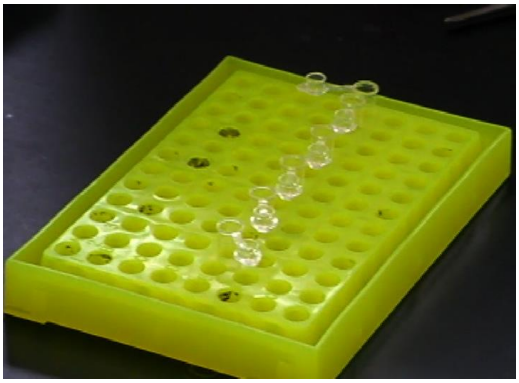
Analysis of DNA

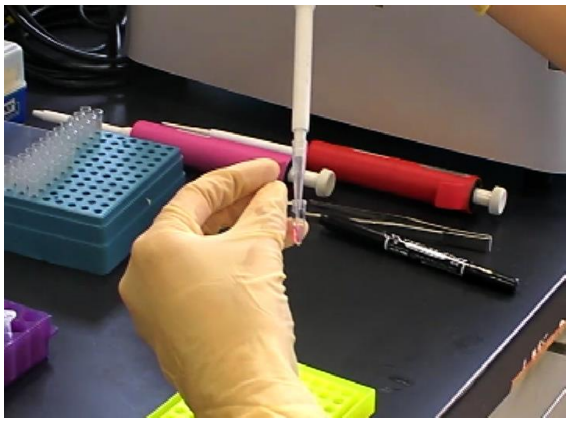
CHIBA University

Protocol for DNA digestions with restriction enzymes

1 Place the DNA solution in a microfuge tube.



2 Mix with sufficient water to give a volume of 18μl.



3 Add 2μl of the 10×restriction enzyme digestion buffer.

4 Mix by tapping the tube.

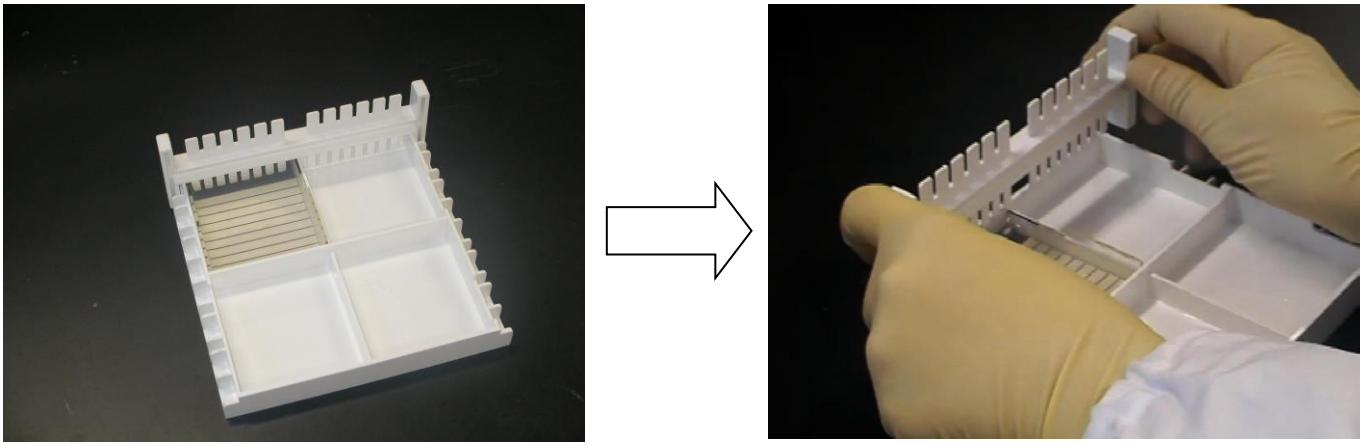
5 Add 2 unit (1μl) of restriction enzyme.

6 Mix by tapping the tube.

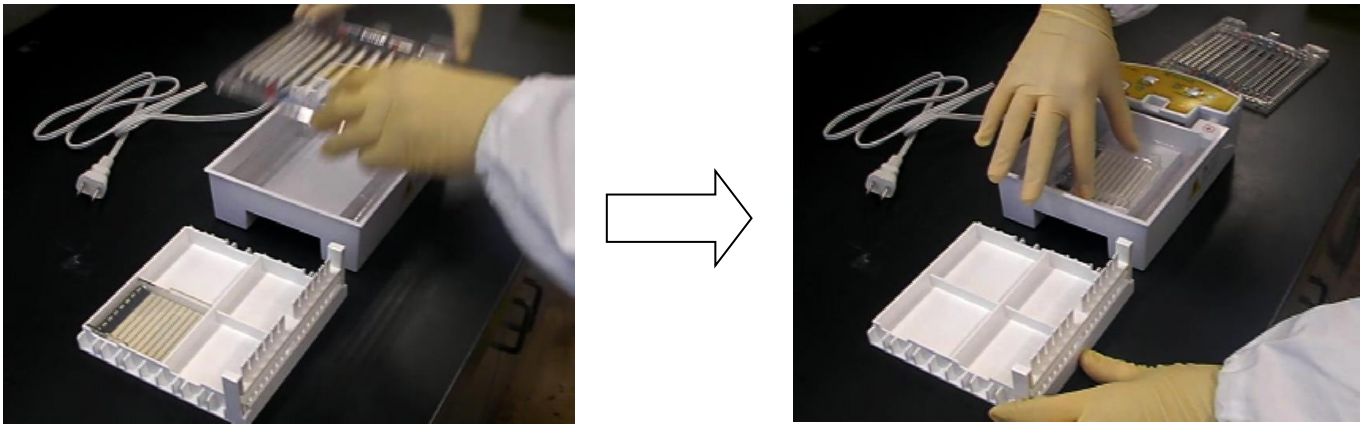
7 Incubate the mixture at 37℃ for 30 minutes.

Protocol for gel electrophoresis of DNA

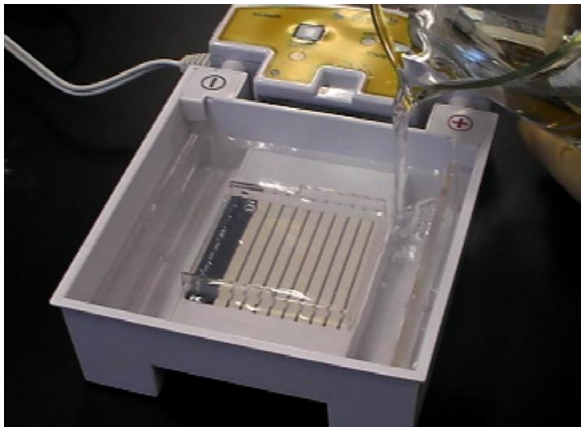
8　Carefully remove the comb.



9　Mount the gel in the electrophoresis tank.

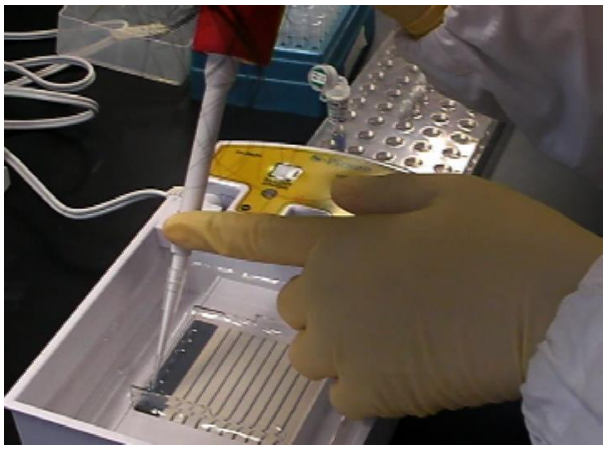


10　Add just enough electrophoresis buffer to cover the gel to a depth of about 1mm.



11　Mix the samples of DNA with 4μl of gel-loading buffer.

12 Slowly load the mixture into the slots of the submerged gel using a disposable micropipette.

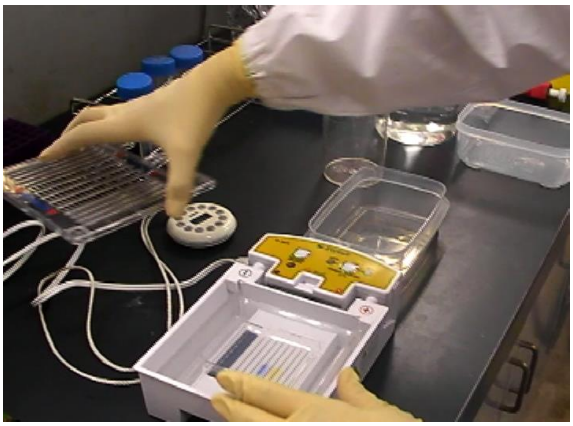


13 Close the lid of the gel tank.

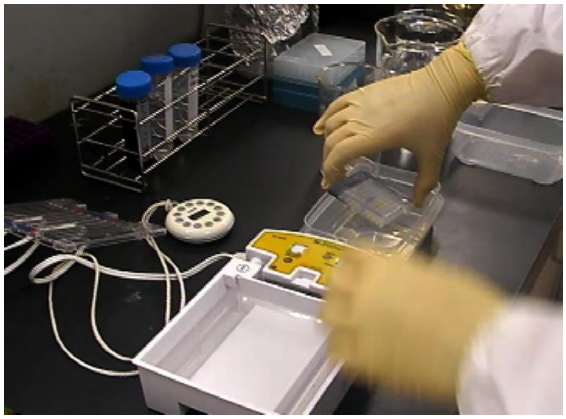
14 Turn on the electric current.

Run the gel about 30 minutes.

15 Remove the lid from the gel tank.

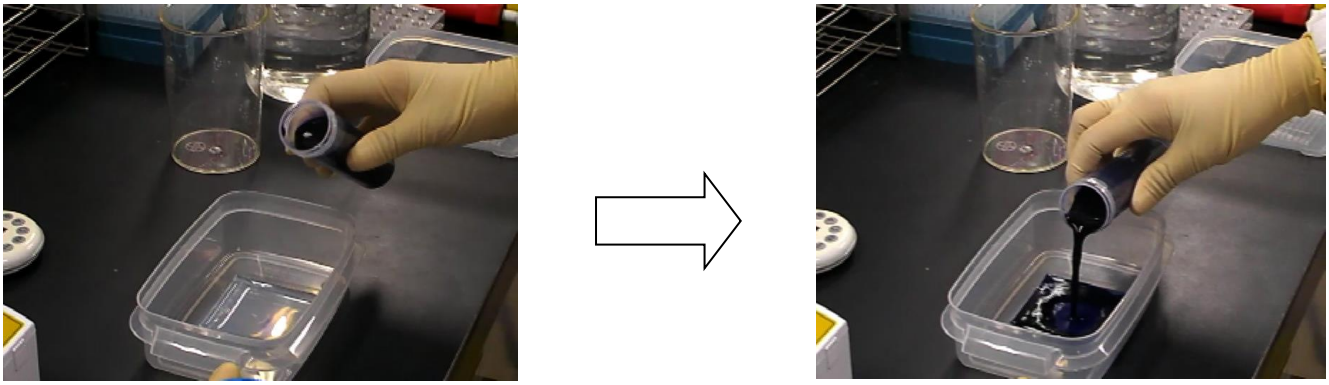


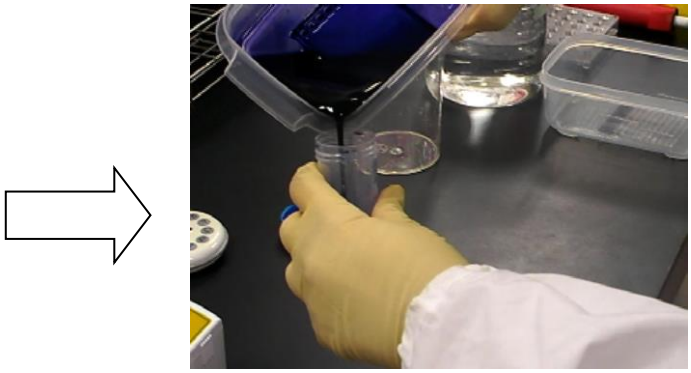
16 Transfer the gel into plastic container.



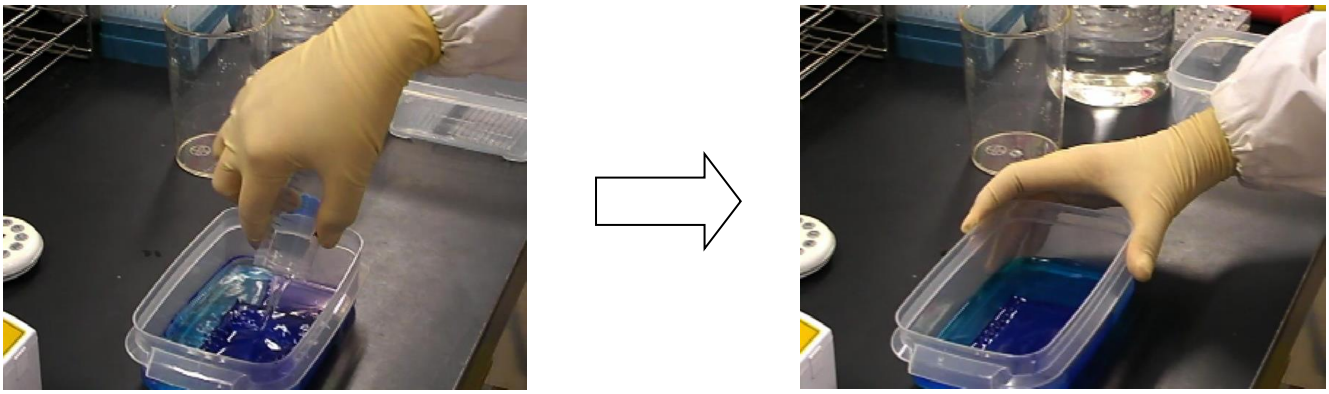
Protocol for the gel staining with Mupid-Blue

17 The gel is immersed in water containing Mupid-Blue for 1 minute.



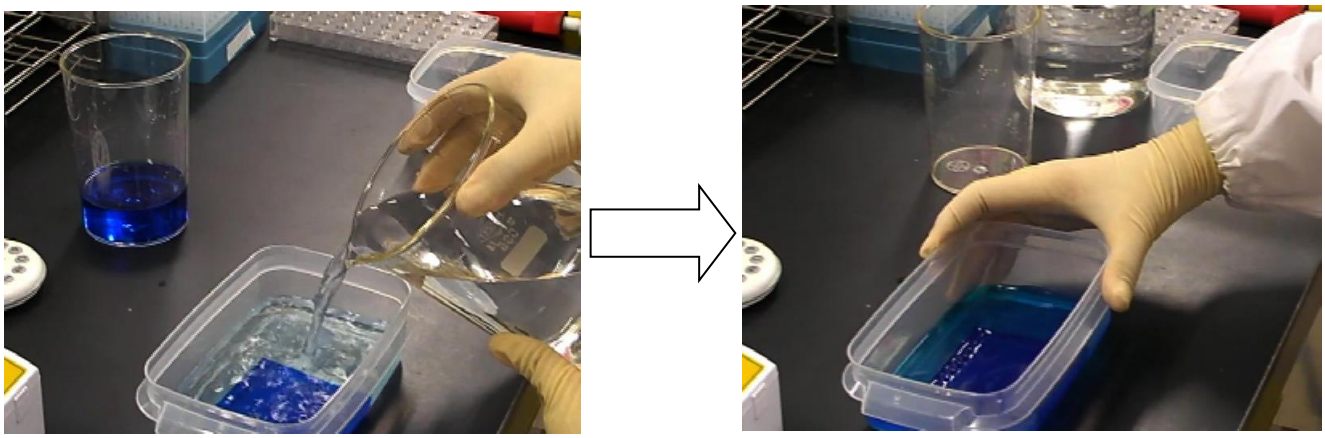


18 Soaking the stained gel in 70% Ethanol for 1 minute.



19 Repeat once.

20 Soaking the gel in water for 2 minutes.



21 Repeat this until blue bands become visible.