Ligation and gel electrophoresis

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This protocol is used for ligation of circular probe

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Preparation

1 Dilute linear DNA into 10μM

2 Dilute T4 ligation primer into 10μM

Protocol of Ligation

3 Add **11 μL** of RNase-free water into a eppendorf

4 Add **3 µL** of 10X T4 Ligase buffer

5 Add **9 µL** of 10µM T4 ligation primer

6 Add **3 µL** of 10µM linear DNA

7 Add **4 µL** of T4 Ligase

8 Spin down after vortex

9 Incubate for **04:30:00** at **Room temperature** 4h 30m

Protocol of Gel Electrophoresis 30m

10 Put 2% agarose gel into the electrophoresis tank.

11 Prepare 0.5X TAE buffer (add **3.5 mL** 50X TAE buffer and **350 mL** ddH2O and mix them), then pour into the electrophoresis tank. Make sure the gel is soaked into the buffer completely.

12 Take **5 µL** of marker and load it in the first well.

13 Take **1 µL** of 6X loading dye and drop on the parafilm separately, pipetting dye with

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5 µL of sample

14 Load samples into each well

15 Run gel at a constant voltage of 100V for **00:30:00** at room temperature

30m

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

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