Pulsed-Field Gel Electrophoresis

Plug preparation

Prepare a log-phase cell culture (OD_{600}=0.5~1.2)

Count cells and collect 2.5 x10^8 cells by centrifugation
Use hemacytometer

Wash cells in 20ml of CSE (suspend cells by vortex and centrifuge to pellet cells)

Cell pellet can be stored at -80˚C (Snap freeze cell pellets in EtOH/Dry ice)
**Remove the sup completely before the sample is frozen

Suspend cells in 1ml of CSE + 1mg/ml Zymolyase 100T. Incubate cells at 37˚C for 2 h

Spin cells down and re-suspend cells at 8x10^8 cell/ml in TSE (300 ul)

Warm the cell suspension to 42˚C

Add 300 ul of 1.1% LMT agarose in TSE (warmed to 42˚C)

Dispense aliquots into plug molds (5 aliquots per sample)

Allow plugs to solidify at 4˚C

Transfer 5 plugs into a 50ml conical tube

Add 3ml of Tris-EDTA-SDS (freshly made)

Incubate plugs at 55˚C for 90 min

Replace the buffer with 3ml of NDS+1mg/ml ProteinaseK (pre-incubated for 30min at 37˚C)

Incubate plugs at 55˚C for 24 h

Replace the buffer with new 3ml of NDS+1mg/ml ProteinaseK (pre-incubated for 30min at 37˚C))

Incubate plugs at 55˚C for 24 h

Equilibrate plugs in 5ml of TE (pH 7.5) three times for 30 min each
(Remove buffer, add TE, wait 30 min, remove buffer)
(Plugs are now ready for electrophoresis)

Store plugs in 5ml of 0.5 M EDTA at 4˚C

Equilibrate a plug in 1ml TE in a microfuge tube as described above before electrophoresis
**use a disposable loop to transfer a plug into a microfuge tube

(Option)
Not I digested PFGE

Equilibrate the plug in 1ml of 1xNEB3 buffer+BSA
(three times for 1h each at 37˚C)
Add NotI and incubate a plug and incubate at 37˚C overnight
Wash the plug in TE twice

Buffers

CSE (SP1)
20mM citric acid
20mM sodium phosphate (Na_2HPO_4 $7H_2O$)
adjust pH to 5.6
add 1.2 M sorbitol
add 40 mM EDTA (pH 8.0)

TSE
10 mM Tris-HCl (pH 7.5)
0.9 M sorbitol
45 mM EDTA (pH 8.0)

Tris-EDTA-SDS
0.25 M EDTA (pH 8.0)
50 mM Tris-HCl (pH 7.5)
1 % SDS

NDS
10mM Tris-HCl
0.5M EDTA (pH 8.0)
Adust pH to 9.5
Then add 1 % lauryl sarcosine