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06 Gel purification

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Protocol status: Working
We use this protocol and it's working

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Guidelines

1. The addition of balanced liquid BL can improve the adsorption capacity of the adsorption column, improve the uniformity and stability of the adsorption column, and eliminate the influence of high temperature, humidity or other adverse environmental factors on the adsorption column.Please check whether there is turbidity in the balance liquid BL before use. If there is turbidity, it can be heated in 37℃ water bath for several minutes, and then the clarification can be restored. 37 °C

2. It is better to use a new electrophoresis buffer to avoid affecting the results.

3. TAE electrophoretic buffer should be used as far as possible as the following step requires higher requirements.

4. When cutting gel, UV irradiation should be as short as possible, so as not to cause damage to DNA.

5. If the recycle rate is low, the pH can be detected after the gel is fully dissolved. If pH is more than 7.5, 10-30ul 3M sodium acetate (pH5.2) can be added to the gel solution containing DNA to adjust the pH to 5-7.

6. When DNA fragments less then 100 bp or more than 10 kb were recovered, the volume of PN should be increased and the adsorption and elution time should be extended.

7. The recycle rate is related to the initial amount of DNA and elution volume. The lower the initial amount and elution volume, the lower the recycle rate.

Materials

MATERIALS

TIANgel Midi Purification Kit **Catalog #**/

Gel with DNA samples **Catalog #**/

Troubleshooting

1 Column balancing step: add 500ul buffer BL to the adsorption column CA2 (the adsorption column is put into the collection tube), centrifuge at 12,000 RPM (~13,400×g) for 1 min, dump the waste liquid in the collection tube, and put the adsorption column back into the collection tube.

500 µL

00:01:00

2 Remove the single target DNA strip from the agarose gel (remove the excess as much as possible) and put it into a clean centrifuge tube, and weigh it.

3 Add buffer PN to the glue block (if the gel weight is 0.1g, its volume can be considered as 100 ul, then add 100 ul PN), put in 50℃ water bath, during which gently turn up and down the centrifugal tube, to ensure that the glue block is fully dissolved. If there is any undissolved glue, continue to leave for a few minutes or add more PN until the glue is completely dissolved. ***Put the tube on ice.***

50 °C

4 Add the solution obtained in the previous step to an adsorption column CA2 (the adsorption column was placed in the collection tube), place at room temperature for 2 min, centrifuge at 12,000 RPM (~13,400×g) for 30-60 SEC, and dump the waste liquid in the collection tube, place the adsorption column CA2 into the collection tube.

5 Add 600ul bleach buffer PW to the adsorption column CA2 (check whether anhydrous ethanol has been added before use), centrifuge 30-60 SEC at 12,000rpm (~13,400×g), dump the waste liquid from the collection tube, and put the adsorption column CA2 into the collection tube.

6 Repeat step 5.

7 Put the adsorption column CA2 back into the collection tube and centrifuge at 12,000 rpm (~13,400×g) for 2 min to remove as much bleach as possible.Place the adsorption column CA2 at room temperature for several minutes to dry thoroughly to prevent the residual rinse fluid from affecting the next experiment.

00:02:00

8 Put the adsorption column CA2 into a clean centrifugal tube, and drop an appropriate amount of ddH_2O(about 30ul) onto the middle position of the adsorption film, and leave it at room temperature for 2 min. Final DNA solution was collected by centrifugation at 12,000 RPM (~13,400×g) for 2 min.

30 µL