Despite M2+ being a complete digest, lane 5 (containing M2+), contained the expected bands at 3,755bp and 1,861bp but additionally contained a band at 2,3222bp and RNA degradation. Additionally, AK+ was a complete digest but yielded 4 bands at 3,755bp, 2,322bp, 1,861bp, and 784bp. This indicates that the plasmid DNA was not successfully purified and isolated. Instead, it indicates that both original plasmids were isolated rather than the desired recombinant DNA nor was the recombinant DNA achieved. The study was a failure due to the lack of isolated recombinant DNA. If repeated, the second digest should go longer to ensure that digestion is complete before final electrophoresis.

References

- Micklos, D. A., Freyer, G. A., & Crotty, D. A. (2003). *DNA science : a first course*. Cold Spring Harbor Laboratory Press. Pages 447-448
- Micklos, D. A., Freyer, G. A., & Crotty, D. A. (2003). *DNA science : a first course*. Cold Spring Harbor Laboratory Press. Pages 448-450
- Micklos, D. A., Freyer, G. A., & Crotty, D. A. (2003). *DNA science : a first course*. Cold Spring Harbor Laboratory Press. Pages 484-486
- Micklos, D. A., Freyer, G. A., & Crotty, D. A. (2003). *DNA science : a first course*. Cold Spring Harbor Laboratory Press. Pages 489-490
- Micklos, D. A., Freyer, G. A., & Crotty, D. A. (2003). *DNA science : a first course*. Cold Spring Harbor Laboratory Press. Pages 490-492
- New England Biolabs. (2015). Neb.com. https://www.neb.com/en-us/products/c2987-neb-5-alpha-competent-e-coli-high-efficiency?srsltid=AfmBOoqZ-fMNCKatpdYGlXvJQSvoAn4iEut7SF_Nc-Q91kKAjQwh7pVi