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（出國類別：訓練研習）

赴香港參加亞太區 PulseNet(細菌病原實驗室即時監測網)研習營報告

服務機關：疾病管制局

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內容摘要: PulseNet爲美國疾病管制中心發展之實驗室分子分型即時監測網，用以監測細菌病原，目前台灣已加入此一國際組織。PFGE(脈衝式電泳)至今在分子分型法中被公認爲黃金標準，也是目前PulseNet主要採用的分型方式 本次在香港舉辦的研習營，主要目的是訓練亞洲地區的實驗室人員，使之俱備製做標準PFGE圖譜的能力，才能與PulseNet的資料庫有效比對，隨時監測細菌病原之發生。議程除了操做訓練外，專題演講部份分別有日本，美國的專家講述PFGE在E.coli O157監測方面的實際應用及PulseNet資料庫運作的現況。隨著國際交流日趨頻繁，傳染病的擴散速度有增無減，PulseNet提供了細菌病原即時的，正確的監測平台，台灣有幸加入此一組織，應僅速整合國內各實驗室建立好自己的PulseNet架構，以備不時之需。

本文電子檔已上傳至出國報告資訊網

目次

摘要

PulseNet 爲美國疾病管制中心發展之實驗室分子分型即時監測網， 用以監測細菌病原， 目前台灣已加入此一國際組織。PFGE(脈衝式電泳)至今在分子分型法中被公認爲黃金標準， 也是目前 PulseNet 主要採用的分型方式 本次在香港舉辦的研習營， 主要目的是訓練亞洲地區的實驗室人員， 使之俱備製做標準 PFGE 圖譜的能力， 才能與 PulseNet 的資料庫有效比對， 隨時監測細菌病原之發生。議程除了操做訓練外，專題演講部份分別有日本，美國的專家講述 PFGE 在 *E.coli* O157 監測方面的實際應用及 PulseNet 資料庫運作的現況。隨著國際交流日趨頻繁， 傳染病的擴散速度有增無減， PulseNet 提供了細菌病原即時的， 正確的監測平台， 台灣有幸加入此一組織， 應僅速整合國內各實驗室建立好自己的 PulseNet 架構，以備不時之需。

目的

PulseNet 爲美國疾病管制中心發展之實驗室分子分型即時監測網， 用以監測細菌病原， 因爲成效卓著， 而計劃推到全世界， 以建立全球監測網爲目標， 2002 年，本實驗室負責人邱乾順博士至美國疾病管制中心了解 PulseNet 的運作方式及學習建立細菌 DNA 指紋資料庫之標準化 PFGE(脈衝式電泳)技術及圖譜分析比對的方法， 並於 2002 年底代表我國參加亞洲地區籌備會議， 同時加入隨後成立之 PulseNet Asia Pacific 組織， 該組織目前計有十一國家/地區/研究機構參與。

2002 年底，本實驗室逐步建立了 PFGE 的操作及分析能力， 同時擬定了「建立重要傳染性病原細菌 DNA 指紋資料庫」的三年計劃。目前此計劃於 2004 年 1 月開始爲本局其它實驗室送來的傳染性細菌株做分型的服務，同時資料庫的網路系統已大致建構完成， 預計在今年內對其它實驗室人員進行 PFGE 的操作訓練並對目前的資料庫系統進行測試， 希望於未來此系統能提供各實驗室遠端電腦傳送，下載圖譜進行比對的需求。

今年， PulseNet 委託香港於 3 月 15 日至 3 月 17 日舉辦亞洲地區的技術訓練營， 爲了解相關技術最新進展， 蒐集亞洲各國之發展資訊， 本人奉派至香港參加此訓練營。

研習過程

此次的訓練營是於 3 月 15 日至 17 日在香港公共衛生檢測中心進行， 參加的學員除了我國還有來自韓國， 中國， 孟加拉， 泰國，馬來西亞，研習營的主要內容包含操作訓練及專題演講。

一. PFGE 標準操作的示範教學及疑難排除

操作訓練 PFGE 是目前 Pulsenet 主要使用的分子分型法，標準化的 PFGE 圖譜之重要性在於必須有良好的再現性，可信度，及即時產生的才能有效偵測群聚情形， 採取適當的防疫措施， 防止疾病的散播。訓練主題是 "Laboratory directions for molecular subtyping of ***Salmonella*** (***E.coli*** O157:H7 and ***Shigella***) by Pulsed- Field Gel Electrophoresis (PFGE)" ，美國疾病管中心微生研究部門的 Dr. Mary Ann Lambert- Fair 負責 PFGE 實驗操作示範， 至於產生之 PFGE 圖譜以 Bionumerics 分析軟體分析， 則由 PulseNet 資料庫負責人 Dr. Kelley B. HISE 指導， 實驗流程詳見附錄 (一)。

疑難排解 Dr. Mary Ann Lambert- Fair 建議維持 PFGE 的實驗品質必須注意常規事項如右， 正確的量取， 計算， 確認所有的儀器準確度， 確認所有試劑無沉澱、變色，、變濁、過期的問題。由於本實驗室在 2002 年已建立了 PFGE 的操作系統， 因此對於技術層次的學習著重在問題圖譜的疑難排除， 將所得資訊整理如表(一)。

表(一) 問題圖譜的疑難排除

問題狀況	可能原因	排除方法
Thick Band	菌液太濃, 染色過久	檢查菌液濃度， 染劑濃度及時間
Smear Band	Plugs 不正確 Lysis 及 wash 限制酵素活性降低	檢查 Lysis, wash buffer 菌液濃度, 水質 增加限制酵素單位
Curved Band	未正確製膠	檢查膠台水平， 防止移動 Plugs
No Band	電泳儀受細菌或黴菌污染	以 2L 5%~10%漂白水清洗再以 2L 純水清洗
Untypeable lanes	發生於某些菌種如: *Salmonella* 特定血清型 *Clostridia* *Vibrio parahaemolyticus* *Pseudomonas aeruginosa* *Klebsiella*	Running buffer 加入 50 μM thiourea 可防止 DNA 被破壞
Not straight lanes	製膠未水平, running buffer 未均勻流動	檢查膠台水平, 電泳儀

在分析 PFGE 圖譜方面，必須注意 light band， intensity bands，圖譜內 intensity band 必須排除菌量， 染色時間的影響， light band 必須以不同濃度的限制酵素處理並重覆測試其再現性來確認，最後仍應以目視爲準， 並充份配合流病資料， 以推估分析出來群聚情形的正確性。

二. PulseNet 用於追踪傳染病的實際情況

日本國家傳染病協會細菌部門的 Dr. Jun Terajima 介紹 PulseNet Japan 追踪傳染病的成效， 日本目前列入監測的有 *Shigella*， *Salmonella* ， *EHEC* O157:H7， 2001 年一次 *E. coli* O157 的流行， 由市售冷凍牛肉分離出與患者同一 PFGE 型別的 *E. coli* O157， 並即時阻止該批牛肉販售防止疾病的傳播。 他認爲 PFGE 圖譜的品質是 PulseNet 的最關鍵要素， 在日本由地區實驗室上傳的圖譜仍有無法比對的情形， 因此技術支援尤爲重要。

美國疾病管制中心 PulseNet 主席 Dr. Bala Swaminathan 介紹 PulseNet 在美國運作及國際推展的現況， PulseNet 現階段配合 PFGE 分型方法用以監測食因性疾病，已開發出標準 PFGE 操作流程的病原菌有 *E.coli* O157:H7， *Salmonella*， *Listeria monocytogens*， *Shigella sonnei*， *Campylobacter jejuni*， *Clostridium perfringens*， *Vibrio cholerae*， *Vibrio parahaemolyticus*， *Yersinia enterocolitica*，

Non-O157 STEC。 PulseNet 最主要的功能是在散發病例中發現群聚的情形而**提早偵測**到流行， 並非只是追踪流行，例如在 2002 年， 由 6 個地區實驗室分別傳回散發病例的 *Listeria monocytogenes* PFGE 圖譜， 經比對發現高度聚集， 之後於某品牌火雞肉發現同 PFGE 型別的 *Listeria monocytogenes*， 而緊急召回該品牌的肉品， 防止疾病繼續擴散。基於疾病傳播已全球化， 及**即時資訊共享**的精神， 而要推廣此計劃， 目前成立的有 PulseNet Canada， PulseNet Europe， PulseNet Asia Pacific， PulseNet Latin America。

心得與建議

在三天既緊湊又充實的研習營議程中， 除了實驗室技術方面獲得與會專家們許多寶貴的經驗及建議， 也對主辦地區香港的用心印象深刻， 個人認爲有許多是值得我們參考的。

一. 重視實驗室安全及整體規劃

香港衛生檢測中心是集中所有公共衛生實驗室的大樓，管理十分嚴謹， 非員工一定要有內部人員帶領才能進入。工作人員進出實驗室均需經過準備室和更衣室， 使實驗室與環境交叉污染的情形降低。而對於類似 ETBR 等有毒物質的操作及處理， 相當謹慎。 另外會議室設計爲多功能式的， 可做爲訓練， 會議， 聚餐等用途， 十分結省空間， 香港地狹人稠， 似乎更擅長於空間的運用。

二. 創造英語環境

此次在香港衛生署接觸到的實驗室人員， 均能以英語與各國人員溝通無礙， 不禁對當地英語之普及感到佩服， 也覺得台灣應更努力營造英語環境才能提升國際競爭力。

三. 整合地區實驗室建立完整的 PulseNet 架構爲當務之急

觀察與會各國均排除各種困難積極參與 PulseNet， 台灣算是實

驗設備及技術建立較完善的， 更應僅速整合好各地區實驗室， 使 PulseNet 能發揮遠端比對的功能， 以備不時之需。

附錄

附錄(一) Laboratory directions for molecular subtyping of *Salmonella*(*E. coli* O157:H7 and *Shigella*) by Pulsed-Field Gel Electrophoresis(PFGE)

LABORATORY DIRECTIONS FOR MOLECULAR SUBTYPING Of *Salmonella* (*E. coli* O157:H7 and *Shigella)* By PULSED-FIELD GEL ELECTROPHORESIS (PFGE)

Monday morning, March 15, 2004

Laboratory Module IA

Preparation of PFGE Agarose Plugs from *Salmonella* Cell Suspensions

BIOSAFETY WARNING: These organisms may have low infectious doses and most have caused human illness. Please handle with caution.

Please read laboratory instructions on pages 1 and 2 before continuing. Treat all plasticware, glassware, pipets, spatulas, etc. that come in contact with the cell suspensions or plugs as contaminated materials and dispose of, or disinfect, according to instructions.

Please put your group initial (A, B, C or D) on all tubes, flasks, etc. that you use. You will be given plates of seven different *Salmonella* strains. Cell suspensions will be made in Cell Suspension Buffer[1] (CSB) from 14-16 h blood agar plates. Handle them carefully and with gloves.

1. Label seven Falcon 2054 tubes at the top with the culture numbers and your initial. Label one Falcon 2054 tube with "Blank." Add ~2 ml of Cell Suspension Buffer (CSB) to each tube.

2. Label seven 1.5-ml microcentrifuge tubes with the culture numbers and your initial (1-A).

3. Label one well of disposable and one well of reusable PFGE plug molds with culture numbers.

4. Adjust concentration of the cell suspensions with the Dade MicroScan Turbidity Meter as described in the following steps:

   a. Check the digital output on the instrument by inserting the CSB "blank" tube in the left position of the turbidity meter and one of the other tubes of CSB in SAMPLE position (right) of the turbidity meter. The reading should be 0.00±0.01. Hold the tubes at the top.
   b. Moisten a sterile disposable loop with sterile CSB and remove growth from the agar plates into the appropriate Falcon tube that contains 2 ml of CSB. Suspend the cells by rubbing against the wall of the tube gently so the cells are evenly dispersed and formation of aerosols is minimized.

[1] Formulas for reagents are on page 10.

c. Insert the tube of the cell suspension into the SAMPLE position to check the reading (desired range is 0 48 - 0.52)

d. If the reading is greater than 0.52, add additional CSB, mix well and check reading again until it is within the desired range of 0.48 - 0.52.

e. If the reading is less than 0.48, add additional growth from the plate to the cell suspension until the desired range is obtained.

5. Transfer 400 µl (0.4 ml) of each cell suspensions to labeled microcentrifuge tubes using the 1000 µl (1 ml) pipet and tip. Place microcentrifuge tubes in plastic holders (float) and incubate in the 37°C water bath for 5 minutes. Keep tubes containing rest of the cell suspensions on ice bath until plugs are made and they are in the shaking water bath.

6. Remove cell suspensions in 1.5 ml tubes from water bath; add 20 µl proteinase K (20 mg/ml stock solution) to each 0.4 ml cell suspension using the 100 µl pipet; mix by closing tubes and tapping side of tube. (The final concentration of Proteinase K [Invitrogen 25530-049] in the cell suspension is 0.5 mg/ml.)

7. Remove 1% SeaKem Gold: 1% Sodium Dodecyl Sulfate (SDS) agarose from 56°C water bath; keep agarose in beaker of water so that it will stay warm while making plugs.

Note: Do not remove melted agarose from water bath until you are ready to make the plugs; mix the agarose with the cell suspensions immediately before the agarose can cool too much.

8. Add 400 µl (0.4 ml) melted 1% SeaKem Gold:1% SDS agarose to one of the 0.4 ml cell suspensions; mix by gently pipetting mixture up and down a few times. The 1000 µl pipetman can be used without changing the setting.

9. Immediately, dispense part of mixture into appropriate well in both plug molds. Do not allow bubbles to form. Repeat for remaining samples. Allow plugs to solidify for 10-15 min at room temperature.

Note: When reusable plug molds (2-cm x 1-cm x 1.5-mm) are used, 2 plugs can be made from these amounts of cell suspension and agarose; when disposable plug molds (1.5 mm x 10-mm x 5-mm) are used, 4-5 plugs can be made.

10. Continue with Step 1 of **Laboratory Module IB**.

Laboratory Module IB
Lysis of Cells in Agarose Plugs

Read instructions in this section before continuing.

1. Label seven 50 ml polypropylene screw-cap tubes with culture numbers and group initial. (One of the tubes contains ~35 ml Cell Lysis Buffer [5 ml x 7 tubes = 35 ml].)

2. Add 175 µl Proteinase K stock solution (25 µl x 7 tubes = 175 µl) to the tube that contains the 35 ml Cell Lysis Buffer using 200 µl pipet; recap tube and invert or swirl gently to mix. (The concentration of Proteinase K stock solution is 20 mg/ml. The final concentration in the Cell Lysis Buffer is 0.1 mg/ml)

3. Dispense 5 ml of Proteinase K/cell lysis buffer to each of the other six labeled 50 ml tubes. (5+ ml will be left in the original tube.)

4. If a flat edge is wanted on the plugs, trim excess agarose from top of plug with scalpel. Dispose of scalpel in red SHARPS container.

a. **Reusable Plug Molds**: Open mold by partially unscrewing the back and removing it. Put back of mold into plastic container that contains chlorox (5,000 ppm). Transfer plugs from mold with wide end of spatula to appropriately labeled tube. Remove tape from reusable mold and put front section of plug mold and the spatula in container with 75% Isopropyl alcohol.

b. **Disposable Plug Molds**: Remove white tape from bottom of molds and use tab from strip to push plugs into appropriate tube. Discard disposable mold, tab and tape in discard pan.

Note: Lyse both the large and small plugs of each strain in the same tube.

5. Confirm that plugs are under buffer and not on side of tube.

6. Place tubes in rack in 54°C shaking water bath incubate for 2 hours with constant agitation. Confirm that the water level is above level of lysis buffer in tubes and tubes are moving freely in the rack. Note the time the tubes went into the incubator or water bath.

7. Put bottle containing ~250 ml of sterile Ultrapure (Reagent Grade, Type 1) water into 50°C water bath.

Monday afternoon, March 15, 2004

Laboratory Module IC
Washing of Agarose Plugs After Cell Lysis
Read instructions in this section before continuing.

1. Remove tubes with plugs from incubator or water bath; remove caps and replace with green screened caps. Carefully pour off lysis buffer into discard pan. Gently tap bottom of tube against lab bench to force plug to bottom of tube.

Note: Touch top of green cap onto an absorbent paper towel so that most of the liquid during this and subsequent washing steps is removed.

2. Add 15 sterile Reagent Grade water that has been pre-heated to 50°C to each tube and screw original cap on top of green screened cap.

3. Confirm that plugs are under water and not on side of tube; return to shaking incubator or water bath (50°C). Shake tubes for 10 minutes.

4. Pour off water into sink and repeat wash steps with pre-heated water (Steps 2 and 3) one additional time.

5. Put one liter bottle of Tris-EDTA Buffer, pH 8.0 (TE) fitted with a dispenser into the 50°C water bath.

6. Pour off water from plugs, add 15 ml pre-warmed (50°C) sterile TE, mix, and shake moderately in shaking incubator or water bath (50°C) for 15 minutes.

7. Pour off TE and repeat wash step three more times (10-15 minutes each).

8. Pour off TE from last (fourth) wash step, add 10 ml TE, and place in rack (provided by instructor) for overnight refrigeration at 4°C. Confirm that plugs are in the TE and not on the side of the tube.

Laboratory Module IIA

Restriction Digestion of DNA in Agarose Plugs with *Xba* I

Tuesday morning (08:45– 09:45am), March 16, 2004

Read instructions in this section before continuing.

1. Label seven 1.5-ml microcentrifuge tubes with culture numbers and group initial; label three 1.5-ml microcentrifuge tubes with H9812 (PulseNet Universal standard strain of *Salmonella* ser. Braenderup) and group initial. Label one Falcon 2057 (17-mm x 100-mm) tube with "H" and group initial.

2. Measure sterile Type 1 water and 10X H Buffer (Roche Molecular Biochemicals) into labeled Falcon 2057 tube according to the following table to make a 1:10 dilution of the buffer. Measure reagents carefully; prepare enough for 11 plug slices. Mix well.

Note: Wear gloves when handling 10X buffer. Keep buffer on ice.

Reagent	µl/Plug Slice	µl/11 Plug Slices
Sterile Type 1 Water	180 µl	1980 µl
H Buffer	20 µl	220 µl
Total Volume	200 µl	2200 µl

3. Add 200 µl diluted H Buffer to labeled 1.5-ml microcentrifuge tubes.

4. Carefully remove plug from tube containing TE with wide end of spatula and place in a sterile disposable petri dish or on the "plug cutter".

5. Cut a 2-mm-wide slice from test samples with a razor blade and transfer to the labeled 1.5-ml microcentrifuge tube containing 200 µl diluted H Buffer. Be sure plug slice is under buffer. The same spatula, razor blade or scalpel, and petri dish can be used for all samples. Replace rest of plug in original tube that contains TE Buffer.

Notes: The shape and size of the plug slice that is cut will depend on the size of the comb teeth that is used for casting the gel. PulseNet recommends that the combs with larger teeth (10-mm-wide teeth) be used to cast the gels because computer analysis of the gel lanes is more accurate and less tedious than analysis of gel lanes cast with combs with the smaller teeth (5.5-mm). The number of slices that can be cut from the plugs will depend on the skill of the operator, condition of the plug, and whether the slices are cut vertically or horizontally (5-mm x 10-mm plug).

6. Cut three 2-mm-wide slices of H9812 *Salmonella* ser. Braenderup standard plug and transfer to tubes of diluted H buffer. Be sure plug slices are under buffer.

7. Place tubes containing sample and control plug slices in float and incubate in a 37°C water bath for 10-15 min.

8. While plug slices are incubating in the buffer, dilute 10X H Buffer 1:10 with sterile Type 1 water according to the following table. Mix in the same Falcon 2057 tube that was used for H Buffer.

Reagent	µl/Plug Slice	µl/11 Plug Slices
Sterile Type 1 Water	175 µl	1925 µl
H Buffer	20 µl	220 µl
Enzyme (10 U/µl)	5 µl	55 µl
Total Volume	200 µl	2200 µl

9. After incubation of plug slices, remove H buffer by inserting pipet fitted with 200-250 µl tip all the way to bottom of tube and aspirating buffer. Be careful not to cut plug slice with pipet tip and that plug slice is not discarded with the tip.

10. Add[2] 55 µl of *Xba*I enzyme (Roche Molecular Biochemicals) to the diluted buffer; mix well.

Note: Keep vial of restriction enzyme on ice or in insulated storage box (-20°C) at all times. Wear gloves.

11. Add 200µl restriction enzyme mixture to each tube. Close tube and mix by tapping gently on bench top; confirm that plug slice is under enzyme mixture.

12. Place sample and control tubes in float and incubate in 37°C water bath for at least 2 hr.

[2] The instructor will dispense the *Xba*I enzyme

Laboratory Module IIB
Casting Agarose Gel and Loading Restricted Plug Slices on the Comb

Tuesday morning (11:30am – 01:00pm), March 16, 2004

Read instructions in Steps 1-9 before continuing.

1. Place 10-well comb in 14-cm-wide gel form; use leveling platform to confirm gel is level. Confirm that front of comb holder and teeth face bottom of gel, and that the teeth of the comb touch the gel platform.

2. Remove restricted plug slices from 37°C water bath; allow to equilibrate to room temperature.

3. Remove enzyme/buffer mixture from plug slices with pipet and tip. Insert pipet fitted with 200-250 µl tip all the way to bottom of tube and aspirate buffer. Be careful not to cut plug slice with pipet tip and that plug slice is not discarded with the tip.

4. Add 200 µl 0.5X TBE to each plug slice.

5. Put comb on bench top or on top of gel form and load the plug slices on the bottom of the comb teeth; put the H9812 *Salmonella* ser. Braenderup standard plug slices on teeth 1, 5, and 10 and the sample plug slices on the remaining teeth.

Note: Load samples in order that is shown on the PFGE report form provided by instructors.

6. Remove excess buffer with edge of tissue; allow the plug slices to air dry for 3 minutes.

7. Position comb in gel form, confirm that plug slices are correctly aligned, and carefully pour 100 ml of melted 1% SKG agarose (55°C-60°C) into the gel form. Remove any bubbles that form with clean pipet tip. Allow gel to solidify for 30 minutes before removing the comb.

8. As soon as gel is poured, continue with **Laboratory Module IIC** below.

Note: Instructor will provide melted 1% SeaKem Gold (SKG) Agarose.

Laboratory Module IIC
Preparation of Electrophoresis Unit for PFGE of Restriction Digests

Read instructions in Steps 1-4 before continuing.

1. Confirm that electrophoresis chamber is level; adjust the leveling screws on bottom of unit, if the chamber is not level. Put black gel frame in electrophoresis chamber; avoid touching the electrodes.

2. Add 2.2 L of 0 5X TBE; close cover of electrophoresis chamber.

3. Turn on power supply and pump; confirm that pump setting is –70 (the buffer flow at this setting is approximately 1 liter/min) and that buffer is circulating through the tubing.

4. Turn on cooling module and confirm that temperature setting is 14°C. (It usually takes approximately 20 minutes for the buffer to cool to 14°C).

Laboratory Module IID
Electrophoresis of Restriction Digests in PFGE Gel

Read instructions in Steps 1-3 before continuing.

1. Unscrew and remove end gates from gel form; remove excess agarose from sides and bottom of casting platform with a tissue. Keep gel on black casting platform and carefully place gel inside casting frame in electrophoresis chamber. Close cover of chamber.

2. Select the following conditions on the CHEF Mapper electrophoresis unit:

   Auto Algorithm
   Low MW = 30 kb, High MW = 700 kb
   Select default values except where noted by pressing "Enter"
   Change run time to 16h.
   (Initial switch time = 2.16 s; final switch time = 63.8 s)

3. Record the milliamp (ma) value on PFGE Report form (normal values = 120-145 ma).

Laboratory Module III
Staining and Documentation of PFGE Agarose Gel

Wednesday morning, March 17, 2004

Read instructions in this section before continuing.

1. Turn off chiller, pump, and CHEF Mapper (3 switches) when electrophoresis run is over.

2a. Remove gel and place it in covered plastic container that contains 40µl ethidium bromide in 400 ml Ultrapure water (Reagent or Type 1 water). The stock solution is 10 mg/ml; it is diluted 1:10,000 for staining.

Notes: Ethidium bromide is toxic and a mutagen. The diluted stain can be reused 3-5 times if it is kept in a dark bottle; discard used ethidium bromide according to the hazardous waste guidelines of your institution (See Section 10 of the PFGE Manual).

3. Place container with gel on rocker for 25-30 min.

4. Attach tubing to right port of electrophoresis chamber and drain buffer into large Erlenmeyer flask.

5. Rinse electrophoresis chamber with 1-2 liters Reagent Grade water; drain into large flask.

6a. Ethidium bromide - After gel has stained for 25-30 minutes, carefully pour into labeled bottle. WEAR GLOVES! Add 400 - 500 ml Reagent Grade water to gel and place on rocker to de-stain for 60-90 minutes; change water every 20-30 minutes, if possible.

7. Capture image on **Gel Doc 2000** (***.1sc** file extension) according to the instruction sheet provided. Name the files according to the appropriate designation:

HKG04001-a
HKG04001-b
HKG04001-c
HKG04001-d

Note: If background interferes with resolution, de-stain for additional 30-60 min.

8. Convert the ***.1sc** file to a ***.tif** file and save on diskette provided by instructor.

Formulas for Reagents Used for PFGE[3]

Cell Suspension Buffer (CSB; 100 mM Tris:100 mM EDTA, pH 8.0)
10 ml of 1 M Tris, pH 8.0
20 ml of 0.5 M EDTA, pH 8.0
Dilute to 100 ml with sterile Type 1 water

Tris:EDTA Buffer (TE; 10 mM Tris:1 mM EDTA, pH 8.0)
10 ml of 1 M Tris, pH 8.0
2 ml of 0.5 M EDTA, pH 8.0
Dilute to 1000 ml with sterile Type 1 water

1% SeaKem Gold:1% Sodium Dodecyl Sulfate Agarose (1% SKG:1% SDS) in TE Buffer
a. Weigh 0.50 (or 0.25) gram SKG into 250 ml screw-cap flask.
b. Add 47.0 (or 23.5) ml TE Buffer; swirl gently to disperse agarose.
c. Remove cap, cover loosely with clear film, and microwave for 30-sec; mix gently and repeat for 10-sec intervals until agarose is completely dissolved.
d. Place flask in 56EC water bath for at least 5 minutes.
e. Add 2.5 (or 1.25) ml of 20% SDS that has equilibrated to 56°C and mix well.
f. Recap flask and return to 56°C water bath until ready to use.

Cell Lysis Buffer (50 mM Tris:50 mM EDTA, pH 8.0 + 1% Sarcosine)
25 ml of 1 M Tris, pH 8.0
50 ml of 0.5 M EDTA, pH 8.0
5 g of Sarcosyl (N-Lauryl-Sarcosine, Sodium salt)
or
50 ml of 10% Sarcosyl (N-Lauryl-Sarcosine, Sodium salt)
Dilute to 500 ml with sterile Type 1 water

Add 25 µl Proteinase K stock solution (20 mg/ml) per 5 ml of cell lysis buffer <u>just</u> <u>before</u> <u>use</u>. The final concentration of Proteinase K is 0.1 mg/ml in the buffer.

1% SeaKem Gold Agarose (1% SKG) in 0.5X TBE Buffer
a. Weigh 1.0 gram SKG into 500 ml screw-cap bottle.
b. Add 100.0 ml 0.5 X TBE Buffer; swirl gently to disperse agarose.
c. Remove cap, cover loosely with clear film, and microwave for 60-sec; mix gently and repeat for 15-sec intervals until agarose is completely dissolved.
d. Place bottle in 54-58°C water bath for at least 15 minutes before pouring gel.

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

[3] See Section 5a of the PulseNet PFGE Manual for detailed information.