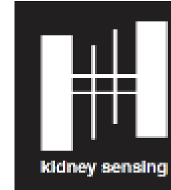
Team Brasil-SP
iGEM 2014



Agarose Gel Electrophoresis and DNA Gel Purification

Materials

- 1X TAE Buffer [40 mM Tris base, 5 mM Sodium acetate, 1mM EDTA (pH 8)]
- Electrophoresis chamber
- Ethidium bromide
- Nitrile gloves
- DNA ladder
- Loading Buffer

Apparatus

- UV transilluminator
- Gel image system

Method

1. Prepare 100 mL of 1X TAE, 0.8% agarose gel and add ethidium bromide in a final concentration of 0.2-0.5µg/mL (about 2-5 µL of lab stock solution (10 mg/mL) per 100 mL gel) .
2. Add the loading buffer to each the sample.
3. Carefully load the DNA ladder into the first well of the gel and the samples into the additional wells.
4. Run the gel for 40-50 minutes at 100 volts.
5. Take a picture with a UV gel image system.
6. Place the gel in a UV transilluminator and excise the band of interest.
7. Purify the gel slice with Wizard SV Gel and PCR Clean-Up System kit (Promega) according to manufacturer's instructions or other similar kit.

Considerations:

- Always use nitrile gloves when handling the agarose gel stained with ethidium bromide.
- If you are working with small DNA fragments, prepare a 1X TAE, 1.2 % agarose gel and be careful with the run time.

For more information, consult the Wizard SV Gel and PCR Clean-Up System kit specifications available on the website below.
[http://www.promega.com.br/~/media/files/resources/protcards/wizard%20sv%20gel%20and%20pcr%20clean-up%20system%20quick%20protocol.pdf]