DNA laddering

Ⅰ、Analytical Gel

1）Composition

-10×TAE 60ml

-Biowest Agarose 0.9g

-SYBRSafe 0.6μL

2）Boil over med-high hit for 1.5 min until the powder completely soluted. Add SYBRSafe(6μL).

3）Pour 30ml solution in per small gel tray. Place 11- well comb. Wait for the gels to solidify (20min) .

1□Running the gel

- Put the gel with the gel tray in enough 10xTAE.

- Load 2μL Marker in one plate holder.

-Add 3μL FD Green buffer to the DNA. Load 3μL into each plate holder.

-Set the timer and voltage to 30min 125V.

2、Autoradiography and Annotation

Ⅱ、Extraction Gel

1、Agarose gel

1）Composition

-10×TAE 60ml

-Agarose 0.9g

-SYBRSafe 0.6μL

2）Boil over med-high hit for 1.5 min until the powder is completely soluted. Add SYBRSafe (6μL).

3）Pour 30ml solution in per small gel tray. Place 6-well comb. Wait for the gels to solidify (20min) .

2□Running the gel(Marker:6μL)

3□Cut out the appropriate bands. Place into Eppendorf tubes.

4□Extraction

- Add 500μL BL into CA2.Spin 12000rpm for 1min.

- Use equal volume Buffer PN to dissolve the gel. Incubate at 55℃ 1300rpm until the it is completely dissolved.

- Add dissolved gel into CA2 column in the collection tube. Wait for 2min at 25℃.Spin at 12000rpm for 1min. Discard the liquid in the collection tube. Repeat..

- Add 600μL Buffer PW into the CA2 column. Spin at 12000 rpm for 1min. Discard the liquid in the collection tube.

- Spin the empty column in collection tube at 12000rpm for 1 min. Discard the collection tube.
- Put the CA2 column in an Eppendorf tube. Incubate at 55℃ for 5min with the cap open.
- Add 50μL Buffer EB. The buffer should be pipetted onto centre of filter .Incubate at 55℃ for 2min. Then spin at 12000rpm for 2min.
- Measure the concentration of DNA.