Gel Extraction:

1. Excise gel slice containing the DNA fragment using a sterile scalpel. Cut as close to the DNA as possible to minimize the gel volume. Place the gel slice into a pre-weighed 1.5 mL tube and weigh. Record the weight of the gel slice.
2. Add 1:1 (volume: weight) volume of Binding Buffer to the gel slice.
3. Incubate the gel mixture at 50-60 °C for 10 min or until the gel slice is completely dissolved. Mix the tube by inversion every few minutes to facilitate the melting process. Vortex the gel mixture briefly before loading on the column.
    a. Check the color of the solution. A yellow color indicates an optimal pH for DNA binding. If the color of the solution is orange or violet, add 10 μL of 3 M sodium acetate, pH 5.2 solution and mix. The color of the mix will become yellow.
4. Optional Step (if DNA is ≤500 bp or >10 kb)
    a. If the DNA fragment is ≤500 bp, add 1 gel volume of 100% isopropanol to the solubilized gel solution (e.g. 100 μL of isopropanol should be added to 100 mg gel slice solubilized in 100 μL of Binding Buffer). Mix thoroughly.
    b. If the DNA fragment is >10 kb, add 1 gel volume of water to the solubilized gel solution (e.g. 100 μL of water should be added to 100 mg gel slice solubilized in 100 μL of Binding Buffer). Mix thoroughly.
5. Transfer up to 800 μL of the solubilized gel solution (from step 3 or 4) to the GeneJET purification column. Centrifuge for 1 min. Discard the flow-through and place the column back into the same collection tube.
6. Optional Step (if DNA will be sequenced)
    a. Add 100 μL of Binding Buffer to the GeneJET purification column. Centrifuge for 1 min. Discard the flow-through and place the column back into the same collection tube.
7. Add 700 μL of Wash Buffer (diluted with ethanol as described on p. 3) to the GeneJET purification column. Centrifuge for 1 min. Discard the flow-through and place the column back into the same collection tube.
8. Centrifuge the empty GeneJET purification column for an additional 1 min to completely remove residual wash buffer.
9. Transfer the GeneJET purification column into a clean 1.5 mL microcentrifuge tube (not included). Add 50 μL of Elution Buffer to the center of the purification column membrane. Centrifuge for 1 min.
    a. For low DNA amounts the elution volumes can be reduced to increase DNA concentration. An elution volume between 20-50 μL does not significantly reduce the DNA yield. However, elution volumes less than 10 μL are not recommended.

b. If DNA fragment is >10 kb, prewarm Elution Buffer to 65 °C before applying to column.
c. If the elution volume is 10 μL and DNA amount is ≤ 5 μg, incubate column for 1 min at room temperature before centrifugation.

10. Discard the GeneJET purification column and store the purified DNA at -20 °C.