Engaging Non-Science Students: Applications of Tools of Molecular Biology

Paula Lessem
Director of Biological Sciences
University of Richmond
Biology Department
Richmond, VA 23173
plessem@richmond.edu

Abstract: Mass media, as they are searching for the attention-catching titles and topics that attract viewers/readers, have a tendency to represent biology-related topics in a sensational manner. This often results in misleading presentations of biological concepts in large and small screen industry productions as well as in printed media and ever growing web sources. From the portrayal of eugenics in GATTACA to numerous uses of DNA technology in TV shows, the public is constantly assaulted with surreal and frightening scenarios. The inability to comprehend the relevance of this information is likely to multiply as the public struggles to keep up with a fast growing body of knowledge in biology – including its implications for advances in health care, the environment, and national security. In this module, the non-science student will be introduced to basic tools in molecular biology using the β-lactamase gene to illustrate PCR, restriction digestion, agarose gel electrophoresis, and induction of antibiotic resistance in bacteria. As a result of these experiments, the students will be more equipped evaluate biologic data as it becomes available.

Funds for this class were provided by the Associated Colleges of the South Reform of Introductory Science Courses for Non-Majors Min-Grant program. Travel funds were provided by the Arts & Sciences Deans office at the University of Richmond.

Contents

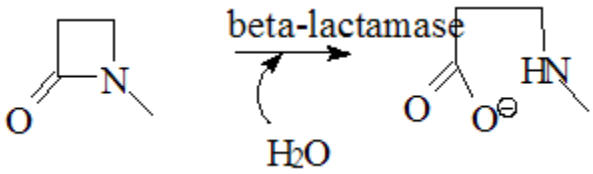
Overview of the workshop

Using the β-lactamase gene, students can be introduced to basic tools of molecular biology that are used in gene testing, paternity and forensic investigations, genetic counseling and used with genetically modified organisms. In addition, use of the β-lactamase gene affords further experimentation allowing students to explore antibiotic resistance in bacteria (and the possible induction of greater resistance). The experiments and applications can be adapted to different student populations – non-science students as well biology majors.

I. β-Lactamase

The generation of antibiotic resistance is a phenomenon exacerbated by overuse and misuse of antibiotics. However, therapeutic antibiotic use was not the underlying cause. The β-lactam antibiotics are manufactured by fungi growing in the soil. These organisms produce the antibiotics extracellularly effectively killing any susceptible microbe. In an effort to stay alive, microbes needed to evolve a mechanism to allow for survival. Though there are diverse β-lactam antibiotics, bacteria have been successful in developing resistance to this class of drugs. There are various mechanisms bacteria employ to resist β-lactam antibiotics but the focus of these experiments is bacterial production of β-lactamase. B-lactamase evolution and production provided the survival mechanism needed by the bacteria.

All β-lactamase enzymes share a common characteristic, a serine residue in the active site. This enzyme is effective by hydrolyzing the β-lactam bind rendering the antibiotic ineffective (Fig. 4)



www.hud.ac.uk/.../images/beta-lactamases01.gif

Fig. 1. β-lactamase hydrolyzes the β-lactam bond

Many diverse genera of bacteria are capable of producing β-lactamase enzymes. This is a very broad term that encompasses both chromosomally encoded enzymes as well as plasmid mediated enzymes. Using the same terminology as the β-lactam antibiotics, the enzymes are classified as narrow, broad and extended spectrum determined by the range of antibiotics they can hydrolyze. Interestingly, these enzymes have a serine residue in the active site similar to the transpeptidase enzymes that catalyze the cross linking reaction (and are the site for inactivation by β-lactam antibiotics).Due to their production many antibiotics cannot be prescribed

In this laboratory module, the β-lactamase gene

II. **Laboratory Protocols:**

A. The Polymerase Chain Reaction (PCR):

Amplification of DNA is the cornerstone for many biological protocols. This is a fundamental tool students need to understand to evaluate information (fictional or non-fiction). In this module, 2 different amplification samples will be evaluated by agarose gel electrophoresis: (1) the PCR mix before amplification (as a control), and (2) a sample removed after 10 cycles. When performing this in the laboratory setting, the suggested sample points are at 0, 10 and 30 cycles. The number of cycles is being adapted to fit into the workshop time period.

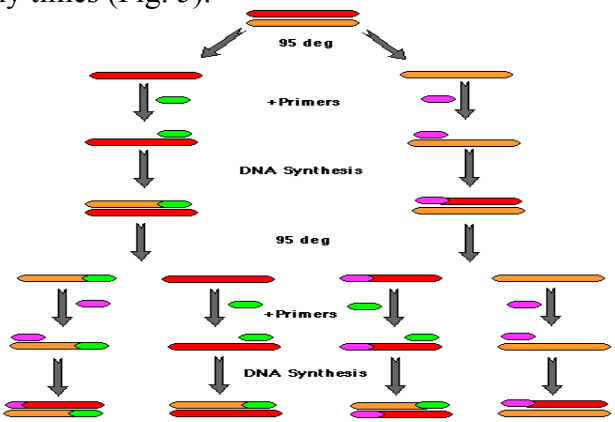
Relevance: DNA amplification is essential for many forensic investigations including RFLP's, DNA fingerprinting and STFR's. In order to understand the data achieved, students need a fundamental understanding of the underlying process.

II.A.1 PCR: Selection of primers

Students have an opportunity to understand the design of the samples to facilitate successful amplification of the selected DNA. The requirements needed for this procedure include the sample of DNA, the dinucleotides as "building blocks", appropriate buffer, the DNA polymerase and the target specific primers. While most of the reagents used are generic, the primers need to be designed specifically for the fragment of DNA.

The primers are essential for successful PCR as they identify the DNA region of interest for the DNA polymerase. Two primers are selected (both 5' to 3') – one for the forward strand and one for the reverse strand. Examination of the plasmid sequence identifies the coding sequence, in this case the β-lactamase gene. The primers chosen must flank this sequence while not being in the sequence.

It is also helpful if the primers are relatively close to the coding sequence desired. Using Primer 3 (http://frodo.wi.mit.edu/) and the entire plasmid sequence of pUR3, the students can experience the challenge (and sometimes difficulty) in obtaining the primers. As a result of successful PCR, the target site is amplified many times (Fig. 5).



http://members.aol.com/BearFlag45/Biology1A/LectureNotes/LNPics/Recomb/pcr.gif

Fig. 2 The Polymerase Chain Reaction

In this simulation the forward primer encroaches over a few base pairs on the forward strand. This simulation results in the students obtaining virtual data that indicates the size (in base pairs) of the successfully amplified product. In wet laboratories, this can be demonstrated by agarose gel electrophoresis.

To illustrate that PCR yields a greater quantity of a set piece of DNA and not a longer piece of DNA, various aliquots can be evaluated by agarose gel electrophoresis. An aliquot can be removed before any amplification, one after approximately 10 cycles and a sample at the conclusion of the process (Fig. 3).

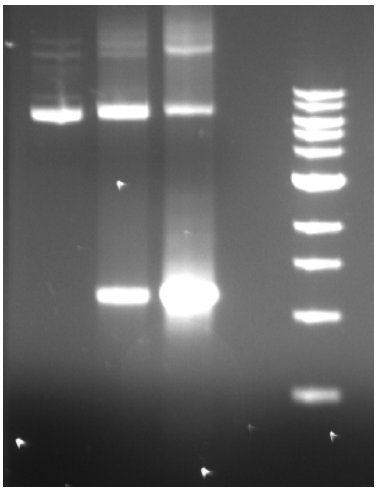


Fig. 3 Results of PCR. Lane 1 is the PCR mix prior to amplification, Lane 2 is after 10 cycles, Lane 3 is at the conclusion of the process and Lane 4 is the DNA marker.

II. B. Plasmid isolation:

Plasmids, extrachromosomal DNA, are used as vectors to transport DNA to a new cell resulting in the generation of a recombinant DNA molecule. They are also involved in transferring antibiotic resistance genes between bacteria. Plasmids are used in genetic engineering resulting in improvement in crops (nutritional value), drug production (insulin) and possible uses in gene therapy.

Relevance: Genetically modified foods and DNA recombinant technology is an integral part of everyday life (e.g. insulin production, corn and foods). Plasmids serve as vectors to transport a desired sequence of DNA into another biological entity.

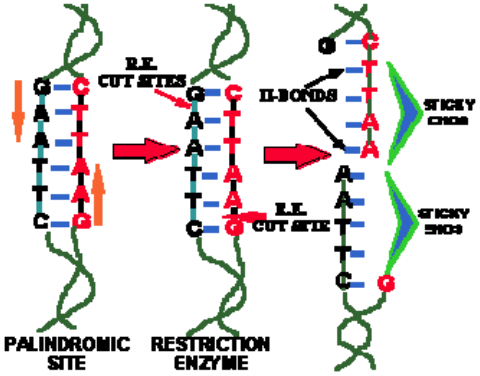
http://www1.qiagen.com/literature/handbooks/literature.aspx?id=1000248

Fig. 4. The Qiagen system is easy to implement and gives dependable results.

II.C. Restriction digestion: This procedure is used to "cut" out the desired fragment of DNA either after PCR or separation of the DNA from the plasmid. Restriction enzymes are used to complete this process. Students will have the opportunity to "cut" out the β-lacatmase gene from the isolated plasmid. There is a virtual program that will be utilized to assist students in restriction enzyme selection.

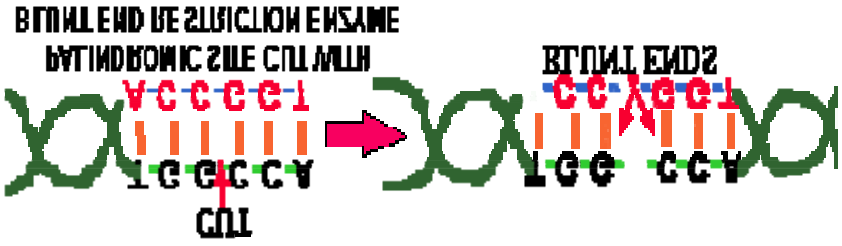
1. Relevance: "Molecular scissors" are instrumental in processing recombinant DNA molecules. These enzymes, which act as an immune system in bacteria, are able to excise out precise fragments of DNA.

Use of NEB cutter to digest the plasmid**.** Restriction digestion is another powerful tool used routinely in molecular biology. This technique successfully "cuts out" the desired piece of DNA. Most bacteria use restriction enzymes as a means of protection from invading DNA by viruses. This process is specific and the enzyme only recognizes a set sequence of DNA base pairs called a palindrome. When the restriction enzymes "cut" the DNA, they can generate sticky ends or blunt ends (Figs. 7 and 8).



http://www.slic2.wsu.edu:82/hurlbert/micro101/images/mol10.gif

Fig. 5. Restriction digestion results in sticky ends



www.slic2.wsu.edu:82/.../images/mol10.gif

Fig. 6. Restriction digestion results in blunt ends

There are hundreds of restriction enzymes available for use when digesting DNA. As with designing primers for PCR, the restriction enzyme should cut the DNA outside the desired sequence allowing isolation of the target fragment of DNA (in this case, the β-lactamase gene) from the rest of the nucleic acid sequence.

The selection of the correct restriction enzyme can be investigated using a web based site, NEB cutter (http://tools.neb.com/NEBcutter2/index.php) which shows a linear map of pUR3 with numerous restriction sites. Students, while referring to the plasmid map and sequence, will select different enzymes (or multiple enzymes) to successfully cut out the β-lactamase gene. Evaluation of the map (and sequence) allows estimation of the size of the gene (in base pairs). This can be confirmed by virtual gel electrophoresis as well as the actual restriction digestion. The virtual digestion (Fig.7 A) indicates a 1000 base pair fragment isolated from the rest of the plasmid and this is confirmed with the actual restriction digestion (Fig. 7 B).

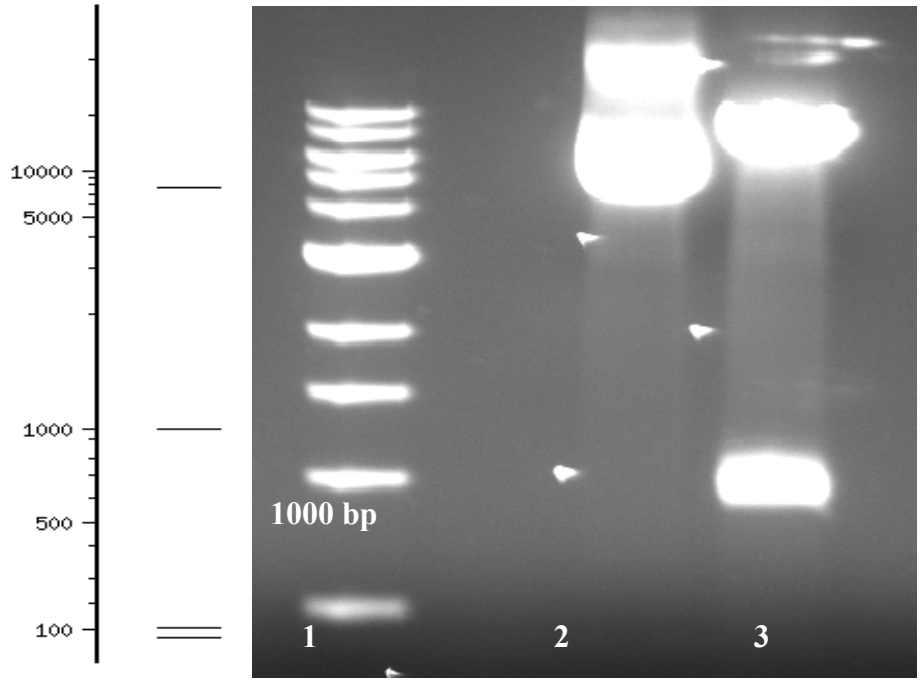


Fig. 7 A and B. Agarose gel electrophoresis: Restriction digestion of pUR3 with BspHI.. A. Virtual digestion, B. Actual restriction digestion (Lane 1 is the DNA marker) in Lanes 2 (uncut control) and Lane 3 (cut)

II.D. Agarose gel electrophoresis: In order to visualize the PCR products, and the isolated and digested plasmid, gel electrophoresis is used. This exercise provides the student with concrete data to analyze. Agarose gel electrophoresis and adaptations are essential in many molecular biological applications.

Relevance: Agarose gel electrophoresis (in various forms) is used to document the results of an experiment. Adaptations of this procedure are used in paternity testing, DNA fingerprinting, and verification (based on size of the DNA fragment) of the recombinant DNA.

Appendices

I. On-Line Sources

Cell wall of bacteria:

http://www.cehs.siu.edu/fix/medmicro/pix/walls.gif
http://www.bact.wisc.edu/Microtextbook/index.php?module=Book&func=displayarticle&art_id=89&theme=Printer

Peptidoglycan biosynthesis: http://student.ccbcmd.edu/courses/bio141/lecguide/unit1/prostruct/ppgsynanim.html
http://images.google.com/imgres?imgurl=
http://www.chemsoc.org/exemplarchem/entries/2002/stanley/04_Site_of_Action/Site_8.jpg&imgrefurl=http://www.chemsoc.org/exemplarchem/entries/2002/stanley/04_site_of_action/site_of_action.htm&h=248&w=474&sz=24&hl=en&start=51&um=1&tbnid=Trze3OTrpkzTxM:&tbnh=67&tbnw=129&prev=/images%3Fq%3Dpeptidoglycan%26start%3D40%26ndsp%3D20%26svnum%3D10%26um%3D1%26hl%3Den%26rls%3Dcom.microsoft:en-us%26sa%3DN

β-lactam antibiotics
http://www.elmhurst.edu/~chm/vchembook/652penicillin.html
www.scielo.br/.../v82n5s0/en_v82n5s0a08f01.gif

β-lactamase activity
www.hud.ac.uk/.../images/beta-lactamases01.gif

PCR:
http://members.aol.com/BearFlag45/Biology1A/LectureNotes/LNPics/Recomb/pcr.gif
http://www.dnalc.org/ddnalc/resources/pcr.html animation
http://learn.genetics.utah.edu/units/biotech/pcr/ animation

Restriction digestion:
http://www.slic2.wsu.edu:82/hurlbert/micro101/images/mol10.gif
http://www.wiley.com/legacy/college/boyer/0470003790/animations/agarose/agarose.htm animation
http://www.slic2.wsu.edu:82/hurlbert/micro101/images/LigaseAnimation6.gif animation
http://www.dnalc.org/ddnalc/resources/restriction.html animation

II. References

Peptidoglycan biosynthesis
http://www.textbookofbacteriology.net/structure.html

Bush, K. 1988. Recent Developments in β-Lactamase Research and Their Implications for the Future. Reviews of Infectious Diseases, Vol. 10, No.4: 681 – 690.

Medeiros, A. 1997 Evolution and Dissemination of β-Lactamases Accelerated by Generations of β-Lactam Antibiotics. Clinical Infectious Diseases, Vol. 24, S1: S19-S45.

Meroueh, S. O, Minasov, G, Lee, W., Shoichet, B., and S. Mobashery. 2003 Structural aspects for evolution of beta-lactamases from penicillin-binding proteins.. Journal of the American Chemical Society, Vol. 125 (32): 9612-9618.

Neu, H. 1992. The Crisis in Antibiotic Resistance. Science, New Series, Vol. 257, No. 5073: 1064 – 1073.

III. Materials

A. Luria Bertani (LB): Broth and / or agar plates
10 g Tryptone
5 g Yeast Extract
10 g Sodium chloride
14 g agar
1 L dH_2O
Autoclave

B. Ampicllin: final concentration 50µg/ml

C. pUR3: available on request and can be sent on an LB – Ampicillin plate

D. Plasmid isolation protocol: Qiagen kit 27104 plasmid min-prep kit for 50 plasmid prepations

E. Restriction digestion: BspHI, RO517S, New England Biolabs

F. Bovine Serum albumin: 200 µg/ml

G . Agarose: prepare 0.8% gels in TBE

H. TBE:

TBE buffer (electrophoresis) 1liter – 5X
54 g Tris base
27.5 g Boric acid
20 ml 0.5M EDTA

I. DNA marker: Quick-Load™ 1 kb DNA Ladder New England Biolabs

L. Loading dye: Blue-Orange 6X loading dye, G1881, Promega

K. Ethidium bromide: 10 mg/ ml for stock, add 1µl/10 ml agarose