DYS SEE

PCR Cleanup.

iGEM Thessaly

Protocols

DNA extraction from agarose gels

Protocol from MN

Estimated time: 30min-1h

Before starting the preparation:

1. Excise DNA fragment/solubilize gel slice

Note: Minimize UV exposure time to avoid damaging the DNA.

- Take a clean scalpel to excise the fragment from an agarose gel. Remove all excess agarose.
- Determine the weight of the gel slice and transfer it to a clean tube.
- For 100mg of agarose gel <2% add 200ul Buffer NTI.
  For gels containing <2% agarose, double the volume of Buffer NTI.
- Incubate sample for 5-10 min at 50 degrees Celsius. Vortex the sample briefly every 2-3 min until the gel slice is **completely dissolved!**

2. Bind DNA

- Place a silica bead column into a collection tube (2mL) and load up to 700uL sample.
- Centrifuge for 20 s at 11,000 x g. Discard flow-through and place the column back into the collection tube.
- Load the remaining sample if necessary and repeat the centrifugation step.

3. Wash silica membrane

- Add **700 ul Buffer NT3(ethanol)** to the column. Centrifuge for **30s** at **11,000 x g.** Discard flow-through and place the column back into the collection tube.
- **Repeat this step (total 2 times).**

4. Dry silica membrane

- Centrifuge for 2 min at 11,000 x g to remove Buffer NT3 completely. Make sure the spin column does not in contact with the flow-through while removing it from the centrifuge and the collection tube.
  Note: Residual ethanol from Buffer NT3 might inhibit enzymatic reactions. Total removal of ethanol can be achieved by incubating the columns for 2-5 min at 70 degrees Celsius prior to elution.

5. Elute DNA

- Place the column into a new 1.5mL microcentrifuge tube. Add 15-30uL Buffer NE (or ddH_2O) and incubate at **room temperature** (18-25 Celsius) for **1 min.** Centrifuge for **1 min at 11,000 x g.**
  Note: DNA recovery of larger fragments (>1000bp) can be increased by multiple elution steps with fresh buffer, heating to 70 degrees and incubation for 5 min.

Protocols