Taglio della banda da gel

Appoggiare il gel sul transilluminatore (intensità luce al 75 %) e tagliare la porzione di gel contenete la banda del prodotto di PCR con una lama pulita con etanolo e trasferirla in una Falcon da 15 mL.

Purificazione dell'inserto da gel utilizzando il GenElute™ Gel Extraction Kit

Procedure
Spin Procedure for Agarose Gels
All centrifugations (spins) are performed at 12,000 to 16,000 x g.

1.Excise band.
Excise the DNA fragment of interest from the agarose gel with a clean, sharp scalpel or razor blade. Trim away excess gel to minimize the amount of agarose.

2.Weigh gel.
Weigh the gel slice in a tared colorless tube.

3.Solubilize gel.
Add 3 gel volumes of the Gel Solubilization Solution to the gel slice. In other words, for every 100 mg of agarose gel, add 300 µL of Gel Solubilization Solution. Incubate the gel mixture at 50-60 °C for 10 minutes, or until the gel slice is completely dissolved. Vortex briefly every 2-3 minutes during incubation to help dissolve the gel.

4a. Prepare binding column.
Preparation of the binding column can be completed while the agarose is being solubilized in step 3. Place the GenElute Binding Column G into one of the provided 2 ml collection tubes. Add 500 µL of the Column Preparation Solution to each binding column. Centrifuge for 1 minute. Discard flow-through liquid.

5a. Add isopropanol.
Add 1 gel volume of 100% isopropanol and mix until homogenous. For a gel with an agarose concentration greater than 2%, use 2 gel volumes of 100% isopropanol.

6a. Bind DNA.
Load the solubilized gel solution mixture from step 6a into the binding column that is assembled in a 2 ml collection tube. It is normal to see an occasional color change from yellow to red once the sample is applied to the binding column. If the volume of the gel mixture is >700 µL, load the sample onto the column in 700 µL portions. Centrifuge for 1 minute after loading the column each time. Discard the flow-through liquid.

7a.Wash column.
Add 700 µLof Wash Solution to the binding column. Centrifuge for 1 minute. Remove the binding column from the collection tube and discard the flow-through liquid. Place the binding column back into the collection tube and centrifuge again for 1 minute without any additional wash solution to remove excess ethanol. Residual Wash Solution will not be completely removed unless the flow-through is discarded before the final centrifugation.

8a. Elute DNA.
Transfer the binding column to a fresh collection tube. Add 25 µL of Elution Solution to the center of the membrane and incubate for 1 minute. Centrifuge for 1 minute.