# Gel Electrophoresis and band extraction

### Introduction

Run a gel electrophoresis for the BBs from the PCR to quantify their length and to extract the bands for purification.

### Materials

- 1% or 2% agarose
- SYBRSafe gel stain
- Ladder
- Dye (6x, 5 parts of sample, 1 part of dye)
- Gel casting tray, comb (with different well sizes), transport tray, scalpel and Blue Light Transilluminator

### Procedure

1. Assemble a gel casting tray based on the number of samples needed.
2. Based on the sample size use the appropriate certain comb, for analytical wells use 5-8 µL, for gel extraction use 33 µL wells.
3. Pour in the desired agarose and pipette 10 µL or 20 µL of SYBR safe stain (for small gels add 10 µL and for the big gels 20 µL) and mix well with the pipette tip.
4. Let it sit and solidify.
5. Meanwhile prepare the samples by adding the dye (for 6x dye: 5 parts of sample 1 part of dye).
6. Once the gel solidifies, transfer it into the gel electrophoresis unit. Make sure the gel is fully submerged in TAE buffer.
7. Load the ladder and the samples in the wells and run at the appropriate voltage and time for the resolution desired. (Reference standard parameters: 100 V for 20 min)
8. For analytical gels: After the gel has run, transfer it to an imaging system and analyze with trans-UV light.
9. For gel band extraction: After the gel has run, transfer the gel to the Blue Light Transilluminator. Extract the desired bands with scalpel and tweezers and transfer to Eppendorf tubes. Remember to use the amber spectacles and clean all material with ethanol in between every sample.

# Gel Band Purification Kit GE Healthcare

## Introduction

Protocol for purification of DNA from TAE and TBE agarose gels after gel band extraction.

## Materials

- Centrifuge
- Weighing scale
- Wash Buffer Type 1
- Elution Buffer Type 4
- Elution Buffer Type 6
- Capture Buffer Type 3
- Collection Tubes and GFX MicroSpin column (All the buffers and materials are supplied by the kit)
- Microcentrifuge tubes

## Procedure

## Sample Capture

1. Weigh a DNase-free 1.5 mL microcentrifuge tube and record the weight.
2. Using a clean scalpel, long-wavelength (365 nm) ultraviolet light and minimal exposure time, cut out an agarose band containing the sample of interest. Place agarose gel band into a DNase-free 1.5 mL microcentrifuge tube (user supplied).
3. Weigh the microcentrifuge tube plus agarose band and calculate the weight of the agarose slice. Note: The sample may now be stored at -20 °C for up to 1 week.
4. Add 10 µL Capture buffer type 3 10 mg of gel slice, for example, add 300 µL Capture buffer type 3 to each 300 mg gel slice. 10 µL Capture buffer type 3 per 10 mg gel slice for each A.
   Note: If the gel slice weighs less than 300 mg, add 300 µL Capture buffer type 3. DO NOT add less than 300 µl Capture buffer type 3 per sample. Note: To save time when purifying multiple samples of gel bands (each weighing less than 500 mg), add 500 µL Capture buffer type 3 to each gel slice. DNA recovery will be unaffected providing the volume of Capture buffer type 3 is in excess of the weight of each gel slice.
5. Mix by inversion and incubate at 60 °C for 15-30 minutes until the agarose is completely dissolved. Mix by inversion every 3 minutes. 60 °C until agarose dissolves
   Note: If sample contains DNA greater than 5 kb, do not vortex, as this may cause shearing of the DNA.
6. Once the agarose has completely dissolved check that the Capture buffer type 3-sample mix is yellow or pale orange in colour.
   Note: If the colour of the binding mixture is dark pink or red, add a small volume (~ 10 µL) of 3 M sodium acetate pH 5.0 and mix. Ensure that the binding mixture turns a yellow or pale orange colour before loading onto the GFX MicroSpin column.

7. For each purification that is to be performed, place one GFX MicroSpin column into one Collection tube.

**Sample Binding**

1. Centrifuge Capture buffer type 3- sample mix briefly to collect the liquid at the bottom of the Pulse tube.
2. Transfer up to 800 µL Capture buffer type 3-sample mix onto the assembled GFX MicroSpin Apply up to 800 µL Capture buffer type 3-sample mix column and Collection tube. Note: the cap of the Collection tube can be used to cap the GFX Microspin column. If the cap is not required, cut if off.
3. Incubate at room temperature for 1 minute.
4. Spin the assembled column and Collection tube at 16 000 x g for 30 seconds.
5. Discard the flow through by emptying the Collection tube. Place the GFX MicroSpin column back inside the Collection tube.
6. Repeat Sample Binding steps b. to e. as necessary until all sample is loaded.

**Wash & Dry**

1. Add 500 µL Wash buffer type 1 to the GFX MicroSpin column.
2. Spin the assembled column and Collection tube at 16 000 × g for 30 seconds.
   Note: If purity is paramount (e.g., if the sample is to be used in a blunt-ended ligation), repeat Wash & Dry step a and perform step b twice. After the first spin, discard flow through, place the GFX Microspin column back inside the Collection tube and centrifuge at 16 000 × g for an additional 30 seconds. This extra wash step may reduce yield by 4%.
3. Discard the Collection tube and transfer the GFX MicroSpin column to a fresh DNase-free 1.5 mL microcentrifuge tube (supplied by user).

**Elution**

1. Add 10-50 µL Elution buffer type 4 OR type 6 assembled GFX MicroSpin column and sample 1 minute to the centre of the membrane in the RT Collection tube.
2. Incubate the assembled GFX MicroSpin column and sample Collection tube at room temperature for 1 minute.
3. Spin the assembled column and sample Collection tube at 16 000 x g for 1 minute to recover the purified DNA.
4. Proceed to downstream application. Store the purified DNA at -20°C.