# Purification of DNA with GeneClean kit II by means of glassmilk

**1. Subject**

The disadvantage of a mini-prep extraction is the bad purity of rDNA. The suspension contains DNA, but also other propagules from the cell and cell debris. These substances can inhibit PCR. A possible solution to improve PCR is a purification step.

**2. Principle**

The principal of this purification procedure is the ability of glassmilk (SiO2) to bind DNA in the presence of a high concentration of NaI (1 M). By alternating steps of centrifugation and washing with NEW-buffer impurities are discarded. The NEW-buffer helps to remain DNA fixed to the glassmilk while washing. After several washes with buffer the DNA is resuspended in sterile ultrapure water or TE-buffer by disconnecting the bond between glassmilk and DNA.

**3. Reagents and material**

3.1 pipets
3.2 pipettips
3.3 eppendorfcups
3.4 microcentrifuge 14.000 rpm (Sigma 112, Eppendorf 5417R)
3.5 GeneClean kit II (containing NaI-solution, glassmilk and NEW washbuffer)
3.6 sterile ultrapure water
3.7 waterbath 48□C
3.8 Tris(-hydroxymethyl)-aminomethane (Merck 8382)
3.9 Na-EDTA, Titriplex III (BioRad 161-0729)
3.10 HCl 1N (Ferak 11448)

**4. Solutions**

4.1 TE-buffer, pH 8.0:
Add 0.12 g Tris [3.8], 0.04 g Na-EDTA [3.9] to 80 ml ultrapure water. Adjust pH to 8.0 with 1N HCl [ 3.10]. Heat the solution if EDTA does not solve very well. Adjust volume to 100 ml with ultrapure water. Make aliquots of 50 ml. Autoclave the buffer during 15 min. at 121□C. Store at roomtemperature.

**5. Protocol**

5.1 Pipet 45-50 µl DNAsample (PCR-amplicon) into an eppendorfcup.
5.2 Add 150 µl NaI (sodiumiodide) and 5 µl glassmilk.
(vortex glassmilk vigorously before use)
5.3 Incubate during 10 min. at roomtemperature.
5.4 Centrifuge shortly (± 3 sec.) at full speed in a microcentrifuge (20.400 rcf).
5.5 Discard the solution by pipeting; pellet should be left undisturbed.
5.6 Add 135 µl NEW-buffer to the pellet, vortex shortly and centrifuge [5.4].
5.7 Discard buffer.
5.8 Repeat steps 5.6-5.7 twice.
5.9 Pipet 25 µl sterile, ultrapure water or TE-buffer [4.1] and resuspend the pellet.
5.10 Incubate 4 min.n at 48° C (e.g. in a waterbath) [6.1].
5.11 Centrifuge 1 min. at full speed in a microcentrifuge.
5.12 Pipet supernatant into a sterile eppendorfcup; it contains the purified DNA.
5.13 Discard eppendorfcup containing glassmilk pellet.

*A.H.G. Gerrits van den Ende*

**6. Remarks**

6.1 In literature [7.1] a method is published with 54°C to get a higher yield especially with amplicons smaller than 1000 bp.

**7. Literature**

7.1 Smith LS, Lewis TL, Matsui SM. Increased yield of small DNA fragments purified by silica binding. BioTechniques 18: 970-975 (1995).

*A.H.G. Gerrits van den Ende*