EDVOTEK®

The Biotechnology Education Company ®

Revised and Updated

EDVO-Kit #
301

Construction and Cloning of a Recombinant DNA

Storage: See Page 3 for specific storage instructions

EXPERIMENT OBJECTIVE:

The objective of this experiment is to use various recombinant DNA technology procedures to clone a DNA fragment, extract and map the resulting fragment from the recombinant plasmid.

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

Table of Contents

IMPORTANT READ ME!

Transformation experiments contain antibiotics which are used for the selection of transformed bacteria. Students who have allergies to antibiotics such as penicilllin, ampicillin, kanamycin or tetracycine should not participate in this experiment.

Experiment Components

Component Quantities:

Experiment # 301 is designed for 5 groups of 2 - 4 students.

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.

None of the experiment components are derived from human sources.

		Storage
Reagents for DNA Ligation		
A	Qualified water (for enzyme reactions)	-20°C Freezer
B	DNA fragments for ligation (linear vector and kan^r gene)-20°C	Freezer
C	T4 DNA ligase reaction tubes	Room Temp
Reagents and Biologicals for Transformation		
D	Kanamycin Sulfate	-20°C Freezer
E	Supercoiled control DNA for transformation	-20°C Freezer
F	*E. coli* HB101 Bacterial slant	Refrigerator
G	0.05M $CaCl_2$	Refrigerator
•	ReadyPour Agar (sterile)	Refrigerator
•	Recovery Broth (sterile)	Refrigerator
•	Growth Medium (sterile)	Refrigerator
Reagents for Plasmid Extraction		
H	Tris-EDTA-Glucose (TEG)	Refrigerator
I	2M NaOH	Refrigerator
J	10% SDS	Refrigerator
K	RNase (DNase free)	Refrigerator
L	Tris-EDTA Buffer concentrate (TE)	Refrigerator
M	Acidified potassium acetate	Refrigerator
N	Resuspension Buffer	Refrigerator
Reagents for Restriction Enzyme Analysis		
O	Restriction Enzyme Reaction Buffer (10x)	-20°C Freezer
P	Restriction Enzyme Dilution Buffer	-20°C Freezer
Q	Standard DNA Fragments (ready for electrophoresis)	-20°C Freezer
R	Supercoiled Plasmid Vector Standard (ready for electrophoresis)	-20°C Freezer
S	*Eco* RI Endonuclease	-20°C Freezer
T	*Pvu* II Endonuclease	-20°C Freezer
U	*Cla* I Endonuclease	-20°C Freezer

continued

Components, continued

Reagents for Gel Electrophoresis

• InstaStain® Ethidium Bromide	Room temp
• 10x Gel Loading Solution	Room temp
• 50x Electrophoresis Buffer	Room temp
• UltraSpec-Agarose™	Room temp

Disposable Supplies

- Screw cap tubes (sterile)
- Microtest tubes (0.5 ml)
- 1 ml pipets (sterile)
- Petri plates (sterile, 60 x 15 mm)
- 1.5 ml microcentrifuge tubes
- Sterile inoculating loops/needles
- 10 ml pipets (sterile)
- Sterile 50 ml culture tubes
  (for Module III, Growth of Kan^r Transformants)

Requirements

- Horizontal Electrophoresis apparatus
- DC power supply
- 37°C and 42°C water baths
- Shortwave ultraviolet light source
- Microcentrifuge
- Table top, clinical centrifuge or floor model centrifuge
- 37°C incubation oven
- Shaking incubator or shaking water bath
- Automatic micropipets and sterile pipet tips
- Pipet pumps
- Balance
- Microwave, hot plate or Bunsen burner
- 95-100% ethanol
- Distilled or deionized water
- Ice
- Photodocumentation system (optional)
- Autoclave

Construction and Cloning of a Recombinant DNA

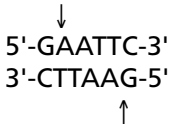
The majority of specialized recombinant DNA molecules used in biotechnology have been constructed by subcloning procedures. Several hundred vectors have been designed to meet specific needs in molecular biology and biomedical research. For example, some vectors have high copy numbers and will produce large amounts of plasmids. Others have been designed to facilitate *in-vitro* transcription, super-expression of proteins *in-vivo*, DNA sequence analysis, replication in both prokaryotes and eukaryotes, facile conversion between double-stranded and single stranded forms of recombinant DNA, the cloning of large DNA fragments and the expression of characteristic marker genes which aid in the selection of cloned DNA.

Subcloning involves the ligation of a previously cloned and purified DNA molecule into a vector. The resulting recombinant molecule is then introduced in the appropriate host cell where it is propagated and expressed. Subsequently, selection procedures are performed and the recombinant DNA is purified.

This experiment involves five distinct experimental modules. They are: 1) the ligation of a kanamycin resistance gene in a plasmid vector; 2) introduction of the recombinant DNA into *E. coli* cells by transformation and selection of kanamycin resistant transformants; 3) growth of the transformants in liquid medium; 4) extraction of supercoiled recombinant plasmid DNA; 5) restriction enzyme analysis. The enzymatic analysis will verify the identity of the recombinant DNA and determine the orientation and multiplicity of the subcloned kanamycin resistance gene.

The kan^r Gene

Kanamycin is an aminoglycoside which interferes with translation by binding to the 70 S prokaryotic ribosome. The drug interferes with translation by causing misreading of messenger RNA. The kanamycin resistance gene is a cloned derivative from a strain of *E. coli* containing the Tn 903 transposon. The transposon encodes resistance to kanamycin and neomycin. The protein product of this gene is a 3'-aminoglycoside phosphotransferase which inactivates the drug by covalent modification. The modified drug can not bind to the ribosomes. The cloned fragment encoding the gene is approximately 1300 base pairs in length and possesses *Eco* RI generated cohesive termini (Figure 1). The *Eco* RI recognition site is:



The staggered cleavage generates a protruding 5' phosphate on the adenine and a recessed 3' hydroxyl group on the guanine. The structural

Background Information

Construction and Cloning of a Recombinant DNA

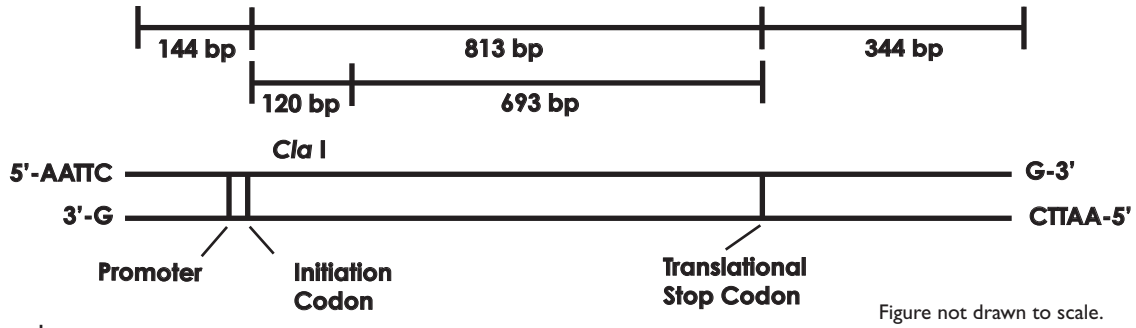


Figure not drawn to scale.

Figure 1:

gene contains 813 bases which would code for a polypeptide consisting of 271 amino acid residues. The DNA fragment contains a promoter for *E. coli* polymerase and has the required ribosomal binding sequences for mRNA transcripts.

Plasmid Vector

The vector is a 3000 base pair plasmid derived from the pUC series. The plasmid possesses a single recognition site for *Eco* RI endonuclease, which is located in a M13 mp derived polylinker. The polylinker region is 44 base pairs in length and contains several unique restriction enzyme sites to facilitate the insertion of foreign DNA. The plasmid also contains an ampicillin resistance gene (codes for beta-lactamase) and undergoes relaxed replication in *E. coli*. The plasmid has been linearized with *Eco* RI endonuclease to produce compatible termini for the subcloning experiment.

Ligation Reaction

The ligation of the 1300 base pair fragment to the linearized vector will be accomplished by the addition of T4 DNA ligase to a buffered mixture of the two DNAs. The enzyme catalyzes the formation of a phosphodiester bond by the condensation of a 5' phosphate and 3' hydroxyl group of adjacent nucleotides occurring in a nick or between cohesive or blunt termini of DNA. The enzyme is purified from T4 phage infected *E. coli*. It requires magnesium and ATP. Each phosphodiester bond formation results in the hydrolysis of ATP to AMP plus pyrophosphate. The catalytic efficiency of the enzyme is optimal at 37°C. However, ligation of DNA fragments having cohesive termini is usually done at temperatures of 4°C to 22°C. Lower temperatures allow for annealing between complementary ends of DNA which is a prerequisite for the ligation of cohesive termini.

Construction and Cloning of a Recombinant DNA

In the simplest and most ideal case, the ligation of a one to one complex between vector and kanamycin insert would result in a circular recombinant plasmid consisting of 4300 base pairs. Phosphodiester bond formation would occur between the guanine 3' hydroxyl group and the adenine 5' phosphate in the *Eco* RI termini.

The stoichiometry of the vector and the kanamycin gene insert in the ligation reaction is complex. It is affected by the lengths and relative concentrations of the two DNA species, the concentration of enzyme and the ionic strength of the reaction conditions. Due to the complimentarity of the *Eco* RI termini, the vector can undergo reclosure without an insert. At high concentrations it can form concatamers, i.e., larger linear arrays consisting of repeating units of full length vector. Circularization

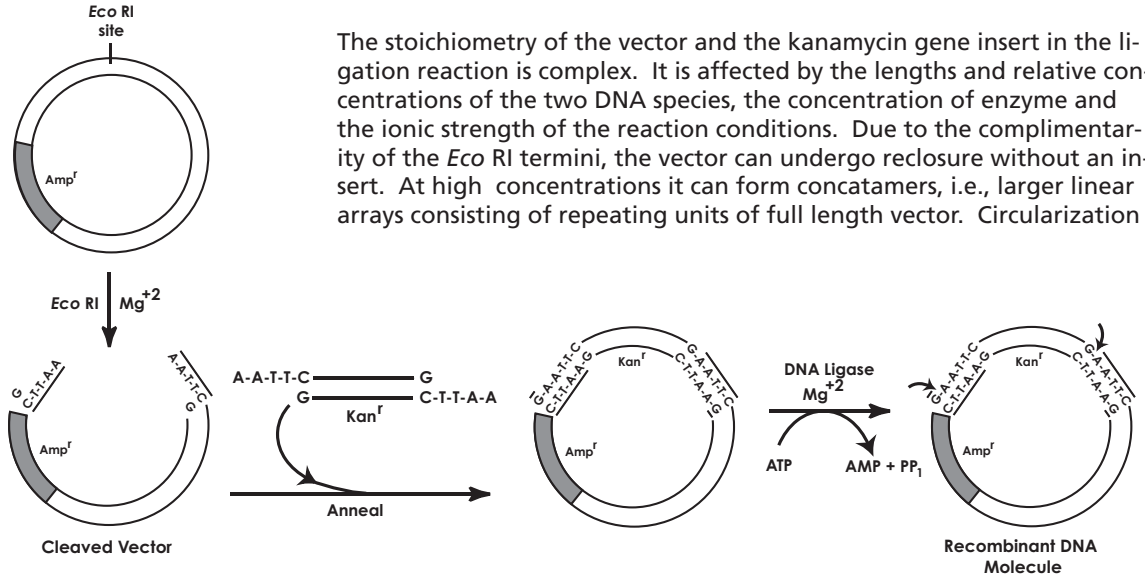


Figure 2:

Figure not drawn to scale.

and concatamer formation can also occur with the kanamycin fragment. Moreover, many combinations and orientations between vector and insert can be envisioned, some of which are shown in Figure 3. Recombinants shown in Figure 3 represent ligated inserts with various orientations. Such examples, unlike that in Figure 2, will not be functional.

The multiple forms of the recombinant DNA explain the complex banding patterns observed during electrophoresis of ligation reaction products. When cloning it is desirable to minimize the number of different ligation forms since transformation of competent E. coli cells is very inefficient with linear DNA molecules. Consequently, production of correct circular molecules should be optimized. Furthermore, large recombinant molecules containing multiple arrays of vector and insert may not replicate efficiently and can complicate DNA mapping. Linearized plasmid vectors can be treated with alkaline phosphatase. This phosphomonoesterase removes the 5' phosphates at DNA termini, yielding

Construction and Cloning of a Recombinant DNA

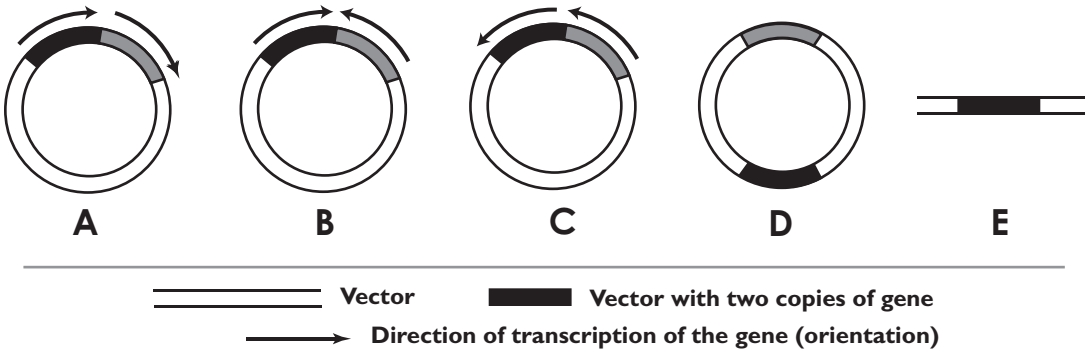


Figure not drawn to scale.

Figure 3:
Examples of possible products obtained for the ligation reactions.

a free 5' hydroxyl group plus inorganic phosphate. Since ligase requires a 5' phosphate for phosphodiester bond formation, problems associated with vector reclosure and concatamers are eliminated. In this case, the ligation of DNA insert to vector will produce nicks at the annealed junctions since only two instead of four phosphodiester bonds can be formed. The nicks are repaired in the transformed host. The selection procedure used in this experiment eliminates any cells containing ligated forms of vector since vector plus insert are required for growth on the kanamycin medium. Circularization and concatamer formation of the kanamycin fragment can still occur but will not be observed in the *E. coli* transformants since this DNA does not have any replication origins. The formation of a disproportionately large amount of these forms will reduce the amount of recombinant DNA produced *in-vitro*. Concatamers of the kanamycin fragment can be reduced by lowering its concentration. Increased yields of circular recombinant molecules can be obtained by adjusting the total DNA concentration and the molar ratio of vector to insert. In summary, the sizes of the DNAs, buffer conditions, the type of DNA termini involved and vector that have been dephosphorylated play a role in the construction of a recombinant.

When the vector and insert contain the same cohesive termini, the orientation of the subcloned insert can vary between individual bacterial colonies that came from the same transformation experiment. This is due to the symmetrical nature of the termini and, statistically, one would expect to find a 50:50 occurrence of the two insert orientations if many colonies were analyzed. A single insert in the recombinant plasmid can be in either one of two directions relative to a fixed point in the vector. In this case, a single Pvu II recognition site in the vector is a convenient reference point to determine direction of ligation (Figure 4).

Construction and Cloning of a Recombinant DNA

Transformation

Competent cells were prepared from cultures of E. coli HB101. This strain does not have any natural antibiotic resistance or plasmids and lacks restriction enzymes. In addition, the strain does not have recA protein which reduces the possibility of intracellular recombination events. All these features make E. coli HB101 an excellent host for cloning and subcloning experiments.

Transformation with the ligation reaction products performs several functions. Biological incorporation of the recombinant DNA allows for its expression, propagation and purification. Transformation acts as a purification step since it separates the complex mixture of ligation reaction products into individual bacterial colonies or eliminates some of them entirely. Linear vector and very large concatamers are not taken up well by competent E. coli. Supercoiled and relaxed circular DNA have the highest transformation efficiencies. Only small amounts of DNA, typically less than 10 nanograms, are required for transformation. In fact, transformation is inhibited by amounts of DNA exceeding 100 nanograms. Despite the small masses of DNA required to produce transformants, only 1 in 10,000 cells successfully incorporate the exogenous DNA. The uptake of two different molecules of DNA by the same cell during a transformation experiment occurs at a lower frequency. The transformation efficiency is defined by the number of transformants obtained per microgram of DNA. For example, 10 nanograms of DNA were used for a transformation and the cells were allowed to recover in a final volume of 1 ml. One tenth of this volume 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 more than sufficient for most subcloning experiments. When the cloning of single copy genes from genomic DNA is done, the required efficiencies are 10 7 to 10 8.

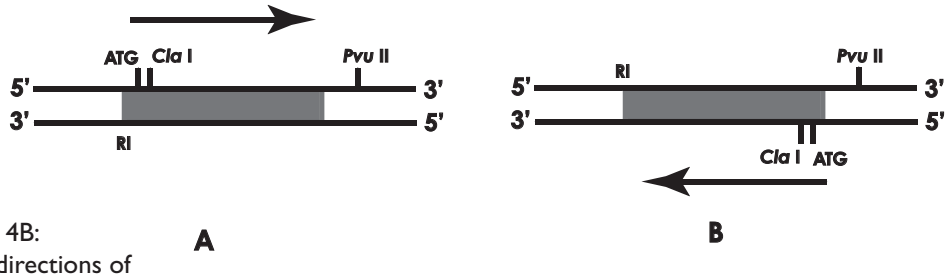


Figure 4A and 4B:
Two possible directions of ligation

Construction and Cloning of a Recombinant DNA

The Experiment

Selection of Recombinants

The cloning or subcloning of an antibiotic resistance gene permits a simple and rapid method of selection. Only transformants that have successfully incorporated and expressed the kanamycin resistance gene will grow on nutrient agar plates containing kanamycin. Most cloning experiments involve genes that do not have any properties that allow for such rapid selection. One or more screening methods must be employed. Screening can often be tedious and time-consuming. Plasmid vectors usually contain antibiotic resistance genes that are used for the positive selection of bacteria containing the plasmid, and hopefully, the cloned DNA segment of interest. In these cases, the vector resistance genes are used to select for transformed cells only. In this experiment, each group will pick a single transformed colony which will be grown in liquid culture in the presence of kanamycin. It is necessary to keep this selective pressure applied since bacteria will often eliminate their plasmids if there is no continued advantage in keeping them.

Extraction of Supercoiled Plasmid DNA

Alkaline-lysis plasmid extraction procedures will be used to partially purify recombinant plasmid. SDS in this solution disrupts the cell membrane and denatures proteins. The high pH causes the degradation of large RNA and aids in protein denaturation. It also irreversibly denatures fragments of chromosomal DNA. Linear and nicked forms of plasmid DNA undergo strand separation. The ionic strength at which this procedure is performed causes the partial removal of the denatured chromosomal DNA from solution. Residual RNA is removed by treatment of RNAse. Supercoiled plasmid does not undergo strand separation since its two strands are topologically linked. Supercoiled plasmid remains free in solution. The addition of acidified potassium acetate to the lysate neutralizes the pH and causes the precipitation of free SDS and associated SDS membrane and protein complexes. Since *E. coli* chromosomal DNA is attached to the cell membrane, the majority of it is entrapped in the SDS-membrane precipitate. Residual protein can be removed from the DNA by organic solvents such as phenol and chloroform. The addition of isopropanol precipitates the plasjid, which is later resuspendedf with Tris buffer.

Restriction Enzyme Analysis of Recombinant DNA

Agarose gel electrophoresis of the uncut, extracted plasmid (and controls) will reveal several DNA bands. Residual, degraded RNA may be observed and will have the fastest migration rate (ahead of the bromophenol blue tracking dye). Supercoiled monomeric plasmid has the fastest migration rate of all the forms of plasmid DNA. A variable

Construction and Cloning of a Recombinant DNA

amount of nicked plasmid and plasmid catenanes (interlocked rings of plasmid molecules) will also be observed. Their rates of migration would be nicked ≥ dimer > trimer> tetramer > etc. Keep in mind that restriction enzyme cleavage of catenanes results in the same fragmentation patterns as produced by the cleavage of the uncatenated DNA.

Cleavage of recombinant plasmid with *Eco* RI endonuclease will generate two fragments having lengths of 3000 base pairs (vector) and 1300 base pairs (kanamycin insert). This reaction verifies the identity and integrity of the recombinant molecule. The plasmid may possess two or more **adjacent** kanamycin inserts (Figure 3, A-C). This can be ascertained by cleaving the plasmid with *Pvu* II endonuclease. The vector contains a single *Pvu* II recognition site located 180 base pairs downstream (in the 3' direction) from the *Eco* RI site in the polylinker. The kanamycin insert does not contain a *Pvu* II site. Consequently, the plasmid will be linearized to unit length. By comparison to standard marker fragments electrophoresed in parallel, the size of the linear ized plasmid can be estimated by agarose gel electrophoresis. For example, if two adjacent inserts were present, the length of the plasmid would be 5600 base pairs.

The orientation of a single kanamycin insert in a single circular vector can also be determined by restriction enzyme analysis. The insert possesses a single *Cla* I recognition site located approximately 120 base pairs downstream from the initiation codon (Figure 4). The unique *Pvu* II site in the vector can act as a fixed reference point. Since the *Cla* I site is off center in the insert, a *Pvu* II–*Cla* I codigest of the recombinant can be used to determine the orientation after estimating the size of the smallest of the two restriction fragments produced. The kanamycin resistance structural gene (813 base pairs) is not flanked by equal lengths of DNA (which comprise the rest of the 1300 base pair insert). There are approximately 144 base pairs of flanking DNA upstream (in the 5' direction) from the initiation codon to the nearest *Eco* RI site. There are approximately 344 base pairs from the translational stop codon to the nearest *Eco* RI site (see Figure 1). With this in mind, an insert orientation that is left to right, Figure 4A, will yield 1217 and 3083 base pair fragments after a *Pvu* II-*Cla* I digest. The opposite orientation will yield 444 and 3856 base pair fragments.

If adjacent inserts are present in a single circular vector, the determination of orientation becomes more complex (Figure 3A-C). Assume the unique *Pvu* II site in the vector is 180 base pairs to the right (3') of *Eco* RI insertion site (going in the clockwise direction) in figures 3A-C. A *Pvu* II-*Cla* I codigest of the recombinant in Figure 3A would yield three fragments having lengths of 1217, 1301 and 3084 base pairs. Keep in mind that the distance from the *Cla* I site to the end of the structural gene is

Construction and Cloning of a Recombinant DNA

693 base pairs. Students are encouraged to predict the *Pvu* II-*Cla* I fragmentation patterns that would result from the recombinants in Figures 3B and 3C. It should be noted that in this system, the orientation of a single insert does not have any large effects on gene expression since the kanamycin fragment has its own promoter. However, when a vector promoter is required for expression, one of the insert orientations can abolish the expression of the subcloned gene.

Experiment Overview

BEFORE YOU START THE EXPERIMENT

1. Read all instructions before starting the experiment.
2. Write a hypothesis that reflects the experiment and predict experimental outcomes.

EXPERIMENT OBJECTIVE:

The objective of this experiment is to use various recombinant DNA technology procedures to clone a DNA fragment, extract and map the resulting fragment from the recombinant plasmid. The experiment has five modules.

Module I:
The ligation of a plasmid vector with a fragment containing the kanamycin resistance gene

Module II:
Introduction of the recombinant DNA into *E. coli* cells by transformation and selection of transformants

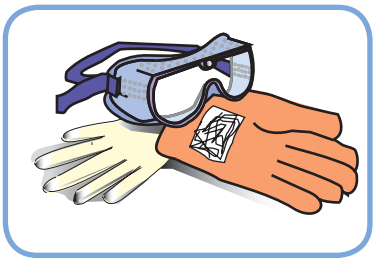
Module III:
Picking and Growth of Kanr Transformants

Module IV:
Extraction of supercoiled recombinant plasmid DNA

Module V:
Restriction enzyme analysis

Laboratory Safety

The Experiment



Important READ ME!

Transformation experiments contain antibiotics which are used for the selection of transformed bacteria. Students who have allergies to antibiotics such as penicilllin, ampicillin, kanamycin or tetracycine should not participate in this experiment.

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

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

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

4. The *E. coli* bacteria used in this experiment is not considered pathogenic. Although it is rarely associated with any illness in healthy individuals, it is good practice to follow simple safety guidelines in handling and disposal of materials contaminated with bacteria.

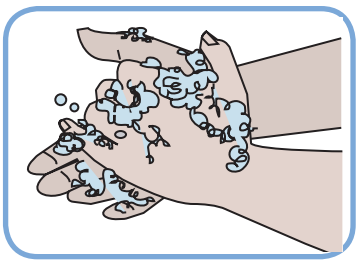
5. Properly dispose materials after completing the experiment:

   A. Wipe down the lab bench with a 10% bleach solution or a laboratory disinfectant.

   B. All materials, including petri plates, pipets, transfer pipets, loops and tubes, that come in contact with bacteria should be disinfected before disposal in the garbage. Disinfect materials as soon as possible after use in one of the following ways:

   - Autoclave at 121° C for 20 minutes.
     Tape several petri plates together and close tube caps before disposal. Collect all contaminated materials in an autoclavable, disposable bag. Seal the bag and place it in a metal tray to prevent any possibility of liquid medium or agar from spilling into the sterilizer chamber.

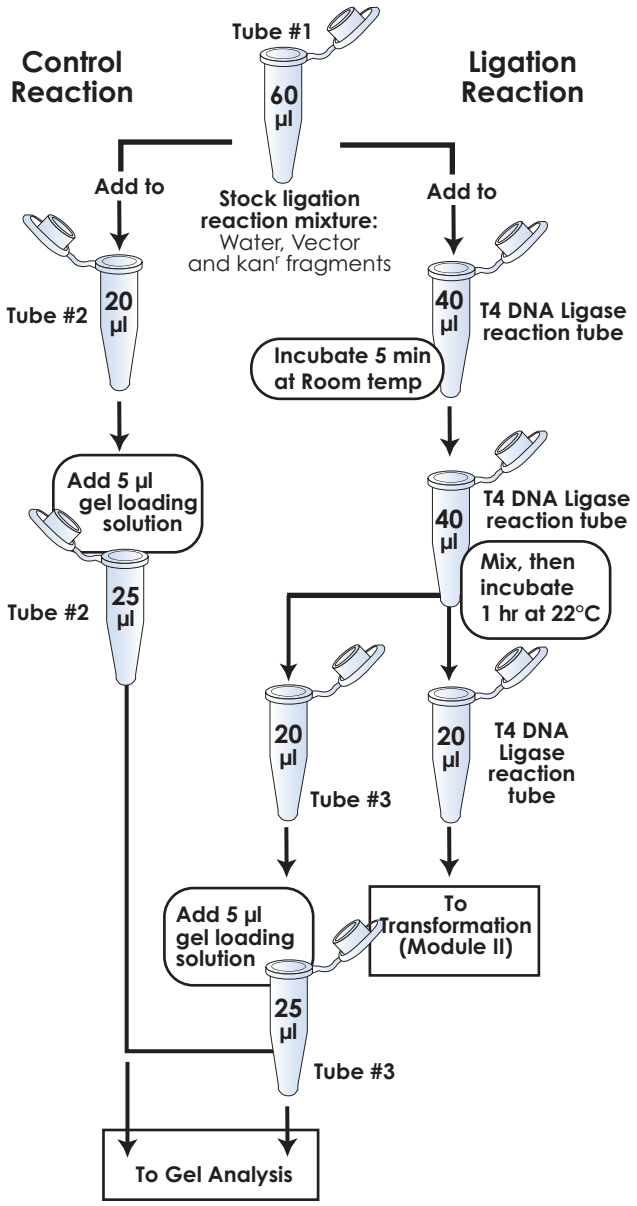
   - Soak in 10% bleach solution.
     Immerse petri plates, open tubes and other contaminated materials into a tub containing a 10% bleach solution. Soak the materials overnight and then discard. Wear gloves and goggles when working with bleach.



6. Wear gloves, and at the end of the experiment, wash hands thoroughly with soap and water.

MODULE I: Ligation of Vector to the Kan^r Gene

MODULE I OVERVIEW



The Experiment

MODULE I: Ligation of Vector to the Kan^r Gene

LIGATION REACTION

1. Label and initial three 1.5 ml microtest tubes 1 - 3:

   "1" stock ligation reaction mixture
   "2" ligation control
   "3" ligation reaction

2. Add ligation reaction components to tube 1.
   - First, add 40 µl of qualified water, followed by 20 µl of vector and kan^r fragments. The total volume is 60 µl.

3. Mix by tapping or briefly vortexing.

4. Prepare the control sample:
   - With a fresh tip, remove 20 µl of the ligation reaction from tube 1 and transfer it to tube 2. Close tube 1.
   - Add 5 µl of 10x gel load to tube 2 and mix. Set the tube aside.

5. Gently vortex or tap the T4 DNA Ligase Reaction Tube (C) on lab bench to collect T4 ligase pellet at bottom of tube.

6. With a fresh tip, add all of the remaining stock ligation reaction mixture from tube 1 (40 µl) to the T4 DNA Ligase Reaction Tube. Incubate for 5 minutes at room temperature.

7. Carefully stir the mixture of DNA and T4 DNA ligase with a pipet tip and gently pipet the solution up and down. Briefly pulse in a micro-centrifuge to collect the solution at the tube bottom.

8. Incubate at room temperature (approx. 22°C) for 1 hour or in a 16°C cool waterbath for 30 minutes by adding ice. Tap the tube to mix, or vortex periodically throughout the incubation period.

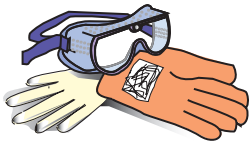
9. Prepare the ligation reaction sample:
   - With a fresh tip, remove 20 µl of the ligation reaction from the T4 DNA Ligase tube and transfer to tube 3.
   - Add 5 µl of 10x gel load to tube "3". Mix.

10. Samples from tubes "2" and "3" will be submitted to electrophoresis or can be stored frozen until needed.

Optional Stopping Point

Continue with the experiment, or freeze the remainder of the ligation reaction (Tube 1) until needed for Transformation in Module II.

MODULE I: Ligation of Vector to the Kan^r Gene



Wear gloves
and safety goggles

EDVOTEK 50x Electrophoresis buffer is Tris-acetate-EDTA (20 mM tris, 6 mM sodium acetate, 1 mM disodium ethylenediamine tetraacetic acid) pH 7.8.

The same buffer concentrate is used for preparing the agarose gel solution and chamber buffer for DNA analysis. The formula for diluting EDVOTEK (50x) concentrated buffer is 1 volume of buffer concentrate to every 49 volumes of distilled or deionized water.

AGAROSE GEL PREPARATION AND ELECTROPHORESIS

- Recommended gel size: 7 x 7 cm or 7 x 15 cm
- Number of sample wells required: 2 per group
  (Each gel can be shared by 2-3 groups)
- Placement of well-former template: first set of notches
- Agarose gel concentration required: 0.8%

CASTING AGAROSE GELS

1. Prepare the gel solution. Add buffer concentrate, distilled water and agarose powder to a 250 ml flask according to Table A.

Table A Individual 0.8% UltraSpec-Agarose™ Gel
DNA Staining with InstaStain® EtBr

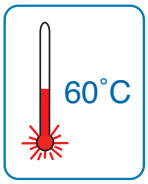
Size of EDVOTEK Casting Tray (cm)	Amt of Agarose (g)	+	Concentrated Buffer (50x) (ml)	+	Distilled Water (ml)	=	Total Volume (ml)
7 x 7	0.2		0.5		24.5		25
7 x 14	0.4		1.0		49.0		50

2. Swirl the mixture to disperse clumps of agarose powder.
3. With a marking pen, indicate the level of the solution volume on the outside of the flask.
4. Heat the mixture (using microwave oven or a hot plate) to dissolve the agarose powder. Boil until all the agarose is completely dissolved. The final solution should appear clear (like water) without undissolved particles.
5. 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 as marked on the flask in step 5.

MODULE I: Ligation of Vector to the Kan^r Gene

DO NOT POUR BOILING HOT AGAROSE INTO THE GEL BED.

Hot agarose solution may irreversibly warp the bed.



After the gel is cooled to 60°C:

6. Pour the cooled agarose solution into the bed. Make sure the bed is on a level surface.
7. Allow the gel to completely solidify. It will become firm and cool to the touch after approximately 20 minutes.

PREPARING THE GEL FOR ELECTROPHORESIS

8. After the gel is completely solidified, carefully and slowly remove the comb and rubber dams or tape from the gel bed. Be especially careful not to damage or tear the gel wells.
9. Place the gel (on its bed) into the electrophoresis chamber, properly oriented, centered and level on the platform.
10. Fill the electrophoresis chamber with the required volume of diluted buffer for the specific unit you are using (see guidelines in Table B). Make sure the gel is completely covered with buffer.

Table B Dilution of Electrophoresis (Chamber) Buffer

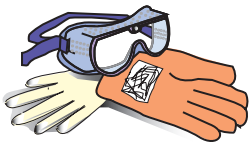
EDVOTEK Model #	Concentrated Buffer (50x) (ml)	+	Distilled Water (ml)	=	Total Volume (ml)
M6+	6		294		300
M12	8		392		400
M36 (blue)	10		490		500
M36 (clear)	20		980		1000

Table C Time and Voltage
Electrophoresis of DNA

Volts	Recommended Time Minimum	Maximum
125	30 min	45 min
70	40 min	1.5 hrs
50	60 min	2.0 hrs

11. Each group should load 25 µl each of the ligation control and reaction samples into adjacent gel wells.
12. Set the power source at the required voltage and conduct electrophoresis according to guidelines in Table C, or as determined by your instructor.
13. Allow the tracking dye to migrate 4.5 cm (7 x 7 cm gel) or 6-7 cm (7 x 14 cm gel) from the wells for adequate separation of the DNA bands. Terminate the electrophoresis before the tracking dye moves off the end of the gel.
14. After the electrophoresis is completed, turn off the power, unplug the power source, disconnect the leads and remove the cover.
15. Remove the gel from the bed for staining with InstaStain® Ethidium Bromide.

MODULE I: Ligation of Vector to the Kan^r Gene



Wear gloves and safety goggles

www.edvotek.com

Visit our web site for an animated demonstration of InstaStain® EtBr.

STAINING WITH INSTASTAIN® ETBR

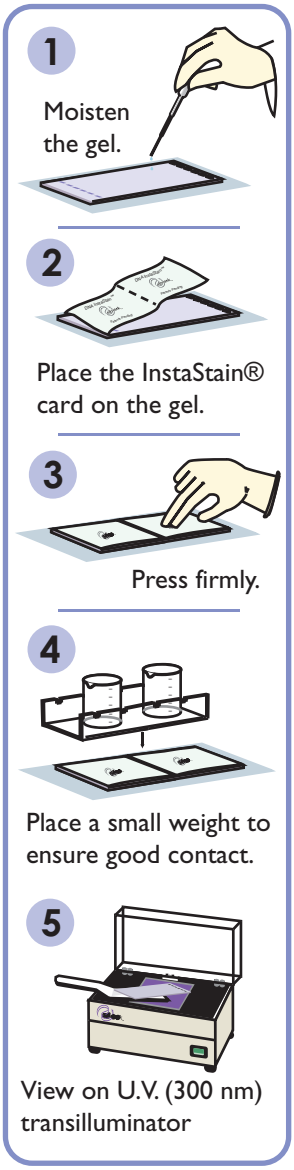
1. After electrophoresis, place the gel on a piece of plastic wrap on a flat surface. Moisten the gel with a few drops of electrophoresis buffer.
2. Wearing gloves, remove the clear plastic protective sheet, and place the unprinted side of the InstaStain® EtBr card on the gel.
3. Firmly run your fingers over the entire surface of the InstaStain® EtBr. Do this several times.
4. Place the gel casting tray and a small empty beaker on top to ensure that the InstaStain® card maintains direct contact with the gel surface.

   Allow the InstaStain® EtBr card to stain the gel for 2 minutes.
5. After 2 minutes, remove the InstaStain® EtBr card. Transfer the gel to a ultraviolet (300 nm) transilluminator for viewing. Be sure to wear UV protective goggles.

DISPOSAL OF INSTASTAIN

Disposal of InstaStain® cards and gels should follow institutional guidelines for chemical waste.

Caution: Ethidium Bromide is a listed mutagen.

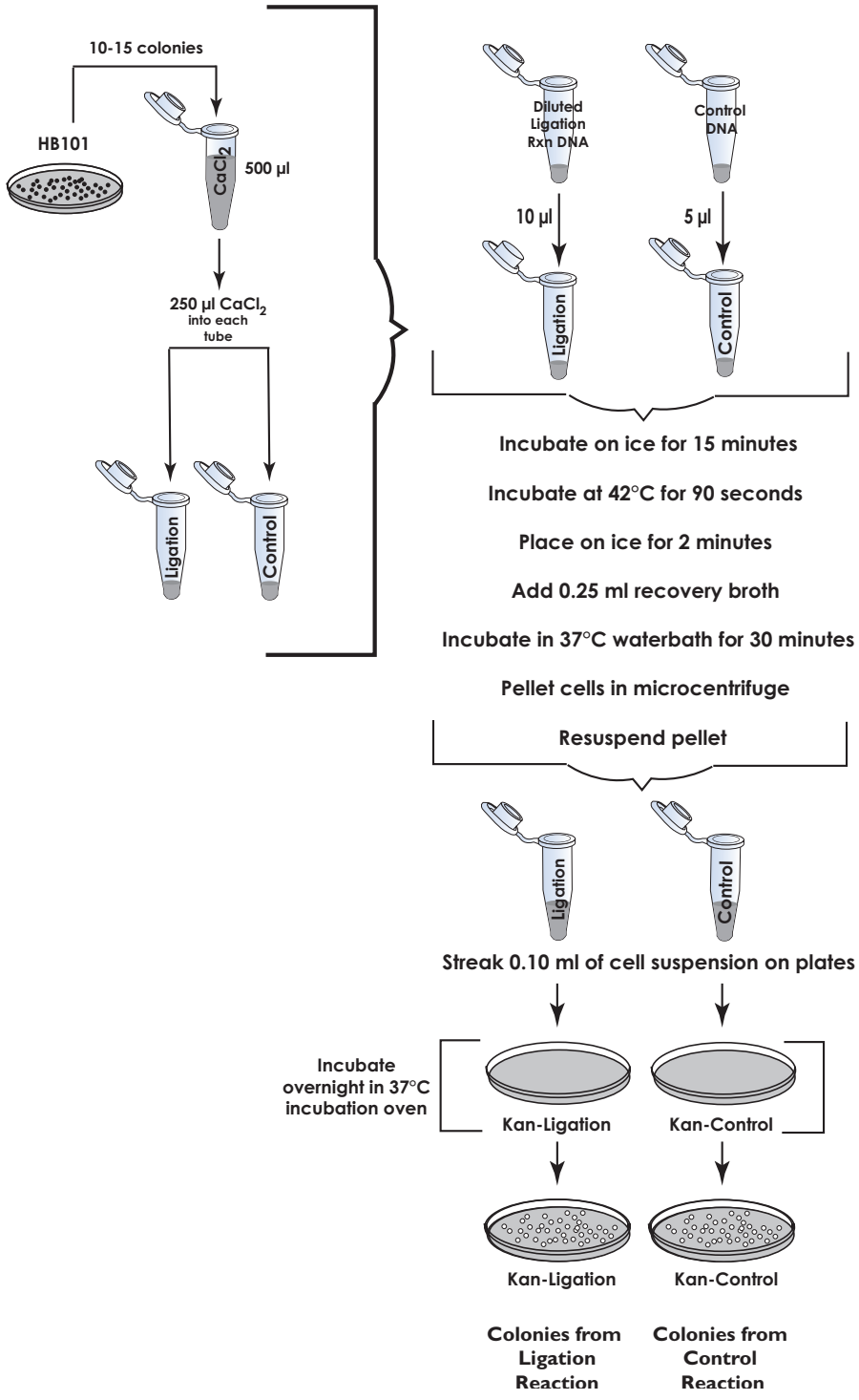


Additional Notes About Staining

- If bands appear faint, or if you are not using EDVOTEK UltraSpec-Agarose™, gels may take longer to stain with InstaStain® EtBr. Repeat staining and increase the staining time an additional 2-10 minutes.
- Standard DNA markers or ladders should be visible after staining even if other DNA samples are faint or absent. If they are not visible, troubleshoot for problems with the electrophoretic separation.

The Experiment

Module II - Transformation and Selection - Overview



MODULE II: Transformation and Selection

SETTING UP THE TRANSFORMATION AND CONTROL EXPERIMENT

1. Label 1.5 ml microcentrifuge tubesf "Ligation" and "Control."
2. Using a sterile 1 ml pipet, add 0.5 ml of ice cold 0.05M $CaCl_2$ into the "Control" tube and place on ice.
3. With a sterile loop, transfer a group of 10-15 single, well-isolated colonies from the plate labeled "HB101" to the "Control" tube. Twist the loop vigorously between your fingers to dislodge the cells.
4. Vortex the cells to mix and fully suspend the cells in the $CaCl_2$.
5. Transfer 250 µl of this cell suspension to the tube labeled "Ligation".
6. Place both the "Control" and "Ligation" tubes on ice. At this point, each tube should have 250 µl of $CaCl_2$ -suspended cells.
7. Dilute the DNA from the ligation reaction by mixing 5 µl of DNA from the T4 DNA Ligase tube (Module I) in 45 µl qualified water (A). Label this tube "DLR DNA" (Diluted Ligase Reaction DNA). Vortex or tap the tube with your finger.

Diluting the DNA helps to minimize the carryover of excess salts from the ligation reaction.

8. Add 10 µl of the diluted ligation reaction DNA to the tube labeled "Ligation". Vortex or tap the tubes with your finger.
9. Add 5 µl of supercoiled control DNA for transformation (E) directly to the tube labeled "Control." Vortex or tap the tube with your finger.
10. Incubate both tubes on ice for 15 minutes.
11. Place both tubes in a waterbath at 42°C for 90 seconds for the heat shock step. This facilitates the entry of DNA in bacterial cells.
12. Return tubes immediately to the ice bucket and incubate for two minutes.
13. Using a sterile pipet, add 250 µl (0.25 ml) of Recovery Broth to each tube and mix.
14. Incubate the cells for 30 minutes in a 37°C waterbath for a recovery period.

Quick Reference:

DNA and competent cells are combined in a suspension and incubated in growth medium (recovery broth). Bacterial cells continue to grow through the recovery process, during which time the cell wall is repaired. Cells recover and begin to express the antibiotic resistance gene.

MODULE II: Transformation and Selection

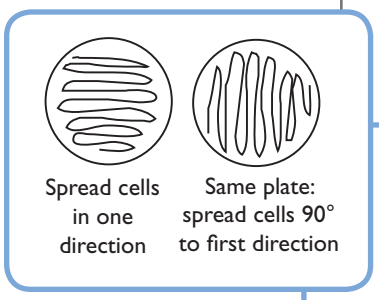
15. After the recovery period, remove the tubes from the waterbath and place them in a microcentrifuge and spin for 5 minutes to pellet the cells.

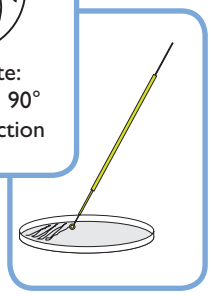
16. Remove and discard 0.4 ml of supernatant from each tube and re-suspend cell pellet in remaining 0.1 ml liquid.

PLATING THE CELLS

17. Obtain two agar plates and label them "Control," and "Ligation" . Also label the plates with your initials or lab group number.

18. Pipet 0.1 ml of the recovered transformed cells to the corresponding plates.





19. Using a sterile loop for each plate, spread the cells evenly and thoroughly over the entire surface. Turn the plate 90° and thoroughly spread again.

20. Cover both plates and allow the liquid to be absorbed (approximately 15-20 minutes).

To avoid contamination when plating, do not set the lid on the lab bench - lift the lid of the plate only enough to allow spreading. Be careful to avoid gouging the loop into the agar.

PREPARING PLATES FOR INCUBATION

21. Stack your group's set of plates on top of one another and tape them together.

22. Put your initials or group number on the taped set of plates.

23. Place the set of plates in a safe place where they will not be disturbed. The plates should be left in the upright position to allow the cell suspension to be absorbed by the agar for 15 - 20 minutes.

24. Place the plates in the **inverted** position (agar side on top) in a 37°C incubation oven for overnight incubation (15-20 hours).

If the cell suspension has not been absorbed into the medium, it is best to incubate the plates upright. The plates are inverted to prevent condensation on the lid, which could drip onto the culture and may interfere with experimental results.

VIEWING PLATES AFTER INCUBATION

25. Proceed to analyzing your results.

26. After analyzing your results, follow proper procedures for disposal of contaminated materials.

MODULE II: Transformation and Selection

DETERMINATION OF TRANSFORMATION EFFICIENCY

Transformation efficiency is a quantitative determination of how many cells were transformed per 1 µg of plasmid DNA. In essence, it is an indicator of how well the transformation experiment worked.

You will calculate the transformation efficiency from the data you collect from your experiment.

1. Estimate the number of transformants on the "Ligation" plate. A convenient method to keep track of counted colonies is to mark the colony with a marking pen on the outside of the plate.

2. Calculate the transformation efficiencies for total transformants and for colonies that contain vectors with inserts (white colonies).

   The final recovery volume of the cells was 0.50 ml. Because the cells were centrifuged, the volume plated is 0.10 ml. The quantity of DNA used was approximately 25 ng.

   Determine the transformation efficiency using the formula:

$$\frac{\text{Number of transformants}}{\text{µg of DNA}} \times \frac{\text{final vol at recovery (ml)}}{\text{vol plated (ml)}} = \text{Number of transformants per µg}$$

Example: Assume you observed 40 colonies:

$$\frac{40 \text{ transformants}}{0.125 \text{ µg}} \times \frac{1.05 \text{ ml}}{0.25 \text{ ml}} = 1344 \ (1.3 \times 10^3) \text{ transformants per µg}$$

Quick Reference for Expt. 301:

25 ng of DNA is used.

The final volume at recovery is 0.50 ml.

The volume plated is 0.10 ml.

OPTIONAL STOPPING POINT

The plates may be wrapped and stored in the refrigerator for one week.

USE ONLY COLONIES FROM THE "KAN-LIGATION" PLATE FOR MODULE III ACTIVITIES. TO PREVENT MIX-UPS, LEAVE "KAN-CONTROL" PLATES IN THE REFRIGERATOR FOR STORAGE.

The Experiment

MODULE III: Picking and Growth of Kan ^{r} Transformants

Shake loop in broth to allow bacteria to come off the loop and enter the broth.

1. Obtain a tube of liquid kanamycin medium and put your initials or lab group number on it.
2. Using a sterile inoculating loop or needle, pick a **SINGLE**, **well-isolated** colony from your **kanamycin** agar plate, labeled "Kan-Ligation" .
3. Inoculate the medium. Tightly cap the tube.
4. Incubate the tubes at 37°C, with shaking (400 rpm) overnight (12-15 hours).

MODULE IV: Extraction of Recombinant Plasmids from Kan ^{r} Transformants

1. Obtain a microcentrifuge tube of suspended *E. coli* cells and put your initials or group number on it. Place the tube on ice.
2. Harvest the cells by centrifugation at full speed (10,000 - 14,000 rpm) for 2 minutes at room temperature.
3. Remove the supernatant and add 200 µl of Resuspension Buffer (N) to the bacterial pellet. To the suspension, add 5 µl of RNAse solution (K). Incubate the suspension at room temperature for 5 minutes.
4. Add 350 µl of freshly prepared Lysis Buffer. Cap the tube and mix well by inverting gently 4 to 6 times. Do not vortex to avoid breaking the plasmid.
5. Add 200 µl of Potassium Acetate Solution (M). Cap the tube and mix thoroughly by inverting the tube. A white precipitate should form. Place the tube on ice for 5 minutes without shaking.
6. Centrifuge the tube at full speed for 5 min. at room temperature.
7. Carefully transfer the supernatant into a new microcentrifuge tube. Avoid transferring the white debris with the supernatant. Discard the tube containing the white pellet.

MODULE IV: Extraction of Recombinant Plasmids from Kan ^{r} Transformants

8. Add 0.6 volume of 100% isopropanol (i.e. 0.6 ml isopropanol for 1 ml of supernatant from step 7). Mix gently by inverting 4 to 6 times and keep at room temperature for 10 minutes.

9. Centrifuge sample at full speed for 5 minutes at room temperature. Remove and discard the supernatant. The plasmid DNA is precipitated in the pellet.

Be careful not to dislodge the pellet and aspirate it into the transfer pipet.

10. Wash the DNA pellet with 350 µl of 70% ethanol. Centrifuge at full speed for 3 minutes at room temperature. Remove the supernatant and air dry the pellet for 5-10 minutes to get rid of the residual ethanol.

11. Resuspend the pellet in 50 µl of 1x TE buffer. Cap the tube and mix by shaking and vortexing. Briefly centrifuge to get all the contents to the bottom of the tube.

OPTIONAL STOPPING POINT

The sample can be frozen until needed or you can proceed to Module V.

Module V - Restriction Enzyme Analysis

The Experiment

Be sure to use a fresh pipet tip before going into enzyme, DNA and buffer stocks. Keep the enzymes on ice.

1. Make a reaction cocktail in a 1.5 ml microtest tube and mix.

150 µl	qualified water (A)
25 µl	restriction reaction buffer (O)
25 µl	resuspended recombinant plasmid

2. Label four (4) 1.5 ml microtest tubes 3-6. (Tubes 1 and 2 are standard DNA fragments and supercoiled vector, respectively.)
3. Transfer 40 µl of the cocktail to each tube.
4. Add 10 µl of qualified water (A) to tube 3.
5. Add 5 µl of qualified water (A) to tubes 4 and 5.
6. Add 5 µl (10-15 units) of diluted *Eco* RI endonuclease (S) to tube 4. Tap or **briefly** vortex to mix.
7. Add 5 µl (10-15 units) of diluted *Pvu* II endonuclease (T) to tube 5. Cap. Mix.
8. Add 5 µl of diluted *Pvu* II endonuclease (T) to tube 6. Then with a fresh pipet tip, add 5 µl (10-15 units) of diluted *Cla* I endonuclease (U) to tube 6. Cap. Mix.
9. Incubate tubes 3 to 6 at 37°C for 1 hour.

Summary of Restriction Enzyme Digestion Reactions

Reaction Tube	Reaction Cocktail (µl)	Water (µl)	*Eco* RI (µl)	*Pvu* II (µl)	*Cla* I (µl)	Final Reaction Volume (µl)
1	(Q) Standard DNA Fragments (ready for electrophoresis)					25
2	(R) Supercoiled Vector Standard (ready for electrophoresis)					25
3	40	10	-	-	-	50
4	40	5	5	-	-	50
5	40	5	-	5	-	50
6	40	-	-	5	5	50

10. After the incubation, add 5 µl of 10x gel loading solution to reaction tubes 3 - 6. Mix.
11. Prepare agarose gel and apply samples to gel analysis as described in the next section.

OPTIONAL STOPPING POINT

DNA samples can be frozen or you may continue with sample preparation and gel analysis.

Module V - Restriction Enzyme Analysis

SEPARATION OF RESTRICTION ENZYME REACTIONS BY ELECTROPHORESIS

- Recommended gel size: 7 x 7 cm or 7 x 15 cm
- Number of sample wells required: 6 per group
- Placement of well-former template: first set of notches
- Agarose gel concentration required: 0.8%

Prepare agarose gels according to previous instructions on page ___.

Equilibrate a waterbath at 65°C for heating the tubes containing Standard DNA fragments before gel loading. At 65°C, non-specific aggregation due to sticky ends generated by restriction enzyme digestions will melt. This will result in sharp individual DNA bands upon separation by agarose gel electrophoresis.

1. Heat the standard DNA fragments (Q) for two minutes at 65°C. Allow the samples to cool for a few minutes.
2. Load samples in consecutive order in the wells.

Lane		
1	25 µl	Standard DNA Fragments (Q)
2	25 µl	supercoiled (nonrecombinant) vector (R)
3	25 µl	restriction enzyme control (recombinant plasmid)
4	25 µl	*Eco* RI digest
5	25 µl	*Pvu* II digest
6	25 µl	*Pvu* II / *Cla* I codigest

3. Set the power source at the required voltage and conduct electrophoresis according to guidelines in Table C, or as determined by your instructor.
4. Allow the tracking dye to migrate 4.5 cm (7 x 7 cm gel) or 6-7 cm (7 x 14 cm gel) from the wells for adequate separation of the DNA bands. Terminate the electrophoresis before the tracking dye moves off the end of the gel.
5. After the electrophoresis is completed, turn off the power, unplug the power source, disconnect the leads and remove the cover.
6. Remove the gel from the bed for staining with InstaStain® Ethidium Bromide.

Table C Time and Voltage
Electrophoresis of DNA

Volts	Recommended Time Minimum	Maximum
125	30 min	45 min
70	40 min	1.5 hrs
50	60 min	2.0 hrs

Module V - Restriction Enzyme Analysis

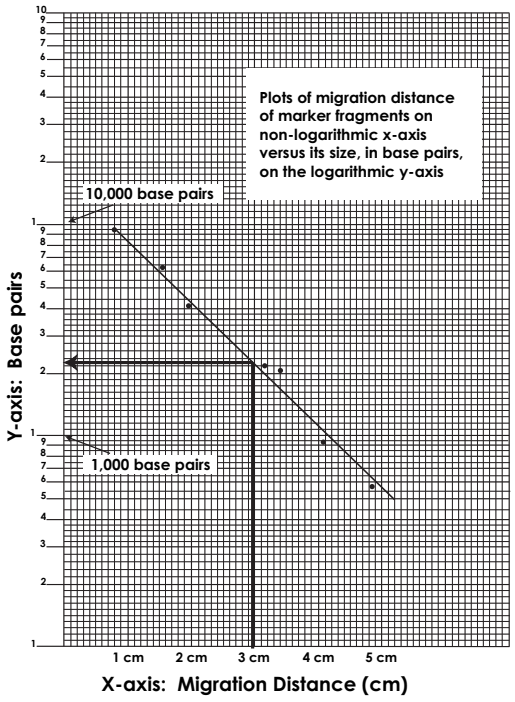
The Experiment

This is the first step for mapping DNA restriction sites, which is to determine the size of the "unknown" DNA fragments generated after electrophoresis. The assignment of sizes for DNA fragments separated by agarose gel electrophoresis can have ± 10% margin of error. The sizes of the "unknowns" will be extrapolated by their migration distances relative to the Standard DNA Fragments (Sample A), for which the size of each fragment is known.

Quick Reference:

Standard DNA fragment sizes - length is expressed in base pairs.

23130	9416	6557
4361	3000	2322
2027	725	570



Example figure

1. Measure and record the distance traveled in the agarose gel by each Standard DNA fragment (except the largest 23,130 bp fragment, which will not fit in a straight line in step 4).

   In each case, measure from the lower edge of the sample well to the lower end of each band. Record the distance traveled in centimeters (to the nearest millimeter).

2. For each Standard DNA fragment, plot the measured migration distance on the x-axis versus its size in base pairs, on the y-axis.

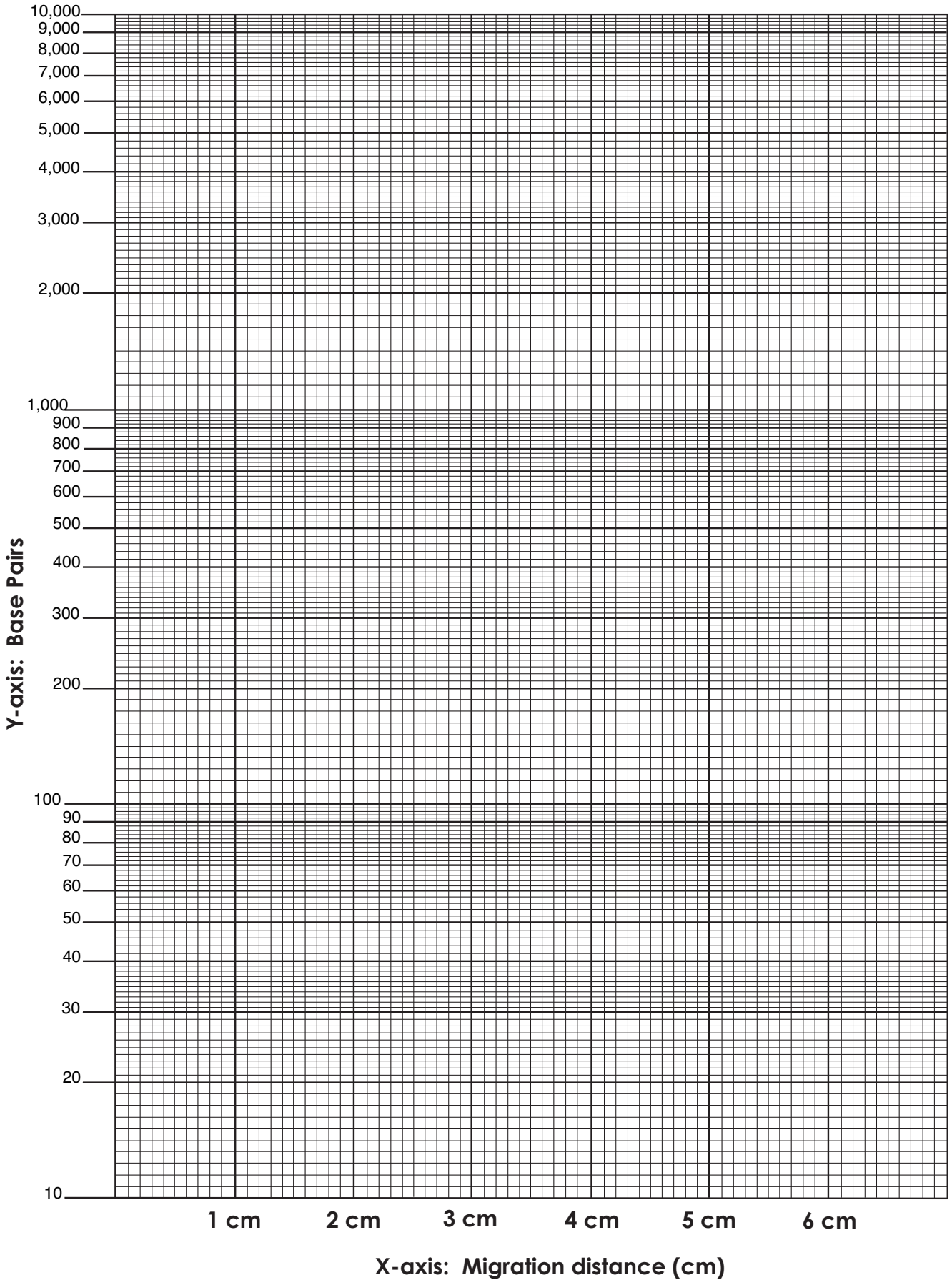
3. Draw the best average straight line through all the points. The line should have approximately equal numbers of points scattered on each side of the line. Some points may be right on the line (see Example figure at left).

4. Measure the migration distance of each of the 3, 4, 5, and 6 fragments from samples.

5. Using the graph of the Standard DNA fragments, determine the sizes in base pairs of each fragment.

   A. Find the migration distance of the fragment on the x-axis - draw a vertical line from that point until the standard graph line is intersected.

   B. From the point of intersection, draw a second line horizontally to the y-axis and determine the approximate size of the fragment in base pairs (refer to example figure at left).


10,000
9,000
8,000
7,000
6,000
5,000
4,000
3,000
2,000
1,000
900
800
700
600
500
400
300
200
100
90
80
70
60
50
40
30
20
10
Y-axis: Base Pairs
1 cm
2 cm
3 cm
4 cm
5 cm
6 cm
X-axis: Migration distance (cm)

Study Questions

Answer the following study questions in your laboratory notebook.

1. Did you observe a discreet band of DNA after the electrophoresis of the ligation reaction products? Explain. Did you observe any bands that migrated faster than the 1300 base pair kan^r fragment? If so, how could these DNA forms have been generated? (Hint: DNA does not always circularize as a relaxed molecule).

2. Which of the following pairs could be ligated together? (All termini are cohesive and complementary.)

   a. 5'-dephosphorylated linear insert DNA + linear vector
   b. Supercoiled vector + linear insert DNA
   c. 5'-dephosphorylated linear vector + linear 5'-dephosphorylated insert DNA
   d. Linear 5'-dephosphorylated vector + linear insert DNA
   e. Nicked vector + linear insert DNA

   In general, which of the above possibilities would be the best approach in a subcloning experiment like the one you have done? Why?

3. Assume the transformants produced with the ligated DNA were also plated on ampicillin medium. Would you expect to see a significant difference in the number of colonies compared to the kanamycin plates? Why? (Hint: the linear vector was not dephosphorylated before the ligation). Why would it be unwise to pick a transformant from an ampicillin plate if you were trying to isolate the recombinant DNA? If you had, is there a step in this series of experiments that would have prevented the propagation of the incorrect plasmid?

4. Did the electrophoretic pattern of your uncut recombinant plasmid contain many forms of DNA like your ligation reaction? Explain.

5. Did your recombinant plasmid have more than one insert? What was the orientation of the insert(s)? Make a rough map of your recombinant plasmid.

6. Can the size of a supercoiled plasmid be calculated by comparison to linear DNA fragments of known size that have been run in parallel?

7. A Kan^r transformant was found to contain the supercoiled pUC vector without an insert in addition to the expected supercoiled recombinant plasmid. How can this be explained?

Instructor's Guide

Notes to the Instructor:

IMPORTANT READ ME!

Transformation experiments contain antibiotics which are used for the selection of transformed bacteria. Students who have allergies to antibiotics such as penicilllin, ampicillin, kanamycin or tetracycine should not participate in this experiment.

ORGANIZING AND IMPLEMENTING THE EXPERIMENT

Class size, length of laboratory sessions, and availability of equipment are factors which must be considered in the planning and the implementation of this experiment with your students.

Prior to commencing this experiment, carefully check that you have all the necessary experiment components and required equipment. Check the lists of Components and Requirements on pages 3 and 4 to ensure that you have a complete inventory to perform the experiment.

The guidelines that are presented in this manual are based on five laboratory groups. The following are implementation guidelines, which can be adapted to fit your specific set of circumstances. If you do not find the answers to your questions in this section, a variety of resources are available at the EDVOTEK web site. In addition, Technical Service is available from 9:00 am to 6:00 pm, Eastern time zone. Call 1-800-EDVOTEK for help from our knowledgeable technical staff.

This experiment has five modules:

I. The ligation of a plasmid vector with a fragment containing the kanamycin resistance gene
II. Introduction of the recombinant DNA into *E. coli* cells by transformation and selection of transformants
III. Picking and Growth of Kan^r Transformants
IV. Extraction of supercoiled recombinant plasmid DNA
V. Restriction enzyme analysis

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Please have the following information:

- The experiment number and title
- Kit Lot number on box or tube
- The literature version number (in lower right corner)
- Approximate purchase date

Instructor's Guide

Notes to the Instructor:

NATIONAL CONTENT AND SKILL STANDARDS

By performing this experiment, students will develop skills necessary to do scientific inquiry, learn new techniques using several types of biotechnology equipment, and will learn standard procedures used in transformation. Analysis of the experiments will provide students the means to transform an abstract concept into a concrete explanation.

Approximate Time Requirements

Module	Pre-Lab	Experiment
I	1 hour	3.5 hrs
II	3 hrs	1 hr
III	2 hrs	15 min
IV	2 hrs	1.5-2 hrs
V	30 min	2.5 hrs

APPROXIMATE TIME REQUIREMENTS FOR THE EXPERIMENT

1. The experiment can be temporarily stopped after the completion of all modules and later resumed. Experimental results will not be compromised if instructions are followed as noted under the heading "Optional Stopping Point" at the end of each module.
2. Module I includes 1 hour for ligation and 1.5 to 2 hours for electrophoresis and gel staining.
3. Module II requires preparation of agar plates, streaking plates for isolated colonies the night before the experiment and approximately 1 hour to perform the transformation experiment. Plates must be incubated overnight to obtain results.
4. In Module III, students will use transformation results from Module II. Broth cultures will be inoculated and grown overnight for use in Module IV.
5. In Module IV, students will perform a mini-prep DNA isolation from the cells grown in Module III. The DNA will be used for restriction enzyme analysis (Module V).
6. Module V includes a 1 hour restriction enzyme analysis and a 1.5 hour electrophoresis and staining step.

PRE-LAB PREPARATIONS

1. Module I pre-lab preparations can be completed on the day that Module 1 is to be conducted.
2. Module II requires preparation of agar plates two days prior to the day of the lab. *E. coli* source plates must be prepared the day before the laboratory and incubated 16-24 hours before the experiment. Other pre-lab preparations should be performed on the day of the laboratory.

Notes to the Instructor:

3. Module III pre-lab preparations should be completed on the day Module III is to be performed.

4. In Module IV, kan^r cells must harvested from liquid growth the day after Module III. Solutions for the extraction of recombinant plasmid must be freshly prepared, preferably within 3 hours of the laboratory.

6. In Module V, restriction enzymes should be prepared within 30 minutes of use. Restriction enzymes and other reagents can be aliquoted and placed on ice.

LABORATORY NOTEBOOKS

It is highly recommended that students maintain a laboratory notebook to formulate hypotheses and to record experimental procedures and results.

- EDVOTEK Cat. # 1401, Laboratory DataBook is recommended.
- Guidelines for keeping a laboratory notebook is available at the EDVOTEK web site.

Pre-Lab Preparations - Module I

MODULE I - LIGATION OF A PLASMID VECTOR WITH A FRAGMENT CONTAINING THE KANAMYCIN RESISTANCE GENE

Enough reagents are provided to perform 5 ligation reactions. You may aliquot the reagents for each lab group as described in step 2. Alternatively, the students can share the stock tubes in a central location. Note that sharing the tubes increases the risk of a spill or contamination.

1. Shortly before the lab begins, thaw and place the water (A) and DNA fragments for ligation (vector and Kan^r insert) (B) on ice:

2. For each lab group, transfer the following volumes into separate, **ice cold** 0.5 ml microtest tubes that are appropriately labeled.

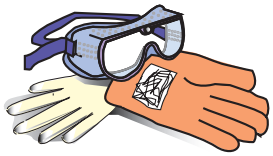
A	50 µl	Water
B	25 µl	DNA fragments (vector and Kan^r insert)

3. Keep the tubes on ice.

4. Each group requires one T4 DNA Ligase Reaction Tube (C).

5. Each group also requires 10 µl of 10x Gel loading solution.

Pre-Lab Preparations - Module II



Wear Hot Gloves and Goggles during all steps involving heating.

MODULE II - INTRODUCTION OF THE RECOMBINANT DNA INTO THE *E. COLI* CELLS BY TRANSFORMATION AND SELECTION OF TRANSFORMANTS

Pour Agar Plates (prior to the Lab experiment)

For optimal results, prepare plates two days prior to plating and set aside the plates inverted at room temperature. If they are poured more than two days before use, they should be stored inverted in the refrigerator. Remove the plates from the refrigerator and store inverted for two days at room temperature before use.

Heat the ReadyPour™ Medium

1. Thaw the kanamycin (D).
2. Equilibrate a water bath at 60°C for step 6 below.
3. Loosen, but **do not** remove, the cap on the ReadyPour medium bottle to allow for the venting of steam during heating.

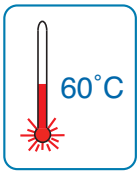
   Caution: Failure to loosen the cap prior to heating or microwaving may cause the ReadyPour medium bottle to break or explode.
4. Squeeze and vigorously shake the plastic bottle to break up the solid agar into chunks
5. Heat the bottle of ReadyPour medium by one of the methods outlined below. When completely melted, the amber-colored solution should appear free of small particles.

   A. Microwave method:
   - Heat the bottle on High for two 30 second intervals.
   - Using a hot glove, swirl and heat on High for an additional 25 seconds, or until all the medium is dissolved.
   - Using a hot glove, occasionally swirl to expedite melting.

   B. Hot plate or burner method:
   - Place the bottle in a beaker partially filled with water.
   - Heat the beaker to boiling over a hot plate or burner.
   - Using a hot glove, occasionally swirl to expedite melting.
6. Allow the melted ReadyPour medium to cool. Placing the bottle in a 60°C water bath will allow the agar to cool, while preventing it from prematurely solidifying.

   When the ReadyPour™ medium reaches approximately 60°C, the bottle will be warm to the touch but not burning hot.

Pre-Lab Preparations - Module II



7. While the ReadyPour medium is cooling, label a total of 20 petri plates. Label these plates on their bottom halves:

   5 plates: HB101
   5 plates: Kan-Control
   10 plates: Kan-Ligation

After the ReadyPour medium has cooled:

8. Pour 8 ml of molten medium into each of the 5 plates labelled HB101. (See Quick Reference: Pouring Agar Plates)

9. Add 0.7 ml of kanamycin (D) to the remaining molten medium with a sterile 1 ml pipet. Swirl the medium to mix. Return the kanamycin to the freezer.

10. Pour 15 plates of medium with kanamycin, 8 ml each. Pour additional plates with any remaining medium for extras.

11. Let the agar cool and resolidify.

    If plates will be used within two days, store at room temperature, inverted without taping.

    If plates are prepared more than two days before use, rewrap in the plastic sleeve and store inverted in the refrigerator.

Quick Reference: Pouring Agar Plates

- Use a sterile 10 ml pipet with a pipet pump to transfer the designated volume of medium to each petri plate. Pipet carefully to avoid forming bubbles.
- Rock the petri plate back and forth to obtain full coverage.
- If the molten medium contains bubbles, they can be removed by passing a flame across the surface of the medium.
- Cover the petri plate and allow the medium to solidify.

Take plates out of the refrigerator and leave inverted at 37°C for several hours prior to use.

Pre-Lab Preparations - Module II

DAY BEFORE THE EXPERIMENT

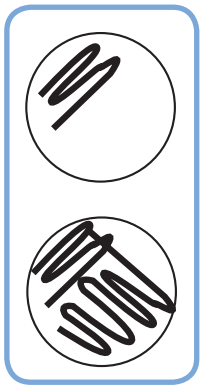
This experiment requires preparation of isolated *E.coli* host transformation colonies 16-24 hours before the laboratory experiment, so plan accordingly.

Important: Do not prepare source plates more than 24 hours before the experiment. Old source plates will compromise the success of the transformation experiment.

Preparation of *E. Coli* Cells

This experiment kit contains a bacterial slant for obtaining isolated colonies for transformation. Store the slant at room temperature. The night before the lab:

1. Use a sterile loop to scrape a small amount of cells from the surface of the slant.
2. Subculture onto growth medium (without antibiotic) by streaking for isolated colonies.
   - Streak the cells into a clean section of the plate.
   - Then streak through the cells once or twice into another clean section of the plate.
3. Cover the plates and label the plates "*E. coli*".
4. Invert the plates and incubate the plates overnight (16-24 hours) at 37°C in an incubation oven.



If growth result on plates is heavy (i.e. few or no isolated colonies), instruct students to touch the toothpick to a small amount of cells.

Pre-Lab Preparations - Module II

Other Preparations for Module II Transformation Experiment:

1. Allow ample time for the equilibration of water baths and incubation ovens.

2. Assemble the plates and materials for 5 lab groups. Each group receives:

   1 HB101 plate (with isolated colonies)
   1 Kan-Ligation plate
   1 Kan-Control plate
   1 tube of 0.05M $CaCl_2$ (1 ml)
   1 tube of control, supercoiled DNA (E) as a positive control for competency, 10 µl of DNA in a 0.5 ml tube labelled "Control".
   1 ligation reaction (tube #1) from Module I
   • sterile inoculating loops
   • qualified water (A)

Pre-Lab Preparations - Module III

MODULE III - PICKING AND GROWTH OF KANAMYCIN TRANSFORMANTS

Preparation of Kanamycin Medium:

Medium should be prepared on the day of the Module III laboratory.

1. Thaw the kanamycin (D).

2. Withdraw 0.25 ml of the kanamycin with a sterile 1 ml pipet

3. Add 0.25 ml of kanamycin to the growth medium. Cap and swirl to mix.

4. Aseptically transfer 10 ml of the medium to each of 5 sterile culture tubes (sterile 50 ml conical tubes). Close the tubes.

   The culture tubes have a substantially larger volume than 10 ml to allow for adequate aeration

Pre-Lab Preparations - Module IV

HARVESTING OF KAN^R CELLS FROM LIQUID GROWTH:

(Harvesting must be done the day after Module III)

1. Centrifuge the cells at 1000-3000 rpm for 15 minutes.

   You may transfer the cells to the appropriate centrifuge tube if required. Sterility is no longer critical. Be sure the lab group number or initials are transferred to the centrifuge tube if this is done.

2. Pour off supernatant.

3. Drain off residual medium by leaving the tubes inverted on paper towels for a few minutes. Take care not to let the cell pellet slip out of the tube.

4. Resuspend each cell pellet in 100 µl of TEG buffer (H). Mix by pipeting, tapping and limited vortexing.

5. Label five (5) 1.5 ml microtest tubes with the student group numbers or initials corresponding to the tubes in which the cells were grown.

6. Transfer the cells from the tube to the corresponding microtest tube using a micro or transfer pipet. Do this for all five, change pipet tips for each use.

If the DNA extractions are going to be done on the same day as the cell harvest (steps 1-6), put the cells on ice or in the refrigerator. If not, freeze them until they are needed.

Pre-Lab Preparations - Module IV

EXTRACTION OF SUPERCOILED RECOMBINANT DNA

The reagents can be aliquoted for each group of students. Alternatively, the reagents can be measured by students directly from the stock preparations - set up a pipetting station with a designated 1 ml pipet for each reagent.

1. Preparation of Cell Lysis Solution
   - In a beaker, add all of the Sodium Hydroxide solution (I) to 8 ml of distilled water.
   - Add all of the SDS solution (J). Mix.
   - Label this "Cell Lysis Solution" and keep at room temperature.
   - Dispense 1.4 ml for each group of students. Label these 5 tubes "Lysis Solution".
2. Dispense 250 µl of Potassium Acetate Solution (M) for each group of students. Label these 5 tubes "Potassium Acetate".
3. Preparation of 1x TE Solution
   - Add 1 ml of Tris Buffer concentrate (L) to 9 ml of distilled water. Mix well.
   - Dispense 100 µl for each group of students. Label these 5 tubes "1x TE".
4. Dispense 10 µl of RNAse Solution (K) for each group of students. Label these 5 tubes "RNAse"
5. Dispense 250 µl of Resuspension Buffer (N) for each group of students. Label these 5 tubes "Resuspension Buffer".

Each group should receive:

1 tube containing 100 µl of resuspended cells
1 clean 1.5 ml snap-top tube

Reagents to be shared by each group of students:

Prepared Cell Lysis Solution	1.4 ml
Potassium Acetate Neutralization Buffer	250 µl
Resuspension Buffer	250 µl
1x TE Solution	100 µl
Isopropanol - 95-100%	1.5 ml
Ethanol - 70%	1.5 ml
RNAse Solution	10 µl
10x Gel Loading Solution	50 µl

Pre-Lab Preparations - Module V

MODULE V -
Restriction Enzyme Analysis

1. Thaw the Restriction Enzyme Reaction Buffer (O), Restriction Enzyme Dilution Buffer (P) Standard DNA Fragments (Q) and Supercoiled Plasmid Vector Standard (R).

2. **Within 30 minutes** of the Module V experiment, remove the restriction enzymes and place them on ice.

3. Gently mix each tube of enzymes by tapping until all of the dense glycerol layer containing enzyme is mixed.

4. Dilute the restriction enzymes:

Change tips after each addition of enzyme to avoid cross-contamination.

- Add 35 µl of **ice cold** dilution buffer (P) to the tube of *Eco* RI (S)
- Add 35 µl of **ice cold** dilution buffer (P) to the tube of *Cla* I (U)
- Add 75 µl of **ice cold** dilution buffer (P) to the tube of *Pvu* II (T).

300	µl	Qualified water (A)	on ice
40	µl	Restriction enzyme reaction buffer (O)	on ice
7	µl	Diluted *Eco* RI	on ice
15	µl	Diluted *Pvu* II	on ice
7	µl	Diluted *Cla* I	on ice
25	µl	Standard DNA Fragments (Q)	
25	µl	Supercoiled plasmid vector standards (R)	

At this point, the enzymes can no longer be stored. They must be used. Keep the tubes on ice.

5. For each lab group, aliquot reagents listed at left into 0.5 ml tubes:

6. Equilibrate a 37°C water bath.

7. Metric rulers and graph paper (semi-log) will be needed if the students are calculating the restriction fragment sizes. However, visual inspection of the gel (or photograph) should be sufficient for adequate size estimates to determine orientation, etc.

Agarose Gel Electrophoresis:

Each group will require a gel with 6 sample wells for electrophoresis: four for the preparation and two for aliquots of standards from tubes Q and R. Samples can be frozen until the electrophoresis run is scheduled.

Instructor's Guide

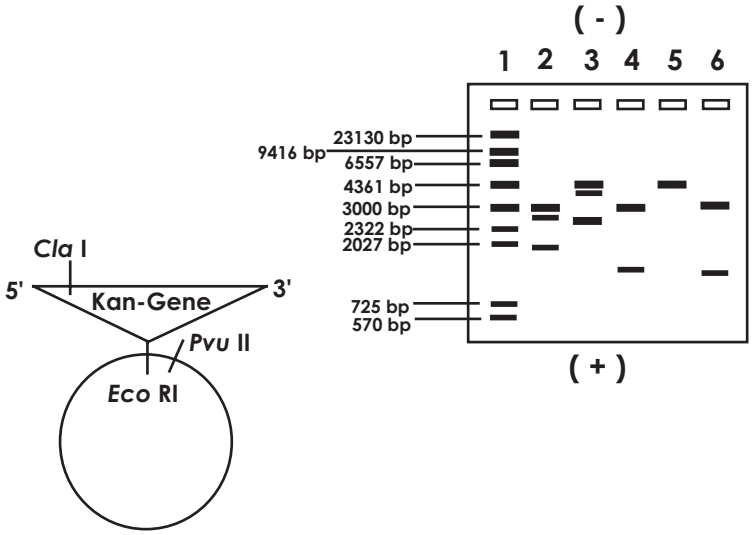
Idealized Schematic of Results

There are at least 4 possible recombinant forms - Examples 1 & 2.

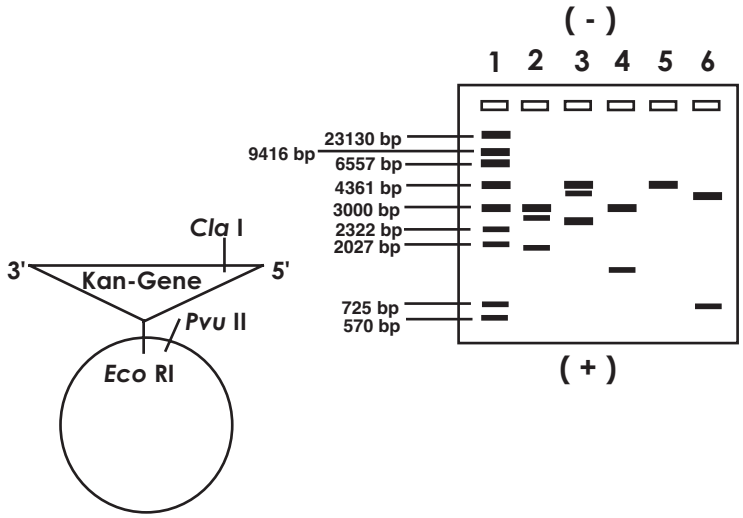
Lane
1 Standard DNA Fragments
2 Plasmid Vector (nonrecombinant)
3 Recombinant Plasmid Vector, uncut (control)
4 Recombinant Plasmid Vector cut with *Eco* RI
5 Recombinant Plasmid Vector cut with *Pvu* II
6 Recombinant Plasmid Vector cut with *Pvu* II and *Cla* I

Additional bands may be visible due to catenated forms.

Example 1: Single Insert 5' - 3' Orientation



Example 2: Single Insert 3' - 5' Orientation



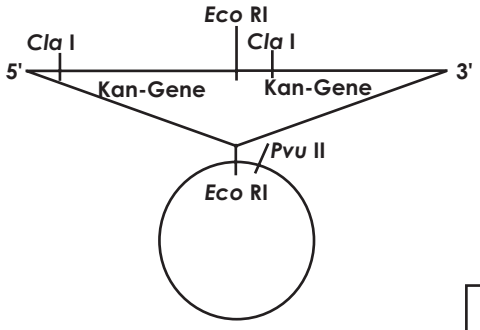
Idealized Schematic of Results

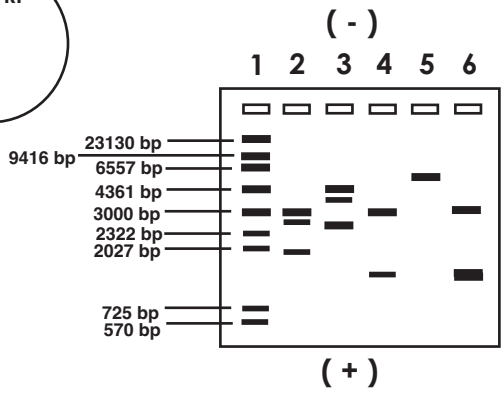
There are at least 4 possible recombinant forms - Examples 3 & 4.

Lane
1 Standard DNA Fragments
2 Plasmid Vector (nonrecombinant)
3 Recombinant Plasmid Vector, uncut (control)
4 Recombinant Plasmid Vector cut with *Eco* RI
5 Recombinant Plasmid Vector cut with *Pvu* II
6 Recombinant Plasmid Vector cut with *Pvu* II and *Cla* I

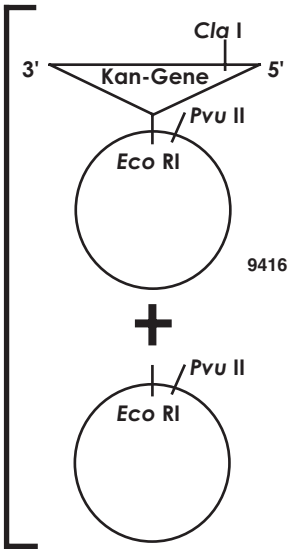
Additional bands may be visible due to catenated forms.

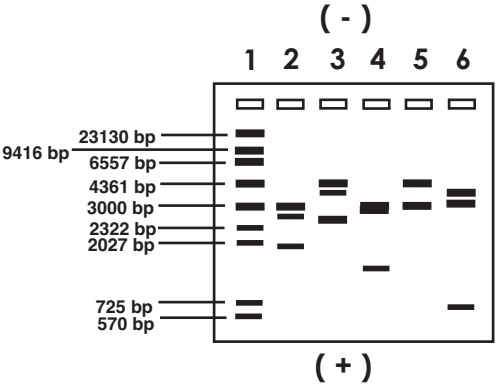
Example 3: Double Insert 5' - 3' Orientation





Example 4: Vector without insert + Recombinant Plasmid with 3' - 5' Single Insert





Please refer to the kit insert for the Answers to Study Questions

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): 301 Component I/NaOH

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: 09-01-05

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)
Sodium Hydroxide	2mg/m^3	2mg/m^3	No data	

Section III - Physical/Chemical Characteristics

Boiling Point	1390°C	Specific Gravity (H_20 = 1)	2.13
Vapor Pressure (mm Hg.)	20°C	Melting Point	318°C
Vapor Density (AIR = 1)	No data	Evaporation Rate (Butyl Acetate = 1)	No data

Solubility in Water: 10%, appreciable

Appearance and Odor: Clear liquid, odorless

Section IV - Physical/Chemical Characteristics

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

Extinguishing Media: Use exstinquishing media appropriate for surrounding fire

Special Fire Fighting Procedures: Wear protective equipment and SCBA. Flood material with water. Do not splatter or splash material.

Unusual Fire and Explosion Hazards: Contact with moisture or water may generate sufficient heat to ignite other materials. Reacts with metals to produce hydrogen gas which can form explosive mixture with air.

Section V - Reactivity Data

Stability			Conditions to Avoid
	Unstable		Moisture
	Stable	X	

Incompatibility: Water, strong acids, combustible materials, organic materials, zinc, aluminum, peroxides

Hazardous Decomposition or Byproducts: None identified

Hazardous Polymerization			Conditions to Avoid
	May Occur		
	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 identified

Carcinogenicity: NTP? IARC Monographs? NO DATA OSHA Regulation?

Signs and Symptoms of Exposure: Ingestion: Severe burns to mouth, throat, and stomach. Inhalation: irritating skin/eye contact: severe irritation or burn.

Medical Conditions Generally Aggravated by Exposure: None identified

Emergency First Aid Procedures: Ingestion: Do not induce vomiting. Give water followed by vinegar, juice, or egg whites. Inhalation: Move to fresh air. Skin/eye contact: flush with water.

Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released for Spilled: Wear SCBA and protective clothing carefully place materials into clean dry container and cover. Dispose of properly.

Waste Disposal Method: Follow all federal, state, and local laws.

Precautions to be Taken in Handling and Storing: Keep container tightly closed. Store in corrosion-proof area. Store in a dry area. Isolate from incompatible materials.

Other Precautions: None

Section VIII - Control Measures

Respiratory Protection (Specify Type): NIOSH/MSHA approved respirator

Ventilation				
	Local Exhaust	Yes	Special	No
	Mechanical (General)	Yes	Other	None

Protective Gloves: Safety gloves

Eye Protection: Safety goggles

Other Protective Clothing or Equipment: Laboratory apron

Work/Hygienic Practices: Avoid 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): 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: 07/01/03

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_20 = 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: 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		
	Will Not Occur	X	None

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				
	Local Exhaust		Special	
	Mechanical (General)	Gen. dilution ventilation	Other	

Protective Gloves: Yes

Eye Protection: Splash proof goggles

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

Work/Hygienic Practices: None

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): Deproteinization Matrix

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: 09-01-05

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)
Trace amounts of diamines may be present.				

Section III - Physical/Chemical Characteristics

Boiling Point	Aprox. 212 °F	Specific Gravity (H_20 = 1)	No data
Vapor Pressure (mm Hg.)	N/A	Melting Point	N/A
Vapor Density (AIR = 1)	N/A	Evaporation Rate (Butyl Acetate = 1)	N/A

Solubility in Water: Dispersed solid-phase supplied is aqueous buffer

Appearance and Odor: Faint yellow suspension. Faint acetic acid (vinegar) odor

Section IV - Physical/Chemical Characteristics

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

Extinguishing Media: Use exstinquishing media appropriate for surrounding fire

Special Fire Fighting Procedures: Use SCBA if exposed to high levels of dust and smoke

Unusual Fire and Explosion Hazards: None

Section V - Reactivity Data

Stability			Conditions to Avoid
	Unstable		
	Stable	X	

Incompatibility: No data

Hazardous Decomposition or Byproducts: No data

Hazardous Polymerization			Conditions to Avoid
	May Occur		
	Will Not Occur	X	

Section VI - Health Hazard Data

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

Health Hazards (Acute and Chronic): If sensitive, physiological effects may include irritation to eyes, respiratory sys. and skin.

Carcinogenicity: NTP? IARC Monographs? NO DATA OSHA Regulation?

Signs and Symptoms of Exposure: No data

Medical Conditions Generally Aggravated by Exposure: No data

Emergency First Aid Procedures: No particular measures after spillage/leakage of small amounts

Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released for Spilled: Wipe surface with detergents

Waste Disposal Method: Material is non-hazardous. Dispose in authorized industrial landfill

Precautions to be Taken in Handling and Storing: If skin is sensitive, wear protective gloves. Work in a well ventilated area.

Other Precautions: The above information is believed to be correct but does not purport to be all inclusive.

Section VIII - Control Measures

Respiratory Protection (Specify Type): No

Ventilation				
	Local Exhaust	No	Special	No
	Mechanical (General)	No	Other	None

Protective Gloves: Safety gloves Eye Protection: Safety goggles

Other Protective Clothing or Equipment: No

Work/Hygienic Practices: Avoid 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): **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: 07/01/03

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_20 = 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		
	Will Not Occur	X	None

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

Protective Gloves: Yes Eye Protection: Safety goggles

Other Protective Clothing or Equipment: None

Work/Hygienic Practices: None

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): 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: 07/01/03
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:	NTP?	IARC Monographs?	OSHA Regulation?
None	No data	No data	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: yes
Eye Protection: 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.

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: 07/01/03
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			Conditions to Avoid
	Unstable		None
	Stable	X	

Incompatibility: Strong oxidizing agents

Hazardous Decomposition or Byproducts: Carbon monoxide, Carbon dioxide, nitrogen oxides, hydrogen bromide gas

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): 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			Special	
	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): Kanamycin Sulfate

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: 09-01-05

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)
Kanamycin Monosulfate from streptomyces CAS # 25389-94-0				

Section III - Physical/Chemical Characteristics

Boiling Point	N/A	Specific Gravity (H_20 = 1)	No data
Vapor Pressure (mm Hg.)	N/A	Melting Point	121°C
Vapor Density (AIR = 1)	N/A	Evaporation Rate (Butyl Acetate = 1)	N/A

Solubility in Water: Water-soluble

Appearance and Odor:

Section IV - Physical/Chemical Characteristics

Flash Point (Method Used)	Flammable Limits	LEL	UEL
N/A	N/A	N/A	N/A

Extinguishing Media: Water spray

Special Fire Fighting Procedures: Wear SCBA and protective clothing

Unusual Fire and Explosion Hazards: Emits toxic fumes under fire conditions

Section V - Reactivity Data

Stability			Conditions to Avoid
	Unstable		
	Stable	X	

Incompatibility: Toxic fumes of carbon monoxide, carbon dioxide, nitrogen oxide

Hazardous Decomposition or Byproducts:

Hazardous Polymerization			Conditions to Avoid
	May Occur		
	Will Not Occur	X	

Section VI - Health Hazard Data

Route(s) of Entry: Inhalation? N/A; Skin? Yes; Ingestion? Yes

Health Hazards (Acute and Chronic): Harmful if swallowed or absorbed through skin.

Carcinogenicity: NTP?; IARC Monographs? N/A; OSHA Regulation?

Signs and Symptoms of Exposure: Prolonged or repeated exposure may cause an allergic reaction.

Medical Conditions Generally Aggravated by Exposure: None

Emergency First Aid Procedures: In case of contact with eye or skin flush with water. If swallowed wash out with water.

Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released for Spilled: Wear gloves, wash area after complete.

Waste Disposal Method: Observe federal, state, and local laws.

Precautions to be Taken in Handling and Storing: Wear protective gloves and safety goggles.

Other Precautions: None

Section VIII - Control Measures

Respiratory Protection (Specify Type): N/A

Ventilation				
	Local Exhaust	N/A	Special	N/A
	Mechanical (General)	N/A	Other	N/A

Protective Gloves: Yes; Eye Protection: Yes

Other Protective Clothing or Equipment: N/A

Work/Hygienic Practices: N/A

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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): Potassium Acetate

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: 09-01-05

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)
Potassium Acetate $C_2H_3KO_2$	No data	No data	No data	No data

Section III - Physical/Chemical Characteristics

Boiling Point	No data	Specific Gravity (H_20 = 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: 200% at 20°C

Appearance and Odor: Clear liquid, vinegar-like 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: Move container from fire area if possible. Avoid breathing vapors

Unusual Fire and Explosion Hazards: None

Section V - Reactivity Data

Stability			Conditions to Avoid
	Unstable		None
	Stable	X	

Incompatibility: None

Hazardous Decomposition or Byproducts: Thermal decomposition may release smoke and irritating fumes.

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): Moderately toxic by ingestion

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

Signs and Symptoms of Exposure: May cause skin/eye irritation. May cause nausea, sore throat, coughing, and abdominal pain

Medical Conditions Generally Aggravated by Exposure: Unknown

Emergency First Aid Procedures: Induce vomiting if ingested. For skin/eye contact, flush with large amounts of water.

Section VII - Precautions for Safe Handling and Use

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

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

Precautions to be Taken in Handling and Storing: Wear eye protection

Other Precautions: None

Section VIII - Control Measures

Respiratory Protection (Specify Type): SCBA with full facepiece

Ventilation				
	Local Exhaust	No	Special	None
	Mechanical (General)	Gen. dilution vent.	Other	None

Protective Gloves: None; Eye Protection: Splash proof goggles

Other Protective Clothing or Equipment: Not required

Work/Hygienic Practices: Avoid 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): RNAse (DNAse-Free)

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: 09-01-05

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)
Nuclease, ribo ~~CAS # 9001-99-4~~				

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: Clear liquid, no odor

Section IV - Physical/Chemical Characteristics

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 SCBA and protective clothing to prevent contact with skin and eyes.

Unusual Fire and Explosion Hazards: None

Section V - Reactivity Data

Stability	Unstable		Conditions to Avoid
	Stable	X	None

Incompatibility: None

Hazardous Decomposition or Byproducts: None

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

Section VI - Health Hazard Data

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

Health Hazards (Acute and Chronic): Prolonged or repeated exposure may cause allergic reaction in some individuals.

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

Signs and Symptoms of Exposure: Unknown

Medical Conditions Generally Aggravated by Exposure: Unknown

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: Follow all federal, state, and local regulations.

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

Other Precautions: None

Section VIII - Control Measures

Respiratory Protection (Specify Type): NIOSH-MSHA approved respirator

Ventilation	Local Exhaust	No	Special	None
	Mechanical (General)	Yes	Other	None

Protective Gloves: Chemical resistant

Eye Protection: Splash proof goggles

Other Protective Clothing or Equipment: Yes

Work/Hygienic Practices: Avoid contact and inhalation

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): Sodium Dodecyl Sulfate (SDS)

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: 09-01-05

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)
Lauryl Sulfate, Sodium	No data	No data	No data	No data
$C_{12}H_{26}O_4S$ CAS# 151-21-3				

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: Clear liquid, no odor

Section IV - Physical/Chemical Characteristics

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

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

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

Unusual Fire and Explosion Hazards: May emit toxic fumes.

Section V - Reactivity Data

Stability	Unstable		Conditions to Avoid
	Stable	X	None

Incompatibility: Strong oxidizing agents

Hazardous Decomposition or Byproducts: Carbon monoxide, carbon dioxide, sulfur oxides

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

Section VI - Health Hazard Data

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

Health Hazards (Acute and Chronic): May cause irritation to eyes, ears and nose.

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

Signs and Symptoms of Exposure: Respiratory tract: burning sensisation, coughing, wheezing, laryngitis, shortness of breath, & headache

Medical Conditions Generally Aggravated by Exposure: No data

Emergency First Aid Procedures: Flush skin/eyes with large amounts of water. If inhaled, remove to fresh air.

Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released for Spilled: Evacuate area. Wear SCBA, rubber boots and rubber gloves. Mop up with absorptive material and burn in chemical incinerator equipped with an afterburner and scrubber.

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

Precautions to be Taken in Handling and Storing: Wear protective gear. Avoid contact/inhalation.

Other Precautions: Strong sensitizers

Section VIII - Control Measures

Respiratory Protection (Specify Type): NIOSH/MSHA approved respirator.

Ventilation	Local Exhaust	No	Special	Chem. fume hood
	Mechanical (General)	No	Other	None

Protective Gloves: rubber

Eye Protection: Splash proof googles

Other Protective Clothing or Equipment: Rubber boots

Work/Hygienic Practices: Avoid prolonged or repeated exsposure.

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): Tris-EDTA Buffer (TE)

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: 09-01-05

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)
CAS # 139-33-3	---------- No data ----------			

Section III - Physical/Chemical Characteristics

Boiling Point	No data	Specific Gravity (H_20 = 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: Clear, no odor

Section IV - Physical/Chemical Characteristics

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

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

Special Fire Fighting Procedures: Move container from fire area if possible

Unusual Fire and Explosion Hazards: Thermal decomposition products may include toxic and hazardous oxides of carbon, nitrogen, and sodium.

Section V - Reactivity Data

Stability			Conditions to Avoid
	Unstable		
	Stable	X	Excessive heat, sparks or open flame

Incompatibility: Acids, aluminum, metals, oxidizers (strong)

Hazardous Decomposition or Byproducts: Thermal decomposition products of toxic and hazardous oxides of C, N, & Na

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

Section VI - Health Hazard Data

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

Health Hazards (Acute and Chronic): Moderately toxic by ingestion. Systemic toxicity may result. May chelate lead magnesium, zinc, trace metals if present in intestine poss. causing incr.absorption.

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

Signs and Symptoms of Exposure: Mucous membrane irritation, eye/skin irritation, irritating to gastrointestinal system.

Medical Conditions Generally Aggravated by Exposure: Renal or heart disease, potassium deficiency, insulin dependent, diabetes, seizures or intracranial lesions.

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 absorptive material. Containerize to dispose or properly

Waste Disposal Method: Observe federal, state, and local laws.

Precautions to be Taken in Handling and Storing: Stores away from strong oxidizers or heat. Avoid skin/eye contact.

Other Precautions: None

Section VIII - Control Measures

Respiratory Protection (Specify Type): Chemical cartridge respirator with full facepiece and organic vapor cartridge

Ventilation				
	Local Exhaust	Yes	Special	None
	Mechanical (General)	Vent. Sys.	Other	None

Protective Gloves	Yes	Eye Protection	Splash proof goggles

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

Work/Hygienic Practices: Emergency eye wash should be available

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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): Tris-Glucose EDTA 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: 09-01-05

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)
Ethylenediaminetetraacetic acid	No data	No data	No data	No data
C10-H14-08-N2.2Na				
CAS # 139-33-3				

Section III - Physical/Chemical Characteristics

Boiling Point	No data	Specific Gravity (H_20 = 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: Clear liquid, no odor

Section IV - Physical/Chemical Characteristics

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

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

Special Fire Fighting Procedures: Move container from fire area if possible.

Unusual Fire and Explosion Hazards: Thermal decomposition products may include toxic and hazardous oxides of carbon, nitrogen, and sodium.

Section V - Reactivity Data

Stability			Conditions to Avoid
	Unstable		
	Stable	X	Excessive heat, sparks or open flame

Incompatibility: Acids, aluminum, metals, oxidizers (strong)

Hazardous Decomposition or Byproducts: Thermal decomposition products of toxic and hazardous oxides of carbon, nitrogen, & sodium

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

Section VI - Health Hazard Data

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

Health Hazards (Acute and Chronic): Moderately toxic by ingestion. Systemic toxicity may result. May chelate lead magnesium,

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

Signs and Symptoms of Exposure: Mucous membrane irritation, eye/skin irritation, irritating to gastrointestinal system.

Medical Conditions Generally Aggravated by Exposure: Renal or heart disease, potassium deficiency, insulin dependent, diabetes, seizures or intracranial lesions.

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 absorptive material. Containerize to dispose or properly

Waste Disposal Method: Observe federal, state, and local laws.

Precautions to be Taken in Handling and Storing: Stores away from strong oxidizers or heat. Avoid skin/eye contact.

Other Precautions: None

Section VIII - Control Measures

Respiratory Protection (Specify Type): Chemical cartridge respirator with full facepiece and organic vapor cartridge

Ventilation				
	Local Exhaust	Yes	Special	None
	Mechanical (General)	Dilution Vent. Sys.	Other	None

Protective Gloves	Yes	Eye Protection	Splash proof goggles

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

Work/Hygienic Practices: Emergency eye wash should be available