GeneCAPSULE™

[Also known as G-CAPSULE]

For Extracting Nucleic Acids and Proteins from Gel

PRINCIPLE

GeneCAPSULE™ is an electro-elution tool for rapid recovery of PCR products, DNA fragments and proteins from agarose and polyacrylamide gels. *GeneCAPSULE*™ consists of mainly two parts, *Gel-PICK*™ - for picking DNA or protein band from the gel, and the *Gene-TRAP*™, which traps the migrating DNA or protein during electroelution. After excising DNA or protein band from the gel with *Gel- PICK*™, the *GeneCAPSULE*™ assembly is then placed in a gel box with current applied. Under the influence of an electrical field, DNA and protein molecules migrate toward the positive (+) terminal of the electrophoresis apparatus and captured by *Gene-TRAP*™ and become bound to the membrane (See Fig 1).

Electroelution through *GeneCAPSULE*™ is a gentle method that eliminates the risks of damage to DNA or protein samples commonly encountered in use of glass milk, spin columns, binding columns and other popular techniques. The *GeneCAPSULE*™ method is simple to perform and eliminates additional steps involving washing, spinning, heating or precipitation etc. The recovered DNA is of highest quality for use in molecular manipulations such as ligation, restriction enzyme digestion, sequencing, amplification, random priming, and other enzymatic reactions.

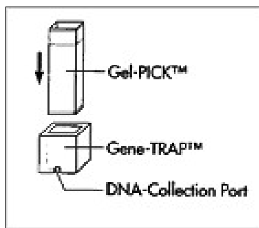

Fig. 1 *GeneCAPSULE*™ *consists of two parts:* ***Gel- PICK***™*- for picking DNA or protein band from the gel, and* ***Gene-TRAP***™*- for trapping migrating DNA or protein during electroelution.*

KIT COMPONENTS

	Cat# 786-001
GeneCAPSULE™ * *(Gel-PICK™+Gene-TRAP™)*	55
Capillary Pipette Tip	55
Pin	55
Plunger	1

ADDITIONAL ITEMS NEEDED

1. Horizontal electrophoresis apparatus
2. TAE buffer for DNA

***GeneCAPSULE*™Accessory Kit (Cat. # 786-004)**– Supplied separately; It prevents *GeneCAPSULE*™ from floating as shown in Fig. 1. The accessory kit consists of one ***GeneCAPSULE*™-Weight** and **plastic Forceps**. The Weight is designed to trap *GeneCAPSULE*™ when submerged under buffer and prevents it from floating or changing direction during electroelution. Forceps can be used for manipulation of *GeneCAPSULE*™ in and out of buffer tank.

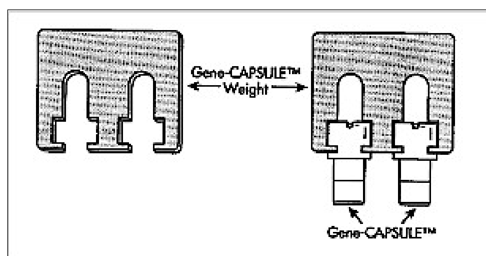

Fig. 1

PROTOCOL SUMMARY
I. Washing: Wash *GeneCAPSULE*™ thoroughly in elution buffer. **II. Excising and Picking up a Gel Band:** Use *Gel-PICK*™ to punch out the gel band. **III. Assembling *GeneCAPSULE*™:** Fill *Gene-TRAP*™ with elution buffer. Assemble *Gel-PICK*™ and *Gene-TRAP*™ together. Use the plunger to push the gel piece close to the membrane. Fill Gel-PICK™ with elution buffer. **IV. Electroeluting DNA:** Submerge *GeneCAPSULE*™ in the gel tank and apply desired current for electroelution. **V. Recovering the Electroeluted DNA:** Reverse current for 10-20 seconds. Remove the entire buffer from *Gel-PICK*™. Puncture a small hole in the DNA-Collection Port, insert the capillary tip and remove the DNA collected against the membrane. The collected DNA is ready for most uses. Read the detailed protocol in the following pages.

PROTOCOL

DNA ELECTROELUTION

Important Notes:

1. *If you are using GeneCAPSULE™ for the first time, we recommend performing a few trial runs on molecular weight marker bands to become familiar with the GeneCAPSULE™ protocol. The critical steps are: (a) removing the free buffer from the Gel- PICK™, and (b) removing the eluted sample from the collection port of the Gene-TRAP™.*
2. *Use TAE buffer as elution buffer for DNA samples to ensure that subsequent ligation, restriction or sequencing reactions are successful. TBE buffer is known to inhibit T4 DNA Ligase and may inhibit the enzyme that catalyzes other recombinant reactions. However, TBE and any other buffer of your choice can also be used, if the enzyme inhibition is not a concern.*

I. WASHING THE *Gene*CAPSULE™ COMPONENTS:

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*™ on a clean surface and fill it with TAE buffer or elution buffer of your choice until the *Gene-TRAP*™ overflows with TAE buffer.

II. EXCISING AND PICKING A DNA BAND FROM THE GEL:

Warning: *Wear protective eyewear, gloves and take all lab safety precautions.*

Note: *To visualize the DNA bands, stain the gel with ethidium bromide and observe the gel under ultraviolet light.*

1. Each *Gel-PICK*™ has two open ends. **Always use the cutting end of the G*el- PICK*™ for picking a DNA band from the gel.** Position the cutting end of the *Gel- PICK*™ on the gel adjacent to the band of interest to ensure that it covers the width of the band (see Fig 2A)
2. Move the *Gel-PICK*™ onto the top of the DNA band of interest and make sure it covers most of the DNA band. Push the *Gel-PICK*™ into the soft gel until you hit the bottom hard surface of the gel tray (see Fig 2B).
3. Excise the gel band by tilting *Gel-PICK*™ to one side and lifting it (see Fig 2C).

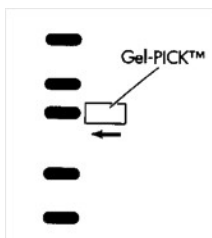

Fig. 2A

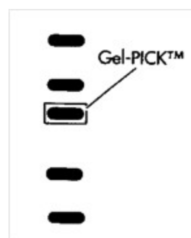

Fig. 2B

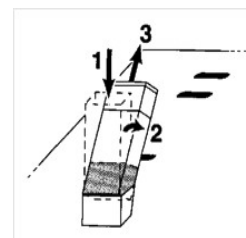

Fig. 2C

NOTE: *If there are still remnants of DNA band left behind in the gel or you fail to pick up the DNA band properly -* ***Turn the Gel-PICK™ around and use the other end to pick up the remnant of the band*** *(SEE TROUBLESHOOTING).*

Alternatively, use a sharp scalpel to trim the remnant of the DNA band and load the trimmed gel pieces containing DNA into the Gel-PICK™ after step 2C.

III. ASSEMBLING THE *GeneCAPSULE*™

Note: *Assemble the GeneCAPSULE™ just before you are ready for electroelution.*

1. Make sure the Gene-TRAP™ is filled with elution buffer (at step **I-4** on page 2). If not, fill it with elution buffer.
2. Position the *Gel-PICK™*, containing the DNA band gel piece, into the *Gene-TRAP™* (see Fig 3). Always introduce the cutting end of the *Gel-PICK™* into the *Gene-TRAP™*. Push until it locks in place.
3. Make sure, there are no air bubbles trapped in the *Gene-TRAP™*. Remove any air bubbles by pipetting buffer into the *GeneCAPSULE™*.
4. Using the plunger provided, push the gel piece(s) close to the membrane (see Fig.4), so that, the gel piece is uniformly in contact with the membrane.

   WARNING: *DO NOT PUSH THE PLUNGER TOO HARD; YOU MIGHT OTHERWISE BREAK THE GEL PIECE OR DAMAGE THE MEMBRANE.*

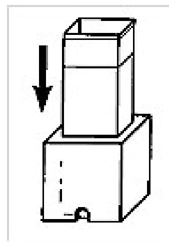
Fig. 3

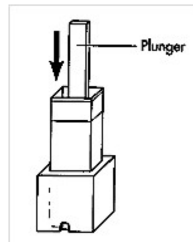

Fig. 4

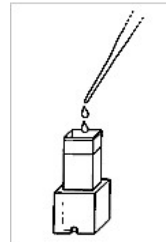
Fig. 5

IV. ELECTROELUTING DNA

1. Fill the *Gel-PICK™* with elution buffer (see Fig.5).
   NOTE: *Make sure there is no air bubbles trapped in the Gel-PICK™ or between gel pieces.*

2. Submerge the assembled *GeneCAPSULE™* in TAE buffer on top of the gel bed of the electrophoresis box such that the *Gene-TRAP™* is facing the (+)-terminal (red color terminal). Add buffer into the gel electrophoresis box just high enough to cover the *Gel-PICK™* (If possible, use pre-chilled buffer). Remove some buffer if the *GeneCAPSULE™* floats (Fig.6).

Optional: *GeneCAPSULE–Weight supplied with the accessory kit (cat# 786-004) can be also be used for holding the GeneCAPSULE when it is submerged in the buffer.*

WARNING: *Make sure the Gene-TRAP™ is facing the (+)-terminal so that the DNA would not migrate out into the tank buffer (see Fig. 6).*

3. Turn on the power supply to electroelute the DNA. For elution time, consult the **Guide For DNA Elution Time** on page 5.

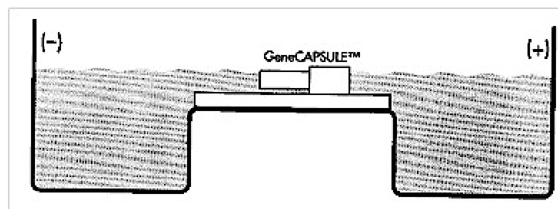

Fig. 6

V. RECOVERING THE ELECTROELUTED DNA FROM THE GeneCAPSULE™:

1. After electroelution is completed, adjust the voltage to ~ 120 V. **Reverse the polarity of the current** for 15-20 seconds to loosen the DNA from the membrane.

2. Turn off the power supply and remove the *GeneCAPSULE*™ from the gel box.

Note: *The DNA must be recovered immediately or maximum within 5-10 minutes after turning off the power supply in order to avoid diffusion of the eluted DNA.*

To minimize loss of the eluted DNA, do not separate the Gel-PICK™ from the Gene-TRAP™.

3. Hold the *GeneCAPSULE*™ horizontally between your fingers and insert a provided pipet tip, until you reach the gel piece and remove the entire free TAE buffer from the *Gel-PICK*™ [Fig. 7]. Any buffer left in the *Gel-PICK*™ will be extracted with the eluted DNA and will result in an increase in the elution volume.

Warning: ***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.***

Note: *Removing the free TAE buffer from the Gel-PICK™ relieves the pressure inside the capsule so that the buffer will not leak and carry away the electroeluted DNA. Once the free buffer is removed, surface tension will keep the electroeluted molecules in the vicinity of the membrane.*

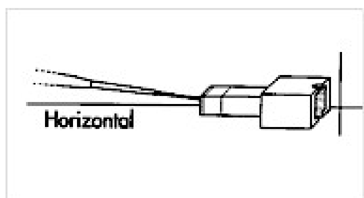

Fig. 7

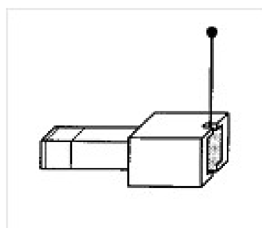

Fig. 8

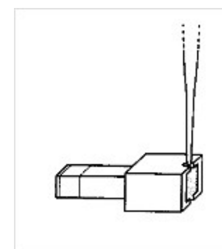

Fig. 9

4. Place the *GeneCAPSULE*™ on a dry surface or hold it between your fingers carefully. Position a pin (provided) in the DNA-collection port and puncture a small hole in the membrane of the Collection Port (see Figure 8, 10). **Warning**: *Do not force the pin downward into the membrane; this will damage the membrane and spill the DNA.*

5. Pre-set a pipetor to 50-60µl, and insert the capillary pipet tip (provided) through the hole in the membrane and gently push the tip downward [See Fig 9 and 11]. 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.

6. Pipet out the DNA collected near the membrane with the capillary pipet tip (Fig.9). **Warning:** *Do not pull out the Gel-PICK™; doing so will create a mess and sample loss.*

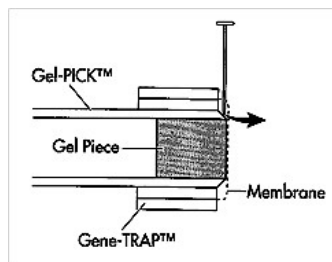

Fig. 10

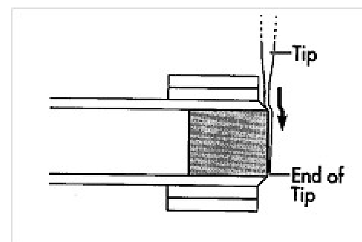

Fig. 11

EXTRACTED DNA IS READY FOR MOST USES:
DNA with molecular weights larger than 4000 is efficiently eluted and recovered using *GeneCAPSULE*™. Recovery for 2µg of DNA is generally between 80%-90%. The DNA recovered from the *GeneCAPSULE*™ is ready for most enzymatic uses. However, some grades of agarose have sulfate and other enzyme inhibitors as contaminants. If you are in any doubt, it is recommended that you precipitate the DNA by any standard method of your choice.

Guide for DNA Elution Time:
Elution of DNA depends on DNA size, concentration of gel and electrical current. It is not possible to provide a universal DNA elution time. However, the following table is simply a guide and will give you some indication of probable elution time in TAE buffer.

Gel (%)	DNA Size (bp)	Voltage	Elution Time (Minutes)
2	4,500	215 V	5
	2,500	215 V	2
	1,500	215 V	1
1	23,000	215 V	8
	9,500	215 V	6
	6,550	215 V	4
	2,500	215 V	1
0.5	23,000	215 V	7
	9,500	215 V	4
	6,500	215 V	3

NOTE: *For appropriate elution time, make test runs with the appropriate size of DNA fragments. If you are not sure of elution time, progress of DNA elution can also be monitored under UV light. Use hand held UV lamps.*

RNA ELECTROELUTION

For RNA use, the *GeneCAPSULE*™ must be made RNase-free by treating with DEPC [diethylpyrocarbonate] or other RNase removing agents. For information on RNase-removing agent RNaseOUT™, call our technical department or visit www.GBiosciences.com for ordering information. After electroelution, reverse the current at 100V for 1 min.

PROTEIN ELECTROELUTION

GeneCAPSULE™ can also be used for electro-eluting protein from the gel.

Use of *GeneCAPSULE*™ for Protein Purification:
GeneCAPSULE™ can be used for purification of protein (>4000 MW) for protein sequencing works, enzyme assays, raising antibodies and other protein studies.

A horizontal electrophoresis tank is required for electroelution. Avoid overheating *(SEE TROUBLESHOOTING)*. If your gel box is not fitted with a cooling device, electroelution should be performed in cold-room and at a low current.

For protein work, it is strongly recommended that *GeneCAPSULE*™ is first soaked for 30 minutes in an appropriate buffer containing 5mM EDTA and 5% BSA solution. Wash extensively with de-ionized water to remove free BSA.

FOR THIN PROTEIN GELS:
Protein gels are often very thin and you might encounter difficulty in picking protein bands into the *Gel-PICK*™. You can overcome this problem by first picking a blank piece of agarose gel.
(SEE TROUBLESHOOTING).

Protein Purification: You can purify protein from both non-denaturing polyacrylamide gel and denaturing SDS polyacrylamide. Use appropriate buffers for electroelution.

Enzyme Study (Assay): After electrophoresis, pick several gel pieces (each piece in a separate *Gel-PICK*™) along the electrophoresis lane and electroelute them for enzyme assay or further analysis. Use appropriate buffer for electroelution.

Final Notes:
After electroelution is completed as per the protocol, almost the entire DNA/protein sample (~99-100%) is collected against the membrane. There is no logical explanation for the sample to go anywhere else. If the recovery has been poor, please read the Trouble Shooting and improve the *GeneCAPSULE*™ handling techniques/steps.

TROUBLESHOOTING:

Fig. 12

Fig. 13

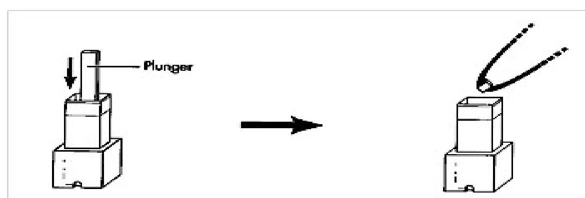

Fig. 14

Fig. 15

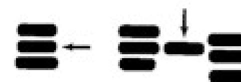
Fig. 16

Problem	Suggestion(s)
The entire band is not excised in a single attempt (Fig.12)	▪ Turn the *Gel-PICK*™ around and use the other open end to pick up the remnants of the band. ▪ **Alternatively:** After punching out a band, position the *Gel-PICK*™ in the *Gene-TRAP*™ and push the gel piece against the membrane with the plunger (Fig.4, 14). Use a scalpel or any sharp instrument to trim the remaining DNA band from the gel. ▪ Cut the gel band into small pieces and load the pieces into the *Gel-PICK*™ (Fig.14). Perform electroelution as usual. The elution time will be longer.
DNA recovery is poor	Check the following: ▪ The gel piece was disturbed while removing buffer from the *Gel-PICK*™. ▪ Electrical current was not reversed or reverse current was allowed to flow longer than 20-25 sec. ▪ The membrane was damaged. ▪ Elution time was not sufficient or was excessive. ▪ DNA was degraded. ▪ The *GeneCAPSULE*™ was vertical for a longer period, and backward flow of DNA occurred **(step V).** ▪ Any manipulation or prolonged observation was performed before removing all free buffer from the Gel-PICK™ **(step V).** ▪ After electroelution, *GeneCAPSULE*™ was placed on an absorbing or wet surface or in a puddle of fluid.
The band is wider than the size of G*el-PICK*™ (Fig.13, 15)	▪ Punch out two or more gel pieces from the band, one on top another. When punching out more than one piece from a gel band, allow some distance between the pieces (as in Fig.15). ▪ Use the other cutting end of the *Gel-PICK*™ and punch out the remnants of the band. Depending on the thickness of the gel, you may be able to punch out 3-10 pieces of gel. ▪ **Alternatively**: Punch out two or more gel pieces as above. Position the *Gel-PICK*™ in the *Gene-TRAP*™ and use the plunger to push the gel pieces against the membrane as described in **Step III** (**Fig.4, 14**). ▪ Use a scalpel or any sharp instrument to trim the remaining DNA from the gel and load the pieces into the *Gel-PICK*™ (Fig.14). Perform electroelution as usual. The elution time will be longer.
The DNA band is crowded by other bands (Fig. 16), which may lead to cross contamination	▪ Use the *Gel-PICK*™ to punch out a blank piece of gel. Position the *Gel-PICK*™ in the *Gene-TRAP*™ and push the blank gel piece against the membrane with the plunger (Fig.4, 14). ▪ Use a scalpel or any sharp instrument to cut out the band you intend to extract. ▪ Cut the gel band into small pieces and load the pieces into the *Gel-PICK*™ (Fig.14). Electroelute as usual. Elution time will be longer.

Gel is too thin, leading to difficulty in picking gel bands with *Gel-PICK*™	▪ **First**, pick a blank piece of agarose gel into the *Gel-PICK*™ from a prepared 4-5mm thick agarose gel in appropriate buffer. ▪ Position the *Gel-PICK*™ in the *Gene-TRAP*™ and push the blank gel pieces against the membrane with the plunger, as in **Step III** (**Fig.4, 14**). ▪ Cut the band with a sharp instrument and load into the *Gel-PICK*™ (Fig.14).
Gel lanes are too close to each other	▪ Load samples in alternate sample wells. Alternatively, do not load samples in the wells adjacent to your most critical samples.
Want to avoid overheating	▪ Before electroelution, replace the buffer in the gel box with pre-chilled buffer. If possible, add a few ice-cubes made of elution buffer (Prepare and store some elution buffer ice-cubes for future use).
Elution volume is larger than 35µl	Check the following: ▪ The electrophoresis gel was thicker than 5-6mm. ▪ The gel piece was damaged, which increased the dead volume of elution buffer in the *Gel-PICK*™. ▪ The entire buffer was not removed from the *Gel-PICK*™. ▪ The gel piece was not pushed close to the membrane **(Step III).**
Buffer is leaking from the *Gene-TRAP*™	*This is normal, since GeneCAPSULE*™ is not watertight and buffer will leak between the plastic parts. Therefore, you must be aware of the following instructions: ▪ Assemble *GeneCAPSULE*™ just before use **(step III)** and quickly submerge in the tank buffer. Keep it there until you begin electrical current for electroelution. ▪ After electroelution is complete and as soon as you remove *GeneCAPSULE*™ from the buffer, **you must first remove all free buffer from *Gel-PICK*™ (step V).** This will relieve pressure so fluid does not leak and carry DNA with it. Once the free buffer is removed, surface tension will keep the electroeluted DNA in the vicinity of the membrane. ▪ Remove drops of buffer around *GeneCAPSULE*™. Use a pipet or tissue paper. Be careful not to suck inside buffer with tissue paper or pipet tip. ▪ Do not place *GeneCAPSULE*™ on an absorbing or wet surface, or in a puddle of fluid; this could create powerful capillary suction through the joining parts of *GeneCAPSULE*™ filled with buffer. ▪ After electroelution, you may place *GeneCAPSULE*™ on a dry surface, provided that you have followed the above instructions.

GeneCAPSULE is *protected by* ***US Patent # 5635045***

RELATED PRODUCTS

1. ***Nucleic dotMETRIC*™ *(Cat # 786-61)*** *– Allows DNA, RNA and oligonucleotide concentrations to be measured using as little as 1 µl of sample. Measurements take 2 minutes and are perfect for geneEXIT*™ *or any other use requiring a minimal waste of sample.*
2. ***Gel Loading Dye-****Ready to use dyes for running agarose gel electrophoresis of DNA and RNA. The dyes for DNA are Ficoll based and are available as Glow Loading Dye and Universal Loading Dye. Visit our web site www.GBiosciences.com for ordering information.*
3. ***Mini Horizontal Gel Electrophoresis System*** *(Cat# MT-108* ***and MT-109).***
4. ***Dot-Blot Enhancer*™ *System (Cat. #. 786-163)****: The device is useful for Nucleic acid and Protein dot-blot procedures. It concentrates the sample in a small area at the point of application and enhances the signal strength.*

For other related products, visit our web site www.GBiosciences.com or contact us at 1-800-628-7730.

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