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The Biotechnology Education Company ®

Revised and Updated

EDVO-Kit #
331

Cloning of a PCR Amplified Gene

Storage: See Page 3 for specific storage instructions

EXPERIMENT OBJECTIVE:

The objective of this experiment is to amplify a DNA fragment by Polymerase Chain Reaction (PCR) and to clone the amplified DNA by using the blue/white cloning system.

This experiment is designed for DNA staining with InstaStain® Ethidium Bromide.

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Components & Requirements

This experiment contains reagents to perform five PCR reactions and five cloning experiments.

Sample volumes are very small. For liquid samples, it is important to quick spin the tube contents in a microcentrifuge to obtain sufficient volume for pipeting. Spin samples for 10-20 seconds at maximum speed.

All components are intended for educational research only. They are not to be used for diagnostic or drug purposes, nor administered to or consumed by humans or animals.

THIS EXPERIMENT DOES NOT CONTAIN HUMAN DNA. None of the experiment components are derived from human sources.

	Reagents for PCR	**Storage**
P1	DNA Template for Amplification	-20°C
P2	Primer Set (two primers)	-20°C
P3	Tubes with PCR Reaction Pellets™ (Each PCR reaction pellet™ contains dNTP Mixture, *Taq* DNA Polymerase Buffer, *Taq* DNA Polymerase and $MgCl_2$)	Room temp.
P4	Enzyme Grade UltraPure Water	-20°C
P5	200 bp DNA ladder	-20°C
•	Wax Beads (for thermal cyclers without a heated lid)	Room temp.
•	10x Gel Loading Solution	Room temp.

	Reagents to Clean and Prepare DNA for Ligation	
C1	Purification Buffer	Room temp.
C2	Wash Buffer	Room temp.
C3	Restriction Enzyme Reaction Buffer	-20°C
C4	Enzyme Grade UltraPure Water	-20°C
C5	*Eco* RI	-20°C
C6	*Hind* III	-20°C
C7	Restriction Enzyme Dilution Buffer	-20°C
•	Spin Columns and Reservoirs	Room temp.

	Reagents for Ligation	
L1	pUC19 Plasmid Vector cut with *Eco* RI and *Hind* III	-20°C
L2	DNA Ligase	-20°C
L3	Ligation buffer	-20°C

	Reagents for Transformation	
T1	Ampicillin	-20°C
T2	IPTG	-20°C
T3	X-Gal in Solvent	-20°C
T4	$CaCl_2$	-20°C
1	Bottle of Recovery Broth	Room temp.
1	Vial of Bacterial LyphoCells™	Room temp.
1	Bottle of Ready Pour Agar	Room temp.

Other Components:

- UltraSpec-Agarose™
- Electrophoresis Buffer (50x)
- InstaStain® Ethidium Bromide
- Microcentrifuge Tubes
- PCR tubes (0.2 ml - for thermal cyclers with 0.2 ml template)
- Petri plates

Requirements

- Thermal cycler (EDVOTEK Cat. # 532 highly recommended) or three waterbaths
- Horizontal gel electrophoresis apparatus
- D.C. power supply
- Balance
- Microcentrifuge
- Water bath (37°C & 65°C)
- Incubation oven (37°C)
- UV Transilluminator or UV Photodocumentation system
- UV safety goggles
- Automatic micropipets (5-50 µl & 0.5-10 µl) with tips
- Microwave, hot plate or burner
- Pipet pump
- 250 ml flasks or beakers
- Hot gloves
- Disposable vinyl or latex laboratory gloves
- Ice buckets and ice
- Distilled or deionized water
- Isopropanol

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- Experiment number and title
- Kit lot number on box or tube
- Literature version number (in lower right corner)
- Approximate purchase date

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Polymerase Chain Reaction, Ligation and Cloning

The Polymerase Chain Reaction (PCR) has made an extraordinary impact on various aspects of biotechnology. The success of utilizing PCR is due to the specificity endowed in the hybridization of nucleic acid and simplicity of the reaction. PCR has also made amplification of genes an alternate approach to traditional cloning experiments. It is currently being used in genome projects, in DNA mapping and sequencing. PCR is also applied in DNA based forensics, paternity and determination of evolutionary relationships.

In a typical PCR reaction, the first step is the preparation of the DNA sample that is extracted from various biological sources. Segments of DNA or genes to be amplified are referred to as the targets and the two synthetic oligonucleotides required for the PCR reaction are referred to as primers. Because PCR is very sensitive, only a few copies of the gene is required. Freshly isolated DNA will give the best amplification results compared to DNA extracted from older specimens that may be degraded. The set of two primers, usually in the range between 15 and 30 nucleotides, are chemically synthesized to correspond to the two ends of the gene or DNA to be amplified. The primer concentrations are always in excess of the DNA target. The nucleotide primer sequences for the DNA amplification reaction are determined to yield the best hybridization.

ABOUT DNA POLYMERASES

DNA Polymerase I is the best studied DNA polymerase. With some exceptions, the mechanism of DNA polymerization is essentially identical for all polymerases. This DNA polymerase is a single polypeptide with a molecular weight of 109,000 (approximately 1000 amino acids). Like other DNA polymerases it requires a primed template, the four deoxynucleotide triphosphates and magnesium for DNA synthesis. Polymerization of the nucleotides occurs in the 5′ to 3′ direction by the addition of a 5′ phosphorylated nucleotide to the free 3′ hydroxyl group of the growing DNA chain with the concomitant production of pyrophosphate. The primer is antiparallel and is base paired to the template strand. In vivo, RNA fragments containing about 10 nucleotides serve to prime DNA synthesis. RNA primers are synthesized by the enzyme primase which is a specialized DNA dependent RNA polymerase.

DNA polymerase I also has a 3′-5′ exonucleolytic activity that cleaves DNA starting with a free terminal 3′ hydroxyl group on the growing chain to yields 5′-deoxynucleotide monophosphates. This activity serves as a proofreading function during DNA synthesis. It recognizes distortions in the 3′ ends of the growing DNA chain caused by mismatched bases between the template and the growing chain. The enzyme also possesses a 5′ to 3′ nuclease activity. This activity is part of a repair enzyme system that removes damaged DNA bases.

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Polymerase Chain Reaction, Ligation and Cloning

Background Information

Limited proteolysis of DNA polymerase I by subtilisin or trypsin produces two polypeptide fragments having molecular weights of 76,000 and 36,000. The larger polypeptide, known as the Klenow fragment contains the polymerization and 3' to 5' exonuclease activities while the smaller fragment contains the 5' to 3' nuclease activity. The Klenow fragment was used in the initial PCR experiments and was subsequently replaced by *Taq* DNA polymerase that is thermally stable. The source of *Taq* DNA polymerase is *Thermus aquaticus*. Severall other thermo-stable polymerases have also been purified from other thermophilic bacteria. Because of its thermal stability, *Taq* DNA polymerase will not be denatured during the high temperature PCR steps described below. In the initial experiments, the Klenow fragment had to be replenished after each DNA denaturation step (94°C). The thermostability of *Taq* DNA polymerase eliminates replenishment, thereby simplifying PCR.

ABOUT THE POLYMERASE CHAIN REACTION

A typical PCR reaction mixture contains DNA, the four deoxynucleotide triphosphates, Mg+2 *Taq* DNA polymerase and the reaction buffer. The total incubation reaction is usually small (10 to 25 µl) in volume. If water baths or a thermal cycler without a heated lid is used, the incubation reaction mixture is layered with mineral oil to minimize evaporation. With thermal cyclers that have heated lids layering with mineral oil is not required.

The PCR reaction mixture is then exposed to sets of three step temperature cycles. The first temperature 94°C melts the hydrogen bonds between the two DNA strands . The temperature is then reduced between 45°C to 60°C to hybridize the two primers to each of target DNA strands. The temperature is then increased to 72°C, the optimum for *Taq* DNA polymerase. During this step DNA is synthesized. The three temperature steps of a cycle are usually repeated 20 to 30 times (Figure1). This process is made efficient by placing the reaction tubes in thermal cyclers that are programmed to alternate and maintain temperatures. Amplified DNA products are detected by gel electrophoresis analysis.

It should be noted that PCR amplification can introduce a small number of mutations in the amplified DNA product. Mutations introduced can be controlled by the use of low nucleotide concentrations. Various undesired amplification products such as short DNA fragments are also synthesized. For best results in subsequent steps such as ligation in plasmids, the amplified DNA is purified from unused dNTPs, primers and *Taq* DNA polymerase prior to further use.

The correct PCR product will be in three forms. These products are due to an intrinsic property of the *Taq* DNA polymerase that appends an extra nucleotide usually a dA, at the 3' ends of blunt double-stranded DNA. This will yield three forms where the first form will be double-stranded with no dA appended at either 3' end. The second form will have a dA on one of the

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Polymerase Chain Reaction, Ligation and Cloning

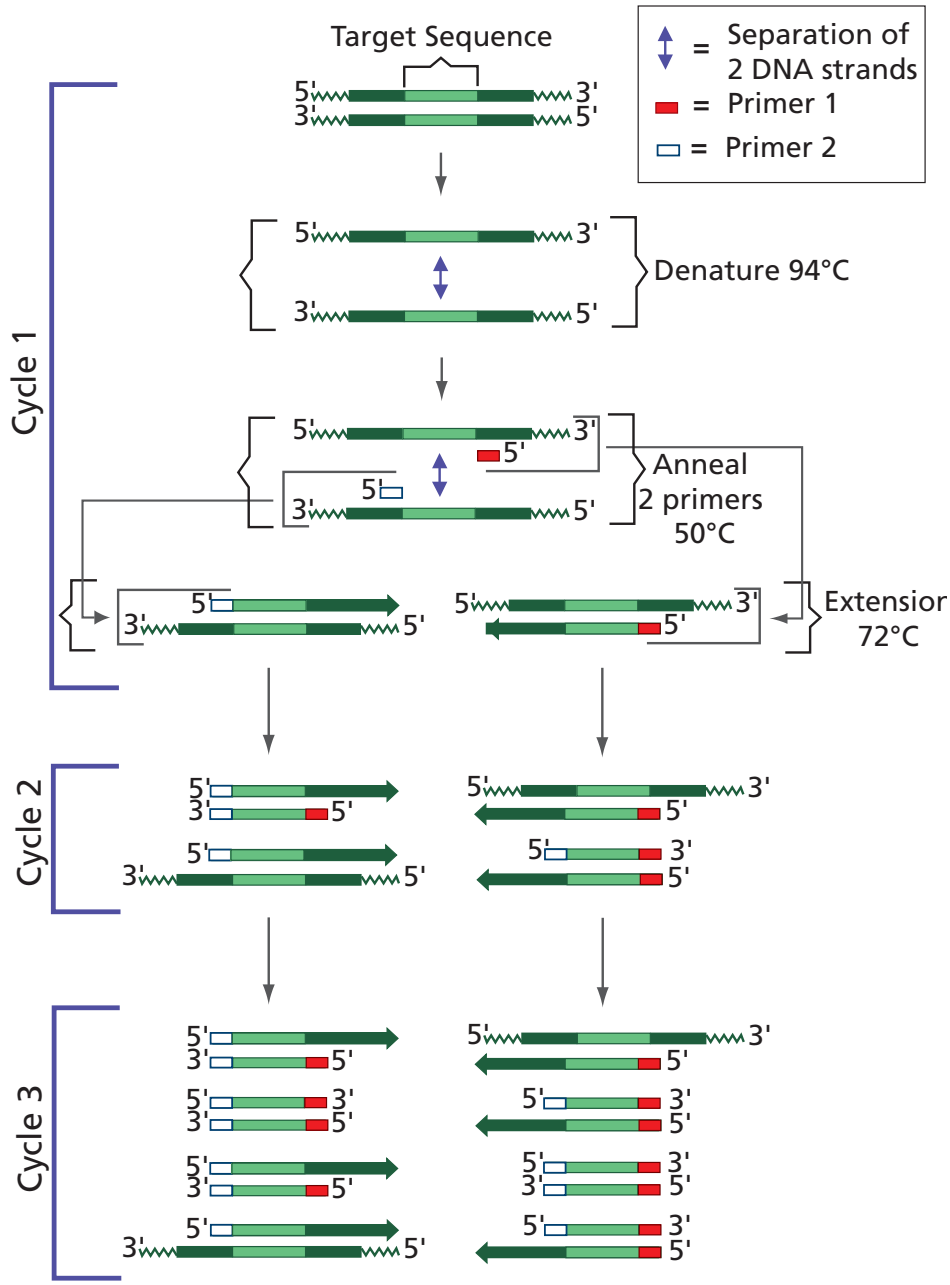

Figure I: The Polymerase Chain Reaction

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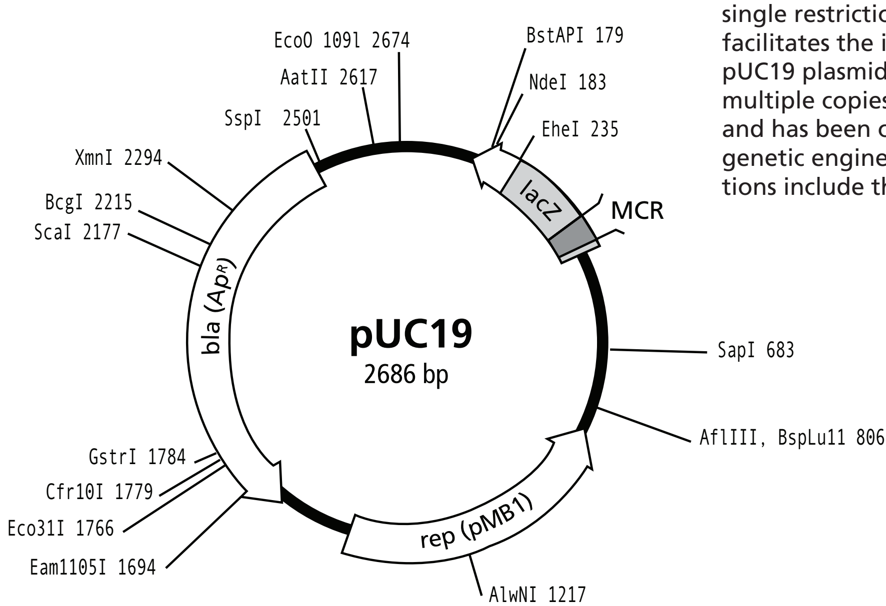
Polymerase Chain Reaction, Ligation and Cloning

two DNA strands and the third form will have a dA residues on both DNA strands. This single nucleotide addition is referred to as "template independent polymerization". The presence of this nucleotide will have a negative impact on a subsequent cloning step. When overhanging end cloning (sticky ends) is desired, the best strategy would be to use primers that flank restriction enzyme sites present at the two ends of the target DNA that can be amplified. The amplified product is then digested by a restriction enzyme.

ABOUT PLASMIDS

The plasmid pUC19 used for this experiment is derived from the pUC series. It has a single recognition site for *Eco* RI and *Hind* III (restriction enzymes), that are located in a polylinker. The polylinker region known as MCR (multiple cloning region) contains several single restriction enzyme sites that facilitates the insertion of DNA. The pUC19 plasmid (Figure 2) is present in multiple copies in a host *E. coli* cell, and has been cleverly modified by genetic engineering. These modifications include the addition of the lac Z gene that codes for beta-galactosidase, an enzyme involved in lactose metabolism. DNA inserted into the MCR interrupts the lac Z' gene and prevents the formation of a functional beta-galactosidase protein. As a result clones of interest will appear as white colonies instead of blue on selection agar plates that contain ampicillin.



MCR:

```
      SacI       SmaI        XbaI       SbfI
   EcoRI      KpnI      BamHI       SalI  PstI  SphI   HindIII
agtgAATTCGAGCTCGGTACCCGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGcgtaatcatggtcat
        •         •         •         •         •         •         •
       400       410       420       430       440       450       460
...S  N  S  S  P  V  R  P  D  E  L  T  S  R  C  A  H  L  S  P  T  I  M  T  M
                                         ←———— lacZα  translational start ——
```

Figure 2: Plasmid pUC19 and MCR Map

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Polymerase Chain Reaction, Ligation and Cloning

CONSTRUCTION OF A RECOMBINANT PLASMID

Ligation of the PCR amplified DNA to the linearized plasmid is catalyzed by T4 DNA ligase. The enzyme catalyzes the formation of phosphodiester bonds by the condensation of a 5′ phosphate and 3′ hydroxyl group of adjacent nucleotides. Each phosphodiester bond formation results in the hydrolysis of ATP to AMP and pyrophosphate. Ligation of DNA fragments having cohesive termini is usually achieved at temperatures between 4°C to 22°C. These temperatures will allow for annealing between complementary DNA ends that serves as a prerequisite for ligation.

When the plasmid and insert have the same cohesive termini, the orientation of the sub-cloned DNA (ligated in the plasmid) will vary due to the symmetrical nature of the termini. Statistically one would expect to find a 50:50 occurrence for the DNA orientation in bacterial colonies obtained from the same transformation reaction. Therefore the insert in the recombinant plasmid can be in either one of two directions relative to a fixed point in the vector (Figure 3A).

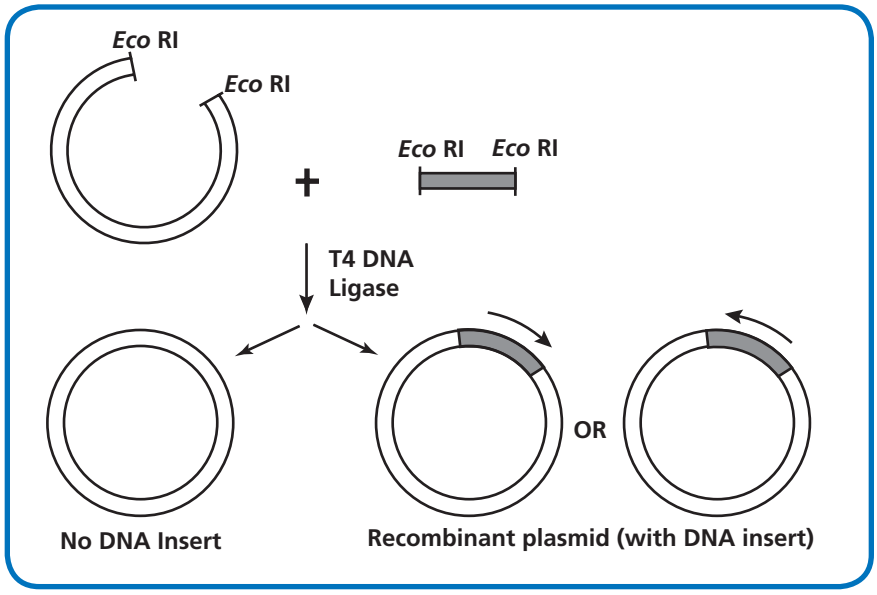

Figure 3A: Use of a single restriction enzyme (*Eco* RI) for the ligation of DNA in an *Eco* RI pre-digested plasmid. As noted by the two arrows the DNA insert is ligated bi-directionally.

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Polymerase Chain Reaction, Ligation and Cloning

Background Information

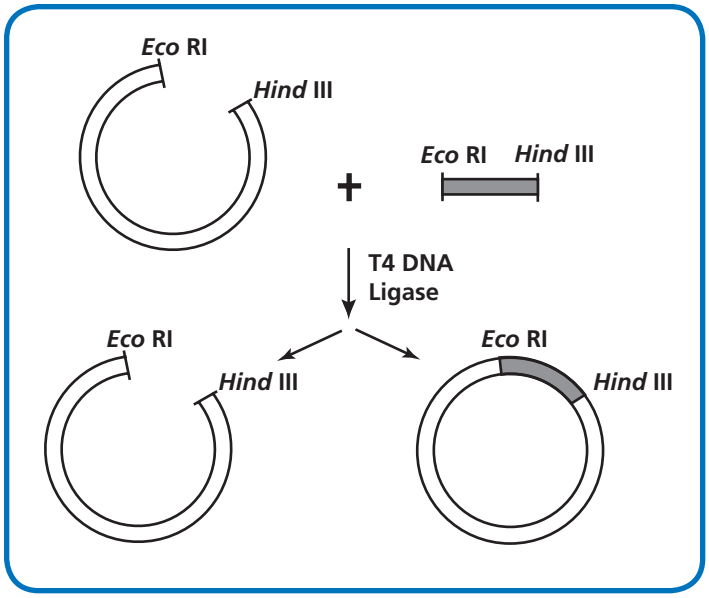

Figure 3B: Demonstrates *Eco* RI and *Hind* III co-digested plasmid and DNA fragment that upon ligation will yield the desired recombinant.

In the simplest reaction, ligation of a plasmid and PCR amplified DNA (cleaved by the same restriction enzyme) will form a circular recombinant plasmid (Figure 3A). The stoichiometry of this reaction is complex and is based on the length and relative concentrations of the two DNAs, the amount enzyme and the ionic strength of the reaction. In this reaction the plasmid (without the amplified DNA) will also circularize (Figure 3A).

To obtain a recombinant with a DNA insert in the desired direction the plasmid is co-digested within the multiple cloning region by two restriction enzymes that will produce cohesive ends that do not match. A similarly co-digested DNA fragment will be ligated in the desired orientation (Figure 3B). In this reaction the plasmid will not circularize because the ends are derived from the co-digestion by two different restriction enzymes. White colonies that may be present would be due to undigested plasmid.

ABOUT TRANSFORMATION:

For this experiment competent cells are prepared from cultures of E. coli, strain JM109. This strain does not have any natural antibiotic resistance or plasmids and lacks restriction enzymes. Transformation with the recombinant DNA allows for its expression, propagation, and purification. Linear plasmids and large concatamers do not transform competent cells, while supercoiled DNA has the highest transformation efficiencies. Only small amounts of DNA, typically less than 10 nanograms, are required for transformation. In fact, transformation is inhibited by DNA exceeding 100 nanograms. Even with this amount of DNA, only 1 in 10,000 cells successfully incorporate the recombinant DNA.

Transformation efficiency is based on the number of transformants obtained per microgram of DNA. As an example to determine transformation efficiency, 10 nanograms of DNA were used for a reaction and cells were allowed to recover in a final volume of 1 ml but only one tenth of this volume

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Polymerase Chain Reaction, Ligation and Cloning

was plated and produced 100 colonies on a selective agar medium. Therefore, 1000 transformants are present per ml. Keeping in mind that each colony grew from one transformed cell, the efficiency would be 1000/0.01ug = 1 x 10^5. Transformation efficiencies of 10^5 to 10^6 are sufficient for most classroom cloning experiments. When cloning of single copy genes from genomic DNA is done, required efficiencies are 10^7 to 10^8.

This experiment has four modules with the following objectives:

1. To amplify a DNA fragment using the PCR reaction.
2. To prepare PCR amplified DNA for ligation.
3. To ligate the PCR amplified DNA in pUC19
4. To transform *E. coli* host cells and determine the number of white colonies (recombinant plasmid).

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Experiment Overview and General Instructions

BEFORE YOU START THE EXPERIMENT

1. Read all instructions before starting the experiment.
2. If you will be conducting PCR using a thermal cycler without a heated lid, also read the Appendix entitled "Preparation and Handling PCR Samples with Wax ".
3. If you will be using three waterbaths to conduct PCR, read the two appendices entitled "Polymerase Chain Reaction Using Three Waterbaths" and "Handling samples with wax overlays".
4. Write a hypothesis that reflects the experiment and predict experimental outcomes.

EXPERIMENT OBJECTIVE:

The objective of this experiment is to amplify a DNA fragment by Polymerase Chain Reaction (PCR) and to clone the amplified DNA by using the blue/white cloning system.

BRIEF DESCRIPTION OF EXPERIMENT:

This experiment has four modules with the following objectives:

1. To amplify a DNA fragment using the PCR reaction.
2. To prepare PCR amplified DNA for ligation.
3. To ligate the PCR amplified DNA in pUC19.
4. To transform *E. coli* host cells and to quantitate the number of white colonies.

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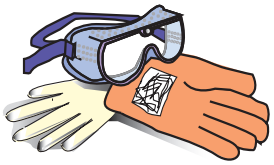
Laboratory Safety

1. Gloves and goggles should be worn routinely as good laboratory practice.

2. Exercise extreme caution when working with equipment that is used in conjunction with the heating and/or melting of reagents.

3. DO NOT MOUTH PIPET REAGENTS - USE PIPET PUMPS.

4. Exercise caution when using any electrical equipment in the laboratory.

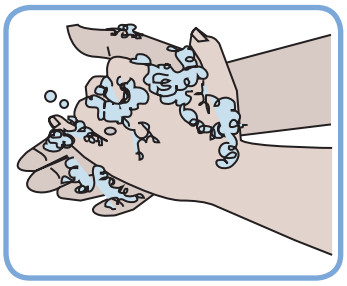


Wear gloves and safety goggles

   - Although electrical current from the power source is automatically disrupted when the cover is removed from the apparatus, first turn off the power, then unplug the power source before disconnecting the leads and removing the cover.
   - Turn off power and unplug the equipment when not in use.

5. EDVOTEK injection-molded electrophoresis units do not have glued junctions that can develop potential leaks. However, in the unlikely event that a leak develops in any electrophoresis apparatus you are using, IMMEDIATELY SHUT OFF POWER. Do not use the apparatus.

6. Always wash hands thoroughly with soap and water after handling reagents or biological materials in the laboratory.



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Module I: Amplification by PCR and Separation by Electrophoresis

The Experiment

The PCR reaction pellet™ contains Taq DNA polymerase, the four deoxytriphosphates, Mg^{+2} and buffer.

Sample volumes are very small. For liquid samples, it is important to quick spin the tube contents in a microcentrifuge to obtain sufficient volume for pipeting. Spin samples for 10-20 seconds at maximum speed.

AMPLIFICATION OF A 270 BP DNA FRAGMENT BY PCR

1. Program thermal cycler for 30 cycles with the following schedule:

Initial Denaturation	30 cycles @	Final Extension
94°C for 5 min.	94°C for 30 sec. 50°C for 30 sec. 72°C for 30 sec.	72°C for 5 min.

2. Obtain one of the tubes (P3) containing the PCR Reaction pellet ™ and label the side of the tube and top of the cap with your lab group number or initials.

3. Add the following to tube P3:

5 µl	DNA Template for Amplification (P1)
10 µl	Primer Set (P2)
15 µl	Enzyme Grade Ultrapure Water (P4).

4. Gently mix the reaction tube and pulse spin it in a microcentrifuge to collect the entire sample at the bottom of the tube.

5. If your thermal cycler has a 0.5 ml template, proceed to step 6.

   If your thermal cycller has a 0.2 ml template, transfer the entire contents of your PCR reaction tube into a clean 0.2 ml PCR tube before proceeding to step 6.

6. Check to see if your thermal cycler is equipped with a heated lid. If it is, proceed directly to placing the tube in the thermal cycler.

   If your thermal cycler **does not** have a heated lid, add one wax bead to the tube before placing the tube in the thermal cycler.

7. After the tubes are placed in the thermal cycler, process the samples for 30 cycles according to the schedule outlined in step 1.

8. To a clean tube add: 3 µl 10x Gel Loading Solution
   12 µl Distilled water.

9. After the final PCR cycle, remove 5 µl of the amplified DNA and transfer it to the tube containing the 10x gel loading solution and water (from step 8). This sample can be analyzed on a 1.0% agarose gel. The remaining amplified DNA (insert) is ready to be cleaned and purified for ligation in Module II.

OPTIONAL STOPPING POINT

Freeze at -20°C the amplified DNA and sample to be analyzed on a 1% gel, or continue with electrophoresis and/or Preparation of Insert for Ligation (Module II).

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Module I: Amplification by PCR and Separation by Electrophoresis

If you are unfamiliar with agarose gel preparation and electrophoresis, detailed instructions and helpful resources are available at **www.edvotek.com**

AGAROSE GEL REQUIREMENTS

- Recommended gel size: 7 x 7 cm
- Placement of well-former template: first set of notches
- Agarose gel concentration: 1.0%

PREPARING THE AGAROSE GEL

1. Close off the open ends of a clean and dry gel bed (casting tray) by using rubber dams or tape.
2. Place a well-former template (comb) in the first set of notches at the end of the bed. Make sure the comb sits firmly and evenly across the bed.
3. To a 250 ml flask or beaker, add agarose powder and buffer as indicated in the Reference Tables (Appendix A) provided by your instructor. Swirl the mixture to disperse clumps of agarose powder.
4. With a marking pen, indicate the level of the solution volume on the outside of the flask.
5. Heat the mixture using a microwave oven or burner to dissolve the agarose powder.
6. Cool the agarose solution to 60°C with careful swirling to promote even dissipation of heat. If detectable evaporation has occurred, add distilled water to bring the solution up to the original volume marked in step 4.

Important Note

Continue heating until the final solution appears clear (like water) without any undissolved particles. Check the solution carefully. If you see "crystal" particles, the agarose is not completely dissolved.

After the gel is cooled to 60°C:

7. Place the bed on a level surface and pour the cooled agarose solution into the bed.
8. Allow the gel to completely solidify. It will become firm and cool to the touch after approximately 20 minutes.
9. After the gel is solidified, be careful not to damage or tear the wells while removing the rubber dams or tape and comb(s) from the gel bed.
10. Place the gel (on its bed) into the electrophoresis chamber, properly oriented, centered and level on the platform.
11. Fill the electrophoresis apparatus chamber with the appropriate amount of diluted (1x) electrophoresis buffer (refer to Table B on the instruction Appendix provided by your instructor).

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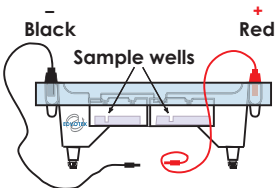
Module I: Amplification by PCR and Separation by Electrophoresis

The Experiment

Reminder:

Before loading the samples, make sure the gel is properly oriented in the apparatus chamber.



BEFORE LOADING THE SAMPLES

This experiment requires a 1.0% agarose gel and is designed for staining with InstaStain® Ethidium Bromide. Make sure the gel is completely submerged under buffer before loading the samples and conducting electrophoresis.

LOADING DNA SAMPLES

1. Load 20 µl of the PCR sample

Lane	
1	200 bp DNA Ladder (P5)
2	Reaction sample after 30 cycles

2. Record the position of your sample in the gel for easy identification after staining.

RUNNING THE GEL

3. After the DNA samples are loaded, properly orient the cover and carefully snap it onto the electrode terminals.

4. Insert the plugs of the black and red wires into the corresponding inputs of the power source.

5. Set the power source at the required voltage and conduct electrophoresis for the length of time determined by your instructor.

6. Check to see that current is flowing properly - you should see bubbles forming on the two platinum electrodes.

7. After the electrophoresis is completed, disconnect the power and remove the gel from the bed for staining.

STAINING AND VISUALIZATION OF DNA

After electrophoresis, agarose gels require staining to visualize the separated DNA samples. Your instructor will provide instructions for DNA staining with InstaStain® Ethidium Bromide.

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Module II: Preparation of Insert for Ligation

The amplified DNA (insert) must be cleaned (the *Taq* DNA polymerase must be removed), then cut with *Eco* RI and *Hind* III before proceeding to the ligation step.

1. Transfer the amplified DNA (~25 µl) to a clean 1.5 ml microcentrifuge tube and label it with your group number or initials.
2. Add 300 µl of the Purification Buffer (C1) to the tube containing the amplified DNA insert. Mix by inversion several times.
3. Place a spin column into a reservoir tube and transfer all of the mixture from step 2 to the column. Close the cap on the column.
4. Balance the tubes in a microcentrifuge and spin at maximum speed for 1 minute. At this point, the DNA product is bound to the membrane in the column.
5. Empty the reservoir tube and replace the spin column.
6. Wash the column.
   - Add 750 µl wash buffer to the spin column.
   - Close the cap on the column.
   - Spin balanced tubes in a microcentrifuge at maximum speed for 1 minute.
7. Empty the contents of the reservoir tube and replace the spin column to the empty reservoir tube. Close the cap on the column and spin balanced tubes in a microcentrifuge at maximum speed for 1 minute.

IMPORTANT!

Make sure that the Enzyme Grade Ultrapure Water (C4) is added directly onto the white membrane of the column (avoid touching the membrane with the pipet tip) for complete elution of the DNA.

8. Recover the DNA insert from the column:
   - Place the column into a clean 1.5 ml snap-top tube.
   - Add 12 µl of Enzyme Grade Ultrapure Water to the center of the column (directly onto the white membrane).
   - Close the cap on the column and let the tube stand for 1 minute.
9. Balance and carefully position the tubes in the microcentrifuge so that the open caps of the snap-top tubes do not interfere with operation of the centrifuge.
10. Spin the tubes for 1 minute at maximum speed. The DNA insert will be recovered in a volume slightly less than 12 µl.
11. Label the tube "PCR insert" and discard the used spin column.

OPTIONAL STOPPING POINT

Freeze the samples at -20°C or continue with restriction enzyme digestion

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Module II: Preparation of Insert for Ligation

The Experiment

RESTRICTION ENZYME DIGESTION

1. To the tube labeled "PCR insert" containing 10-12 µl the purified PCR mixture, add the following:

   - 5 µl Restriction Enzyme Reaction Buffer
   - 10 µl Enzyme Grade Ultrapure Water
   - 10 µl *Eco* RI enzyme
   - 10 µl *Hind* III enzyme

2. Mix the solution by gently pipeting up and down several times.

3. Incubate at 37°C for 60 minutes. Near the end of the incubation, prepare a separate 65°C waterbath.

After the 60 minute restriction enzyme digestion, the DNA digestion requires an incubation at 65°C to heat-inactivate the enzymes prior to being ligated into the vector.

4. Heat-inactivate the restriction enzymes by incubating the completely digested DNA at 65°C for 20 minutes.

5. Label the tube "Cut PCR Insert". After this step, the DNA insert is now ready to be ligated into the vector.

OPTIONAL STOPPING POINT

Freeze the samples at -20°C or continue with the ligation.

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Module III: Ligation of the PCR Amplified 270 bp DNA into pUC19

1. Equilibrate an ice water bath at 16°C for Step 4.

2. In a clean microcentrifuge tube, carefully mix together:

   - 9 µl "Cut PCR Insert" DNA
   - 3 µl pUC19 Plasmid Vector cut with *Eco* RI and *Hind* III
   - 3 µl Ligation buffer
   - 12 µl Enzyme Grade Ultrapure Water
   - 3 µl DNA Ligase

   30 µl Total

   Label this tube "PCR Insert+Vector/Lig".

3. In another clean microcentrifuge tube, carefully mix together:

   - 3 µl pUC19 Plasmid Vector cut with *Eco* RI and *Hind* III
   - 3 µl Ligation buffer
   - 21 µl Enzyme Grade Ultrapure Water
   - 3 µl DNA Ligase

   30 µl Total

   Label this tube "Vector Control/Lig".

4. Incubate the reaction tubes in a 16°C ice-water bath for 30 minutes.

   Alternatively, the reaction tubes can be incubated for 2 hours at room temperature.

OPTIONAL STOPPING POINT

Freeze the samples at -20°C or continue with the transformation.

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Module IV: Transformation

The Experiment

1. Label one microcentrifuge tube "PCR+Vector/Transf". (This will be the transformation tube with the PCR amplified Gene.)
2. Label a second microcentrifuge tube "Vector Control/Transf". (This will be the transformation tube with the vector control.)
3. Using a sterile pipet, add 500 µl (0.5 ml) of ice cold $CaCl_2$ solution to one of the tubes from step 1 or 2.
4. Pick colonies from the source plate of *E. coli* cells.
   - Use a sterile toothpick to transfer 5 colonies (2-4 mm) from the source plate to the tube containing the $CaCl_2$.
   - Between your fingers, twist the tooth pick vigorously in the $CaCl_2$ solution to dislodge the cells.
5. Suspend the cells in the tube by tapping or vortexing (preferred).

   At this point, the $CaCl_2$ cell suspension should look cloudy and slightly turbid. If it does not, add a few additional colonies and/or make sure the cells are completely suspended by mixing and vortexing.

Note:
Sufficient colonies that have been completely suspended in $CaCl_2$ are critical to the success of the transformation portion of the experiment.

6. After the cells are completely resuspended, transfer half (0.25 ml) of the cell suspension to the other labeled tube.
7. Add 10 µl "PCR+Vector/Lig" DNA to the tube labeled "PCR+Vector/Transf" and vortex.
8. Add 10 µl "Vector Control/Lig" DNA to the tube labeled "Vector Control/Transf".
9. Incubate the two tubes on ice for 20 minutes.
10. Briefly vortex the tubes, then float both tubes at 42°C for 90 seconds for the heat shock step. This facilitates the entry of DNA in bacterial cells.
11. Return both tubes immediately to ice and incubate for 2 minutes.
12. With a sterile pipet, add 250 µl of Recovery Broth to each tube & vortex.
13. Incubate cells for 30 minutes in a 37°C waterbath for a recovery period.
14. While the tubes are incubating, label 2 agar plates as follows:
    - "PCR insert + Vector"
    - "Vector Control"
    - Put your initials or group number on both plates.
15. After the recovery period, remove the tubes from the waterbath and place them on the lab bench.

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EVT 003157K

Module IV: Transformation

PLATING CELLS

16. Balance and centrifuge both tubes at maximum speed for 1 – 2 minutes to pellet the cells.

Place the prepared plates inverted in a 37°C incubation oven to warm them before they are needed .

17. After the centrifuge has stopped, use a different pipet to carefully remove 0.4 ml of supernate from each tube. Vortex the tubes to completely resuspend the cells in the remaining liquid (0.1 ml).

18. Use a sterile pipet to transfer all of the suspended cells from the tube labeled "PCR Insert + Vector/Transf" to the middle of the plate "PCR Insert + Vector".

19. Use a sterile pipet to transfer the suspended cells from the tube labeled "Vector Control/Transf" to the middle of the plate "Vector Control".

20. Spread the cells with a sterile inoculating loop.

21. Cover both plates and allow the liquid to be absorbed.

PREPARING PLATES FOR INCUBATION

22. Stack your group's set of plates on top of one another and tape them together. Put your initials or group number on the taped set of plates.

23. Leave the plates in an upright position to allow the cell suspension to be absorbed by the agar. Place the set of plates in a safe place designated by your instructor.

24. After the cell suspension is absorbed by the agar for approximately 15-30 minutes, you or your instructor will place the plates in the inverted position (agar side on top) into a 37°C bacterial incubation oven for overnight incubation (15-20 hours).

    Note: The plates are inverted to prevent condensation on the lid, which could drip onto the culture and interfere with experimental results.

AFTER OVERNIGHT INCUBATION

25. Observe the plates and estimate the number of transformants (both white and blue colonies) on each plate. Keep track of the counted colonies by putting a dot over them on the outside of the plate with a lab marker.

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Module IV: Transformation

26. Calculate the transformation efficiencies for total transformants and for colonies that contain vectors with inserts (white colonies). The final volume of cells was 50 µl and the volume plated was 50 µl.

Number of transformants µg of DNA	x	Final volume of cells (ml) volume plated (ml)	=	Number of transformants per µg

The quantity of DNA plasmid used for the vector control was approximately 0.012 µg. Estimate the PCR+Vector DNA to be approximately 0.05 µg.

Study Questions

Answer the following study questions in your laboratory notebook or on a separate worksheet.

1. Why does this cloning experiment yield both blue and white colonies?
2. Do all the white and blue colonies contain a plasmid?
3. Why is there a purification step prior to the restriction enzyme digestion step?
4. Why are there two different sticky ends on the PCR amplified DNA?
5. What is a diagnostic step to analyze the success of the various steps in this experiment?

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EDVO-Kit # 331

Material Safety Data Sheets

Full size (8.5 x 11") pdf copy of MSDS available at www.edvotek.com or by request.

Material Safety Data Sheet

EDVOTEK®

May be used to comply with OSHA's Hazard Communication Standard. 29 CFR 1910.1200 Standard must be consulted for specific requirements.

IDENTITY (As Used on Label and List): Agarose

Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.

Section I

Manufacturer's Name: EDVOTEK, Inc.

Address (Number, Street, City, State, Zip Code): 14676 Rothgeb Drive, Rockville, MD 20850

Emergency Telephone Number: (301) 251-5990

Telephone Number for information: (301) 251-5990

Date Prepared: 10/05/06

Signature of Preparer (optional):

Section II - Hazardous Ingredients/Identify Information

Hazardous Components [Specific Chemical Identity; Common Name(s)]	OSHA PEL	ACGIH TLV	Other Limits Recommended	% (Optional)
This product contains no hazardous materials as defined by the OSHA Hazard Communication Standard.				
CAS #9012-36-6				

Section III - Physical/Chemical Characteristics

Boiling Point: For 1% solution 194 F		Specific Gravity (H 0 = 1): 2	No data
Vapor Pressure (mm Hg.)	No data	Melting Point	No data
Vapor Density (AIR = 1)	No data	Evaporation Rate (Butyl Acetate = 1)	No data

Solubility in Water: Insoluble - cold

Appearance and Odor: White powder, no odor

Section IV - Physical/Chemical Characteristics

N.D. = No data

Flash Point (Method Used)	Flammable Limits	LEL	UEL
No data		N.D	N.D.

Extinguishing Media: Water spray, dry chemical, carbon dioxide, halon or standard foam

Special Fire Fighting Procedures: Possible fire hazard when exposed to heat or flame

Unusual Fire and Explosion Hazards: None

Section V - Reactivity Data

Stability			Conditions to Avoid
	Unstable		None
	Stable	X	

Incompatibility: No data available

Hazardous Decomposition or Byproducts:

Hazardous Polymerization			Conditions to Avoid
	May Occur		None
	Will Not Occur	X	

Section VI - Health Hazard Data

Route(s) of Entry: Inhalation? Yes Skin? Yes Ingestion? Yes

Health Hazards (Acute and Chronic): Inhalation: No data available Ingestion: Large amounts may cause diarrhea

Carcinogenicity: NTP? IARC Monographs? OSHA Regulation?

Signs and Symptoms of Exposure: No data available

Medical Conditions Generally Aggravated by Exposure: No data available

Emergency First Aid Procedures: Treat symptomatically and supportively

Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released for Spilled: Sweep up and place in suitable container for disposal

Waste Disposal Method: Normal solid waste disposal

Precautions to be Taken in Handling and Storing: None

Other Precautions: None

Section VIII - Control Measures

Respiratory Protection (Specify Type): Chemical cartridge respirator with full facepiece.

Ventilation			Special
	Local Exhaust		
	Mechanical	Gen. dilution ventilation	Other

Protective Gloves		Eye Protection	
Yes		Splash proof goggles	

Other Protective Clothing or Equipment: Impervious clothing to prevent skin contact

Work/Hygienic Practices: None

Material Safety Data Sheet

EDVOTEK®

May be used to comply with OSHA's Hazard Communication Standard. 29 CFR 1910.1200 Standard must be consulted for specific requirements.

IDENTITY (As Used on Label and List): 50x Electrophoresis Buffer

Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.

Section I

Manufacturer's Name: EDVOTEK, Inc.

Address (Number, Street, City, State, Zip Code): 14676 Rothgeb Drive, Rockville, MD 20850

Emergency Telephone Number: (301) 251-5990

Telephone Number for information: (301) 251-5990

Date Prepared: 10/05/06

Signature of Preparer (optional):

Section II - Hazardous Ingredients/Identify Information

Hazardous Components [Specific Chemical Identity; Common Name(s)]	OSHA PEL	ACGIH TLV	Other Limits Recommended	% (Optional)
This product contains no hazardous materials as defined by the OSHA Hazard Communication Standard.				

Section III - Physical/Chemical Characteristics

Boiling Point	No data	Specific Gravity (H_2O = 1)	No data
Vapor Pressure (mm Hg.)	No data	Melting Point	No data
Vapor Density (AIR = 1)	No data	Evaporation Rate (Butyl Acetate = 1)	No data

Solubility in Water: Appreciable, (greater than 10%)

Appearance and Odor: Clear, liquid, slight vinegar odor

Section IV - Physical/Chemical Characteristics

N.D. = No data

Flash Point (Method Used)	Flammable Limits	LEL	UEL
No data		N.D.	N.D.

Extinguishing Media: Use extinguishing media appropriate for surrounding fire.

Special Fire Fighting Procedures: Wear protective equipment and SCBA with full facepiece operated in positive pressure mode.

Unusual Fire and Explosion Hazards: None identified

Section V - Reactivity Data

Stability			Conditions to Avoid
	Unstable		None
	Stable	X	

Incompatibility: Strong oxidizing agents

Hazardous Decomposition or Byproducts: Carbon monoxide, Carbon dioxide

Hazardous Polymerization			Conditions to Avoid
	May Occur		None
	Will Not Occur	X	

Section VI - Health Hazard Data

Route(s) of Entry: Inhalation? Yes Skin? Yes Ingestion? Yes

Health Hazards (Acute and Chronic): None

Carcinogenicity: None identified NTP? IARC Monographs? OSHA Regulation?

Signs and Symptoms of Exposure: Irritation to upper respiratory tract, skin, eyes

Medical Conditions Generally Aggravated by Exposure: None

Emergency First Aid Procedures: Ingestion: If conscious, give large amounts of water

Eyes: Flush with water Inhalation: Move to fresh air Skin: Wash with soap and water

Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released for Spilled: Wear suitable protective clothing. Mop up spill and rinse with water, or collect in absorptive material and dispose of the absorptive material.

Waste Disposal Method: Dispose in accordance with all applicable federal, state, and local enviromental regulations.

Precautions to be Taken in Handling and Storing: Avoid eye and skin contact.

Other Precautions: None

Section VIII - Control Measures

Respiratory Protection (Specify Type):

Ventilation			Special	
	Local Exhaust	Yes	Special	None
	Mechanical (General)	Yes	Other	None

Protective Gloves		Eye Protection	
Yes		Safety goggles	

Other Protective Clothing or Equipment: None

Work/Hygienic Practices: None

Material Safety Data Sheet

EDVOTEK®

May be used to comply with OSHA's Hazard Communication Standard. 29 CFR 1910.1200 Standard must be consulted for specific requirements.

IDENTITY (As Used on Label and List): Gel loading solution concentrate, 10x

Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.

Section I

Manufacturer's Name: EDVOTEK, Inc.

Address (Number, Street, City, State, Zip Code): 14676 Rothgeb Drive, Rockville, MD 20850

Emergency Telephone Number: (301) 251-5990

Telephone Number for information: (301) 251-5990

Date Prepared: 10/10/06

Signature of Preparer (optional):

Section II - Hazardous Ingredients/Identify Information

Hazardous Components [Specific Chemical Identity; Common Name(s)]	OSHA PEL	ACGIH TLV	Other Limits Recommended	% (Optional)
This product contains no hazardous materials as defined by the OSHA Hazard Communication Standard.				

Section III - Physical/Chemical Characteristics

Boiling Point	No data	Specific Gravity (H_2O = 1)	No data
Vapor Pressure (mm Hg.)	No data	Melting Point	N/A
Vapor Density (AIR = 1)	No data	Evaporation Rate (Butyl Acetate = 1)	No data

Solubility in Water: soluble

Appearance and Odor: Blue liquid, no odor

Section IV - Physical/Chemical Characteristics

Flash Point (Method Used)	Flammable Limits	LEL	UEL
No data	No data	No data	

Extinguishing Media: Dry chemical, carbon dioxide, water spray or foam

Special Fire Fighting Procedures: Use agents suitable for type of surrounding fire. Keep upwind, avoid breathing hazardous sulfur oxides and bromides. Wear SCBA.

Unusual Fire and Explosion Hazards: Unknown

Section V - Reactivity Data

Stability			Conditions to Avoid
	Unstable		None
	Stable	X	

Incompatibility: None known

Hazardous Decomposition or Byproducts: Sulfur oxides and bromides

Hazardous Polymerization			Conditions to Avoid
	May Occur		None
	Will Not Occur	X	

Section VI - Health Hazard Data

Route(s) of Entry: Inhalation? Yes Skin? Yes Ingestion? Yes

Health Hazards (Acute and Chronic): Acute eye contact: May cause irritation No data available for other routes

Carcinogenicity: None NTP? No data IARC Monographs? No data OSHA Regulation? No data

Signs and Symptoms of Exposure: May cause skin or eye irritation

Medical Conditions Generally Aggravated by Exposure: None reported

Emergency First Aid Procedures: Treat symptomatically and supportively. Rinse contacted area with copious amounts of water.

Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released for Spilled: Rinse contacted area with copious amounts of water.

Waste Disposal Method: Observe all federal, state, and local regulations.

Precautions to be Taken in Handling and Storing: Avoid eye and skin contact.

Other Precautions: None

Section VIII - Control Measures

Respiratory Protection (Specify Type): Chemical cartridge respirator with organic vapor cartridge.

Ventilation			Special	
	Local Exhaust	Yes	Special	Yes
	Mechanical (General)	Yes	Other	None

Protective Gloves		Eye Protection	
yes		Splash proof goggles	

Other Protective Clothing or Equipment: None required

Work/Hygienic Practices: Do not ingest. Avoid contact with skin, eyes and clothing. Wash thoroughly after handling.

EDVOTEK®

Material Safety Data Sheet

May be used to comply with OSHA's Hazard Communication Standard. 29 CFR 1910.1200 Standard must be consulted for specific requirements.

IDENTITY (As Used on Label and List): InstaStain® Ethidium Bromide

Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.

Section I

Manufacturer's Name: **InstaStain, Inc.**
P.O. Box 1232
West Bethesda, MD 20827

Emergency Telephone Number: **(301) 251-5990**

Telephone Number for information: **(301) 251-5990**

Date Prepared: 10/05/06

Signature of Preparer (optional):

Section II - Hazardous Ingredients/Identify Information

Hazardous Components [Specific Chemical Identity; Common Name(s)]	OSHA PEL	ACGIH TLV	Other Limits Recommended	% (Optional)
Ethidium Bromide		Data not available		
(2,7-Diamino-10-Ethyl-9-Phenylphenanthridinium Bromide)				
CAS# 139-33-3				

Section III - Physical/Chemical Characteristics

Boiling Point	No data	Specific Gravity (H_2O = 1)	No data
Vapor Pressure (mm Hg.)	No data	Melting Point	No data
Vapor Density (AIR = 1)	No data	Evaporation Rate (Butyl Acetate = 1)	No data

Solubility in Water: Soluble

Appearance and Odor: Chemical bound to paper, no odor

Section IV - Physical/Chemical Characteristics

N.D. = No data

Flash Point (Method Used)	Flammable Limits	LEL	UEL
No data		N.D.	N.D.

Extinguishing Media: Water spray, carbon dioxide, dry chemical powder, alcohol or polymer foam

Special Fire Fighting Procedures: Wear protective clothing and SCBA to prevent contact with skin & eyes

Unusual Fire and Explosion Hazards: Emits toxic fumes

Section V - Reactivity Data

Stability	Unstable		Conditions to Avoid: None
	Stable	X	
Incompatibility	Strong oxidizing agents		
Hazardous Decomposition or Byproducts	Carbon monoxide, Carbon dioxide, nitrogen oxides, hydrogen bromide gas		
Hazardous Polymerization	May Occur		Conditions to Avoid: None
	Will Not Occur	X	

Section VI - Health Hazard Data

Route(s) of Entry: Inhalation? Yes Skin? Yes Ingestion? Yes

Health Hazards (Acute and Chronic): Chronic: May alter genetic material
Acute: Material irritating to mucous membranes, upper respiratory tract, eyes, skin

Carcinogenicity: No data available NTP? IARC Monographs? OSHA Regulation?

Signs and Symptoms of Exposure: Irritation to mucous membranes and upper respiratory tract

Medical Conditions Generally Aggravated by Exposure: No data

Emergency First Aid Procedures: Treat symptomatically and supportively

Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released for Spilled: Wear SCBA, rubber boots, rubber gloves

Waste Disposal Method: Mix material with combustible solvent and burn in a chemical incinerator equipped afterburner and scrubber

Precautions to be Taken in Handling and Storing: Use in chemical fume hood with proper protective lab gear.

Other Precautions: Mutagen

Section VIII - Control Measures

Respiratory Protection (Specify Type): SCBA

Ventilation	Local Exhaust	Yes	Special	Chem. fume hood	
	Mechanical (General)	No	Other	None	
Protective Gloves	Rubber		Eye Protection	Chem. safety goggles	

Other Protective Clothing or Equipment: Rubber boots

Work/Hygienic Practices: Use in chemical fume hood with proper protective lab gear.

EDVOTEK®

Material Safety Data Sheet

May be used to comply with OSHA's Hazard Communication Standard. 29 CFR 1910.1200 Standard must be consulted for specific requirements.

IDENTITY (As Used on Label and List): Ampicillin

Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.

Section I

Manufacturer's Name: **EDVOTEK, Inc.**

Address (Number, Street, City, State, Zip Code): **14676 Rothgeb Drive**
Rockville, MD 20850

Emergency Telephone Number: **(301) 251-5990**

Telephone Number for information: **(301) 251-5990**

Date Prepared: 02/08/07

Signature of Preparer (optional):

Section II - Hazardous Ingredients/Identify Information

Hazardous Components [Specific Chemical Identity; Common Name(s)]	OSHA PEL	ACGIH TLV	Other Limits Recommended	% (Optional)
Ampicillin				
CAS# 7177-48-2	No data			

Section III - Physical/Chemical Characteristics

Boiling Point	No data	Specific Gravity (H_2O = 1)	No data
Vapor Pressure (mm Hg.)	No data	Melting Point	No data
Vapor Density (AIR = 1)	No data	Evaporation Rate (Butyl Acetate = 1)	No data

Solubility in Water: Slightly soluble

Appearance and Odor: Odorless, white crystaline powder

Section IV - Physical/Chemical Characteristics

N.D. = No data

Flash Point (Method Used)	Flammable Limits	LEL	UEL
No data		N.D.	N.D.

Extinguishing Media: Dry chemical, carbon dioxide, water spray or regular foam

Special Fire Fighting Procedures: Move container from fire area if possible. Do not scatter spilled material with water streams.

Unusual Fire and Explosion Hazards: Avoid breathing vapors.

Section V - Reactivity Data

Stability	Unstable		Conditions to Avoid: Incompatibles
	Stable	X	
Incompatibility	Strong oxidizers		
Hazardous Decomposition or Byproducts	Toxic oxides of carbon, nitrogen and sulfur		
Hazardous Polymerization	May Occur		Conditions to Avoid: Incompaticles
	Will Not Occur	X	

Section VI - Health Hazard Data

Route(s) of Entry: Inhalation? Yes Skin? Yes Ingestion? Yes

Health Hazards (Acute and Chronic): Sensitizers may result in allergic reaction

Carcinogenicity: No data NTP? IARC Monographs? OSHA Regulation?

Signs and Symptoms of Exposure: Repeated exposure may result in sensitization and possible anaphylactic shock.

Medical Conditions Generally Aggravated by Exposure: No data

Emergency First Aid Procedures: Ingestion: Allergic symptoms.
Eyes/Skin: Flush with water Inhalation: Move to fresh air

Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released for Spilled: Wear suitable protective clothing. Sweep up and place in suitable container for later disposal. Do not flush spilled material down sink.

Waste Disposal Method: Observe all federal, state, and local regulations

Precautions to be Taken in Handling and Storing: Keep away from incompatible substances

Other Precautions: None

Section VIII - Control Measures

Respiratory Protection (Specify Type):

Ventilation	Local Exhaust	Yes	Special	None	
	Mechanical (General)	No	Other	None	
Protective Gloves	Yes		Eye Protection	Splash or dust proof	

Other Protective Clothing or Equipment: Eye wash

Work/Hygienic Practices: Wear protective clothing and equipment to prevent contact.

EDVOTEK®

Material Safety Data Sheet

May be used to comply with OSHA's Hazard Communication Standard. 29 CFR 1910.1200 Standard must be consulted for specific requirements.

IDENTITY (As Used on Label and List): IPTG

Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.

Section I

Manufacturer's Name: **EDVOTEK, Inc.**

Address (Number, Street, City, State, Zip Code): **14676 Rothgeb Drive**
Rockville, MD 20850

Emergency Telephone Number: **(301) 251-5990**

Telephone Number for information: **(301) 251-5990**

Date Prepared: 02/08/07

Signature of Preparer (optional):

Section II - Hazardous Ingredients/Identify Information

Hazardous Components [Specific Chemical Identity; Common Name(s)]	OSHA PEL	ACGIH TLV	Other Limits Recommended	% (Optional)
Not applicable				

Section III - Physical/Chemical Characteristics

Boiling Point	None	Specific Gravity (H_2O = 1)	Unknown
Vapor Pressure (mm Hg.)	None	Melting Point	109-110C
Vapor Density (AIR = 1)	None	Evaporation Rate (Butyl Acetate = 1)	None

Solubility in Water: Moderate

Appearance and Odor: White crystals/slight odor thiophenol

Section IV - Physical/Chemical Characteristics

Flash Point (Method Used)	Flammable Limits	LEL	UEL
Unknown			

Extinguishing Media: Water, carbon dioxide, or dry chemical

Special Fire Fighting Procedures: None

Unusual Fire and Explosion Hazards: None

Section V - Reactivity Data

Stability	Unstable		Conditions to Avoid: None
	Stable	X	
Incompatibility	Strong oxdizing agents		
Hazardous Decomposition or Byproducts	Carbon dioxide and sulfur dioxide		
Hazardous Polymerization	May Occur		Conditions to Avoid
	Will Not Occur	X	

Section VI - Health Hazard Data

Route(s) of Entry: Inhalation? Yes Skin? Not studied Ingestion? Not studied

Health Hazards (Acute and Chronic): Toxcity has not been studied

Carcinogenicity: Unknown NTP? No data IARC Monographs? No data OSHA Regulation? No data

Signs and Symptoms of Exposure: Unknown: avoid dust

Medical Conditions Generally Aggravated by Exposure: Unknown

Emergency First Aid Procedures: External: flush with water Internal: Induce vomiting, consult physician

Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released for Spilled: Cover and sweep up with inert carrier

Waste Disposal Method: Dissolve in a combustible solvent and burn in a chemical incinerator with afterburner and scrubber, or sweep up and return inoriginal container.

Precautions to be Taken in Handling and Storing: Avoid dust store cool

Other Precautions: Information CAS #367-93-1

Section VIII - Control Measures

Respiratory Protection (Specify Type): Filter mask

Ventilation	Local Exhaust	Yes	Special	None	
	Mechanical (General)	Yes	Other	None	
Protective Gloves	Rubber or vinyl		Eye Protection	Face mask or goggles	

Other Protective Clothing or Equipment: Lab apron

Work/Hygienic Practices: Avoid dust or contact with skin

Material Safety Data Sheets
Full size (8.5 x 11") pdf copy of MSDS available at www.edvotek.com or by request.

EDVO-Kit # **331**

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Material Safety Data Sheets

Full size (8.5 x 11") pdf copy of MSDS available at www.edvotek.com or by request.

EDVOTEK®

Material Safety Data Sheet

May be used to comply with OSHA's Hazard Communication Standard. 29 CFR 1910.1200 Standard must be consulted for specific requirements.

IDENTITY (As Used on Label and List): Enzyme Reaction/ Dilution Buffer

Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.

Section I

Manufacturer's Name: EDVOTEK, Inc.

Address (Number, Street, City, State, Zip Code): 14676 Rothgeb Drive, Rockville, MD 20850

Emergency Telephone Number: (301) 251-5990

Telephone Number for information: (301) 251-5990

Date Prepared: 11/07/06

Signature of Preparer (optional):

Section II - Hazardous Ingredients/Identify Information

Hazardous Components [Specific Chemical Identity; Common Name(s)]	OSHA PEL	ACGIH TLV	Other Limits Recommended	% (Optional)
This product contains no hazardous materials as defined by the OSHA Hazard Communication Standard.				

Section III - Physical/Chemical Characteristics

Boiling Point	No data	Specific Gravity (H_2O = 1)	No data
Vapor Pressure (mm Hg.)	No data	Melting Point	N/A
Vapor Density (AIR = 1)	No data	Evaporation Rate (Butyl Acetate = 1)	No data

Solubility in Water: soluble

Appearance and Odor: Clear liquid, no odor, dry chemical, carbon dioxide, water spray or foam.

Section IV - Physical/Chemical Characteristics

Flash Point (Method Used)	Flammable Limits	LEL	UEL
No data		No data	No data

Extinguishing Media: Use extinguishing media appropriate to surrounding fire

Special Fire Fighting Procedures: Remove container from fire if possible.

Unusual Fire and Explosion Hazards: May produce toxic gases

Section V - Reactivity Data

Stability			Conditions to Avoid
	Unstable		None
	Stable	X	

Incompatibility: Copper, iron, silver salts, hydrogen peroxide, phenol, picric acid Formaldehyde, ether, alcohol, nitrogen oxide, strong bases, oxidizing agents.

Hazardous Decomposition or Byproducts: Toxic gases: Carbon monoxide, carbon dioxide, nitrogen oxides, chlorine, hydrogen chloride

Hazardous Polymerization			Conditions to Avoid
	May Occur		None
	Will Not Occur	X	

Section VI - Health Hazard Data

Route(s) of Entry:	Inhalation?	Skin?	Ingestion?
	Yes	Yes	Yes

Health Hazards (Acute and Chronic): Toxicity has not been quantified. Sensitivity reactions (allergic) may occur by skin penetration including anaphylactic shock.

Carcinogenicity:	NTP?	IARC Monographs?	OSHA Regulation?
None	No data	No data	No data

Signs and Symptoms of Exposure: May cause irritation to skin/eye, mucous membranes and upper respiratory tract.

Medical Conditions Generally Aggravated by Exposure: Respiratory conditions

Emergency First Aid Procedures: Treat symptomatically and supportively

Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released for Spilled: Mop up with absorbant material. Dispose of properly.

Waste Disposal Method: Mix with vermiculite and dry caustic, wrap in paper and burn in a chemical incinerator equipped with afterburner and scrubber. Ignite in presence of sodium carbonate and slaked lime (CaOH)

Precautions to be Taken in Handling and Storing: Wear protective gear to avoid skin/eye contact

Other Precautions: None

Section VIII - Control Measures

Respiratory Protection (Specify Type): Chemical cartridge respirator with organic vapor cartridge.

Ventilation			Special	
	Local Exhaust	Yes		None
	Mechanical (General)	No	Other	Process Encl.Vent. Sys.

Protective Gloves	Eye Protection
yes	Splash proof goggles

Other Protective Clothing or Equipment: Protection to avoid skin contact

Work/Hygienic Practices: Do not ingest. Avoid contact with skin, eyes and clothing. Wash thoroughly after handling.

EDVOTEK®

Material Safety Data Sheet

May be used to comply with OSHA's Hazard Communication Standard. 29 CFR 1910.1200 Standard must be consulted for specific requirements.

IDENTITY (As Used on Label and List): Enzyme Reaction Buffer

Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.

Section I

Manufacturer's Name: EDVOTEK, Inc.

Address (Number, Street, City, State, Zip Code): 14676 Rothgeb Drive, Rockville, MD 20850

Emergency Telephone Number: (301) 251-5990

Telephone Number for information: (301) 251-5990

Date Prepared: 10/10/06

Signature of Preparer (optional):

Section II - Hazardous Ingredients/Identify Information

Hazardous Components [Specific Chemical Identity; Common Name(s)]	OSHA PEL	ACGIH TLV	Other Limits Recommended	% (Optional)
This product contains no hazardous materials as defined by the OSHA Hazard Communication Standard.				

Section III - Physical/Chemical Characteristics

Boiling Point	No data	Specific Gravity (H_2O = 1)	No data
Vapor Pressure (mm Hg.)	No data	Melting Point	N/A
Vapor Density (AIR = 1)	No data	Evaporation Rate (Butyl Acetate = 1)	No data

Solubility in Water: soluble

Appearance and Odor: Clear liquid, no odor, dry chemical, carbon dioxide, water spray or foam.

Section IV - Physical/Chemical Characteristics

Flash Point (Method Used)	Flammable Limits	LEL	UEL
No data		No data	No data

Extinguishing Media: Use extinguishing media appropriate to surrounding fire

Special Fire Fighting Procedures: Remove container from fire if possible.

Unusual Fire and Explosion Hazards: May produce toxic gases

Section V - Reactivity Data

Stability			Conditions to Avoid
	Unstable		None
	Stable	X	

Incompatibility: Copper, iron, silver salts, hydrogen peroxide, phenol, picric acid Formaldehyde, ether, alcohol, nitrogen oxide, strong bases, oxidizing agents.

Hazardous Decomposition or Byproducts: Toxic gases: Carbon monoxide, carbon dioxide, nitrogen oxides, chlorine, hydrogen chloride

Hazardous Polymerization			Conditions to Avoid
	May Occur		None
	Will Not Occur	X	

Section VI - Health Hazard Data

Route(s) of Entry:	Inhalation?	Skin?	Ingestion?
	Yes	Yes	Yes

Health Hazards (Acute and Chronic): Toxicity has not been quantified. Sensitivity reactions (allergic) may occur by skin penetration including anaphylactic shock.

Carcinogenicity:	NTP?	IARC Monographs?	OSHA Regulation?
None	No data	No data	No data

Signs and Symptoms of Exposure: May cause irritation to skin/eye, mucous membranes and upper respiratory tract.

Medical Conditions Generally Aggravated by Exposure: Respiratory conditions

Emergency First Aid Procedures: Treat symptomatically and supportively

Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released for Spilled: Mop up with absorbant material. Dispose of properly.

Waste Disposal Method: Mix with vermiculite and dry caustic, wrap in paper and burn in a chemical incinerator equipped with afterburner and scrubber. Ignite in presence of sodium carbonate and slaked lime (CaOH)

Precautions to be Taken in Handling and Storing: Wear protective gear to avoid skin/eye contact

Other Precautions: None

Section VIII - Control Measures

Respiratory Protection (Specify Type): Chemical cartridge respirator with organic vapor cartridge.

Ventilation			Special	
	Local Exhaust	Yes		None
	Mechanical (General)	No	Other	Process Encl.Vent. Sys.

Protective Gloves	Eye Protection
yes	Splash proof goggles

Other Protective Clothing or Equipment: Protection to avoid skin contact

Work/Hygienic Practices: Do not ingest. Avoid contact with skin, eyes and clothing. Wash thoroughly after handling.

EDVOTEK®

Material Safety Data Sheet

May be used to comply with OSHA's Hazard Communication Standard. 29 CFR 1910.1200 Standard must be consulted for specific requirements.

IDENTITY (As Used on Label and List): X-Gal in solvent

Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.

Section I

Manufacturer's Name: **EDVOTEK, Inc.**

Address (Number, Street, City, State, Zip Code): **14676 Rothgeb Drive Rockville, MD 20850**

Emergency Telephone Number: **(301) 251-5990**

Telephone Number for information: **(301) 251-5990**

Date Prepared: 02/08/07

Signature of Preparer (optional):

Section II - Hazardous Ingredients/Identify Information

Hazardous Components [Specific Chemical Identity; Common Name(s)]	OSHA PEL	ACGIH TLV	Other Limits Recommended	% (Optional)
This product contains no hazardous materials as defined by the OSHA Hazard Communication Standard.				

Section III - Physical/Chemical Characteristics

Boiling Point	at 760mm Hg	189C	Specific Gravity (H_2O = 1)	No data
Vapor Pressure (mm Hg.)	20C	0.46	Melting Point	18C
Vapor Density (AIR = 1)		2.7	Evaporation Rate (Butyl Acetate = 1)	N/A

Solubility in Water: Complete (100%)

Appearance and Odor: Colorless liquid, faint odor

Section IV - Physical/Chemical Characteristics

Flash Point (Method Used)	Flammable Limits	LEL	UEL
(closed cup) 88C (192F)		3%	43%

Extinguishing Media: Water spray, carbon dioxide, dry chemical, ordinary foam

Special Fire Fighting Procedures: Wear SCBA with full facepiece operated in positive pressure mode. If possible, move container from fire area

Unusual Fire and Explosion Hazards: Vapors may flow along surfaces to distant ignition sources and flashback. Closed containers exposed to heat may explode. Contact w/ strong oxidizers may cause fire.

Section V - Reactivity Data

Stability			Conditions to Avoid
	Unstable		Heat, flame, other sources of ignition
	Stable	X	

Incompatibility: Strong oxidizing agents, active halogen compounds, alkali metals

Hazardous Decomposition or Byproducts: Oxides of sulfur, mercaptane, active halogen compounds, alkali metals

Hazardous Polymerization			Conditions to Avoid
	May Occur		None
	Will Not Occur	X	

Section VI - Health Hazard Data

Route(s) of Entry:	Inhalation?	Skin?	Ingestion?
	Yes	Yes	Yes

Health Hazards (Acute and Chronic): Chronic affects: kidney damage, liver damage

Carcinogenicity:	NTP?	IARC Monographs?	OSHA Regulation?
None	No	No	No

Signs and Symptoms of Exposure: Skin/eye irritation, headache, nausea, vomiting, dizziness

Medical Conditions Generally Aggravated by Exposure: Skin disorders

Emergency First Aid Procedures: Flush skin/eyes with large amounts of water. If ingested do not induce vomiting.

Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released for Spilled: Shut off ignition sources. Wear protective clothing. Use water spray to reduce vapors. Take up with sand or other noncombustible absorbent material and dispose of properly

Waste Disposal Method: Dispose in accordance with all applicable federal, state, and local environmental regulations

Precautions to be Taken in Handling and Storing: Store in cool, dry, well ventilated flammable liquid storage area or cabinet, store above 20C

Other Precautions: Product may solidify at room temperature

Section VIII - Control Measures

Respiratory Protection (Specify Type): Chemical cartridge respirator w/ organic vapor cartridge.

Ventilation			Special	
	Local Exhaust	Yes		None
	Mechanical (General)	Yes	Other	None

Protective Gloves	Eye Protection
Butyl rubber gloves	Splash Proof Safety goggles

Other Protective Clothing or Equipment: Uniform or apron

Work/Hygienic Practices: Avoid contact with skin/eyes Keep container tightly closed.