DNA Gel Extraction

This protocol allows DNA extraction from a gel

Materials

- Gel with DNA sample of interest
- Zymoclean Gel DNA Recovery Kit (ZYMO research)

Equipment

- Scalpel
- 55ºC heat block
- 1.5 mL Eppendorf tubes
- Thermocycler (Eppendorf Mastercycler Nexus Gradient)
- Analytical balance
- Pipettes and pipette tips (0.5 - 10, 2-20, 20-200, 100 - 1000 µL)

Protocol

1. Weigh an empty Eppendorf tube
2. Cut the desired gel band and place it in an Eppendorf tube
3. Weigh the extracted gel, subtracting the weight of the empty Eppendorf
4. For the remaining steps, follow the Zymoclean Gel DNA Recovery Kit protocol

Miniprep

This protocol allows the isolation of the plasmid of interest from E. coli

Materials

- 5-10 mL overnight bacterial liquid culture
- QIAprep Spin Miniprep Kit (QIAGEN)

Equipment

- 1.5 mL Eppendorf tubes
- Benchtop centrifuge
- Thermocycler (Eppendorf Mastercycler Nexus Gradient)
- Pipettes and pipette tips (20-200, 100 - 1000 µL)

Protocol

1. Follow the QIAprep Spin Miniprep Kit protocol from QIAGEN

Yeast Genome Extraction

This protocol allows for the extraction of DNA from yeast

Materials

- 2mL Yeast Culture
- 0.2 M LiAc
- 1% SDS (w/v) solution.
- 100% ethanol
- NAE buffer (Macherey-Nagel)

Equipment

- 1.5 mL Eppendorf tubes
- 70℃ heat block
- Benchtop centrifuge
- Pipettes and pipette tips (0.5-10, 2-20 µL)

Protocol

1. Pick a small amount of the colony of interest.
2. Resuspend the colony in a 1.5 ml Eppendorf with 0.2 M LiAc + 1% SDS (w/v) solution.
3. Incubate the cell suspension at 70℃ for 5 min in a heat block.
4. Add 3 volumes of 100% ethanol to 1 volume of cell mixture and vortex thoroughly.
5. Centrifuge at 15,000g for 3 min.
6. Wash the resulting pellet once with 100% ethanol.
7. Carefully remove the supernatant, then resuspend the pellet in AE buffer (Macherey-Nagel).
8. Isolate/collect the supernatant from this mixture for downstream use by pelleting for 30 seconds at 15,000g