G-Biosciences ♦ 1-800-628-7730 ♦ 1-314-991-6034 ♦ technical@GBiosciences.com

A Geno Technology, Inc. (USA) brand name

Electroelution

Teachers Handbook

(Cat. # BE-602)

MATERIALS INCLUDED WITH THE KIT

This kit has enough materials and reagents for 24 students (six groups of four students).

- 1 pack Agarose
- 1 vial DNA Stain (500X)
- 4 vials DNA Ladder (1kb)
- 1 vial DNA Loading Buffer (6X)
- 1 bottle TAE Buffer (50X)
- 30 Centrifuge Tubes (2ml)
- 12 GeneCAPSULE™ Electroelution Devices

SPECIAL HANDLING INSTRUCTIONS

- Store DNA ladder (1kb) frozen at -20°C.
- DNA Loading Buffer (6X) store at 4°C
- All other reagents can be stored at room temperature.

The majority of reagents and components supplied in the BioScience Excellence™ kits are non toxic and are safe to handle, however good laboratory procedures should be used at all times. This includes wearing lab coats, gloves and safety goggles.
For further details on reagents please review the Material Safety Data Sheets (MSDS).

The following items need to be used with particular caution.

Part #	Name	Hazard
D161	DNA Stain (500X)	Flammable

ADDITIONAL EQUIPMENT REQUIRED

- Agarose Electrophoresis Equipment

TIME REQUIRED

- **Day 1**: 3 hours

AIMS

- Separate DNA fragments by agarose electrophoresis.
- Purify a gene of interest from the agarose gel.

BACKGROUND

Electrophoresis is routinely used as an analytical tool for the examination of DNA, RNA and protein; however an equally important use is in the separation and purification of the respective molecules.

Several methods exist for the purification of DNA, RNA and protein fragments from agarose gels (DNA and RNA) or polyacrylamide gels (Protein). The choice of technique is a matter of preference and cost, but a highly versatile technique that is compatible with DNA, RNA and protein is electroelution.

Electroelution of DNA or RNA from agarose or protein from polyacrylamide uses a current to migrate the molecules out of the gel matrix. Originally, the excised piece of gel is normally placed into a small piece of dialysis tubing, an electric current is passed across the dialysis tubing that causes the molecules of interest to migrate out of the gel piece. The purified molecules can then be removed from the dialysis tubing. Today several specialized apparatus based on this technique are routinely available, which allow for easier excision and electroelution of the DNA. G-Biosciences manufactures GeneCAPSULE™, an electroelution device for rapid purification of proteins and nucleic acids from electrophoresis gels. Figure 1 depicts a schematic of how the G-CAPSULE™ works.

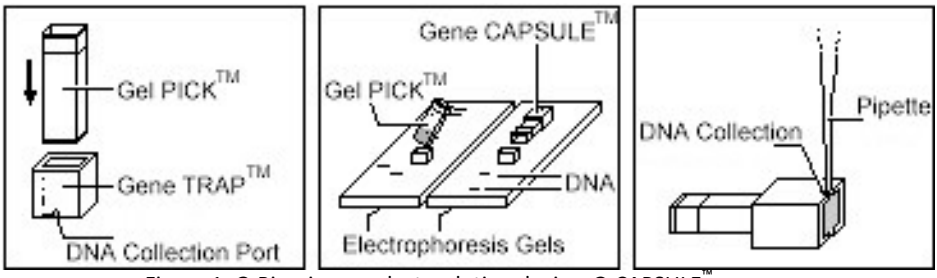


Figure 1: G-Biosciences electroelution device, G-CAPSULE™

Electroelution is routinely used to elute RNA from agarose gels and proteins from polyacrylamide gels.

This kit allows students to understand the electroelution principle and will use an electroelution device to elute DNA fragments from an agarose gel.

TEACHER'S PRE EXPERIMENT SET UP

Preparation of agarose gel

Make 1-2 hours before the experiment.

Wear heat protective gloves throughout the agarose melting and pouring procedure.

1. Prepare running buffer: In a clean two liter container, add the entire contents of the 50X running buffer and add two liters of ultra pure water to make a 1X running buffer solution. Stir until thoroughly mixed.
2. Supply each group with 2ml 1X running buffer.
3. Prepare agarose: Two gels are required; one to separate the DNA fragments for electroelution and one to examine the electroeluted products. 2 wells are required for each group. In a clean, glass 1000ml container add the entire contents of the agarose pack and add 500ml of the running buffer from step 1.
4. Heat the solution in a microwave on full power, using 10 second bursts, or use a boiling waterbath. Check to see if all the agarose has dissolved. Continue until agarose has dissolved.

   DO NOT BOIL. The agarose gets very hot, very quickly and can cause severe burns. Wear heat protective gloves throughout the melting and pouring procedure.
5. Once the agarose has cooled to the point it can be held comfortably in your hand, pour the agarose into the gel casting mould as per the manufacturer's instructions.
6. Once the gels have set, remove the comb, transfer to the running apparatus and cover with the running buffer until ready to use.

PREPARE DILUTE DNA STAINING SOLUTION

The DNA stain solution is a safe alternative to the carcinogenic ethidium bromide routinely used in laboratories. Ethidium bromide is more sensitive, but is a strong carcinogen and requires exposure to UV radiation. The DNA stain contains methylene blue.

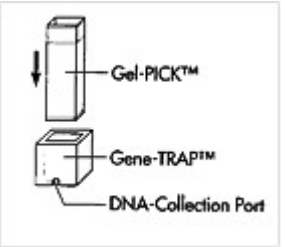
1. Add 100ml 1X TAE buffer to a 1 liter container and add 900ml deionized water to make a 0.1X TAE buffer. Stir until thoroughly mixed. The 0.1X TAE buffer is used to dilute the DNA Stain and also as the destaining solution.
2. To 500ml 0.1X TAE buffer, add 1ml 500X DNA Stain. Stir until thoroughly mixed.

PREPARE THE REFERENCE MARKERS

1. Add 25µl ultra pure water to each the lyophilized 1kb DNA ladder, dissolve by
2. Add 5µl 6X Loading Buffer, mix by gently pipetting up and down 5-6 times. Supply each group with 20µl DNA Ladder.

PREPARE THE GENECAPSULE™

1. Disassemble the GeneCAPSULE unit and fill the Gene-TRAP™ with ~ 500µl TAE buffer. Submerge both Gel-PICK™ and Gene-TRAP™ in TAE buffer for 10-15 minutes to wash the components and to equilibrate its membrane.



2. Take out the Gel-PICK™ and Gene-TRAP™ from the buffer and wash the membrane of the Gene-TRAP™ 2-3 times with TAE buffer.
3. Washed Gene-TRAP™ can be kept in TAE buffer until use. If not used right away, store it at 4°C in 0.5% sodium azide solution.
4. Just before use, place the Gene-TRAP™ in a small dish and fill with TAE buffer.

MATERIALS FOR EACH GROUP

Supply each group with the following components. Several components are shared by the whole class and should be kept on a communal table.

- 20µl DNA Ladder
- 1 vial Running Buffer
- 2 centrifuge tubes (2ml)
- 2 GeneCAPSULE™ Electroelution Device (each includes GeneCAPSULE™, 1 pin, 1 plunger, 1 Pipette Tip)

PROCEDURE

This kit is designed for each group of 4 to work as two pairs.

1. Each group of 4 loads 20µl DNA Ladder onto a 1% agarose gel as instructed by your supervisor. If possible leave at least one empty well between samples for easier excision.
2. Once the samples are all loaded apply a current and migrate at 12-15V/cm. For an 8cm long gel run at 96-120 volts. Once the blue dye front has migrated ¾ the length of the gel, turn off the power and carefully transfer the gel to a staining tray.

CAUTION: Agarose gels are very fragile, handle with extreme care.

3. Add sufficient diluted DNA Stain to cover the gels and place on a **slow** (less than 60rpm) shaker for 1-4 hours at room temperature or overnight at 4°C.

If shaker is too fast the gels will break. As an alternative, leave the gel at room temperature for 2-4 hours or overnight at 4℃ without shaking.

4. If DNA bands are hard to see after staining, due to a high background, then destain with 0.1X running buffer (0.1X TAE) for 30-60 minutes. To help visualize bands, place gel on a sheet of white paper.
5. Taking it in turns, each pair of students uses the gel pick (Figure 1) to carefully excise a band from the DNA Ladder.

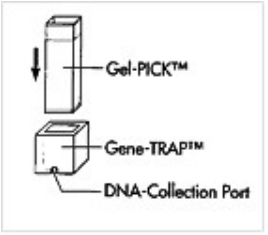


Figure 1: GeneCAPSULE™

6. Using the cutting edge of the gel pick push into the agarose gel until it hits the gel tray (Figure 2 (1)). Remove by tilting and pulling up (Figure 2 (2&3))

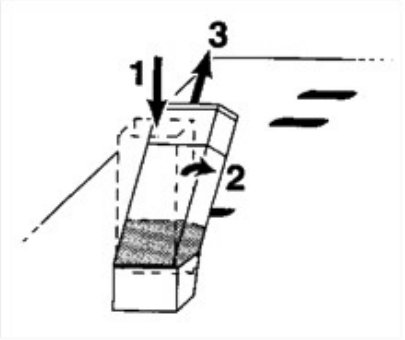


Figure 2: How to use gel pick.

7. Make sure the Gene-TRAP™ is filled with Running Buffer. If not, fill with Running Buffer.
8. Insert the Gel-PICK™, cutting edge first, into the Gene-TRAP™. Push until it locks in place.
9. Ensure there are no air bubbles in the Gene-TRAP™. Remove any air bubbles by pipetting buffer into the GeneCAPSULE™.
10. Using the plunger provided, slowly push the gel piece close to the membrane, so that the gel piece is uniformly in contact with the membrane.

Do not push the plunger too hard or far as you may damage the gel piece of more importantly the membrane.

11. Fill the Gel-PICK™ with Running Buffer.

Make sure there is no air bubbles trapped in the Gel-PICK™.

12. Submerge the assembled GeneCAPSULE™ in the Running Buffer on top of the gel bed of the electrophoresis box such that the Gene-TRAP™ is facing the (+)-terminal (red color terminal) (Figure 3). Add buffer into the gel electrophoresis box just high enough to cover the Gel-PICK™. Remove some buffer if the GeneCAPSULE™ floats.

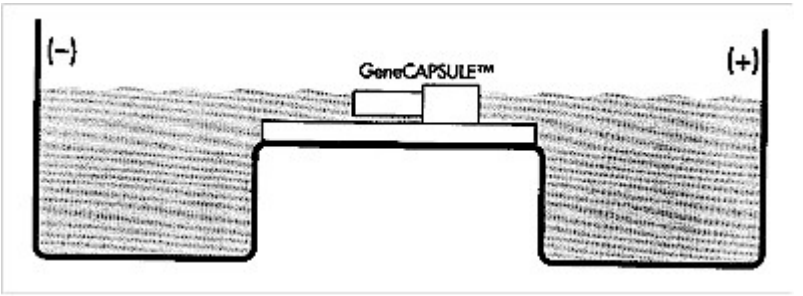


Figure 3: GeneCAPSULE™ Orientation.

13. Turn on the power supply and run the power at 215 volts for 1 minute.
14. Following the 1 minute electroelution, reduce the power to 120 volts and immediately turn off the power. Reverse the polarity of the current by placing the red power lead into the black terminal and the black lead into the red terminal of the powerpack.
15. Turn on the power for 15-20 seconds to loosen the DNA from the membrane. Turn off the power.
16. Immediately, holding the GeneCAPSULE™ horizontal, insert the provided pipette tip, until you reach the gel piece and remove the free TAE buffer from the Gel-PICK™ (Figure. 4). Any buffer left in the Gel-PICK™ will be extracted with the eluted DNA and will result in an increase in the elution volume and dilution of your sample.

Be careful not to hit or disturb the gel piece in the GeneCAPSULE™. If the gel band is disturbed, the DNA in the vicinity of the membrane may flow backward and be lost.

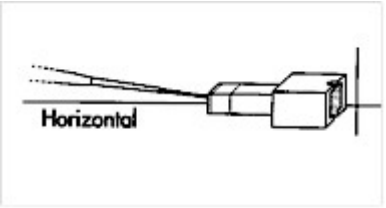


Figure 4: Remove excess running buffer.

17. Place the GeneCAPSULE™ on a dry surface. Puncture a small hole in the membrane at the DNA collection port (see Figure5) with provided pin. Do not force the pin downward into the membrane; this will damage the membrane and spill the DNA.

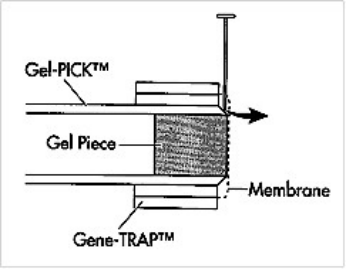


Figure 5: Pierce the membrane.

18. Pre-set a pipettor to 50-60µl, and insert the provided capillary pipette tip through the hole in the membrane and gently push the tip downward (Figure 6). The capillary tip slides over the cutting edge (slope) of the punch. The tip pushes the wet membrane forward as it moves downward into the collection port.

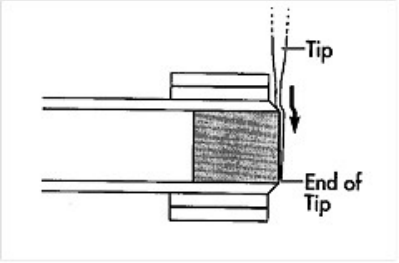


Figure 6: Insert capillary tip.

19. Pipette out the DNA collected near the membrane with the capillary pipette tip and transfer to a clean tube.
20. Add 10µl DNA loading to the buffer and load 20-30µl of the eluted DNA onto a 1% agarose gel.
21. Migrate and stain the agarose gel as in steps 2-4.

RESULTS, ANALYSIS & ASSESSMENT

What is the role of the membrane on the GeneCAPSULE™?

The membrane allows liquid to pass through, but acts as a barrier to stop the DNA escaping.

Describe your results.

A single band should be visualized on the agarose gel.

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A Geno Technology, Inc. (USA) brand name

Ion Exchange Chromatography

Student's Handbook

(Cat. # BE-415)

AIMS

- Separate DNA fragments by agarose electrophoresis.
- Purify a gene of interest from the agarose gel.

BACKGROUND

Electrophoresis is routinely used as an analytical tool for the examination of DNA, RNA and protein; however an equally important use is in the separation and purification of the respective molecules.

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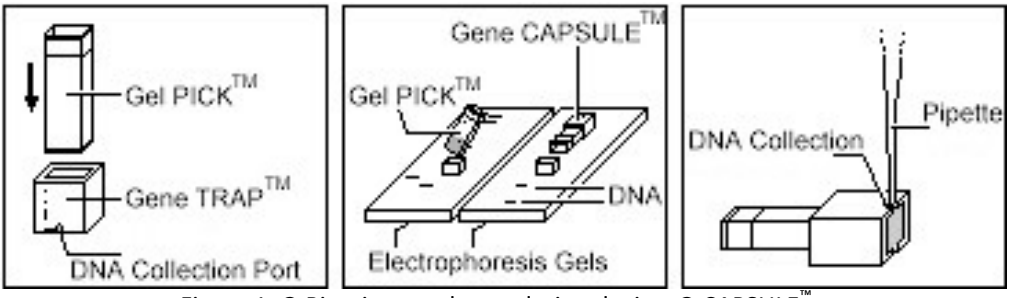

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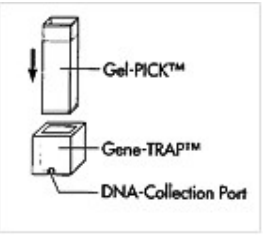
   Remove by tilting and pulling up (Figure 2 (2&3))



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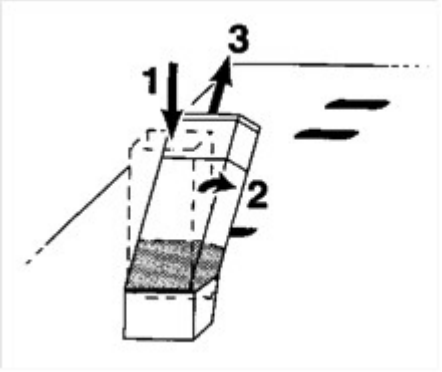

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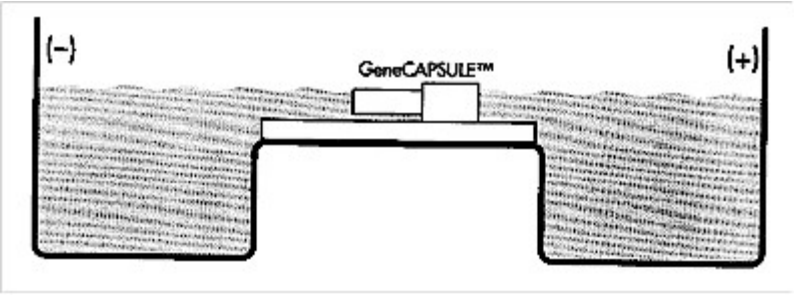

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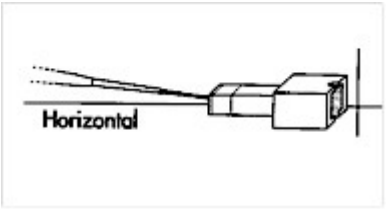

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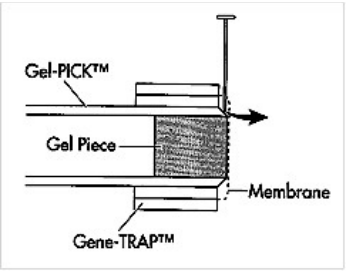

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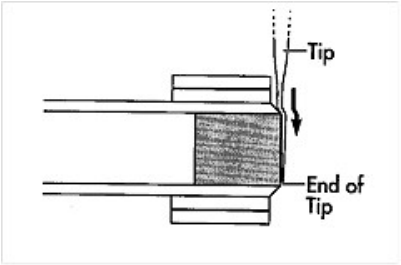

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RESULTS, ANALYSIS & ASSESSMENT

1. What is the role of the membrane on the GeneCAPSULE™?

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2. Describe your results.

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