NEW JETQUICK Gel Extraction Protocol

September 2003

VACUUM MANIFOLD PROTOCOL INSERT

Very Important!

Before starting the procedure, make sure that solution L2 is reconstituted as indicated on the bottle's label.

Either high or low-melting-point agarose can be used.

Complete dissolution of the agarose gel slice is critical. Once the gel slice appears dissolved, an additional 5 min incubation period at 50°C is required.

The use of 100 mg agarose gel slices per extraction is recommended. The maximum weight of gel slices should not exceed 400 mg.

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

PROTOCOL

1.) **Gel Slice Excision:** Cut the area of gel containing the DNA fragment of choice using a clean, sharp blade. Minimize the amount of surrounding agarose excised with the fragment.

2.) **Estimation of Buffer L1 requirement:** Weigh the gel slice.

- **a) For ≤2% agarose gels:** Place up to 400 mg of gel into a suitable 1.5-ml polypropylene reaction tube. Divide gel slices exceeding 400 mg among additional tubes. Add **300 µl** of **Gel Solubilization buffer L1** for every **100 mg of gel**.
- **b) For ≥2% agarose gels:** Place up to 400 mg of gel into a suitable 5-ml polypropylene reaction tube. Divide gel slices exceeding 400 mg among additional tubes. Add **600 µl** of **Gel Solubilization buffer L1** for every **100 mg of gel**.

3.) **Gel Solubilization:** Incubate at **50°C for 15 min**. Mix every 3 min by flicking or vortexing the tube to ensure complete gel dissolution.

Important note: *Larger gel slices or high concentration gels (≥2% agarose) may take longer to dissolve. In such a case increase the incubation time to 20-30 min. Cutting larger gel slices into smaller pieces will enhance solubilization.*

4.) **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.

5.) **Column Loading:** Load the sample from step 3 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.

Important note: *Load no more than 400 mg agarose per cartridge. Do not overload the JETQUICK columns.*

6.) **(Optional column wash):** Add **500 µl** of **Gel Solubilization Buffer L1** to the micro-spin column. Let stand for 1 min. Apply vacuum (-600 to -650 mbar) until all liquid has been pulled through the cartridge. Then turn off the vacuum source.

Important note: *This wash is recommended when ≥250 mg of gel has been loaded onto a cartridge, when the initial agarose concentration was ≥2%, or when higher purity is required for applications such as DNA sequencing or in vitro transcription.*

7.) **Column Wash:** Add **700 µl** of **reconstituted Wash Buffer L2** 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.

8.) **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.*