Pulsed-field Gel Electrophoresis Typing of Gram-negative Bacteria (*E. coli*)

Zhenying Liu

School of Medicine, University of Pennsylvania

Materials and Reagents

1. Chromosomal low melting agarose (Lonza InCert Agarose or Bio-Rad Laboratories)
2. Restriction enzymes (New England Biolabs)
3. Proteinase K (Promega Corporation)
4. 10x Tris-borate EDTA (TBE) buffer (Promega Corporation)
5. Agarose for pulsed field gel (Bio-Rad Laboratories)
6. Lamda ladder for pulsed field gel (Bio-Rad Laboratories)
7. Ethidium bromide (10 mg/ml) (Life Technologies, Invitrogen™)
8. Agar media
9. TE
10. NaCl
11. EDTA
12. Sarkosy l
13. *Sma* I
14. *Xba* I
15. Restriction enzyme buffer
16. Running buffer (0.5x TBE)
17. Suspension buffer (see Recipes)
18. Lysis buffer (see Recipes)

Equipment

1. CHEF-Mapper™ apparatus (Bio-Rad Laboratories)
2. Spectrophotometer (Bio-Rad Laboratories)
3. UV light and photograph
4. Water bath
5. Glass rod
6. 1.5 ml Eppendorf tube

Procedure

A. Chromosomal DNA preparation:

1. Subculture strains onto agar media. Grow under appropriate conditions for 1-3 days.
2. Harvest cells and suspend into 2 ml of suspension buffer. Adjust cell density to OD_{600}: 1.5 to 2.0. Standard cell OD ensures that each sample contains approximately the same amount of DNA.
3. Prepare 1.2% chromosomal grade low melting agarose for making bacterial DNA plugs and cool it to 56 °C in water bath.
4. Add 200 µl molten chromosomal grade agarose suspensions to 200 µl of bacterial suspension. Mix and dispense into 3 chambers of the mould. Then place the plug in the fridge (4 °C) for about 10 min until the gel is solidified.
5. Remove agar blocks by inserting a blunt-tipped glass pipette and place each set of 3 plugs into a 10 ml plastic tube containing 2 ml lysis buffer. Add 1 mg/ml Proteinase K and incubate at 55 °C overnight. This leaves only DNA embedded in the solid agar matrix. The DNA will not shear when handled further and remains stable for analysis by electrophoresis.
6. Discard lysis buffer from samples and wash four times, each for at least 20 min periods, in 2 ml of 10:1 TE (10 mM Tris-1 mM EDTA) buffer at 55 °C. Store plugs at 4 °C.

B. DNA digestion:

1. Remove an agar/DNA plug using a glass rod and place it into a 1.5 ml Eppendorf tube. Add 300 µl restriction enzyme buffer and incubate for 30 min at room temp. Incubation in digestion buffer to makes DNA more amenable to digestion.
2. Remove buffer and add 250 µl of restriction enzyme buffer which contains 20 units of enzyme (*Sma* I or *Xba* I) for 5 h at 37 °C.

C. DNA electrophoresis:

1. Boil 100 ml 1.0% pulsed gel agarose in microwave for separation of DNA fragments. Cool in water bath to 50-56 °C.
2. Assemble gel mould and pour gel into mould. 15 or 30 (Bio-Rad equipment) well-forming comb must be inserted into the assembly before the agarose is poured in.
3. Remove gel well-forming comb and slide DNA plugs into wells using a clean plastic scalpel (be sure that each slice is straight and adheres onto the front side of the well). Also add lambda ladder into the first, last and middle wells fragments.

4. Add 2 L running buffer (0.5x TBE) to electrophoresis chamber and run through pump and cooling unit (14 °C) about 30 min before use. Place gel into electrophoresis tank and close cover.
5. Set electrophoresis conditions on power pack and computer. Program run according to the instructions under two-state mode, and start run.
   Block 1: angle: 60+, 60-; pulse time: 1 sec to 30 sec; ramping factor: 0(linear); run time: 17 h; voltage gradient: 6.0 V/cm.
   Block 2: 5 sec to 9 sec for 6 h, the rest is the same as the block 1.

D. Staining and visualizing the gel:

1. Switch off electrophoresis unit. Remove gel from base and gently place into 400 ml, 0.5x TBE buffer with 40 µl ethidium bromide. Stain 30 min, distain with water for 5 min.
2. View under UV light & photograph. Make sure the top of the MW marker should always be visible and outer edges of the first and last samples are in the printed image.

Recipes

1. Suspension buffer
   1 M NaCl
   10 mM Tris (pH 8)
   10 mM EDTA
2. Lysis buffer
   0.5 M EDTA
   1% Sarkosy l