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ADN PURIFICATION FROM AGAROSE GELS WITH GENELUTE GEL EXTRACTION KIT

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ABSTRACT

Protocol for DNA purification from DNA purification from agarose gels with GeneElute Gel Extraction Kit

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MATERIALS TEXT

GenElute Gel Extraction Kit (NA1111, Sigma-Aldrich):

- Column Preparation Solution
- Gel Solubilization Solution
- Wash Solution Concentrate G
- Elution Solution
- GenElute Binding Column G
- Collection Tubes, 2 mL

Isopropanol
Ethanol
Sodium Acetate 3M, pH 5.2
Thermoblock at 50-60°C.
Electrophoresis equipment for agarose gel
Tubes of 1.5 mL

1 Previous electrophoresis : Realization of an electrophoresis in a agarose gel of the product of ADN, charging the maximum amount of DNA per well (if necessary, use more than one well)

2 Extraction of the band of interest: Extract the band of interest of the gel, removing the excess of agarose

3 Weighting the band: Weight the piece of gel removed in a tarated tube.

4 Gel Solubilization: Add 3 volumes of Gel Solubilization Solution to the piece of gel, meaning, for each **100 mg** of agarose gel, add **300 µL** of Gel Solubilization Solution. Incubate at 50-60°C for **00:10:00** minutes or until the piece of gel has completely dissolved. Give Vortex every 2 or 3 minutes in this incubation time. 10m

5 Preparation of the column (This can be done alongside step 4). Add GenElute Binding Column G in a recolectation tube of 2 mL. Add **500 µL** of the Column Preparation Solution to each Binding Column. Centrifugate for **00:01:00** minute at 12,000 to 16,000 x g. Discard liquid 1m

6 Check the color of the sample: Once the gel had dissolved completely, make sure that the color is yellow before continuing. If the color is red, add **10 µL** of the Sodium Acetate 3M, pH 5.2 buffer and mix. Add until the color is yellow.

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7 Add isopropanol: Add a volume of the gel of isopropanol at 100% and mix until homogenized.

8 Bind ADN: Charge the sample of step 7 to the binding column prepared in step 5. Is normal a change of color from yellow to red once the sample is charged. If the volume of the sample is bigger than **700 µL** , charge the sample in proportions of **700 µL** . Centrifugate for one minute at 12,000-16,000 x g every time after the sample is charged. Discard the liquid obtained from the centrifugation.

9 Wash the column: Add **700 µL** of Wash Solution to the binding column. Centrifugate for one minute at 12,000-16,000 x g. Remove the binding column of the tube and discard liquid. Colocate again the binding column in the recollection tube and centrifuge one more time for one minute to eliminate the excess of ethanol

10 Elute the DNA: Transfer the binding column to a clean, esteril tube. Add **50 µL** of the Elution Solution to the center of the membrane and incubate for **00:01:00** minute. Centrifuge for **00:01:00** minute at 12,000-16,000 x g. In case of purifying plasmid DNA or linear fragmented of DNA with a weight exceeding 3Kb, preheat the Elute Solution at 65 °C 2m

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