PCR/Gel clean up

For the clean-up of PCR-products and samples of the gel-electrophoresis with agarose gels, the Wizard SV Gel and PCR Clean-Up System by Promega was used.

1.1. Gel Slice and PCR Product Preparation

- Following electrophoresis, excise DNA band from gel and place gel slice in a 1.5ml microcentrifuge tube.
- Add 10µl Membrane Binding Solution per 10mg of gel slice. Vortex and incubate at 50–65°C until gel slice is completely dissolved. Add an equal volume of Membrane Binding Solution to the PCR amplification.

1.2. Binding of DNA

- Insert SV Minicolumn into Collection Tube.
- Transfer dissolved gel mixture or prepared PCR product to the Minicolumn assembly. Incubate at room temperature for 1 minute.
- Centrifuge at 16,000 × g for 1 minute. Discard flowthrough and reinsert Minicolumn into Collection Tube.

1.3. Washing

- Add 700µl Membrane Wash Solution (ethanol added). Centrifuge at 16,000 × g for 1 minute. Discard flowthrough and reinsert Minicolumn into Collection Tube.
- Repeat Step 6 with 500µl Membrane Wash Solution. Centrifuge at 16,000 × g for 5 minutes.
- Empty the Collection Tube and recentrifuge the column assembly for 1 minute with the microcentrifuge lid open (or off) to allow evaporation of any residual ethanol.

1.4. Elution

- Carefully transfer Minicolumn to a clean 1.5ml microcentrifuge tube.
- Add 50µl of Nuclease-Free Water to the Minicolumn. Incubate at room temperature for 1 minute. Centrifuge at 16,000 × g for 1 minute.
- Discard Minicolumn and store DNA at 4°C or –20°C.