Technische Universiteit
Eindhoven
University of Technology

**iGEM TU/e 2018**
Biomedical Engineering

Eindhoven University of Technology
Room: Ceres 0.04
Den Dolech 2, 5612 AZ Eindhoven
The Netherlands
Tel. no. +31 50 247 55 59
2018.igem.org/Team:TU_Eindhoven

# Gel purification

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# 1 Gel purification

## 1.1 Making agarose gel

**Estimated bench time:** 30 minutes
**Estimated total time:** 60 minutes
**Purpose:** To construct an agarose gel for gel purification

It is essential to work with Nitrile gloves when adding SybrSafe.

Select the right percentage agarose gel depending on the DNA size.

| Percent agarose gel (w/v) | DNA Size Resolution (kb=1000) |
|---|---|
| **0.5%** | 1 kb to 30 kb |
| **0.7%** | 800 bp to 12 kb |
| **1.0%** | 500 bp to 10 kb |
| **1.2%** | 400 bp to 7 kb |
| **1.5%** | 200 bp to 3 kb |
| **2.0%** | 50 bp to 2 kb |

### 1.1.1 Materials

- 1X TAE buffer
- Agarose
- Agarose gel comb
- Agarose gel tray
- Autoclave tape
- Balance
- Erlenmeyer
- Microwave
- SybrSafe

### 1.1.2 Setup & Protocol

- Tape the outsides of the gel tray, so that the gel can be placed inside the tray.
- Measure out the correct amount of agarose.
- Pour agarose powder into an Erlenmeyer along with 130mL of 1xTAE.
- Weigh the Erlenmeyer containing the agarose-TAE.
- Microwave for 1-3 minutes, swirl every 30 seconds (until the agarose is completely dissolved and there is a nice rolling boil).
- Weigh the Erlenmeyer again and add $H_2O$ to correct for the volume loss caused by heating.
- Let agarose solution cool down (for 5 minutes) to 50-60°C
- Add 13 µl SybrSafe (10,000x stock). Wear gloves and swirl/mix well.
- *If there is a lot of DNA, some well combs can be merged together.*
- Put the well comb in and pour the agarose gel, for loading large amounts of DNA, tape multiple wells of the comb together.

- Let sit at room temperature for 20-30 minutes, until it has completely solidified.
- When the gel is ready to use, load the sample and a fitting ladder.

## 1.2 Preparing samples

**Estimated bench time:** 5 minutes start-up + 1 minute per sample
**Estimated total time:** 5 minutes start-up + 1 minute per sample
**Purpose:** To mix the DNA with a dye such that it becomes visible on the gel.

Wear gloves, as you are working with DNA.

### 1.2.1 Materials

- 6X Loading Dye
- DNA Ladder
- DNA Sample(s)
- Pipette and tips

### 1.2.2 Setup & Protocol

- Prepare the samples with 6X loading dye, i.e. add 1 volume of loading dye to 5 volumes of sample. Pipette up and down to mix the loading dye with the sample.

## 1.3 Loading of the ladder and samples

**Estimated bench time:** 5 minutes start-up + 1 minute per sample
**Estimated total time:** 5 minutes start-up + 1 minute per sample
**Purpose:** Loading the DNA samples within the wells created by the well comb

Wear gloves, as you are working with DNA.

### 1.3.1 Materials

- 1X TAE buffer
- Agarose gel electrophoresis system
- DNA ladder
- Pipettes and tips
- Prepared samples
- Solidified agarose gel

### 1.3.2 Setup & Protocol

- Remove the autoclave tape from the solidified gel. Place the gel on the gel tray within the electrophoresis system. Make sure that the comb is located at the negative electrode.

- Add TAE buffer to the gel electrophoresis system until the gel is completely submerged by the TAE buffer.
- Carefully remove the gel comb from the agarose gel.
- Load 5 µl of the DNA ladder and ~20 µl per well of the samples within the wells. Make sure that the samples and ladder sink to the bottom of the well.

## 1.4 Running gel:

**Estimated bench time:** 1 minute
**Estimated total time:** 60 minutes
**Purpose:** Letting the DNA move through the gel.

- Run the gel for approximately 60 minutes at 100V.
- Turn OFF power, disconnect the electrodes from the power source, and then carefully remove the gel from the gel box.

## 1.5 Cut DNA out of gel

**Estimated bench time:** 15 minutes
**Estimated total time:** 15 minutes
**Purpose**: Purifying the DNA by cutting out the correct bandwidth of DNA.

Wear gloves, as you are working with DNA.

### 1.5.1 Materials

- Blue lamp
- Blue lamp protecting glasses
- Falcon Tube
- Gloves
- Scale
- Sharp knife

### 1.5.2 Setup & Protocol

- Mark and weigh a Falcon tube.
- Place the gel on a blue lamp. Use protecting glasses
- Cut out the band from the gel. Try to cut away all non-DNA containing gel parts.
- Transfer the gel fragment to the Falcon tube.
- Weigh the Falcon tube also after transferring the gel to this tube to calculate the volume of the gel.
- Optional: store the gel in a -20 °C fridge.

## 1.6 Gel extraction

**Estimated bench time:** 30 minutes
**Estimated total time:** 30 minutes
**Purpose:** To extract the DNA out of the agarose gel.

It is essential to work with gloves at all times to protect the DNA from DNase activity.

### 1.6.1 Materials

- Autoclaved $H_2O$)
- Eppendorf tubes
- MiniSpin Centrifuge
- Pipettes and tips
- QIAquick Gel Extraction Kit

### 1.6.2 Setup & Protocol

- Add 3 volumes Buffer QG to 1 volume gel cut-out (100 mg ~100 µL). For >2% agarose gels, add 6 volumes Buffer QG.
- Incubate the samples at 50 °C for 10 minutes, vortexing every few minutes until the gel is fully dissolved. Solute color should be yellow. Make sure the gel is well dissolved.
- Add 1 volume of isopropanol to the sample and vortex.
- Transfer a maximum of 800 µl to the spin column and centrifuge for 60 sec at 13,400 rpm, weight balance well. Repeat this until the entire sample has been centrifuged. Keep adding solution from the same tube to the same spin column. Each time discard flow-through.
- Add 750 µl of Buffer PE to the column and centrifuge for 60 sec at 13,400 rpm. Discard the flow through and repeat 2 times.
- Dry spin the column for 60 sec at 13,400 rpm
- Place QIAquick column into a clean 1.5 ml Eppendorf tube.
- To elute DNA, add 42 µl $dH_2O$ to the center of the QIAquick membrane and let the column stand for 1 minute.
- Centrifuge 1 minute at 13,400 rpm.

## 1.7 Nanodrop

**Estimated bench time:** -
**Estimated total time:** 5 minutes start-up + 2 minutes per sample
**Purpose:** Determine the concentration of DNA in water.

It is essential to work with gloves at all times to protect the DNA from DNase activity.

### 1.7.1 Materials

- Autoclaved $H_2O$ (nuclease free water)
- NanoDrop Photospectrometer
- Pipettes and tips

### 1.7.2 Setup & Protocol

- Select the DNA measurement (Nucleic Acid) in the Nanodrop menu.
- Clear the surface of the Nanodrop with $dH_2O$.
- Preform a calibration and blank measurement by entering one drop of 2 µl $dH_2O$.
- Clean the surface again and place 2 µl per sample on the Nanodrop, and measure the amount of DNA in the Nanodrop.