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Protocol for Isolating a Transgene from a Piece of an Agarose Gel by Using the EZ-10 Spin Column DNA Gel Extraction Kit
(Modified 4/28/21)

Materials needed:

1. SeaKem GTG agarose (Cambrex Bio Science, Cat # 50071, 25g/$67.00) for preparative gels.
2. We use BioExpress GeneMate LE Quick Dissolve Agarose (E-3119-500) for minigels to check DNA integrity.
3. EZ-10 Spin Column DNA Gel Extraction Kit (Bio Basic Inc, Ontario, Canada. Web: biobasic.com
   Catalog # BS353- 50 preps for $35
   Catalog # BS654- 250 preps for $140
4. Prepare **Injection Buffer** (10mM Tris, 0.25mM EDTA, pH 7.4) with sterile milliQ water. Filter the solution through a 0.2μ filter.
5. Ethanol (96-100%).

General considerations:

Rinse all the microfuge tubes you plan on using with filter-sterilized **Injection Buffer**. This is done in an attempt to eliminate lint and other small debris from the tubes. Any inert material that remains in the tube containing the solution of DNA may interfere with the microinjection process and this will have a negative impact on the viability of the embryos.

DNA digestion:

1. Digest 20-40 μgs of DNA with the appropriate restriction enzymes. Run the digest on the appropriate % SeaKem GTG preparative agarose gel until the bands (transgene and vector) are clearly resolved and separated. See below.

2. When using this kit for eluting DNA it is best to use TAE based running buffers rather than TBE.

3. This method is best for fragments between 140bp to 40kb.The DNA gel fragment may be stored at 4 °C in a foil wrapped tube until it is ready to be used.

Running a preparative gel:

1. The goal of a preparative gel is to resolve a large quantity of digested DNA into bands of the distinct, desired sizes. In a typical prep gel you are trying to resolve up to 40 ug of digested DNA into at least 2 bands.

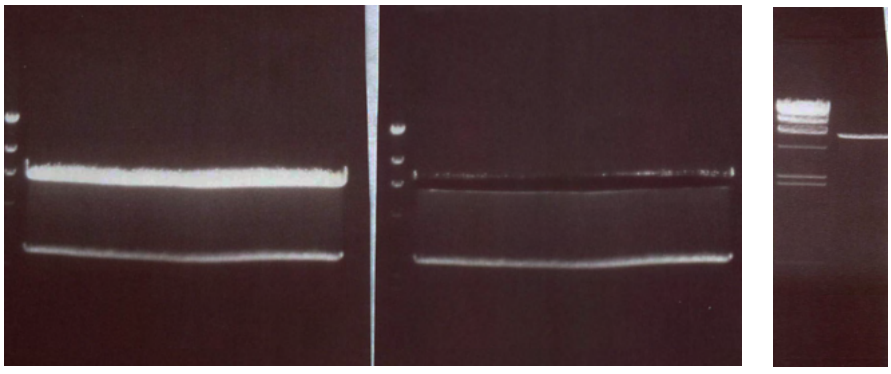
2. The easiest way to run a preparative gel is to pour an agarose gel using a preparative comb. Typically this is a comb that creates a single well that is long and thin. The well may be as big as 8 cm long. Using a comb this size allows the user to resolve fragments of DNA that are similar in size.

3. Run the gel very slowly, typically for several hours in TAE buffer containing ethidium bromide. Be sure to use fresh buffer when running the gel.

4. Once the bands have been resolved, place the gel on a transilluminator and cut out the DNA band you want to isolate using a scalpel blade or a razor blade.

5. Roll the gel fragment around on the transilluminator and remove as much needless agarose (non DNA containing) as possible from the gel fragment. This will help in the elution process by keeping the gel fragment to be processed as small as possible.

A typical preparative agarose gel is shown below revealing the two resolved DNA bands. In the “before excision” photo, the two bands can easily be resolved and in the “after excision” photo you can see the band that was cut out of the preparative gel. The final prep of the resolved, EZ-10 purified DNA fragment of interest is shown along with lambda HindIII markers.



Before Excision After Excision Final prep

Preparation before using columns:

1. Before beginning the procedure, add 80 mls of 96 -100% ethanol to 20 mls of Wash solution for BS353 columns, add 160 mls of 96 to 100% ethanol to 40 mls Wash solution for BS354; add 320 mls of 96 - 100% ethanol to 80mls Wash Solution for BS654. For other volumes of solution, simply add enough ethanol to make a 4:1 ratio (volume of added ethanol: volume of Wash Solution = 4:1).

2. Elution Buffer is 2mM Tris-HCl pH 8.0~ 8.5. Although TE buffer pH 8.0 or water may be substituted, the resulting yields may be up to 20% lower.

Protocol for agarose gel elution:

1. When you are ready to begin the elution procedure, remove the gel slice from the tube, weigh the gel slice, and place back in tube.

2. Add 400ul of Binding Buffer II for each 100mg of gel weight (for example, a gel slice weighing 125mg would require 500μl of Binding Buffer II).

3. Incubate at 60° C for ~10 minutes and shake occasionally until the agarose is completely dissolved. For high concentration gels (1.5-2.0%), 700ul of Binding Buffer II per 100mg of agarose gel are needed.

4. Note: After addition of binding buffer, carefully monitor the color of the binding mixture. If the binding mixture is yellow, then optimal pH has been obtained; continue with the rest of the extraction steps. However, if the biding mixture turns a blue or purple color, adjust the pH by adding a small volume of 3M sodium acetate (pH 5.0) until optimal pH is reached. Proceed with the rest of the extraction steps.

5. Use one (1) EZ-10 column/20ugs of restricted DNA. This means that if your transgene constitutes ~1/2 of the total DNA that was cut then you will only use one column to elute the transgene (10ug of DNA) from the gel. Add 400ul the above mixture to the column, then using a P1000 pipet, press the liquid through the column.

    a. Discard liquid and repeat unil all of the Binding Buffer/Agarose mixture has been pressed through the column.
    b. After final “push-through”, discard liquid and then centrifuge at 10,000 rpm for 2 minutes to remove any residual Binding Buffer. Discard liquid.
    c. Add 750μl of Wash Solution and centrifuge at 10,000rpm for one minute. Discard the solution in the tube.
    d. Repeat step 5c. Centrifuge at 10,000rpm for one minute to remove any residual Wash Buffer.

6. Place the column in a clean 1.5 ml microfuge tube. Add 30ul of Elution Buffer (pre-warmed at 60°) to the center of the column and incubate at 60° for 2 minutes. Centrifuge at 10,000rpm for 2 minutes to elute the DNA.

7. Precipitate the DNA by adding 1/20th volume of 3M sodium acetate plus 2.5 volume of 100% ethanol. Place the tube into the freezer at -20°C overnight.

8. Spin the tube for 15 min, 4C at 14,000rpm. Pour off the EtOH and save the pellet.

9. Wash the pellet with 500ul of 4°C 70% EtOH, gently roll the tube and dump the EtOH.

10. Let the pellet dry in air until just visibly wet and resuspend the pellet in 30 μl of **Injection buffer**.

    a. If there is a visible "white substance" at the bottom of the tube after adding the buffer and resuspending the DNA, spin the tube for 5 minutes, 4C at 14,000rpm. Remove supernatant (DNA) and place in new, clean 1.5 ml Eppendorf tube. The "white substance" appears to be a product of the precipitation and can be discarded once the concentration and integrity of the DNA has been determined.

11. Determine the DNA concentration and run ~ 100ng on a mini agarose gel to assess the integrity of the prep.

12. **Note**: It is extremely important to add the Elution Buffer to the center of the column. Incubating the column at higher temperatures (37°C to 50°C) may slightly increase the yield. Pre-warming the Elution Buffer at 55°C to 80°C may also increase elution efficiency.

13. Store the purified DNA at 4°C.