Pulsed Field Gel Electrophoresis

Bio-Rad CHEF Mapper Systems

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Outline

Part I Introduction of PFGE

Identification and Phylogeny application

- Bacterial, fungal and viral epidemiological typing
- Bacterial source tracking
- Mutation detection and analysis
- Plant and animal breeding
- Phylogenetic inference and evolution

傳統鑒定方法

傳統鑒定方法，是相對於現代的分類鑒定方法而言

形態：
- 菌落，在半固體或液體培養基中的生長狀態等
- 個體，細胞形態，染色反應，各種特殊構造等

生態特性：
- 生長溫度，對氧的需要，宿主種類等

營養要求：
- 能源，碳源，氮源，生長因數等
- 生理、生化反應　(API, Biolog, Vitec, etc.)

血清學反應

藥物感受性

分子鑒定方法

利用現代的遺傳學特性的鑒定細胞的基因或組成成分：

基因定序
- nucleotide, amino acid sequences, MLST, MLVA

一維電泳
- PCR
- AFLP, amplified fragment length polymorphism
- RAPD, random amplification of polymorphic DNA.
- DGGE, denaturing gradient gel electrophoresis
- PFGE, pulsed field gel electrophoresis

組成成分:
- 紅外光譜、氣相色譜和質譜分析等

Accessory machines in Bio-rad

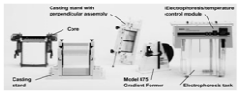
PFGE

CHEF Mapper XA system

AFLP, MLVA

Amplication and electrophoresis

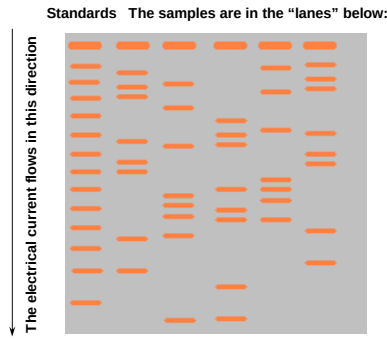
DGGE/TGGE



DCode Universal Mutation Detection System

Imager

Electrophoresis of DNA



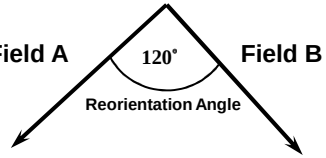
- After the electrophoresis, the gel must be stained so that the DNA can be seen.
- The researcher will have loaded some size standards as well as the samples so that the size of the unknown samples can be accurately estimated
- The gel will look like this illustration when illuminated by UV light.

Pulsed Field Gel Electrophoresis

- DNA in the size range of **30 kb and larger** will no longer migrate in a conventional agarose gel arrangement. It is simply too big and has stretched out too wide.
- In 1984, Schwartz and Cantor devised a solution for this problem - they found that DNA could be coaxed through an agarose gel in a stepwise fashion, by pulsing the electrical fields in different directions. This is why this technique is called **pulsed field gel electrophoresis (PFGE)**.

How does PFGE work?

- When the first electrical field is applied to the gel, the DNA elongates in the direction of the electrical field and begins to migrate in the gel.
- The first electrical field is then switched to the second field according to the run specifications. The DNA must change conformation and reorient before it can migrate in the direction of this field.
- As long as the alternating fields are equal with respect to voltage and pulse duration, the DNA will migrate in a straight path down the gel.



How does PFGE work?

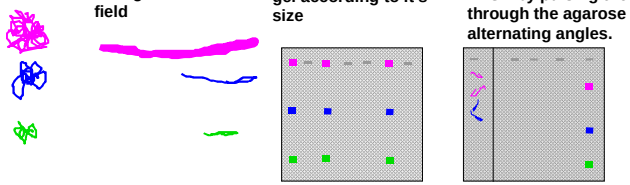
Conventional electrophoresis uses a single electrical field to cause biomolecules to migrate through a matrix according to the mass:charge ratio present in the biomolecule. Thus the migration distance of this biomolecule is indicative of its mass.

DNA in a Native State

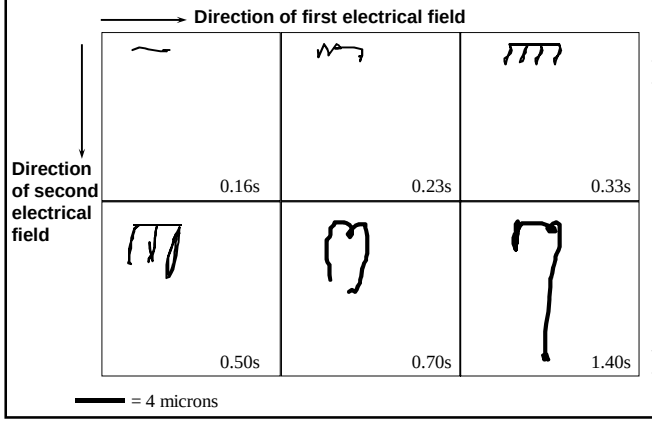
When an electrical field is applied, the DNA stretches out to align with the field

DNA that is less than 25kb will migrate in a conventional agarose gel according to it's size

Larger DNA, 25kb - 10+MB in size will migrate according to it's size in PFGE by pulsing the DNA through the agarose gel at alternating angles.



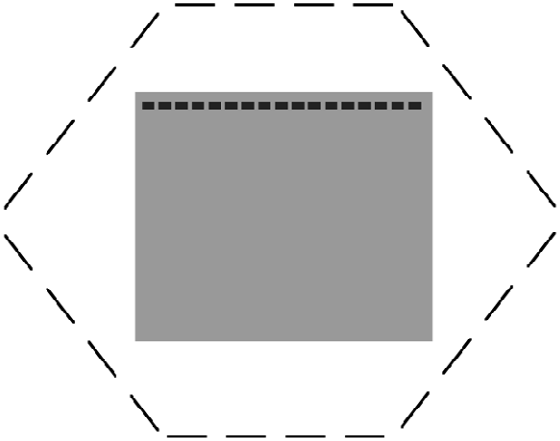
Microscopy of PFGE in action:



You can see here the "kinking" of the DNA in the gel during the electrophoresis process.

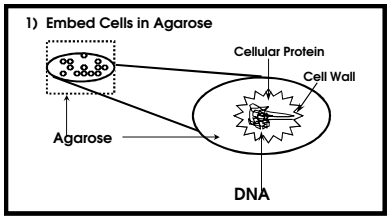
As the fields are switched, several kinks emerge. Eventually one kink "wins out" and pulls the whole molecule through the gel.

How does PFGE work?



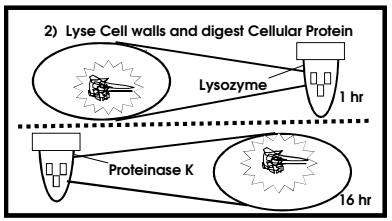
Pulsed Field Gel Electrophoresis Makes Genomic Analysis Possible

- This new method of electrophoresis enabled progress in a whole new group of researchers to make progress in analyzing DNA fragments.
- The PFGE instruments are now in use in a variety of applications :
  - **Cancer Research**, the effectiveness of radiation and chemical therapies is measured and evidence of programmed cell death is monitored using PFGE
  - **Food Safety and Public Health**, spread of various bacteria and fungi are tracked using PFGE
  - **Genome Mapping** of many organisms, including human and the tracking of chromosomal rearrangements



Whole cultured cells are embedded in agarose. This prevents shearing of the genomic DNA during the lysis and digestion steps.

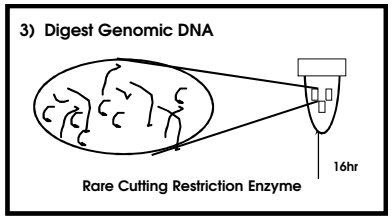
The agarose/cell mixture is poured into a small mold (1.5mm x 10mm x 5mm). Once solidified, the "plug" is extruded from the mold.



The plug preparation and gel electrophoresis can be done in as little as 24 hours or as long as 4 days.

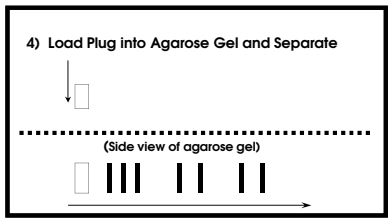
The agarose "plugs" are bathed first in a lysozyme (or appropriate) solution for 1hr. Then they are bathed in Proteinase K solution overnight.

PFGE Sample Preparation



The rare cutting enzymes produce about 15-20 bands that can identify distinct subtypes.

http://insilico.ehu.es/digest/

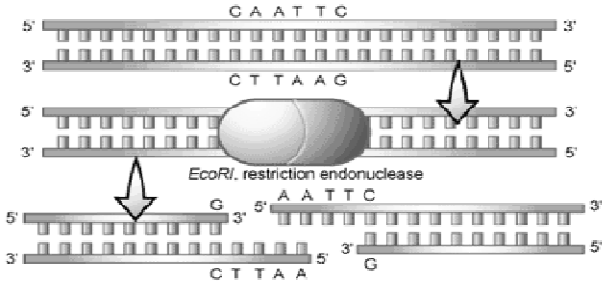


Pulsed Field Gel Electrophoresis (PFGE) can separate very large fragments of DNA. This is achieved by delivering the electric current in short pulses and alternating the direction of the electrical field. Separations in PFGE usually take 14-24 hours.

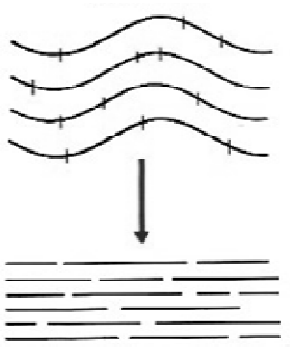
PFGE Sample Preparation

限制酶

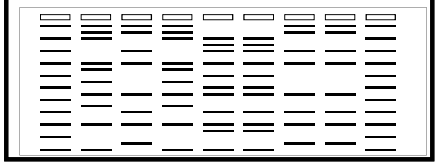
Restriction enzymes **DNA-cutting enzymes**

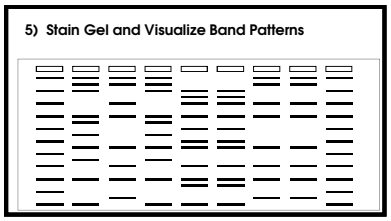


Genome digest with Restriction enzymes

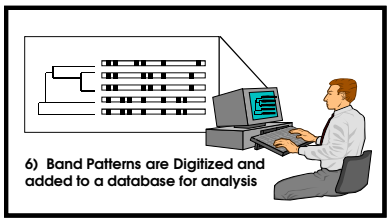


Separate fragments with PFGE





The DNA is visualized by staining the gel with Ethidium Bromide and illuminating the stained gel on a UV light source.



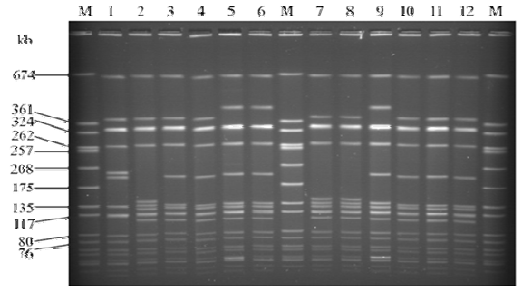
The gel image can be captured by CCD camera or scanner and the digitized gel is ready for comparison in a band pattern database. These comparisons can be easily shared between labs electronically.

PFGE Sample Preparations

院內感染應用

- RCW最常見的菌株：
  - 綠膿桿菌 (Pseudomonas aeruginosa)
  - 大腸桿菌 (E. coli)
  - 克雷伯氏桿菌 (Kp菌,Klebsiella pneumoniae)
  - 鮑氏不動桿菌 (AB菌,Acinetobacter baumannii)
  - Methicillin抗藥性金黃色葡萄球菌 (MRSA)
  - 其他格蘭氏陰性菌

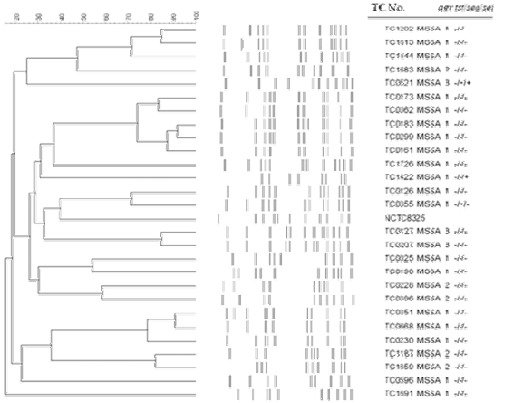
金黃色葡萄球菌之分子分型分析



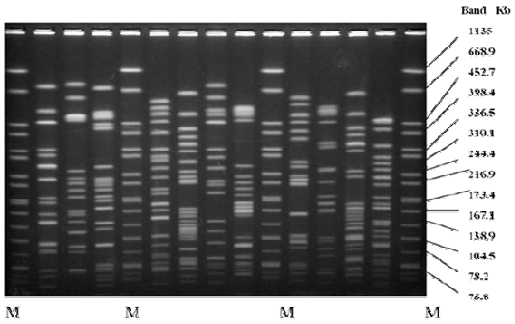
SmaI-Staphylococcus aureus

- 金黃色葡萄球菌包埋處理過的DNA以SmaI 限制酶做處理，並利用CHEF mapper 系統之脈衝式電泳進行大片段分子分型，lane M為S. aureus NCTC8325 作為分子量標準。條件為Switch Time 5~40 sec，6 V/cm，120°，0.5x TBE 2500ml，14℃，1% SeaKem Gold agarose，21 hrs, EtBr staining。

MSSA Phylogenetic Tree analysis



臨床分離克雷伯氏肺炎桿菌的基因分型



XbaI-Klebsiella pneumoniae

- lane 1、5、10、15 是美國CDC 標準菌株H9812 作為當次電泳的internal reference,其它為臨床分離菌株

Journal of General Microbiology (1991), **137**, 2531–2538. *Printed in Great Britain*

Electrophoretic karyotypes of clinically isolated yeasts of *Candida albicans* and *C. glabrata*

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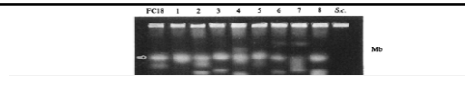
(*Received 5 February 1991; revised 25 June 1991; accepted 25 July 1991*)

One-hundred-and-four isolates of yeast were collected from the vaginas of 97 outpatients. The isolates were identified by their characteristics in a carbohydrate assimilation test, a serological test and from their morphology. *Candida albicans* and *Candida glabrata* were the major isolates (75% and 20%, respectively). The karyotypes of the isolates were analysed by pulsed-field gel electrophoresis and almost all the karyotypes were distinguishable from one another when the band mobilities were carefully compared. Characteristics and karyotypes were not directly correlated, but seven *C. albicans* isolates (from six patients) had a common atypical karyotype and shared the same phenotype. These isolates are inferred to be generated by a wide genomic reorganization and mutation and the phenotypic changes may be advantageous for survival. The karyotypes of the isolates recovered from individual patients after intervals of 1–6 months were all identical except for one or two highly variable bands which were identified with an rDNA probe. This suggests that the variable bands are too variable to be useful for distinguishing strains, but from the patterns of the identical bands (i.e. except for the variable bands) we concluded that strains from individual patients do not change, at least over short periods. This, coupled with the extensive inter-isolate variability in karyotype, will be useful for *Candida* source determination and epidemiological studies.



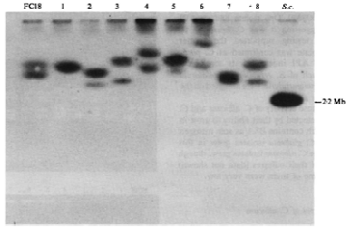
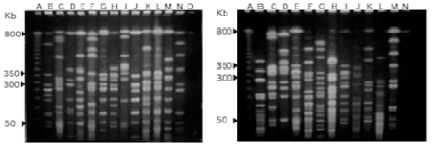


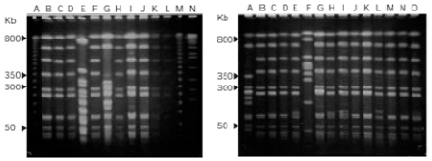
Fig. 2. Assignment of the variable chromosome bands by use of the rrDNA probe. The chromosomal DNAs were separated under the conditions used in Fig. 1(a) and hybridized by rDNA probe. The samples were prepared from *C. albicans* strains PCI8, TCH215 (lane 1), TCH231 (lane 2), TCH145 (lane 3), TCH13 (lane 4), TCH71 (lane 5), TCH168 (lane 6), TCH4 (lane 7), TCH219 (lane 8), from *C. glabrata* strains TIMM1053 (lane 9), TCH1 (lane 10), TCH26 (lane 11), TCH58 (lane 12), TCH157 (lane 13), TCH 168 (lane 14), TCH201 (lane 15), TCH220 (lane 16), and from *S. cerevisiae* (*S.c.*). The pointer on the right margin indicates chromosome XII of *S. cerevisiae*.

[illegible] then 200 for 20 min 140 V. The gels were 0.7% agarose and were run using the Pulsaphor system (Pharmacia-LKB). The samples were prepared from *C. albicans* strains PCI8, TCH215 (lane 1), TCH231 (lane 2), TCH145 (lane 3), TCH13 (lane 4), TCH71 (lane 5), TCH168 (lane 6), TCH4 (lane 7), TCH219 (lane 8), and from *Saccharomyces cerevisiae* (*S.c.*). The API index of TCH168 is 2144174 and the index of the other *C. albicans* strains is 2576174. Arrows on the left indicate the 7 common bands of *C. albicans*; pointers on the right indicate chromosomal DNA molecular size markers from *S. cerevisiae*.



A. PFGE of reference strains of *Leptospira* spp; lanes A, Lambda DNA markers; B, serovar Autumnalis; C, Australis; D, Bataviae; E, Canicola; F, Grippotyphosa; G, Icterohaemorrhagiae; H, Javanica; I, Pomona; J, Pyrogenes; K, Bratislava; L, Copenhageni; M, Bangkok; N, clinical strain and lane O, *S. cerevisiae* markers.

B. PFGE of reference strains of *Leptospira* spp; lanes A, Lambda DNA markers; B, serovars Sejroe, C, Hardjo; D, Hebdomadis; E, Dasimani; F, Ballum; G, Saigon; H, Celledoni; I, Rachmati; J, Sarmin; K, Patoci; L, Panarum; M, clinical strain and N, *S. cerevisiae* markers.



C. PFGE of clinical strains ; lanes A and M, Lambda DNA markers; B, isolate 1; C, isolate 2; D, isolate 3; E, isolate 4; F, isolate 5; G, isolate 6; H, isolate 7; I, isolate 8; J, isolate 9; K, isolate 10; L, isolate 11; lane N, *S. cerevisiae* markers.

D. PFGE of clinical strains: lanes A, reference serovar Autumnalis ; B, isolate 12; C, isolate 13; D, isolate 14; E, isolate 15; F, isolate 16; G, isolate 17; H, isolate 18; I, isolate 19; J, isolate 20; K, isolate 21; L, isolate 22; M, isolate 23; N, isolate 24; O, isolate 25.

Fig 1–Pulse field gel-electrophoresis patterns of clinical isolates and reference strains of *Leptospira* spp. Experienta conditions are described in Materials and Methods.

IDENTIFICATION OF CLINICAL ISOLATES OF *LEPTOSPIRA* SPP BY PULSED FIELD GEL-ELECTROPHORESIS AND MICROSCOPIC AGGLUTINATION TEST

Pimjai Naigowit[1], Sathapana Charoenchai[2], Mayurachat Biaklang[2], Umaporn Seena[2], Piyada Wangroongsarb[3], Pathom Sawanpanyalert[2] and Paijit Warachit[4]

[1]Technical Office, [2]Medical Biotechnology Center, [3]National Institute of Health, Department of Medical Sciences, [4] Department of Medical Sciences, Ministry of Public Health, Nonthaburi, Thailand

Abstract. Twenty five clinical isolates of *Leptospira* spp were characterized by microscopic agglutination test (MAT) and pulsed field gel-electrophoresis (PFGE) in comparison with 23 reference *Leptospira* serovars and with the saprophytic *L. biflexa* serovar Patoc. PFGE DNA profiling was more specific and reliable than MAT.

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BRAZILIAN ARCHIVES OF BIOLOGY AND TECHNOLOGY
AN INTERNATIONAL JOURNAL

Pulsed Field Gel Electrophoresis Reveals Chromosome Length and Number Differences in Brazilian Strains of *Metarhizium Anisopliae*

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黑殭菌 *(Metarhizium anisopliae)*

為一種可以殺死昆蟲的真菌，藉由分生孢子的傳播於田間行成重複有效的感染源。

應用範圍

鱗翅目：斜紋夜蛾(黑肚蟲)、甜菜夜蛾(管仔蟲)、小菜蛾(吊絲蟲)、玉米穗蟲(青蟲)等。

鞘翅目：金龜子(雞母蟲)、叩頭蟲(金針蟲)等。

直翅目：蝗蟲等。

https://zh.wikipedia.org/zh-tw/%E9%BB%91%E6%AE%AD%E8%8F%8C

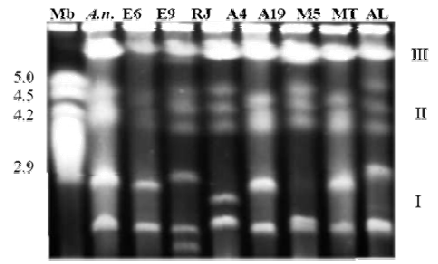
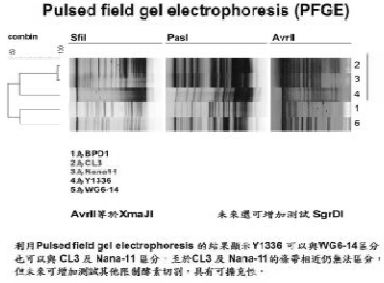


Figure 1 - Separation of *Metarhizium anisopliae* intact chromosomal DNAs on a CHEF gel. Gel (0.6% w/v) was electrophoresed in A condition. Group I) two to three bands corresponding to molecules between 0.9 and 2.8Mb; Group II) three bands corresponding to molecules between 3.6 and 4.9 Mb and, Group III) with these parameters presents two bands corresponding to molecules between 6.0 and 7.7 Mb. *A.n.* shows the *Aspergillus nidulans* chromosome size standards.

農業用微生物資材之基因型鑑定技術開發



利用 Pulsed field gel electrophoresis 的結果顯示Y1336 可以與WG6-14區分，也可以與 CL3 及 Nana-11 區分，至於CL3 及 Nana-11的播帶相近的無法區分，但未來可增加測試其他限制酵素切割，具有可擴充性。

http://www.coa.gov.tw/view.php?catid=2502501

Pulsed-field gel electrophoresis

Jill Herschleb, Gene Ananiev & David C Schwartz

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This protocol describes pulsed-field gel electrophoresis (PFGE), a method developed for separation of large DNA molecules. Whereas standard DNA gel electrophoresis commonly resolves fragments up to ~50 kb in size, PFGE fractionates DNA molecules up to 10 Mb. The mechanism driving these separations exploits the fact that very large DNA molecules unravel and "snake" through a gel matrix, and such electrophoretic trajectories are perturbed in a size-dependent manner by carefully oriented electrical pulses. PFGE has enabled the rapid genomic analysis of microbes and mammalian cells, and motivated development of large-insert cloning systems such as bacterial and yeast artificial chromosomes. As such, this protocol includes descriptions of two types of PFGE instrumentation (not commercially available), along with detailed instructions for their operation. Additionally, this protocol provides basic instructions for the preparation of intact chromosomal DNA from several types of organisms. PFGE takes 2–3 days, excluding sample preparation.

nature protocols

BAC Clone analysis

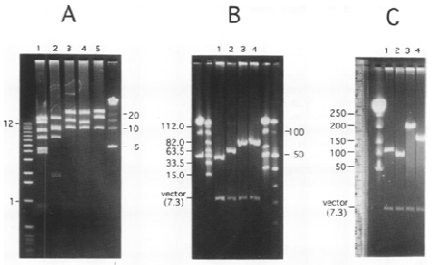
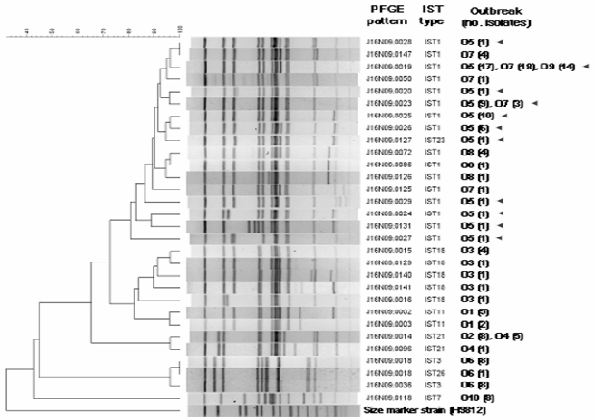


Figure 4. Separations of experimental samples using low reorientation angles. (**A**) 2.5 h separation of fragments up to 25 kb. Fosmid DNAs from mini-preps were cleaved with *Hin*dIII and loaded onto a 1% LE agarose gel in 0.5×TAE. Markers included a 1 kb ladder on the left, lane 1, and a 5 kb ladder on the right. The gel was run at 14°C for 2.5 h using fields of 9 V/cm, a switch interval of 0.4 s, and a reorientation angle of 90°. The 1 kb marker fragment has migrated 8.1 cm from the well. (**B**) 3.5 h separation of molecules up to 100 kb. Mini-prepped BAC DNA was cleaved with *Not*I to excise the inserted segment of human DNA. Digested DNA was run at 14°C, for 3.5 h in a 0.8% gel of SeaKem LE in 0.5×TAE, with a 90° reorientation angle using fields of 9 V/cm and a switch interval of 2 s. The far left lane contains lambda ladders, and the Mid-Range I marker of New England BioLabs is immediately adjacent. The far right lane contains the Low Range marker, with the Mid Range II immediately adjacent. Lanes 1–4 contain DNA from different BAC clones. The BAC vector band of 7.3 kb is visible in all lanes, and has migrated 7.8 cm from the wells. (**C**) 3.5 h separation of molecules up to 250 kb. Mini-prepped BAC DNA was cleaved with *Not*I to excise the inserted segment of human DNA. Digested DNA was run for 3.5 h in a 0.8% gel of FMC Gold Agarose in 0.5×TAE, with 100° reorientation angles using fields of 9 V/cm and a switch interval that was linearly ramped from an initial value of 3 s to a final value of 5 s. Lambda ladders were run in the left lane as size markers. Lanes 1–4 contain BACs of ~120, 100, 150 and 150 kb. The BAC vector band of 7.3 kb is visible in all lanes. A ruler indicates distance (cm).

Nucleic Acids Research, 1994, Vol. 22, No. 24

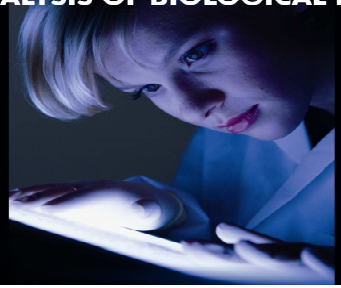
APPLIED MATHS
BioNumerics

Phylogenetic Tree



BioNumerics

A UNIVERSAL PLATFORM FOR DATABASING AND ANALYSIS OF BIOLOGICAL DATA



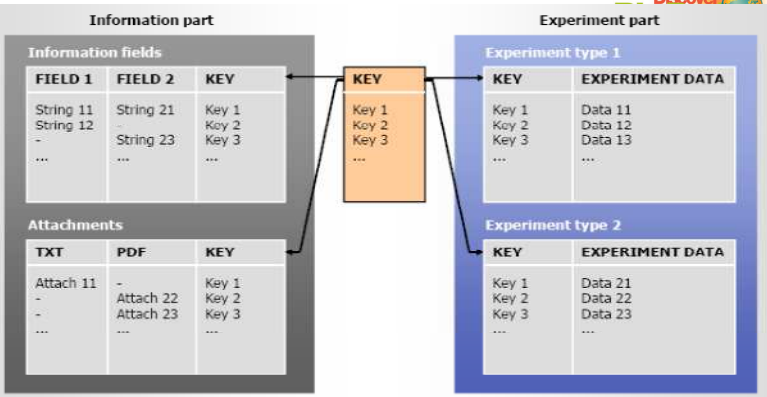
What you can store

Information fields
- Up to 100 fields (each up to 80 characters)
- Link with external databases

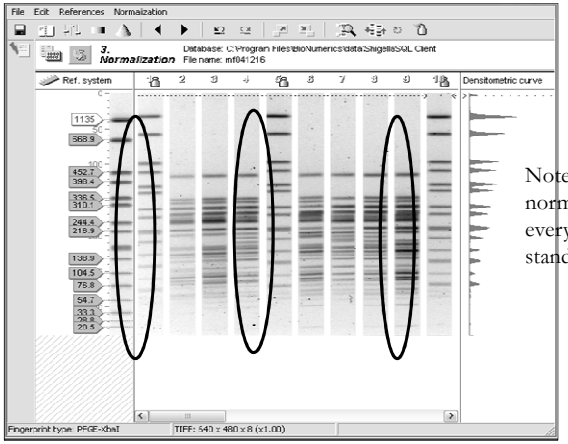
Attachments
- Bitmap images
- HTML and hyperlinks
- Word documents
- Excel spreadsheets
- PDF files
- Text documents

Fingerprints
- 1-D electrophoresis gels scanned as bitmaps (RFLP, PFGE, Ribotyping, RAPD, DGGE & TGGE, etc.)
- Sequencer chromatogram files (AFLP, VNTR, HDA, etc.)
- Spectrophotometric files
- MALDI & SELDI profiles
- All other kinds of densitometric profiles

What you can store

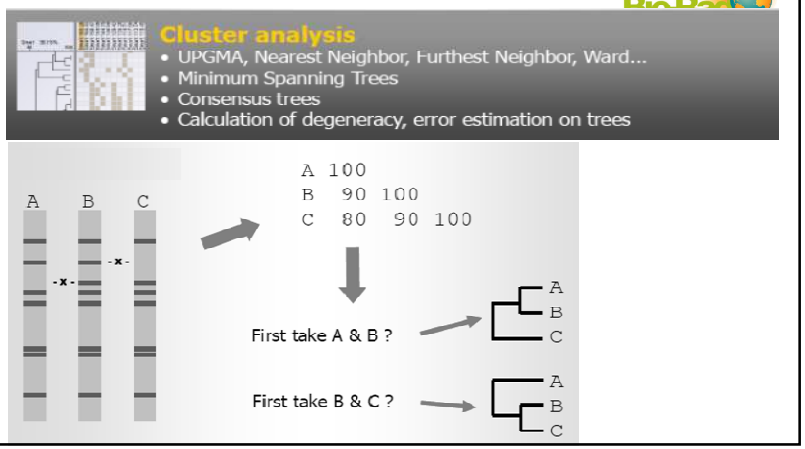


Gel analysis with Normalization

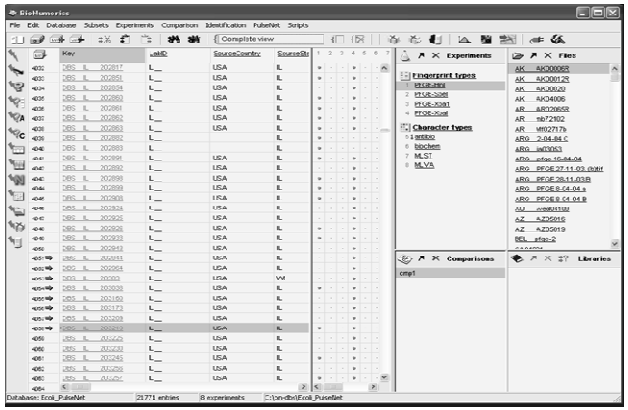


Note: At minimum for proper normalization, the first, last, and every 4th or 5th lane should be a standard lane!!

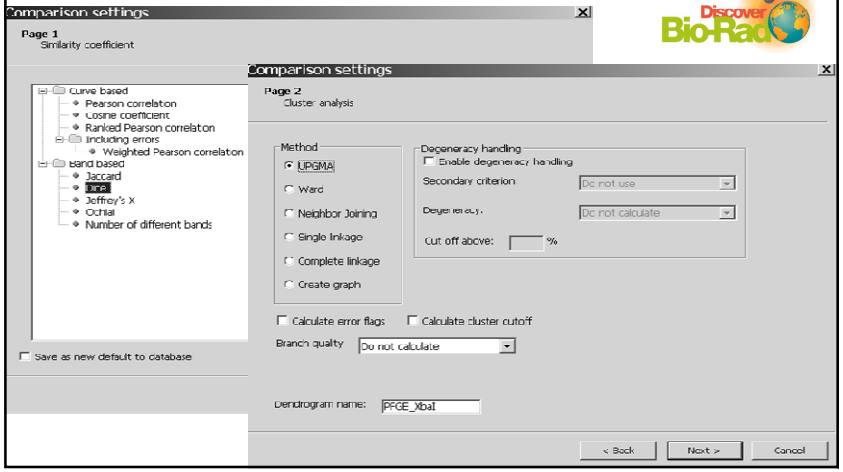
What can you do with Tree and Network Inference (Clustering)



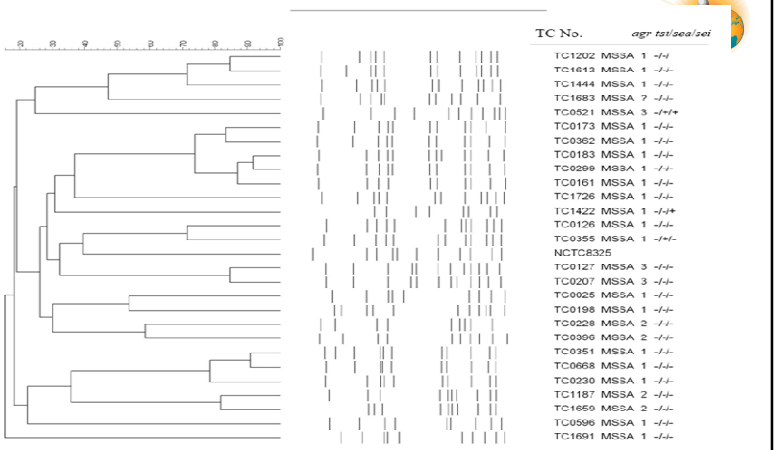
Selection of entries for comparison



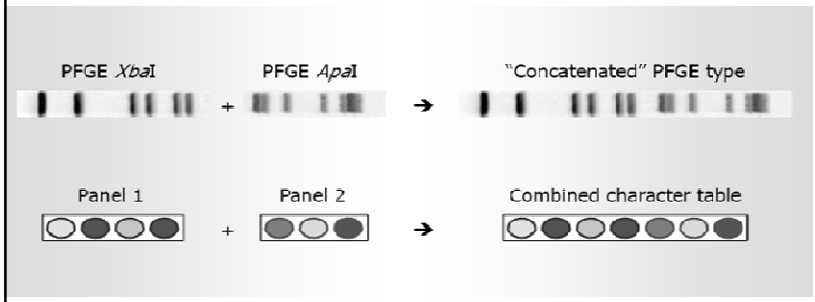
Cluster analysis



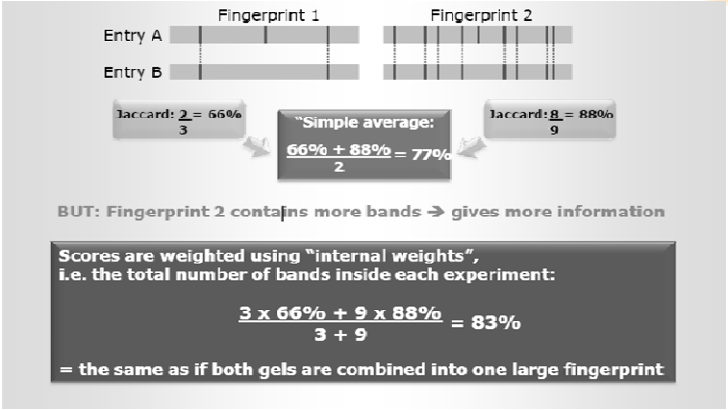
Phylogenetic Tree analysis



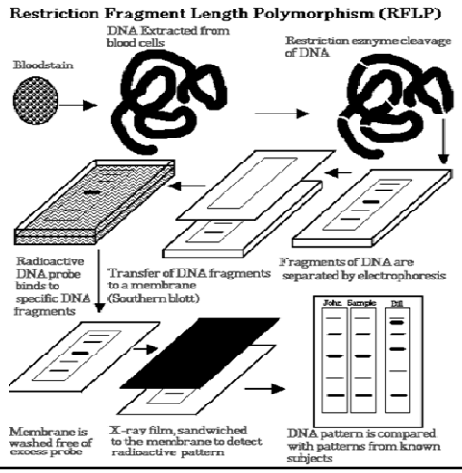
Composite Data sets

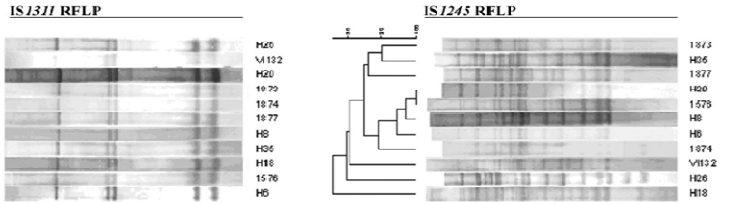


Option 1: averaging similarity matrices

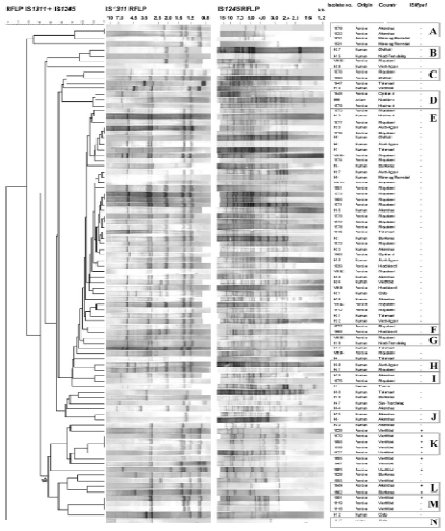


RFLP & Probes





11 isolates identical in their banding pattern by IS*1311* RFLP, which could be further separated by IS*1245* RFLP.



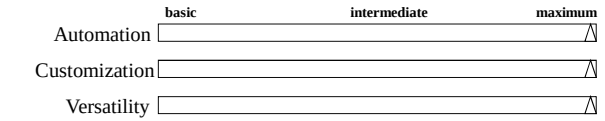
A dendrogram showing the cluster analysis of the composite dataset of RFLP experiments using IS*1311* and IS*1245* as probes
A total of 14 clusters (defined by 80% similarity) are grouped in rectangular frames and labelled A-N.

Part II Bio-Rad CHEF Systems

The CHEF-Mapper XA system.

The CHEF Mapper System

- Optimal DNA fragments in the size range of 100bp - 10Mb.
- Eight contiguous blocks of programming are possible.
- User can specify any angle of electrophoresis from 0° - 360° as well as specify runs in which the angles are asymmetrical.
- Stores 99 simple programs or 20 complex programs with up to 8 blocks of programming each.
- True nonlinear switch time ramping for maximum flexibility in achieving resolution.
- Set clock delay for runs as well as battery backed up RAM for runs that are interrupted.
- Secondary pulsing, and multistate flexibility to enhance separations.
- "Built-in" expertise in two algorithms.



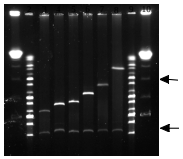
Mapper Feature

Feature	CHEF Mapper
Included Angles	0-360^0
Blocks	8
Battery Backed Up RAM	YES, up to 3 hr of interruption
Program Storage	YES - up to 99
Algorithm	YES
Multi-State Separation	YES
Non-Linear Ramping	YES
Asymmetric FIGE	YES
Voltage Interrupts	YES

Part III Critical Parameters in optimizing your PFGE sample resolution

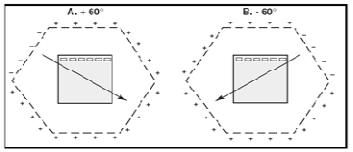
Critical Parameters in optimizing your PFGE sample resolution

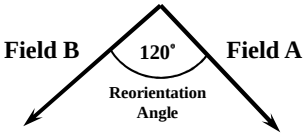
- Pulse / Reorientation Angle
- Switch Time and Switch Time Ramping
- Voltage Gradient
- Buffer Type and Temperature
- Agarose Type and Concentration
- Run Time



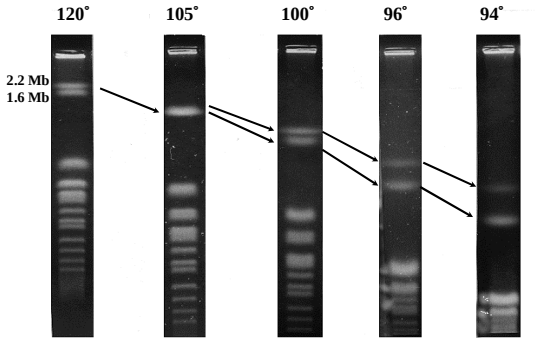
Pulse Angle and Sample Resolution

- Angle is an important factor in achieving optimal resolution
- Using a smaller included angle for large fragments of DNA (over 1Mb in size) results in a 50% savings in run time.
- As the angle is decreased, the resolution of the largest fragments generally improves and the smaller fragments become crowded
- **Most CHEF protocols are optimized for the 120˚ angle**





Effects of different angles on electrophoresis results:

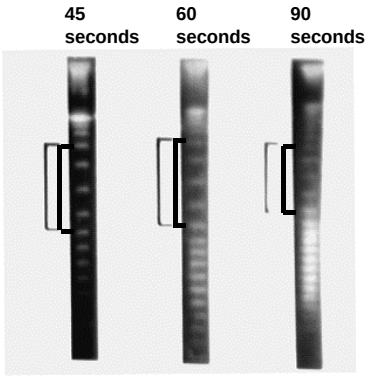


As we see here, the space between the largest two fragments has increased, but the remaining 14 fragments are becoming compressed. If your total analysis was in the range of these largest two fragments, you might choose a smaller angle of electrophoresis.

Switch Time and Sample Resolution

- Switch time is the critical parameter in achieving best PFGE resolution.
- The switch time is the length of time the electrical field is pulsed in a single direction. Thus a 60s switch time means that the electrical field will be pulsed in one direction for 60s and then switched to the other direction for 60s.
- The switch time is generally shorter for samples with smaller DNA fragments and longer for samples with larger DNA fragments.

Effects of switch time on sample resolution



As the switch time increases, the larger DNA fragments move farther into the gel during the same time period.

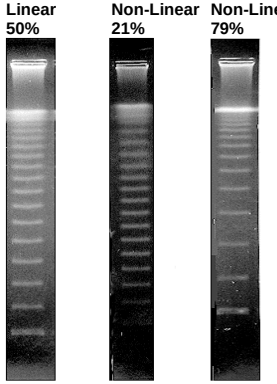
The brackets indicate the same region of the lane for each gel but the sizes of the fragments in this region get larger as the switch time increases.

As you can see Switch Time modification is the most powerful parameter in PFGE.

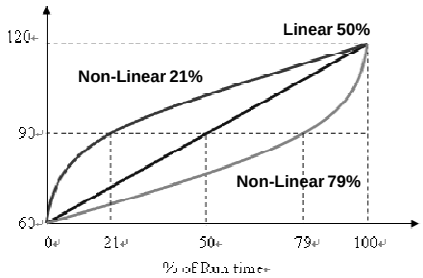
Switch Time Ramping and Sample Resolution

- **Samples with a large range of DNA fragments sizes, for example the *S.cerevisiae* chromosomes, can be resolved by changing the switch time over the course of the run.**
- **This is referred to as Switch Time Ramping because the switching time will "ramp up" from a short switch time to a longer one.**
- **The "ramp" of the switch time can be linear, that is increasing in exact increments over the course of the run; or non-linear, so that you can concentrate the switch times on the largest or smallest regions as is necessary to achieve better resolution.**

Effects of Linear & Non-Linear Switch Time Ramping



The non-linear ramps are described by the amount of the total run time passed (%) when you hit the mid-point of the range of switch times.

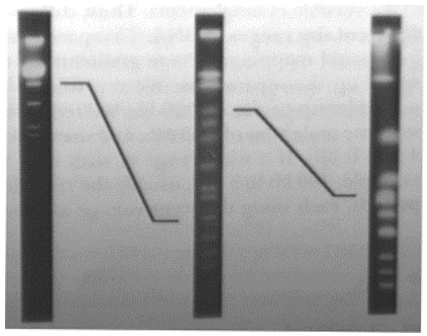


Voltage Gradient and Sample Resolution

- The voltage gradient describes the strength of the electrical field and is represented as **V/cm**, where the total voltage is divided over the distance between two electrodes. Since that distance in a CHEF gel box is approximately 33cm, a 200V run is really about 6V/cm.

- Typically the best resolution of chromosomal sized DNA fragments (> 3 MB) is achieve when using low voltage gradients, 1.5-3 V/cm combined with a narrow angle of electrophoresis.
- High resolution of DNA fragments up to 250 kb can be achieve by using high voltage gradients, 9 V/cm. This voltage can be combined with a narrow angle of electrophoresis to resolve samples in very short run times, 4 hours or less.
- **Most CHEF protocols are optimized at 6V/cm**

Effects of Voltage Gradient on sample resolution



The largest resolved fragment in each gel is identified as well as the position of that fragment in the next gel.

The 3V/cm gel has only separated fragments of about 400kb, whereas the 6V/cm gel has separated upto 850kb and the 9V/cm has separated upto 1.2Mb in the same V*h. All gels used the same switch times.

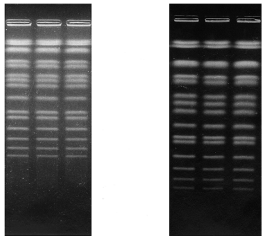
The resolution of the gel is diminished even if the range is greater in the 9V/cm gel.

Voltage alone will not improve resolution - Switch times must be changed to produce best results.

Buffer type & concentration and sample resolution

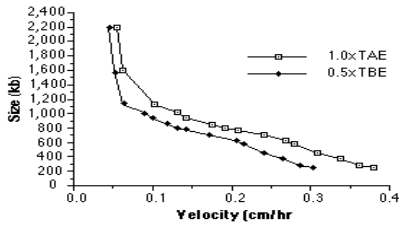
- In general, the lower the ionic strength of the buffer, the faster the run.
- The run time advantage of low ionic strength buffer must be weighed against the limited buffering capacities of dilute buffers and the loss of resolution due to buffer break down.
- The two buffers most frequently used for PFGE of DNA are **0.5X TBE** (Tris Borate EDTA) and **1.0X TAE** (Tris Acetate EDTA).
- **1X TAE** is very useful when separating MB sized DNA fragments (**>3MB**).
- **0.5X TBE** is useful for most separations **up to MB** sized DNA and need not be changed, even over multi-day runs. **This is the most commonly used buffer in CHEF gels.**

Effects of buffer on sample resolution



0.5X TBE

1.0X TAE

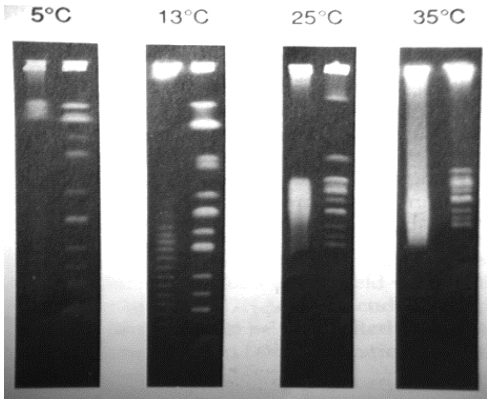


- **Notice that fragments of all sizes migrate farther, having a greater velocity, in the TAE gel than that of the TBE gel.**
- **The TAE buffer is lower in ionic strength (40 mM buffering agent) vs 45 mM in the 0.5 x TBE.**

Buffer Temperature and sample resolution

- Buffer temperature will affect run time. The warmer the buffer temperature, the faster the run. The resolution decreases with the temperature increase.
- Buffer temperatures at room temperature will decrease the run time by about 50% over chilling to 14°, but the resolution loss will be so great, it might not be worth the time savings.
- Buffer temperatures at 4° will provide the sharpest resolutions but at a greatly increased run time.
- **Cooling the buffer to 14℃ is most common and the best compromise between run time and resolution.**

Effects of Buffer Temperature on sample resolution



We can see here very high resolution on the right lane of the 5˚ gel, however, few bands have migrated into the gel.

This is greatly improved in the 13˚ gel.

At 25˚, some resolution is maintained in the right lane but the left lane has become compressed.

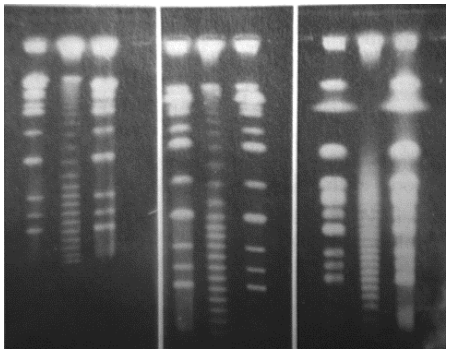
This effect is even greater in both lanes of the 35˚ gel.

Agarose and sample resolution

- Agarose type affects both sample migration and ease of handling for PFGE gels.
- Agaroses for PFGE must be very pure, have a high tensile strength, and have a minimum of charged contaminants (usually described by the mr.)
- Generally **0.8%-1%** agarose is ideal for PFGE separations of up to **3MB** and **0.5% - 0.9%** is useful for the range above **3MB**.
- Agaroses are available specifically for use with PFGE that provide superior resolution (Bio-Rad's PFGE Certified) as well as agaoroses designed for decreasing run time while maintaining good resolution Bio-Rad's Molecular Biology Certified and Chromosomal Grade.)

Effects of agarose on sample resolution

0.6% agarose **1.4% agarose** **1.8% agarose**



The DNA fragments in the gels here migrated nearly four times faster (the gel runs are not all the same lengths) in the 0.6% gel as in the 1.4% gel.

The guideline is that as the % of agarose decreases the DNA migrates faster, a larger total range of fragments can be separated but that some resolution will be lost.

Typically 1% gels are used for CHEF.

Finally, different agaroses yield different migration rates. For example, Bio-Rad PFC Agarose runs 10%+ slower than Molecular Biology Grade Agarose.

Run Time and other notes:

- As we have seen, several strategies may be employed to minimize or extend run time. In general, it is best to optimize for the shortest run time that allows clear sample resolution. This will maximize the efficiency of your PFGE related experiments.
- It is safe to leave the gel after run completion for several hours if necessary as the DNA is much too large to diffuse out (>10kb) and the presence of EDTA in the buffer and the cool buffer will inhibit nucleases.

	DNA 1-100kb	DNA 0.1-2.0mb	DNA 2-4mb	DNA >4mb
%Agarose	1.0-1.2%	0.8-1.2%	0.6-1%	0.5-0.8%
Beffer	0.5xTBE	0.5xTBE	1.0xTAE	1.0xTAE
Temperature	14°C	14°C	14°C	14°C
Voltage	6-9V/cm	4.5-6V/cm	2-3V/cm	1.5-2.5V/cm
Pulse parameters	0.05-10sec	10-200sec	200-1,800sec	10-60min
Rum times	2-15hr	15-30hr	24-72hr	72-144hr
Angle	0°+180°,120°	120°	120°,106°	106°

表一、針對DNA片段大小對電泳條件之設定

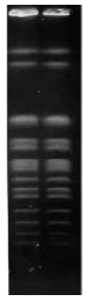
Auto Algorithm

Molecular weight Low [200k] High [2200k]
Calibration Factor [1.0], ENTER for NC
no change

S. Cerevisiae standard plug **200–2,200 kb**

Condition : 1.0% PFGE,0.5x TBE,14℃
Voltage : 6 V/cm
Switch Time : Int. Sw. Tm = 26.31s
Fin. Sw.Tm = 3m48.48s,
Ramping factor: a = [Linear]
Run Time : 28:59 (hr)

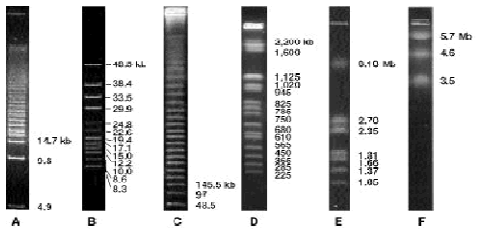
S. cerevisiae



Part IV Bio-Rad PFGE Accessories

Pulsed Field Standards and Markers

A. 170-3624 5 kb ladder standard.
B. 170-3707 8–48 kb standard.
C. 170-3635 Lambda ladder standard.
D. 170-3605 *S. cerevisiae* marker.
E. 170-3667 *H. wingei* marker.
F. 170-3633 *S. pombe* marker.



CHEF Genomic DNA Plug Kits

170-3591 CHEF **Mammalian** Genomic DNA Plug Kit

170-3592 CHEF **Bacterial** Genomic DNA Plug Kit

170-3593 CHEF **Yeast** Genomic DNA Plug Kit

Pulsed Field Other Accessories

161-0733 10x Tris / Boric Acid / EDTA (TBE), 1L
161-0770 10x Tris / Boric Acid / EDTA (TBE), 5L
161-0743 10x Tris / Acetic Acid / EDTA (TAE), 1L
161-0773 10x Tris / Acetic Acid / EDTA (TAE), 5L

161-3108~10 Certified Megabase Agarose, 25 g, 125g, 500g

170-3713 50 Well Disposable Sample Plug Mold, 5
170-3622 10 Well Reusable Sample Plug Mold, 1

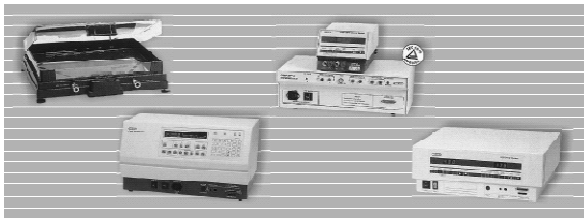
PFGE Application Workflow

Sample Preparation → Sample Electrophoresis → Detection and Analysis

CHEF Genomic DNA Plug Kit

CHEF Mapper XA system

Gel Doc XR+ system



Thank You !

正茂生物科技有限公司