Gel Extraction

Introduction

Get started by giving your protocol a name and editing this introduction.

Materials

- QIAgen gel extraction kit
  - Purple columns
  - Buffer QG
  - Buffer PE
  - Buffer EB
- Isopropanol
- 3M Sodium Acetate, pH=5.0
- Microcentrifuge tubes

Procedure

Setup

1. Set a dry bath to 50°C and fill the holes in the metal block with water. Transfer some Buffer EB (30 ul for each purification plus an extra 30 ul) to a microcentrifuge tube and place it in the dry back to warm.

Excise gel

2. On the blue-light transilluminator, cut out the gel band and put it in an eppendorf tube.

3. Weigh the gel band.

   TARE the balance with an empty eppendorf tube.
   Weigh the tube with the gel band in it.

Gel extraction

4. Add 6 volumes of Buffer QG to 1 volume of gel. (Compute volume assuming 100 mg of gel ~ 100 ul.)

5. Incubate at 50°C until the gel is disolved, about 10 minutes; vortex briefly every 2-3 minutes.

6. Add 10 μl 3M sodium acetate pH 5.0. Vortex briefly.

7. Add 1 gel volume of isopropanol.

8. Apply a maximum of 750 μl of the disolved gel to the QIAquick column and centrifuge for 30 seconds at maximum speed.

9. Pipette the flow-through back into the column and centrifuge again. Discard the flow-through.

10. If needed, apply the rest of the disolved gel to the column. Centrifuge; then re-apply and centrifuge again. Discard flow-through.

11. Add 500 μl of buffer QG and centrifuge 30 seconds. Discard flow-through.

12. Add 750 μl of Buffer PE to the column. Incubate at room temperature for 3-5 minutes, then centrifuge for 30 seconds.

13. Discard flow-through and centrifuge again for 1 minute.

14. Move column to a clean microcentrifuge tube.

15. Add 30 μl of the **warm EB buffer** to the center of the column.

16. Incubate in the 50-degree dry bath for 3 minutes.

17. Centrifuge for 1 minute.

18. Measure the concentration using the nanodrop (make sure to vortex your samples before).