



Global Salm-Surv

A global *Salmonella* surveillance and laboratory support project
of the World Health Organization

Laboratory Protocols

Level 5 Training Course

**Pulsed Field Gel Electrophoresis for *Salmonella* and
*E.coli***

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Introduction to Pulsed-Field Gel Electrophoresis for *Salmonella*.

Introduction

In the past, foodborne outbreaks were detected when cases caused by the same pathogen were clustered in time and space. The investigation was based on epidemiological methods comparing exposures among cases and non-cases. Some outbreaks were missed because geographically dispersed cases or cases separated in time were not be obviously linked to each other. Other outbreaks were identified when none existed because most foodborne pathogens, such as *Salmonella*, are very common. It was often difficult to fully investigate an outbreak. Although the pathogen may have been detected in the suspect food, it was not possible to say whether it was the outbreak strain. Without confirmation of the outbreak strain, it was difficult to trace back the implicated food to identify the initial source of contamination.

Subtyping of microbiological isolates has led to a great improvement in our ability to identify, investigate and control outbreaks of foodborne pathogens. A widely used method of molecular typing in foodborne pathogens is pulsed-field gel electrophoresis (PFGE). It involves cutting genomic DNA using restriction enzymes to generate a small number of large restriction fragments and their differential migration through agarose gel by constantly changing the direction of the electrical field during electrophoresis.

PFGE allows the identification of clones or isolates with a similar ancestry, potentially emerging from the same source. Routine comparison of PFGE patterns from human isolates from across a region, a country or globally allows us to link apparently unrelated cases. Outbreaks that were not recognised because of geographic dispersion or dispersion in time, can now be identified. The comparison of PFGE patterns from human and food or animal isolates allows us to identify and confirm potential sources of disease. Once the epidemiological investigation has identified a potential source, PFGE can help confirm the outbreak strain is found in the various stages of food processing. This leads to a better understanding of the means of contamination and spread and therefore to better control interventions.

PFGE may be too discriminatory or not discriminatory enough for certain pathogens or subtypes. For example, although PFGE is commonly used to differentiate clones of *S. Typhimurium*, it is not as useful for *S. Enteritidis* where most isolates have the same PFGE pattern. In the latter case, PFGE can still be used if a rare pattern is identified. PFGE provides evidence that two cases may share a common source but is not proof that the isolates have a common ancestry. PFGE and other microbiological tools should never replace the epidemiological investigation, but rather enhance it.

There are many methods to conduct PFGE. The one presented in this protocol is the one used by a growing number of laboratories including those, which are member of PulseNet, the US-based network of laboratories that conduct PFGE and share patterns. The use of a standard method across labs and countries is important to allow comparison of PFGE patterns and identification of clusters of cases and potential sources of disease. In order for PFGE to work as a public health tool, data generated by laboratories must be shared among clinical, food and veterinary labs and with public health officials. PFGE and other subtyping methods have led to an increased collaboration between microbiologists and epidemiologists and thus to a better understanding and control of foodborne diseases.

References:

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Materials

Equipment

- Spectrophotometer 620nm
- Waterbath 54°C
- Microwave oven
- Refrigerator
- Pulsed-field gel electrophoresis apparatus e.g CHEF Mapper, CHEF DR. III, CHEF DR II: Bio-Rad
- Incubator 37°C
- UV-transilluminator and photo camera or Gel Doc 2000 or equivalent.

Materials

- Non selective agar plates
- Gloves
- Slides
- Milli-Q water (sterile distilled water)
- CSB-Buffer. (Tris / EDTA)
- Lysis buffer (Tris, EDTA, Sarcosyl)
- 70% Ethanol
- Soap water
- TE buffer 10:1 (Tris, EDTA)
- NaOH-tabs and HCL for pH-adjustments
- Electrophoresis buffer 10 X TBE (Tris, Boric acid, EDTA): Bio-Rad
- Proteinase K (20mg/ml)
- Ethidium bromide solution (1%)
- Enzyme buffer e.g. buffer 10X Tango: Fermentas
- Enzyme e.g. Xba I: Fermentas
- Loops 1ul / 10µl
- 96-well plate / Elisa-plate
- Disposable centrifuge tubes 10ml
- Plug molds
- 50 ml crew-cap tubes with e.g. screened caps: Bio Rad
- 2ml tubes e.g. Cryo tubes.
- Agarose e.g. SeaKem Gold agarose: FMC Bioproducts
- 100 ml Erlenmeyer flask
- Pipettes 20ul, 200ul, 1000ul
- Tips for pipettes
- Tape
- 6 mm spatula or similar device
- Scalpel
- Casting stand
- Platform
- Comb
- Staining bath

Strains

- *Salmonella* Braenderup H9812: Intern standard.

Safety

Carry out all procedures in accordance with the local codes of safe practice.

For staining the DNA, ethidium bromide is used. This dye is carcinogenic. Therefore gloves and proper clothes should be worn (appendix 1: laboratory safety).

Visualisation of the stained DNA is done by use of UV transilluminator. UV light is harmful for skin and eyes. Therefore proper protection (facemask, glasses) should be worn (appendix 1: laboratory safety).

Acid is used to prepare the TBE-buffer Boric. This chemical may impair fertility. Gloves should be worn while working with this chemical. In case of an accident or if you feel unwell, seek medical advice immediately (appendix 1: laboratory safety).

Note

Bear in mind that this manual is NOT a PulseNet protocol and does NOT qualify or certify you as a PulseNet member.

Procedure

Day 1:

Streak the culture with a loop onto non-selective agar plates in a way to obtain single colonies. Incubate the plates at 37°C / 18h.

Day 2: Purification of DNA / plugs

Turn on a shaker waterbath at 54°C and a spectrophotometer if such is used.

Prepare a 1% agarose solution e.g. SeaKem Gold agarose, FCM Bioproducts

For 15-20 samples weigh 0,2g of agarose and add to a 100 ml Erlenmeyer flask. Add 20 ml of Tris- EDTA buffer (10:1).

Cover the flask loosely with clear film and melt the agarose in a microwave at 600 W for 30 sec, swirl gently and repeat for 10 sec. intervals until the agarose is fully dissolved.

Place the solution in the waterbath at 54°C.

Add 2/5 ml of CSB- buffer to each 10 ml centrifuge tube.

Label all tubes with the number of the culture.

Make a suspension of cells from the agar plates using a polyesterfiber or cotton swab moistened with CSB-buffer to eliminate aerosols and clumping of cells. If cells do clump together, use a disposable plastipipette to disperse the cells evenly.

Transfer 200 µl suspension to a 96-well plate. Remember to add CSB-buffer to the blank wells.

Read the absorbency of the samples in a spectrophotometer or on a Dade Microscan turbidity meter
Measure the samples at a wavelength of 620nm. The OD (absorbency) of the samples needs to be 0,12 abs.

Theory / comments

It is much easier to detect contaminants from a plate with single colonies than from confluent growth.
Save the culture on plates until the end of the DNA purification as a backup in case you need to re-purify the DNA.

The gel strength of the plugs could be increased up to 1.6% without causing a major difference in the result. It is easier to slice the plugs if the gel strength is higher than 1%, but we recommend 1%.

Use heat resistant gloves when handling heated glass, which has been in the microwave oven.

CSB buffer prevents further growth of the culture. The volume of CSB-buffer depends on the equipment used.

It is crucial to keep track of your samples.

Clumping cells may result in poor lysis of the cells and an incorrect OD value.

Do not use a normal pipette and tip, since the pressure put on the cells through a narrow tip, may cause unwanted lysis of the cells/shear of DNA.

Remember to use CSB-buffer as blank on the spectrophotometer aswell as on the Dade Microscan turbidity meter.

This concentration gives a satisfactory result at DFVF processed with our instruments. The results may vary if run with other instruments. We suggest optimising this step by calculating at each institute in order to have the best intensity of the bands.

Procedure

When measuring the concentration of cells on a Dade Microscan turbidity meter adjust concentration to 0,48-0,52 (Falcon 2054 tubes) or 0,68-0,72 (Falcon 2057 tubes).

If the concentration of cells is too high, dilute by adding sterile CSB-buffer.

If the concentration is too low, add more cells to your suspension.

Assemble the plug moulds and wipe with 70% ethanol.

Note the position of the culture on tape placed on the side of the mould.

Transfer 400 µl of the cell suspension to 1,5 ml microcentrifuge tubes.

Add 20 µl of proteinase K (20mg/ml) and mix gently with the pipet tip.

Add 400 µl of the melted 1% agarose solution to the 420 µl cell suspension/proteinase K, and mix by gently pipetting up and down a couple of times. Avoid creating airbubbles.

While casting plugs keep the flask of agarose in a beaker of 54°C warm water or in a stationary waterbath at 54°C.

Immediately dispense the mixture into the plug moulds by using a pipette.

700 µl are sufficient to cast 2 plugs.

Check the purity of each culture. After casting the plugs, dip a 1 µl loop into each tube and inoculate the culture onto a non-selective agar plate. Incubate at 37°C over night.

Allow the plugs to solidify at roomtemperature for 10-15 min. or in the refrigerator for 5 min.

Prepare a mastermix of lysis-buffer according to the following concentrations per plug:

- 5 ml of Lysis buffer
- 25 µl of Proteinase K (20mg/ml)

The concentration of Proteinase K in each tube is 0,1mg/ml.

Theory / comments

This is recommended by CDC, and will in short time be implemented.

Cell lysis is optimized if you incorporate Proteinase K in the plugs.

The cell suspension should reach room temperature before being mixed with the agarose.

Air bubbles in the plugs will make it difficult to slice the plug later on in the procedure.

Remember to check the plates for contaminants the following day.

Unused plug-agarose can be stored at room temperature and re-used 1-2 times. Melt on low-medium power for 10-15 sec. and swirl gently and repeat or 5-10 sec. intervals until the agarose is completely dissolved.

This agarose will melt rapidly.

Proteinase K inactivates DNase's.

Proteinase K (20mg/ml) is commercially available or you can prepare a stock solution of proteinase K from powder in sterile distilled water and aliquote in 300-500 µl amounts and store frozen.

Discard any unused thawed non-commercial Proteinase K.

When using proteinase K powder, avoid inhalation of the powder as it may harm your lungs.

Procedure

Mix gently by inverting the mastermix a few times, and transfer 5 ml of the Lysis-buffer/Proteinase K-mix to each 50 ml crew-cap tube.

Make sure each tube is clearly marked with the name or number of the sample.

Disassemble the plug moulds and gently transfer the plugs to the corresponding 50 ml crew-cap tube containing the Lysis buffer using a 10 µl inoculating loop, or a 6mm spatula.

Incubate the tubes in a shaker waterbath at 54°C for 1½-2h (150-175 rpm).

Preheat enough sterile distilled water to wash the plugs 2 times with 10-15 ml.

Wash the moulds with soap and leave to air-dry after cleaning.
Optional: The mould

After incubation, decant the Lysis buffer either by using screened caps or by holding back the plugs with a scalpel while decanting the buffer.

Preheat enough sterile TE-buffer 10:1 to wash the plugs 4 times with 10-15 ml

Lower the temperature of the waterbath to 50 °C.

Add 10-15 ml sterile double distilled preheated water (50°C) to the tubes and incubate for 10-15 min in a shaker waterbath.

Repeat the wash step once.

Wash the plugs four times for 10-15 min with 10-15 ml TE buffer (10:1) at 50°C.

Discard the wash buffer and carefully transfer the plugs to smaller 2 ml Cryo-tubes. Add TE buffer (10:1) until the plugs are covered. The plugs are ready for digestion or storage.

Theory / comments

Be careful not to damage the plugs when transferring them from the mould to the tubes.

Make sure the plugs are submerged in the buffer and not attached to the side of the tube or the lid.

Make sure the tubes are immersed in the water and the level of the water is above the level of buffer. This step leaves only DNA embedded in the solid agarose matrix. The DNA will not shear when handled further and will remain stable for electrophoresis.

Some methods use the chemical PMSF (PhenylMethylSulfonylFluorid) to neutralize Proteinase K and remove cell debris instead of washing the plugs repeatedly. The chemical is however highly toxic and is therefore rarely used.

TE is used because it contains EDTA that binds the divalent ions needed by enzymes.

The plugs could be stored in TE buffer 1:10 for up to 6 months. For a longer storage period use an EDTA solution. If EDTA is used, washing in TE buffer 10:1 is needed before the DNA can be digested.

Procedure

The plugs are stored in a refrigerator at a temperature of 4°C.

Day 3: Restriction digestion

Check the plates from the day before for contaminations, before digesting the plugs.

Dilute the enzyme buffer 10X conc. (e.g. Tango) 1:10 with sterile double distilled water to a final volume of 200 µl per plug, and add to eppendorf- or microcentrifuge tubes. Remember to cut 4 slices of the *S. braenderup* H9812 standard, leaving you with 12 lanes to run your samples.

Place the plug on a glass-slide or a fabricated mould for cutting a 2 mm slice of the plug.

Drain the drops of TE buffer with a Kleenex.

Trim excess gel of the plug.
Use a scalpel or razorblade to cut the 2 mm broad slice from the end of the plug resulting in a 10 mm wide slice.

Using the scalpel or a spatula, transfer the slice to a pre-labelled eppendorf-or microcentrifuge tube.
Replace the rest of the plug in the original storage tube containing TE-buffer.

Make sure the slices are covered by buffer and pre-incubate the plugs at the required temperature of the enzyme for 5-10 min. or at room temperature for 10-15 min.

Discard the pre-incubation buffer with a pipette.

Prepare a mastermix of the restriction enzyme mixture as follows per sample/plug (see page 16 for dilutions)

- 20 µl of enzyme buffer 10X Tango.
- 40 U of restriction enzyme Xba I (10 U/µl)
- 2 µl of BSA for restriction reactions.
- 174 µl. sterile double distilled water

Theory / comments

Never store the plugs in a freezer, as the plugs will turn into jelly.

Incubation in buffer equilibrates the samples and makes the DNA more amenable to digestion.

If Bionumerics is to be used to analyse the captured image, it is necessary to run the internal standard H9812 at every 5th lane in order to be able to align the bands.

Slices cut too thick could result in hazy / diffuse bands.
Slices with a width of 10 mm gives clearer bands compared to 5,5 mm wide slices.

Make sure you do not damage the plug-slice with the tip of the pipette and be careful not to discard the thin slice along with the buffer in the tip.

Several restriction enzymes aimed for Gram Neg. PFGE could be used for the digestion of bacterial DNA. In our example, we use Xba I which is the most common used enzyme for Gram Neg. PFGE, but Spe I, Ssp I, Dra I, Asn I, Bln I and Not I are used as second enzymes in cases where Xba I does not discriminate enough between the selected strains.

Procedure

Add 200 µl of the restriction enzyme mixture to the pre-labelled eppendorf tubes and mix gently by tapping.

Make sure the plugs are covered by the solution and incubate the plugs at the required temperature for 1½-2 hours (Xba I require 37°C).

Preparation of the agarose gel.

Switch on a waterbath and set the temperature at 54°C.

Prepare a 1% SeaKem Gold agarose in 0,5 X TBE solution.

For a comb of 15 x 10mm wells use 150 ml 1% SeaKem agarose solution:

- 1,5 g agarose
- 7,5 ml 10X TBE
- 142,5 ml sterile double distilled water

Gently swirl the mixture to disperse the agarose.

Microwave the solution for 60 sec., swirl gently and repeat for 15 sec. Intervals until the agarose is completely dissolved.

Place the agarose in a 50-60°C water bath.

Approx. 1 hour before loading the gel prepare 2,5 l of 0,5X TBE buffer:

- 125,0 ml of 10X TBE buffer
- sterile double distilled water to a final volume of 2.5 l.

Transfer 4 ml of the 0,5X TBE buffer to a tube and save it for pre-incubation of the plugs.

Add the 2.5 l of freshly prepared 0,5X TBE buffer to the pulsed-field gel electrophoresis chamber.

Turn on the pump with a flow of 1 L/min (pump setting = 70) and the cooling module set to 14°C.

Theory / comments

TBE contains boric acid and may impair fertility. Gloves should be worn while working with this chemical (Appendix 1: laboratory safety).

Weigh the agarose into a 500 ml erlenmeyer-flask. Add 7,5 ml 10X TBE to a measuring flask and add sterile distilled water to a total of 150 ml. Invert the flask a few times and transfer to the erlenmeyer-flask.

Use 150 ml 1% agarose solution for a comb/frame with 22 slim teeth.

Use 100 ml 1% agarose solution for a comb/frame with slim teeth for 10-15 samples.

Use heat resistant gloves when handling heated glass, which has been in the microwave oven.

Procedure

After end digestion of the plugs remove the enzymemixture from the tubes with a pipette, and add 200µl 0,5X TBE buffer and incubate at room temperature for 5 min..

Attention: If using different restriction enzymes on the S.Braenderup H9812 standard and the samples, make sure to switch pipette tip according to the enzymes.

Clean the tray in alcohol before assembling the gel tray with stand, frames and the black platform. The teeth of the comb should just be touching the black platform.
Make sure the gel form is level.

Position the comb on the table facing the side of the comb, which will allow the longest run in the gel.

Remove the plug slices from the tubes with a bent pasteurpipette or a spatula.
Position the spatula right in front of the tooth of the comb and gently push the slice onto the tooth. Make sure the slice is positioned at the bottom of the tooth,

Attention: Make sure that the S.Braenderup H9812 standards are loaded onto teeth/lane: 1, 5, 10, and 15.

Load the remaining samples onto the remaining teeth.

Remove liquid from the slices on the comb with a piece of Kleenex, and allow the slices to air-dry to the teeth of the comb for 5 min.

Optional: Seal the slices to the comb with a few drops of the 1% agarose solution made to cast the gel and let solidify for a few min.

Place the comb in the frame, and carefully pour the agarose into the frame, and let solidify for approx. 30 min.

Optional: Close the wells with leftover melted 1% agar.

Avoid trapping air in the wells.

Remove the comb and unscrew and remove the ends from the frame and release the black platform from the stand.

Theory / comments

There is an alternative way to load the gel:

Pour the agarose into the gel tray with the comb in place and let the gel solidify for 60 minutes.

Remove the comb.

Remove the plug slices from the tubes with a device such as a bent pasteurpipette or a spatula.

Position the blade right in front of the well and gently push the slice gently into the bottom of the well.
Make sure the slice is positioned with no space/air-bubbles between the slice and the side of the gel facing forward.

Close the wells with unused melted agar, either 1% or 1.6%. Avoid trapping air in the wells.
Trapped air-bubbles will cause irregular bands or even split bands.

Procedure

Carefully remove excess agarose from the bottom and the sides of the platform. Insert the black platform with the gel into the black gel frame in the electrophoresis chamber.

Close the lid of the chamber.

Select the desired unit programme (Appendix 2: Example of a PFGE set up) and press the button "RUN".

Day 4: Staining and image capture

Prepare a staining bath by diluting 100µl of the Ethidium bromide stock solution (10mg/ml) with 1000 ml of sterile distilled water to a final concentration of 1 µg/ml Ethidium bromide in a non-transparent staining box.

When the run is completed, turn off the power supply and open the unit lid. Release the black platform from the gel and place the gel in the staining-bath for about 20-30 minutes.

Drain the TBE buffer from the electrophoresis chamber and discard.

Rinse the chamber with 2 l of sterile double distilled water for 30 min.

Remember to flush the lines by activating the pump.

If the chamber is not to be used for a longer period, be sure to empty the lines, by detaching the line in the front to the left from the chamber.

De-stain the gel for 60-90 min in approx. 500 ml sterile distilled water; changing the water every 20 min. before visualising the gel / bands.

Place the gel on top of the UV-transiluminator or in the chamber of a Gel Doc 2000 unit from Biorad.

Make sure to zoom in on the gel and set the focus.

Theory / comments

Gel debris from the bottom or elsewhere may jam the lines and interfere with the pumpflow and cooling unit.

Other programmes could work as well as the one described, but many institutes run PFGE for *Salmonella* using this programme. It makes it easier to match PFGE patterns between institutions.

Wear suitable protection for working with Ethidium bromide (gloves, clothes). (Appendix 1: laboratory safety).

Ethidium bromide intercalates in the double stranded DNA and is visualised under UV light.

The staining bath should only be used for PFGE-gels and stored in a dark place.

The staining solution can be re-used 3-5 times.

The PFGE chamber and lines will last longer and the TBE buffer will not get spiked with old evaporated TBE, which may result in a too high salt concentration if they are cleaned properly.

It is important to de-stain the gel to get the exact balance between the background and the illuminated bands.

UV light is harmful to the skin and eyes. Wear proper protection e.g. facemask (Appendix 1: laboratory safety).

Make sure to focus on the bands whether you use a Polaroid camera or the Gel Doc unit in order to get sharp pictures.

Procedure

Visualise the results by switching on the UV-lamp and pressing autoexposure. Check the box “saturated pixels” so see if the gel is overexposed.

If the background interferes with the resolution, destain for additional 30-60 min.

Freeze the picture, analyse, save and export to .tiff-image according to the software.

If the gel has not run as far as wanted you can extend the run-time by 1 hour. Do not change the switch-times.

If the isolate has autolysed the DNA, you will see a smear in the lane instead of bands. This can be avoided by adding 125 µl 1M Thiourea to the 2,5 l electrophoresis buffer 0,5X TBE.

Theory / comments

When capturing the image do not expose the gel to UV-light for a longer period than necessary.

The gel might need to be further de-stained, and the UV-light will destroy the signal of the bands over time, and hence the signal will have faded afterwards, and not be stronger after further de-staining.

TIFF files can be e-mailed for further analysis and comparison if the same PFGE parameters have been used e.g. programme, enzyme, standards etc.

Steps in the procedure which can be prolonged

- The lysing of the plugs can be prolonged to 3-16h.
- Washing of the plugs with TE buffer 10:1 can be prolonged for 30-45 min at a lower temperature (37°C or roomtemperatur).
- The washing can also be paused until the next day, by storing the tubes containing TE buffer 10:1 in the refrigerator at 4°C over night.
- The digestion of the plugs can be prolonged to 3-16h.

Composition and preparation of media and reagents

Reagents can be made as described below and/or are commercially available from companies like Bio-Rad, BioLabs and Amersham.

TBE (Tris-Borate EDTA) buffer: (pH 8,0)

Working solution

- 0,89 M Tris borate
- 0,89 M boric-acid
- 20 mM EDTA

Concentrated stock solution 10 X (pH 8,0 - Do not adjust with HCl).

- Tris base 107,6 g.
- Boric acid 55,03 g.
- 0.5 M EDTA 7,44 g.
- Water to 1000 ml.

0,5X TBE Buffer

- 125 ml 10X TBE Buffer, pH 8,0
- Dilute with water to a total volume of 2500 ml

1M Tris solution: (pH 8,0)

- Tris 121,14 g.
- Water 700 ml.
- Adjust pH to 8,0 with HCL
- Add water to a final volume of 1000 ml.

0,5M EDTA solution: (pH 8)

- EDTA 93,06 g.
- Water 400 ml.
- Add approx. 10 g of NaOH tabs as EDTA dissolves at a pH of 8. Adjust pH to 8,0.
- Add water to a final volume of 500 ml.

CSB (Cell suspension buffer):

Working solution

- 100 mM Tris
- 100 mM EDTA

- 1 M Tris, pH 8,0 10 ml
- 0,5 M EDTA, pH 8,0 20 ml
- Water 70 ml

Cell Lysis buffer:

Working solution

- 50 mM Tris
- 50 mM EDTA
- 1% Sarcosyl

- 1 M Tris, pH 8,0 50 ml.
- 0,5 M EDTA, pH 8,0 100 ml.
- 1% Sarcosyl 10 g.
- Water to 1000 ml.

Tris-EDTA buffer (TE 10:1): (pH 8,0)

- 1 M Tris, pH 8,0 10 ml.
- 0,5 M EDTA, pH 8,0 2 ml.
- Water to 1000 ml.

Proteinase K solution:

Working solution

- 20 mg/ml

Dissolve 100 mg in 5 ml distilled water and aliquot to 400 µl.

Store at -20°C.

Ethidium bromide (10mg/ml)

- Add 1 g of ethidium bromide to 100 ml of H₂O. Stir on a magnetic stirrer for several hours to ensure that the dye has dissolved. Wrap the container in aluminum foil or transfer to a dark bottle and store at 4°C.
- **Staining-bath (1µg/ml)**
 - Transfer 500 ml of sterile distilled water to a dark container.
 - Add 100 µl of Ethidium bromide 10mg/ml.
 - Add 500 ml of sterile distilled water.

The staining solution can be reused for 4-6 gels.

Sample sheet - PFGE

Purpose:	
PFGE Analysis Performed by:	
Isolates Received From:	
Date Isolates Received:	
Date PFGE was Performed:	

Well	Isolate	Buffer	Enzyme	Units	Temp	Comments
1	S. Braenderup H9812					
2						
3						
4						
5						
6	S. Braenderup H9812					
7						
8						
9						
10	S. Braenderup H9812					
11						
12						
13						
14						
15	S. Braenderup H9812					

10 x Tango Buffer			Xbal - 40U (10U/ul)			Lot No.	Exp date
	1X			1X		ProK	
Water	180	0	Water	174	0	SeaKem	
Buffer	20	0	Buffer	20	0	BSA	
BSA	-	-	BSA	2	0	Xbal	
Enzyme	-	-	Enzyme	4	0	Tango	

Run conditions	
Voltage Gradient	6 V/cm
Included Angle	120o
Ramping	Linear
Initial Switch Time	
Final Switch Time	
Run Time	
Initial Milliamps	mA
Mapper	

Table for 10X buffer dilution:

Reagent	μl / plug slice	μl / 10 plug slices	μl / 15 plug slices
Sterile distilled water	180	1800	2700
10X Tango buffer	20	200	300
Total volume	200	2000	3000

Table for restriction enzyme mixture:

Reagent	μl / plug slice	μl / 10 plug slices	μl / 15 plug slices
Sterile distilled water	176	1760	2640
10X Tango buffer	20	200	300
XbaI (10 U/ μl)	4	40	60
Total volume	200	2000	3000

APPENDIX 1

Safe Work Procedure ETHIDIUM BROMIDE

Use

Gels are stained in ethidium bromide to visualise the nucleic acids.

Hazards

Class 6 – Toxic. Potent mutagen.

Risk Control Measures

Only use Ethidium bromide (EtBr) after receiving safety training (Laboratory Induction / Authorisation). Wear safety glasses when using ethidium bromide. Avoid skin contact, ethidium bromide may be absorbed through the skin. Wear Latex gloves, laboratory coat. Always dispose of gloves after use. Don't touch equipment, door handles, phone, keyboard, etc.

Weighing solid - Powder may cause irritation when inhaled - wear dust mask and use in ventilated area. Use designated micropipette only when dispensing the liquid.

Engineering / Ventilation Controls

Ensure access to a safety shower and eyewash in areas where ethidium bromide is used. Preferably weigh the solid in a fume hood.

Storage Requirements

Store in a cool, dry place away from strong oxidizing agents. Keep containers tightly closed. Use with adequate ventilation.

First Aid / Spill Control Procedures

Wash off immediately with copious amounts of cold water (at least 10 minutes). Ethidium bromide is absorbed through the skin so follow the cold water washing with a thorough washing with warm water and soap. Contaminated clothing should be removed as soon as possible and thoroughly washed.

In case of contact with eyes, immediately flush eyes with copious amounts of water for at least 15 minutes (eye wash).

Seek medical attention.

If the spill is on equipment, use ultraviolet light (wear appropriate eye protection) to locate spill, then use the decontamination procedure outlined below.

Wear protective clothing.

Small spill:

If in solution, absorb freestanding liquid using vermiculite or Polyzorb from Spill Kit. Use ultraviolet light to locate spill. Follow instructions on Spill Kit.

Large Spill: Notify others in the area of spill. Evacuate area. Barricade area with tape (in Spill Kits) to prevent entry until arrival of response personnel. Provide assistance and information to spill clean up crew.

Waste

Store waste: Liquid - In proper waste container

Solid - In proper waste container

Ethidium Bromide Liquid Disposal

1. Add 10g activated charcoal per 2.5L waste.
2. Leave for 1 hour, with occasional shaking.
3. Filter contents through Whatman Number 1 filter paper.
4. Filtrate may be disposed of down the sink.
5. Charcoal & paper are treated as solid hazardous waste and disposed of in the EtBr Solid Waste Bucket.

If Using 'Green Bag' (Bio-101 Cat. No. 2350-200):

- 1 For 10mg Ethidium Bromide (max) add 1 'Green Bag' to the waste bottle with a magnetic flea.
2. Place waste bottle onto a magnetic stirrer and mix the solution for 24hours.
3. Dispose of the 'Green Bag' in the dry Ethidium Bromide waste. The remaining solution may be disposed of in the sink.

Applicable Standards and Regulations

[University of Melbourne Environment and Safety Manual - Poisons and Drugs](#)

[University of Melbourne Environment and Safety Manual - Carcinogens](#)

Occupational Health and Safety (Hazardous Substances) Regulations 1999

Safe Work Procedure ULTRA VIOLET SOURCE

Ultraviolet Light

Ultraviolet Radiation is that portion of the electromagnetic spectrum that falls in the region of 100 to 400nm. This spectrum has been divided into three regions:

A: 400nm to 315nm known as Near UV or UV-A,

B: 315nm to 280nm known as Mid UV or UV-B,

C: 280nm to 100nm known as Far UV or UV-C.

Hazards

Two categories of hazard are involved in the use of high intensity UV lamps: those inherent in the radiation itself and those associated with operation of the lamps. All radiation of wavelength shorter than 250 nm should be considered dangerous.

- Damage to eyes and skin caused by exposure to UV radiation. Repeated overexposure of skin to UV has been linked with premature aging, wrinkles and most seriously, skin cancer. Eye damage can result in corneal scarring and cataract formation.
- Burns caused by contact with a hot UV lamp.
- Fire ignited by hot UV lamp.
- Interaction of other nearby chemicals with UV radiation.

Damage caused to apparatus placed close to UV lamp.

Risks

Damage to vision is likely following exposure to high intensity UV radiation.

Who is likely to be injured?

The user or anyone exposed to the UV light as a result of faulty procedure. Injuries may be slight to severe.

Control Measures Operating Precautions

Lab-coats, gloves and safety glasses or other appropriate eye/skin protection such as UV protective glasses or a UV protective face shield must be worn.

Reactions using UV lamps: external irradiation sources

- These operations must never be attempted by an untrained person.
- These operations must never be attempted by a single person.
- These operations must never be attempted out of normal working hours.
- Use of UV lamps must be carried out in a fume hood with boarded up windows.
- As far as possible, the UV source should be contained in a closed radiation box.
- The fume hood sash must remain closed while the UV lamp is switched on.
- The fume hood must contain only the UV lamp and associated apparatus and chemicals. No other chemicals are to be stored in the fume hood and no other reactions are to be performed in the fume hood.

- Reaction vessels containing flammable solvents must be at least 20 cm away from the lamp to avoid excessive heating.
- Flammable equipment (*e.g.* rubber/plastic tubing) must be positioned at least 10 cm away from the lamp.
- After the UV lamp is switched off, unless the reaction mixture requires immediate attention, the fume hood sash should remain closed for 30 minutes to allow the UV lamp to cool.

Reactions using UV lamps: low/medium pressure Hg lamps in an immersion well.

- These operations must never be attempted by an untrained person.
- These operations must never be attempted by a single person.
- Low/ Medium pressure lamps are to be used ONLY in approved, water-cooled immersion well apparatus.
- The UV lamp power supplies must incorporate an electrical cut-out that activates in the event of disruption to cooling water.
- The UV lamp must not be switched on until:
 - The glassware is shrouded in Al foil,
 - The immersion well set-up is shielded by the appropriate metal case,
 - The boarded up fume-hood doors are closed.
- The UV lamp must NEVER be switched on/connected outside of the shrouded immersion well apparatus.

Training

For the use of high intensity UV sources, new users must be trained by another member of the laboratory who, in the opinion of the member of staff in charge of the laboratory, is sufficiently competent to give instruction on the correct procedure. Newly trained users should be supervised for some time by a competent person.

Emergency Procedures

UV exposure: Call a First Aider.

Burns: See Basic First Aid. Call a First Aider

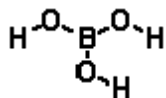
Hazard sheet **BORIC ACID**

Synonyms: Boric Acid, MB Grade (1.12015); Boron; Boricacidhighpurity; Boricacidwhitextl

Molecular Formula: H_3BO_3

Formula Weight: 61.83

Registry number: 10043-35-3



Registry number: 10043-35-3

Density: 1.43

Melting point: 169 °C

Hazard Symbol



Toxic

Risk Description

R60 May impair fertility.

Safety Description

S45 In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

S53 Avoid exposure - obtain special instructions before use.

IR

Analysis

Result

Miscellaneous	545 (29.51); 641 (30.64); 813 (26.79); 1193 (21.05); 1456 (17.55); 3215 (15.75)
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Appendix 2

Example of a PFGE set up

Purpose:	S.Rissen
PFGE Analysis Performed by:	bku
Isolates Received From:	Denmark and Thailand
Date Isolates Received:	10-01-2007
Date PFGE was Performed:	19-01-2007

Well	Isolate	Buffer	Enzyme	Units	Temp	Comments
1	S. Braenderup H9812	Tango	Xbal	40 U	37 C	Standard
2	7134985-7	Tango	Xbal	40 U	37 C	PIG
3	7164867-1	Tango	Xbal	40 U	37 C	PIG
4	7220697-1	Tango	Xbal	40 U	37 C	PIG
5	7274087-2	Tango	Xbal	40 U	37 C	HUMAN
6	S. Braenderup H9812	Tango	Xbal	40 U	37 C	Standard
7	7274089-4	Tango	Xbal	40 U	37 C	PIG
8	7281829-4	Tango	Xbal	40 U	37 C	HUMAN
9	7321579-4	Tango	Xbal	40 U	37 C	HUMAN
10	S. Braenderup H9812	Tango	Xbal	40 U	37 C	Standard
11	7621597-6	Tango	Xbal	40 U	37 C	PIG
12	7649785-5	Tango	Xbal	40 U	37 C	HUMAN
13	7654938-2	Tango	Xbal	40 U	37 C	PIG
14	7698216-4	Tango	Xbal	40 U	37 C	PIG
15	S. Braenderup H9812	Tango	Xbal	40 U	37 C	Standard

10 x Tango Buffer			Xbal - 40U (10U/ul)			Lot No.	Exp date
	1X	15		1X	15	ProK	
Water	180	2700	Water	174	2610	SeaKem	
Buffer	20	300	Buffer	20	300	BSA	
BSA	-	-	BSA	2	30	Xbal	
Enzyme	-	-	Enzyme	4	60	Tango	

Run conditions	
Voltage Gradient	6 V/cm
Included Angle	120o
Ramping	Linear
Initial Switch Time	
Final Switch Time	
Run Time	
Initial Milliamps	
Mapper	