Plasmid Prep. For Two-Dimensional Gel

1. Wash the cells carrying Rep* plasmids in PBS.

2. Resuspend cells in buffer A (10mM Tris, pH8.0, 10mM EDTA,100mM NaCl) at 0.5ml per 10^7 cells.

3. Add equal volume of buffer B (10mM Tris,pH8.0, 10mM EDTA,,100mM NaCl 1.2% SDS), mix gently and incubate for 10' at RT.

4. Add PK(proteinaseK) at 100ug/ml, incubate at 37o for 1hour

5. Bring the lysate to 1M NaCl. And stand on ice for >12hours.

6. Spin down the chromosomal DNA at 17K(25000g) for 2hours at 4oC in a Ti50 rotor(or 13.1k in SA-600 rotor,#04,in Beckman centrifuge).

7. Treat the supernatant with 100ug/ml proteinase K at 37oC for 30'.

8. Extract (invert gently 10min) with chloroform twice, and add 1 vol. of 2-propanol and Spin down the DNA pellet immediately at 3000 rpm in the table top centrifuge for 30min at 4oC.

9. Dissolve in 0.3M NaOAC made in TE and add 2.5 vol. of ethanol, -20oC o/n.(1 to 2x10^8 per ep. tube)

10. Wash the DNA pellet in 70% ehthanol once and resuspend in 980ul of 1xNEB buffer#3 per 10^8 cells plus 100ug/ml RNaseA. Digest the DNA with 100units of NcoI for 1hour at 37oC, then add another 100units for 1 more hour.

11. BND column (2x10^8 per 0.5ml of BND)

12. The pellet is resuspended in 25ul TE (from 4x10^8 cells) and loaded onto 0.4% TAE agarose without EtBr. (11cmx13.5cm, 4mmx0.125mm well, run at 0.8/cm for 22 hours,16v,120ml gel)). Take a picture of the marker and the gel with the slice missing. Record the exact length of the gel slice.

13. Stain the gel for 30' in 0.5ug/ml EtBr in TBE. Cut a 10cm gel slice (from 1kb to the top) Run the second D. in 1.1% TBE agarose (125ml, 0.5ug/ml etbr) at 5v/cm for 12.5hours. (11cmx13.5cm gel) in the presence of 0.5ug/ml EtBr in cold room without buffer circulation.