

Gel Extraction

BEFORE YOU BEGIN:

- All centrifugation steps should be carried out at **16,000 x g** (~13,000 RPM)

Please note: **column holds 800 μ l**

PROTOCOL STEPS:

1. **Excise the DNA fragment** from the agarose gel, taking care to trim excess agarose. Transfer to a **1.5 ml microfuge tube**. (Minimize exposure to UV light)
2. Add **Gel Dissolving Buffer** to the gel slice so that the slice is **covered by buffer**.
3. Incubate the sample at **50°C, vortexing periodically** until the gel slice is **!COMPLLETELY DISSOLVED!** (generally 5-10 minutes). (For DNA fragments >8 kb, an additional 1.5 volumes of water should be added after the slice is dissolved to mitigate the tighter binding in larger pieces of DNA)
4. Insert column into collection tube and **load sample onto the column. Spin for 1 minute**, then **discard flow-through**.

5. Re-insert **column into collection tube**. Add **200 μ l DNA Wash Buffer** and **spin for 1 minute**. Discarding flow-through is optional.

6. **Repeat step 5**.

7. **Transfer column** to a **clean 1.5 ml microfuge tube**. Use care to ensure that the **tip of the column does not come into contact with the flow-through**. If in doubt, re-spin for 1 minute.

8. Add **20 μ l of DNA Elution Buffer** to the **center** of the column **matrix**. **Wait for 1 minute**, then **spin for 1 minute**.

9. **Measure the DNA-concentration** with **NanoDrop** and write it on the side of the Eppi.