Restriction enzyme & gel electrophoresis

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1 *Works for me* This protocol is published without a DOI.

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Preparation

1 Set the dry bath incubator, **37 °C**

2 Take 10x buffer from the **-20 °C fridge** to thaw.

Protocol-Restriction enzyme

3 To add **500 ng** DNA for each sample into each eppendorf, calculate the required DNA volume for each sample. The volume is decided by the formula below:
Volume(sample)= **500 ng** / concentration measured by spectrophotometer(ng/uL)

4 Calculate the required ddH2O volume for each sample. The volume is decided by the formula below:
Volume(ddH2O)=20uL - 2uL - 0.5uL - 0.5uL - Volume(sample) uL

5 Take sufficient eppendorfs and mark them for each sample. First, add ddH2O based on previous calculation in each eppendorf. Next, add DNA samples based on previous calculation in each eppendorf.

6 Take an eppendorf and mark as CT(cocktail). Add 10x buffer to the eppendorf. The volume is decided by the formula below:
Volume(10x buffer)=2uL x (the number of samples +1)

7 Take the determined restriction enzyme from the **-20 °C fridge** . Add RE to the previous eppendorf respectively. Remember to put restriction enzyme **On ice** during the whole process and take RE back to the **-20 °C fridge** right after the process ASAP. The volume is decided by the formula below:
Volume(RE 1)=0.5uL x (the number of samples +1) Volume(RE 2)=0.5uL x (the number of samples +1)

8 Add **3 µl** of CT in each eppendorf. Vortex and spin down each eppendorf to make the liquid stay at the bottom.

9 Put each eppendorf in the **37 °C** dry bath incubator for **02:00:00** or even more.

Protocol-Gel electrophoresis

10 Put a gel into the electrophoresis tank.

11 Take a duran youtility. Add **3.5 mL** TAE and **350 mL** ddH2O into it and then mix it. Put it into the electrophoresis tank. Make sure the gel is soaked into the liquid completely.

12 Take **5 µl** of marker (1kb) and load it in the first well.

13 Take loading dye and drop on the parafilm separately. The volume is decided by the formula below:
Volume(loading dye)=1uL x (the number of samples)

14 Take **5 µl** of sample reacted with RE for **02:00:00** . After pipetting with **1 µl** loading dye, add it into each well. Repeat this step until all the samples are filled into each well.

15 Connect wires with the power supply. Set time for **00:30:00** and click "start" button. Make sure there are bubbles appearing next to anode and cathode.

16 After gel electrophoresis, put the gel into gel reading machine and report the result.