

- Suspend organisms in 0.5mL SE buffer to a turbidity of 2.5 McFarland.
- Keeping samples in waterbath, mix 350 μ L suspension with 350 μ L agarose in a 1.5-mL eppendorf tube, mix by pipetting and immediately transfer to moulds (two/sample). Allow moulds to set at 4°C.
- Transfer plugs to sterile 5 mL bijoux, add 3 mL Gram-positive lysis buffer + 0.5 mg/mL lysozyme, and incubate overnight at 37°C.

Day 3

- Remove G-positive lysis buffer, add 3 mL Gram-negative lysis buffer + 500 μ g/mL proteinase K, and incubate overnight at 56°C.

Day 4

- Wash plugs in 3 mL TE for *c.* 30 min at 4°C. Repeat three times.
- Store plugs at 4°C for 3-6 months, with regular changes of TE.

B. DNA Restriction

Day 1

- Prepare 1x restriction enzyme reaction buffer from 10x buffer supplied with sterile distilled water.
- Cut 2 mm portion of plug, cover with 100 μ L 1x reaction buffer in 0.5 mL eppendorf, incubate at 4°C for \geq 30 min.
- Replace with 100 μ L fresh 1x buffer and 30 U *Spe* I and incubate at 37°C for 4 h/overnight.

C. Separation of DNA fragments

- Prepare 1.5 L 0.5x TBE
- Prepare 1.2% agarose gel in 100 mL 0.5x TBE.
- Load digested plugs into wells, with standard strain either end of gel and every 5 lanes. Seal wells with 1.2% agarose.
- Run conditions: 12°C; initial start time 1 s; final start time 50 s; gradient 6V; run time 30 h.
- DNA fragments generated from *Pseudomonas aeruginosa* strain C15 should be run in every 6th lane to allow normalisation of gels and comparisons with the ARPAC database.

Day 3

- Stain gel for 1 h in TBE + ethidium bromide.
- Destain for 1 h in distilled water.
- Visualise under UV illumination, photograph and save image.