

# Equipment & Reagents Required for PFGE

## Equipment and Supplies Needed for the Molecular Subtyping of Foodborne Bacterial Pathogens by PulseNet's Standardized PFGE Protocols

(Approximate Cost in US\$ - June 2, 2010)

### Electrophoresis and Documentation Equipment:

- Electrophoresis Equipment
- Bio-Rad CHEF Mapper XA System with pump and chiller module (~\$31,200, part number 170-3670)  
OR
- Bio-Rad CHEF-DR III System with pump and chiller module (~\$24,250, part number 170-3700)
- OR
- CHEF-DR II System (not desirable) (~\$12,500)
- Bio-Rad Gel Doc XR (~\$12,000)
- Thermal printer for Gel Doc XR (~\$1,100)

### Other Equipment

- Incubator (\$1,000 to \$5,000)
- Water baths (two are desirable) (~\$650.00 for one)
- Orbital/shaker water bath or shaker incubator (lysis and washing plugs)(~\$3,500 to \$7,000)
- Optical density instruments (cell suspension adjustment):
- Dade Microscan Turbidity Meter (Dade Behring; cat# B1018-66) (~\$1,000)  
OR
- Spectrophotometer (~\$1,200 to \$3,000) OR
- bioMérieux Vitek Colorimeter with standards (~\$600.00)
- Scales/balances to measure solid reagents (~\$650 to \$1,200)
- Microwave with turntable (melt agarose)(\$100 - \$200)

### Supplies and Selected Reagents

- SeaKem Gold Agarose (Cambrex Research Products)
- 25 grams (catalog number 50152) (~\$138)
- 125 grams (catalog number 50150) (~\$505)
- PFGE plug molds (Bio-Rad)
- 50-well disposable (1.5-mm x 10-mm x 5-mm, Bio-Rad 170-3713) (~\$45) OR
- Three to five 10-well reusable (2-cm x 1-cm x 1.5-mm; Bio-Rad 170-3622): (~\$120 each)
- Combs (Bio-Rad)
- Combination comb holder (number 170-3669) (~\$110)
- 10 well comb (170-4326) (~\$50)
- 15 well comb (170-4324) (~\$50)
- Standard Casting Stand (14 x 13-cm frame and platform; Bio-Rad 170-3689) (~\$195)
- Wide/Long Combination casting stand (21 x 14-cm frame and platform; Bio-Rad 170-3704) (~\$270)
- Leveling Table (for casting gels) (~\$230)

- Proteinase K powder (Roche Molecular Biochemicals #745 723) (~\$82) or
- Proteinase K liquid (Amresco, E195-5ml) (~\$82)
- 10X TBE (Sigma, T4415; Bio-Rad 161-770)(~\$87 for 4 liters; ~\$80 for 5 liter cube)
- 1M Tris-HCl, pH 8.0 (Invitrogen, 15568-025) (~\$38 for 1 liter)
- 0.5M EDTA, pH 8.0 (Invitrogen, 15575-020; Amresco, E177) (~\$48 for 400 ml (4 x 100 ml); ~\$27 for 500 ml)
- N-Lauroylsarcosine Sodium Salt (Sigma, L9150) (~\$34 for 100 g; ~\$63 for 250 g)
- Ethidium bromide, 10 mg/ml (Bio-Rad 161-0433; Sigma E1510) (~\$45 for 10 ml; ~\$39 for 10 ml)

### Restriction Enzymes and appropriate Buffers

- Available from Roche Molecular Biochemicals, New England Biolabs, Promega, or other suppliers
- *E. coli* O157:H7, *Salmonella*, and *Shigella* spp:
- *Xba*I Roche: 20,000 units for ~\$385
- *Bln*I/*Avr*II Roche: 1,000 units/New England Biolabs 500 units): ~\$350/\$300
- *Spe*I Roche: 1,000 units for ~\$280
- H Buffer (Roche enzymes): 5 ml for ~\$26
- *L. monocytogenes*:
- *Asc*I (New England Biolabs) 2,000 units for ~\$280
- *Apa*I (Roche) 5000 units for ~\$65
- *Clostridium perfringens*, *Campylobacter jejuni/coli*
- *Sma*I (Roche) 5,000 units for ~\$205
- *Campylobacter jejuni/coli*
- *Kpn*I (Roche) 10,000 units for ~\$160
- *Vibrio cholerae/V. parahaemolyticus* (the enzymes below are being considered for PFGE analysis)
- *Not*I (Roche) 1,000 units for ~\$281
- *Sfi*I (Roche) 1,250 units for ~\$105
- Other miscellaneous items: ~\$5,000 to \$8,000 (for spatulas, weighing paper, paper towels, gloves, scalpels/blades, glassware, staining dishes, test tubes, pipets and tips, culture media, etc).

**Use of trade names and commercial sources is for identification only and does not imply endorsement by CDC, the U.S. Department of Health and Human Services or any PulseNet International participant.**