This protocol is based on the Qiagen Miniprep instruction manual. This document is version 1.00: last updated 8.12.11.

ØØØØ DNA Purification (PCR/Digest)

This protocol will purify DNA based on the QIAgen PCR purification system. DNA must be dissolved in a binding buffer, then run through the column. This protocol can operate for the purification of both digests and PCR, though PCR purification is its primary function.

Solutions
PCR/Digest solution
Buffer PB
Buffer PE
Buffer EB

Materials 100,1000µl Pipettes Centrifuge tubes Spin columns with flow-through containers

You will also need access to a Centrifuge

Procedure

- 1. Add **5x(**volume of DNA solution)μ**L Buffer PB** to each centrifuge tube. Mix by vortexing.
- 2. Add the contents of the tube to a **spin column** with a **1000**μ**L pipette.**
- 3. Place the column in a **flow-through container**. Centrifuge at **13 000 rpm** for **1 minute**. Decant the flow-through.
- 4. Add **750**μ**L Buffer PE** with a **1000**μ**L pipette.** Centrifuge at **13 000 rpm** for **1 minute.** Decant the flow-through.
- 5. Centrifuge at **13 000 rpm** for **1 minute.** This will remove any residual buffer in the column. It is not necessary to decant the flow-through, as the column collection tube will be discarded.
- 6. Remove the column from its collector and insert into a fresh, labeled **1.5mL centrifuge tube.**
- 7. Add **30-50** μ **L Buffer EB** to the center of the column's filter with a **100** μ **L pipette**. Ensure the buffer is absorbed by the filter.
- 8. Centrifuge at **13 000 rpm** for **5 minutes.** The flowthrough will contain the desired DNA.