Polymerase Chain reaction (PCR)-Clean up

Aim of the experiment

This protocol can be used for the quick purification of an exponentially PCR amplified DNA of interest. The protocol described is based on :

Monarch PCR-Clean up Kit

Materials

- PCR amplified DNA of interest
- NEB-Monarch PCR-clean up kit
- nuclease-free H_2O (nf H_2O, Sigma Aldrich, Germany)
- Centrifuge (1.5 ml Tubes, 16.000 rcf)

Procedure

1. Add to a column, collection tube combination the following dilution of your sample:

Table 1: PCR-clean up initial set up

Volumes	Chemicals
1	DNA of interest
2-5	DNA binding buffer

2. Spin for 1 min at 16.000 rcf and room temperature
3. Trash flow-through
4. Add 200 µl of DNA wash buffer to column
5. Spin for 1 min at 16.000 rcf and room temperature
6. Trash flow-through

7. Repeat Step 4-6
8. Make sure no ethanol touches the column when discarding the ethanol flow-through for the second time
9. Transfer the column into a fresh 1.5 ml tube
10. Add 6-20 µl of either Elution buffer or nuclease-free water to the column
11. Let the elutant incubate for at least 1 min
12. Elute by spinning for 1 min at 16.000 rcf and room temperature

Possible follow up protocols

The following protocols are the next steps of a possible cloning cycle after a PCR-clean up:

1. Restriction digest
2. Agarose-Gel-electrophoresis
3. Gibson-Assembly
4. Golden-Gate Assembly