# DYSSEE

## Gel Extraction.

iGEM Thessaly

Protocols

# DNA extraction from agarose gels

## Storage conditions and preparation of working solutions

**Attention:** Buffer NTI contains chaotropic salt. Wear gloves and goggles!
**Caution:** Buffer NTI contains guanidinium thiocyanate which can form highly reactive compounds when combined with bleach (sodium hypochlorite). DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

The NucleoSpin® Gel and PCR Clean-up kit should be stored at room temperature and is stable for at least one year.

Before starting any NucleoSpin® Gel and PCR Clean-up protocol prepare the following:
- **Wash Buffer NT3**: Add the indicated volume of ethanol (96–100 %) to **Buffer NT3 Concentrate**. Mark the label of the bottle to indicate that ethanol was added.
- **Wash Buffer NT3** is stable at room temperature (18–25 °C) for at least one year.

**Before starting the preparation:** check if Wash Buffer NT3 was prepared according to section 3.

## 1. Excise DNA fragment / solubilize gel slice

*Note: Minimize UV exposure time to avoid damaging the DNA. Refer to section 2.5 for more tips on agarose gel extraction.*
- Take a clean scalpel to excise the DNA fragment from an agarose gel.
- Remove all excess agarose.
- Determine the weight of the gel slice and transfer it to a clean tube.
- For each **100 mg of agarose gel < 2 %** add **200 µL Buffer NTI**.
- For gels containing > 2 % agarose, double the volume of Buffer NTI.
- Incubate sample for **5–10 min** at **50 °C**.
- Vortex the sample briefly every 2–3 min until the gel slice is **completely** dissolved!

## 2. Bind DNA

- Place a **NucleoSpin® Gel and PCR Clean-up Column** into a Collection Tube (2 mL) and load up to 700 µL sample.

- Centrifuge for **30 s** at **11,000 x *g***.
- Discard flow-through and place the column back into the collection tube.
- Load remaining sample if necessary and repeat the centrifugation step.

## 3. Wash silica membrane

- Add **700 µL Buffer NT3** to the NucleoSpin® Gel and PCR Clean-up Column.
- Centrifuge for **30 s** at **11,000 x *g***.
- Discard flow-through and place the column back into the collection tube.

*Recommended: Repeat previous washing step to minimize chaotropic salt carry-over and low A260/A230.*

## 4. Dry silica membrane

- Centrifuge for **1 min** at **11,000 x *g*** to remove Buffer NT3 completely.

Make sure the spin column does not come in contact with the flow-through while removing it from the centrifuge and the collection tube.

*Note: Residual ethanol from Buffer NT3 might inhibit enzymatic reactions. Total removal of ethanol can be achieved by incubating the columns for 2–5 min at 70 °C prior to elution.*

## 5. Elute DNA

- Place the NucleoSpin® Gel and PCR Clean-up Column into a **new** 1.5 mL microcentrifuge tube (not provided).
- Add **15–30 µL Buffer NE** and incubate at **room temperature** (18–25 °C) for **1 min**. Centrifuge for **1 min** at 11,000 x *g*.

*Note: DNA recovery of larger fragments (> 1000 bp) can be increased by multiple elution steps with fresh buffer, heating to 70 °C and incubation for 5 min.*

# Protocols