NEW JETQUICK PCR Purification Protocol

September 2003

VACUUM MANIFOLD PROTOCOL INSERT

Very Important!

Before starting the procedure, make sure that solution H2 is reconstituted as indicated on the bottle's label.

Removal of mineral oil is **<u>not</u>** rquired.

The JETQUICK procedure is **<u>not</u>** sensitive to detergents (i.e. Triton X-100, Nonidet NP40) or gelatin in the assay. Nonetheless, final concentrations of >0.1% of Tween-20 should be avoided.

All centrifugation steps are carried out at top speed (approx. 13,000 rpm) in a conventional table-top microfuge.

PROTOCOL

1.) **Sample Preparation:** Add **400 µl** of binding **buffer H1** to **100 µl of amplification reaction** and **<u>mix</u> <u>thoroughly</u>**.

__Important note:__ Make sure, that the volume of the PCR assay does not exceed 100 µl (WITHOUT any oil overlay). If PCR assays with a volume >100 µl are to be processed, remove most of the oil overlayer with a pipette, and adjust the volume of binding buffer H1 proportionally to a ratio of buffer H1 : amplification reaction of 4:1 (v/v). In such a case multiple successive loading steps (see step 3) are necessary.

2.) **Vacuum Manifold Preparation:** Attach the vacuum manifold to a vacuum source. Attach as many micro-spin columns as required to the female luer extensions on the vacuum manifold.

3.) **Column Loading:** Load the sample from step 1 into a prepared micro-spin column. Apply vacuum (-200 to -650 mbar) until all liquid has been pulled through the cartridge. Then turn off the vacuum source.

4.) **Column Wash:** Add **700 µl** of **reconstituted Wash Buffer H2** to the micro-spin column. Apply vacuum (-600 to -650 mbar) until all liquid has been pulled through the cartridge. Then turn off the vacuum source.
Place the micro-spin column into a 2 ml wash tube and centrifuge at top speed for 1 min to remove any residual wash buffer.

5.) **DNA Elution:** Place the JETQUICK micro-spin column into a new 1.5 ml recovery tube and add **50 µl** of sterile water (or TE buffer or 10 mM Tris-HCl [pH 7-8]) directly onto the center of the silica matrix of the spin column. Centrifuge at top speed for 2 min.

__Important:__ Higher DNA concentrations will be obtained if the elution is carried out with only 30 µl of elution buffer. In this case preheat the elution buffer to 65-70 °C, add the buffer onto the center of the silica matrix of the spin column and let stand for 1 min before centrifugation. DNA eluted in water should be generally stored at –20 °C.