Gel Extraction (to miniprep)

Goal- to isolate plasmid DNA from bacteria.

Materials-

DNA isolation kit

Molecular biology

Protocol

Dilute Wash Buffer (concentrated) by adding 5:1 volume of ethanol (96-100%).

Procedure:

All centrifugations should be carried out in a table-top microcentrifuge at >12000g (10000-14 000 rpm)

1. Excise gel slice containing the DNA fragment using a clean scalpel or razor blade. Cut as close to the DNA as possible to minimize the gel volume. Place the gel slice into a pre-weighed 1.5 mLtube and weigh. Record the weight of the gel slice.
Note. If the purified fragment will be used for cloning reactions, avoid damaging the DNA through UV light exposure by minimizing UV exposure time.
2. Add 1:1 volume of Binding Buffer to the gel slice (volume: weight).
3. Incubate the gel mixture at 50-60°C for 10 min until the gel slice is completely dissolved. Mix the tube by inversion every few minutes to facilitate the melting process. Ensure that the gel is completely dissolved. Vortex the gel mixture briefly before loading on the column. 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 solution (pH 5,2) and mix. The color of the mix will become yellow.
4. For DNA fragments ≤500 and >10.
If the DNA fragment is ≤500 bp, add 1 gel volume of 100% isopropanol to the solubilized gel. Mix thoroughly.
If the DNA fragment is >10 kb, add 1 gel volume of water to the solubilized gel solution. Mix thoroughly.
5. Transfer up to 800 µL of the solubilized gel solution (from step 3 or 4) to the purification column. Centrifuge for 1 min. Discard the flow-through and place the column back into the same collection tube.
6. Additional binding step only if the purified DNA will be used for sequencing. Add 100 µL of Binding Buffer to the 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) to the purification column.

Centrifuge for 1 min. Discard the flow-through and place the column back into the same collection tube.

8. Centrifuge the empty purification column for an additional 1 min to completely remove residual wash buffer.

9. Transfer the purification column into a clean 1.5 mL microcentrifuge tube. Add 50 µL of water to the center of the purification column membrane. Centrifuge for 1 min.

Note. For low DNA amounts the elution volumes can be reduced to increase DNA concentration. Elution volumes less than 10 µLare not recommended.

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