V. Protocol for DNA Extraction from Agarose Gels

1. Excise DNA fragment

Take a clean scalpel to excise the DNA fragment from agarose gel. Excise gel slice containing the fragment carefully to minimize the gel volume. Determine the weight of the gel slice and transfer it to a clean tube.

2. Gel lysis

For each **100 mg** agarose gel add **200 *µl* buffer NT.**

For gels containing > 2% agarose, double the volume of buffer NT. The maximum amount of gel slice per NucleoSpin® Extract II column is 400 mg or 200 mg of a high percentage gel > 2%. In this case 2 loading steps are required (step 3).

Incubate sample at **50°C** until the gel slices are dissolved **(5-10 min).** Vortex the sample briefly every 2 3 min until the gel slices are dissolved **completely!**

3. Bind DNA

Place a NucleoSpin® Extract II column into a 2 ml collecting tube and load the sample.

Centrifuge for **1 min at 11,000 x g.** Discard flow-through and place the NucleoSpin® Extract II column back into the collecting tube.

4. Wash silica membrane

Add **600 *µl* buffer NT3.** Centrifuge for **1 min** at **11,000 x g.** Discard flow-through and place the NucleoSpin® Extract II column back into the collecting tube.

V. Protocol for DNA Extraction from Agarose Gels *continued*

5. Dry silica membrane

Centrifuge for **2 min** at **11,000 x g** to remove **buffer NT3** quantitatively. Make sure the spin column doesn't come in contact with the flow-through while removing it from the centrifuge and the collecting tube.

Residual ethanol from buffer NT3 would inhibit subsequent reactions and has to be removed in this step. In addition to centrifugation, total removal can be achieved by incubation of NucleoSpin® Extract II columns for 2-5 min at 70°C prior to elution.

6. Elute DNA

Place the NucleoSpin® Extract II column into a **clean** 1.5 ml microcentrifuge tube. Add **15-50 *µl* elution buffer NE**, incubate at **room temperature** for **1 min** to increase the yield of eluted DNA. Centrifuge for **1 min** at **11,000 x g.**

Yield of larger fragments (> 5-10 kb) can be increased by using prewarmed elution buffer (70°C): For elution, add prewarmed elution buffer and incubate at room temperature for 1 min before collecting eluate by centrifugation.