DNA Electrophoresis protocol

A. Theory：

Electrophoresis is a separation method frequently used to analyze DNA fragments generated by restriction enzymes, and it's an analytical method for observing and determining plasmid size and concentration. Because there are many phosphates in DNA structure, it is usually negative charged. With this characteristics, we can put our DNA samples in DNA agarose and use an electrical field to separate different size of DNAs. DNA fragments with small size can run faster in the gel while DNA fragments with large size only run in little distance at the same time.

B. Materials：

- DNA agarose gel (different percentage for different use)
- TAE/TBE buffer
- Ethidium bromide (EtBr)

C. Laboratory procedures：

1. Produce DNA agarose gel
   - Add proper amount of agarose in TAE/TBE buffer (we usually produce 0.5, 0.75, 1.5% gel) and use a microwave oven to make the agarose completely dissolve in buffer (transparent)
   - Let agarose solution cool down to about 50°C (about when you can comfortably keep your hand on the flask), about 5 mins, and pour the agarose into a gel tray with the well comb in place
   - Let sit at room temperature for 20-30 mins, until it has completely solidified.
2. Add TAE/TBE buffer into gel electrophoresis tank
3. Add EtBr
   - 8~1000 μl
   - Mix well
4. Add dye into sample
   - Since the dye is 6x, so sample amount divide by 6
   - Prepare tape, mix sample with dye
5. Prepare marker：5 μl
6. Electrophoresis
   - First well load marker
   - Voltage 125V, 20 min
   - Marker run to 3rd last row
7. Photo-taking
   - Dry the gel before photo-taking
   - use UV light to observe the results and do photo-taking

*Our protocol is modified from

https://www.addgene.org/protocols/gel-electrophoresis/