Minimal preparation (Mini-Prep) of DNA

Aim of the experiment

This experiment can be used to extract DNA from bacterial overnight cultures with minimal effort. Typical concentrations reachable with this method vary between 50-350 ng/μl of DNA out of a overnight culture, depending on the volume of the culture and the replication level of the used vector. There are several different kits available from Biotech suppliers, which all follow the same workflow. The workflow and the names provided here are based on the Mini-Preparation kit of Quiagen. Names of buffer and incubation times can vary slightly to different kits.

Materials

- Overnight culture of bacterial strain with vector of interest
- QIAprep Spin Miniprep Kit (Qiagen, Canada)
- Nuclease-free water (nf-water, Carl Roth)
- Centrifuge for 1.5 ml tubes
- Centrifuge for 15 ml or 50 ml falcons

Procedure

1. Spin down the cells of the overnight culture at 6000 rcf for 5 min (4 °C advised for more pellet stability)
2. Trash the supernatant
3. Resuspend the cell pellet with the resuspension buffer (with added RNAse) and transfer into 1.5 ml tube
4. Add lysis buffer and mix quickly by inverting the tube
5. Add neutralisation buffer and mix by inverting the tube (let the lysis reaction not go longer than 5 minutes)

6. Spin down cell fragments at 16.000 rcf for 10 minutes in a table-top centrifuge
7. Apply supernatant to column from the kit
8. Perform typical Ethanol column cleaning protocol for DNA
9. Elute DNA from column by adding elution buffer or nuclease-free water and spinning the sample down at 16.000 rcf (let the elutant slowly sink into the column for bigger DNA elution, elution further goes down with small volumes smaller than 30 μl)

Possible follow up protocols

The following protocols are the next steps of a possible cloning cycle after a restriction digest:

1. Concentration measurement
2. Sequencing
3. Restriction digest
4. Transformation