AGARASE

1. Perform electrophoresis of DNA in a low melting point (LM) agarose gel prepared in TAE 0.5X TBE, TBE or TPE buffer, stain the gel if it is necessary.
2. Cut out the desired DNA band from the agarose gel with a clean scalpel under UV light. Cut out only agarose as necessary. **NOTE: The bottom of the excised agarose slice is free of DNA and should be removed.**
3. Determine the weight of the slice. To facilitate melting, cut gel slices larger than 200 mg into smaller pieces.
4. Incubate the tube at 70°C for approx. 10 min. Ensure that the agarose is completely molten.
5. Transfer the tube to a 42°C water bath and equilibrate for 5 min.
6. Add 1 u of Agarase (#EO0461_Fermentas) per 100 mg (approx. 100 µL) of molten 1% LM agarose. Increase the amount of enzyme proportionally for higher percentage agarose, gently mix and incubate at 42°C for 30 min.
7. Add ammonium acetate to a 2.5 M final concentration, chill on ice for 5 min. **NOTE: For 100 µL of molten agarose add 20 µL of 12.5 M (Stock ammonium acetate [12.5 M].**
8. Centrifuge at 10,000 rpm for 10 min to pellet undigested carbohydrates. Transfer the supernatant to a clean tube.
9. Add 2.5 volumes of ethanol or 0.8 volume of isopropanol, mix gently and incubate at room temperature for 1 h. If DNA fragments are smaller than 500 kb or if the DNA concentration is lower than 0.05 µg/mL, incubate at room temperature for 2 h.
   NOTE: After addition of 20 µL ammonium acetate to 100 µL of molten agarose the volume is 120 µL. Therefore add 300 µL of ethanol or 96 µL of isopropanol. If you decide to add isopropanol, do absolutely not incubate the reaction at -20°C as salts would precipitate as well.
10. Centrifuge at 10,000 rpm for 15 min, remove supernatant and dry the pellet. Resuspend the pellet in TE or another appropriate buffer for subsequent manipulation.
    NOTE: If you would like to add glycogen to the reaction, do so after step 8 (i.e. after the centrifugation). Addition of 4 µL of glycogen (20 mg/mL) will yield a final concentration of 0.6 µg/µL, which is within the recommended range (0.05-1 µg/µL). If you want to wash the pellet after precipitation, use 70% ethanol instead isopropanol.