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| Time: 4h  Gel electrophoresis 1h  Make gel 30min  Run gel 30min  Gel extraction 2~3h | 3. Purification of Bisulfite converted DNA by gel electrophoresis  I. Gel electrophoresis  1% agarose gel, 100V for 23 min  Use 11 comb, load 30µL per lane  Replace gel machine with fresh TBE before running.  Loading dye contains 3 marker dyes (bromophenol blue, xylene cyanol, and orange G) that facilitate estimation of DNA migration distance and optimization of agarose gel run time.  Do not let sample runs pass the middle of the gel, as cyberSafe/EB runs at opposite direction, it will look dim if cyber safe concentration is low at the sample location.  Migration Distance of Gel Tracking Dyes   |  |  |  |  | | --- | --- | --- | --- | | %TBE  agarose gel | Xylene cyanol (light blue) | Bromophenol blue (dark blue) | Orange G (orange) | | 1.0 | 3000 bp | 400 bp | <100 bp | | 1.5 | 1800 bp | 250 bp | <100 bp | | 2.0 | 1000 bp | 200 bp | <100 bp |   II. Gel extraction  Kit: QIAquick Gel Extraction Kit  Ref. QIAquick Gel Extraction Kit Protocol  1. Excise the DNA fragment from the agarose gel with a glass lid for microscopy, tape on one edge so it is easy to locate the edge of the lid when cut the gel.  2. Weigh the gel slice in a colorless tube (1.5 Eppendorf tube). Add 3 volumes of Buffer QG to 1 volume of gel (100mg~100µL).  The maximum amount of gel slice per Eppendorf tube/QIAquick column is 400mg; for gel slices >400 mg use more than one QIAquick column.  3. Incubate at 50°C for 10 min (or until the gel slice has completely dissolved). To help dissolve gel, mix by invert/vortexing the tube every 5 min during the incubation.  4. After the gel slice has dissolved completely, check that the color of the mixture is  yellow (similar to Buffer QG without dissolved agarose).  If the color of the mixture is orange or violet, add 10 μl of 3 M sodium acetate, pH 5.0, and mix. The color of the mixture will turn to yellow.  The adsorption of DNA to the QIAquick membrane is efficient only at pH ≤7.5. Buffer QG contains a pH indicator which is yellow at pH ≤7.5 and orange or violet at higher pH  5. Add 1 gel volume of isopropanol to the sample and mix.  Precipitation may appear after adding isopropanol, invert tubes several times to mix thoroughly, the precipitation will dissolve.  6. Place a QIAquick spin column in a provided 2 ml collection tube. The newer version already has it put in.  7. To bind DNA. Apply the sample to the QIAquick column, and centrifuge for 1 min.  The maximum volume of the column reservoir is 800 μl, use 700 instead avoid splatter. For sample volumes of more than 800 μl, simply remove the flow through, load and spin again.  Recommended: Use a vacuum to remove the flow through, pure often leads to extra liquid on the edge, increase the risk of contamination.  8. Discard flow-through and place QIAquick column back in the same collection tube.  9. Recommended: Add 0.5 ml of Buffer QG to QIAquick column and centrifuge for 1 min.  This step will remove all traces of agarose. It is only required when the DNA will subsequently be used for direct sequencing, in vitro transcription, or microinjection.  10. To wash. Add 0.75 ml of Buffer PE to QIAquick column and centrifuge for 1 min.  Recommended: let the column stand 2–5 min after addition of Buffer PE, before centrifuging.  11. Discard the flow-through and centrifuge the QIAquick column for an additional 1 min at 17,900 x g (13,000 rpm).  IMPORTANT: Discard the flow-through before this additional centrifugation to completely remove residual ethanol.  12. Place QIAquick column into a clean 1.5 ml microcentrifuge tube.  13. To elute DNA. Add 30 μl of Buffer EB (10 mM Tris·Cl, pH 8.5) or water (pH 7.0–8.5) to the center of the QIAquick membrane. Let the column stand for 1 min, and then centrifuge for 1 min.  IMPORTANT: Ensure that the elution buffer is dispensed directly onto the QIAquick membrane for complete elution of bound DNA. The average eluate volume is 48 μl from 50 μl elution buffer volume, and 28 μl from 30 μl.  Elution efficiency is dependent on pH. The maximum elution efficiency is achieved between pH 7.0 and 8.5. When using water, make sure that the pH value is within this range, and store DNA at –20°C as DNA may degrade in the absence of a buffering agent. The purified DNA can also be eluted in TE (10 mM Tris·Cl, 1 mM EDTA, pH 8.0), but the EDTA may inhibit subsequent enzymatic reactions. |