GenePath® Group 3 Reagent Kit

Instruction Manual

Catalog Numbers

Reagent Kit Group 3: 310-0113

Standard Module Group 3: 310-0066
Enzyme Module Group 3: 310-0065
Universal Module: 310-0060

For Technical Service

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Table of Contents

Intended use

For the qualitative determination of genetic strain typing based on restriction enzyme digestion.

Caution: Please read thoroughly and familiarize yourself with the entire contents of this instruction manual before using the product for the first time.

Section 1
Introduction

A chronic problem in all health care facilities is the occurrence of nosocomial infections, usually bacterial or fungal in nature, which can lead to exacerbated illness, and even death of hospitalized patients.[1, 2] Numerous microbiological methodologies have been developed to identify both the occurrence and source of nosocomial outbreaks, such as plasmid typing, DNA fingerprinting, ribotyping, Southern hybridization, and multilocus enzyme electrophoresis.[3, 4, 5] While these methods provide additional information, they do not supply reliable, definitive epidemiological data. Recently, Pulsed Field Gel Electrophoresis (PFGE) has been shown to resolve restriction fragments in the genomes of bacteria isolated from nosocomial outbreaks, yielding "DNA fingerprints" of these strains.[6, 7] These fingerprints can be compared to determine if the bacteria are genetically similar or clonal. Clonal isolates from different patients may be an indication of a nosocomial outbreak. PFGE has been shown to be the most discriminatory and reproducible strain typing technique available.[19]

Pulsed Field Gel Electrophoresis allows the separation of DNAs ranging in size from a few hundred base pairs to over 10 million base pairs.[8, 9, 10] Because of the large size of these molecules, simple pipetting shears the chromosomal DNA, resulting in unacceptable quality for PFGE

separations. To prevent breakage of large DNA molecules, intact cells are embedded in agarose, lysed, and deproteinized *in situ*.[11,12] The agarose matrix protects the embedded DNA from shear forces and provides an easy way to manipulate samples. The DNA agarose plugs can then be loaded into the sample wells of agarose gels for electrophoresis.

The GenePath group reagent kits are based on PFGE technology in which isolates from a nosocomial infection are embedded in plugs, digested with a specific restriction enzyme to generate DNA fingerprints, and separated on an agarose gel using the GenePath instrument. The GenePath instrument is based on the pulsed field method of CHEF (Contoured Clamped Homogeneous Electric Field).[13,14,15] Analysis of the gel will show distinct DNA fragments or bands, that resulted from the restriction digestion. This band pattern is specific to each species and can therefore be used for genetic strain identification.[16]

The GenePath Group 3 Reagent Kit will analyze 50 different isolates (or tests). Each of the 50 isolates are prepared in duplicate, creating two sample plugs (100 total plugs). There is enough restriction enzyme to digest 50 samples, therefore, one sample plug from each isolate can be digested. After the restriction digestion step, the sample plug is cut into thirds to fit into the sample well of the agarose gel.

1.1 Organisms to Use with this Kit

Pseudomonas
Enterobacter

1.2 Kit Components

Reagent	Amount	Storage	Cap Color
Universal Module			
Cell Suspension Buffer	12 ml	4 °C	Green
Embedding Agarose	12 ml	4 °C	Green
Proteinase K Buffer	30 ml	4 °C	Red
Proteinase K (> 600 U/ml)	1.3 ml	4 °C	Red
10x Wash Buffer	60 ml	4 °C	Green
Plug Molds	2	RT	
Enzyme Module Group 3			
Lysis Buffer I	30 ml	4 °C	Blue
Lysozyme (25 mg/ml)	1.6 ml	-20 °C	Blue
Spe I Buffer	65 ml	4 °C	Clear
Restriction Enzyme *Spe* I (3 U/µl)	500 µl	-20 °C	Clear
Standard Module Group 3			
Lambda Ladder Standard	3 plugs	4 °C	Green
Control Plugs—Group 3	5 plugs	4 °C	Green
Culture Control—Group 3	1 vial	4 °C	Green

1.3 Additional Items Required—Available from Bio-Rad

Catalog Number	Item
310-0007	**GenePath Gel Kit**
223-9480	**Microcentrifuge Tubes,** sterile (1.5 ml)
223-9430	**Microcentrifuge Tubes,** sterile (2.0 ml)
195-7514	**Microcentrifuge with Rotor**
196-1016	**Adjustable Temperature Water Bath**
223-9550	**Sterile Disposable Transfer Pipets**

1.4 Required Items not Available from Bio-Rad

Inoculating Loop
Shaker incubator
Sterile water
5 and 10 ml sterile culture tubes
Microwave
Pipettes and sterile tips
Hematology Rocker

1.5 Precautions

The reagents in this kit are for research use only.

Ethidium bromide is a possible carcinogen and a known mutagen. Wear gloves and safety glasses at all times and wash contaminated skin with water. Clean up

spills with absorbent material. Spray the area with a 10% bleach solution, clean up with absorbent material, rinse with water, and dry the area with paper towels.

Do not mouth pipette any of the reagents!

All materials which come in contact with the samples being tested should be considered potentially infectious. Appropriate biosafety precautions and disposal of biohazardous waste should be used.

1.6 Quality Control

In keeping with good laboratory practice, digested genomic plugs prepared from the Culture Control—Group 3 and the Control Plugs—Group 3, should be run each time the test is performed. The Lambda ladder standard should always be included each time an electrophoresis gel is run as a guide for fragment size ranges.

Failure to obtain appropriate band patterns for the controls may indicate incorrect manipulations, improper sample handling, or deterioration of reagents. See Section 5 for troubleshooting.

1.7 Control Reagents

Several reagents in the GenePath standard module Group 3 allow you to functionally test the procedures used in this kit. The use of these control reagents is discussed in Section 2.2 and 2.3. The results one can expect using these

control reagents can be seen in Section 3.4. These controls should be used each time this procedure is performed.

Culture Control—Group 3. This lyophilized culture is a strain of *Pseudomonas aeruginosa* (strain 27853). This control is used to test the sample plug preparation procedure and the restriction digestion procedure. When this control is used in conjunction with the control plugs, one can insure that both the sample plug procedure and restriction digestion procedure were performed correctly. This sample can also be used to test all components of the kit before committing valuable pathogenic samples.

Control Plugs—Group 3. The control plug is a strain of *Pseudomonas aeruginosa* (strain 27853), embedded in an agarose plug and ready for restriction digestion (this control is used to test the restriction digestion procedure). For this control, the cells are grown overnight in appropriate media, and the resuspended cells are embedded in agarose, lysed, deproteinized, and washed.

Section 2
Sample Preparation

2.1 Reagent Preparation

Solutions	Recipe
Culture Control—Group 3	Add 500 µl of appropriate media, such as LB or Tryptic Soy Broth to the pellet. Allow the pellet to rehydrate at room temperature for 15 minutes. Resuspend and transfer to a microcentrifuge tube. Use this to inoculate broth media for Culture Control. The remainder of this standard can be stored at -70 °C by adding 100 µl 100% glycerol (20% final concentration).
1x Wash Buffer	Add 50 ml 10x Wash buffer and 450 ml sterile water to a 500 ml sterile bottle. Mix.
0.1x Wash Buffer	Add 5 ml 1x Wash buffer and 45 ml sterile water to a 50 ml sterile bottle or sterile tube. Mix.

2.2 Preparation of Agarose Embedded DNA

Organisms Grown in Broth Culture

1. The samples to be tested should be grown overnight on petri plates with the appropriate media to isolate a single colony.
2. Inoculate one colony into 3 ml of appropriate media in a 10 ml sterile culture tube. Prepare one tube per sample. Grow the samples in a shaking incubator or water bath with agitation (250 rpm) at 37 °C or optimum

growing temperature for 16–20 hours (overnight). For slow growing organisms, longer incubations may be needed to obtain enough cells for sample preparation.

Note: As a control for the kit, 10 µl of the Culture Control—Group 3 should be added to a 10 ml sterile culture tube with 3 ml appropriate broth. The purpose of this control is to insure that each step in the kit performs correctly. See Section 2.1 for resuspending the Culture Control.

3. Melt the Embedding agarose solution using a microwave (about 20 seconds on high). Be careful not to let the agarose boil over. Place the agarose at 50–55 °C to equilibrate. Leave the agarose at 50–55 °C until you are finished making plugs. The agarose will start to solidify below 50 °C.
4. Pipette 90 µl of each overnight culture sample into a separate microcentrifuge tube. Centrifuge for 1 to 2 minutes in a microcentrifuge (10,000–12,000 x rpm) to pellet the cells. Using a pipet, aspirate off the supernatant. Be careful not to aspirate any of the cell pellet.

   Note: Slow growing organisms may require a larger volume of the overnight culture to achieve enough cells for the desired pellet size.
5. Compare the pellet size of your samples with the pellet sizes in Figure 2.1. A pellet size between the size range in Figure 2.1 will give a sufficient number of cells for sample preparation. It is advisable to keep the pellet size within the size ranges provided below.

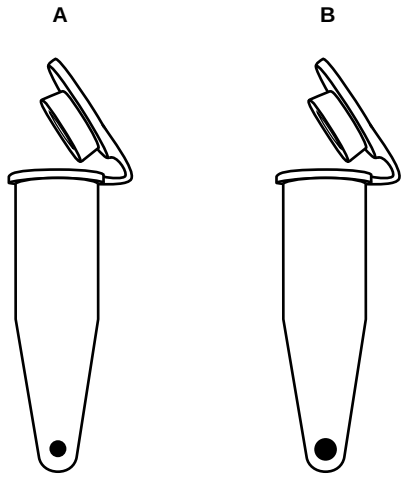

Fig. 2.1. Pellet sizes.

Due to differences in the growth rate between species and strains, it may be necessary to adjust the cell suspension volume in order to harvest the appropriate number of cells. If the pellet is either smaller than that in tube A or larger than that in tube B above, adjust the procedure according to the following:

a. If the pellet is too small, add more of the overnight culture to the tube. Pellet the cells and aspirate off supernatant with a pipet. Repeat this step until the pellet size is between A and B in Figure 2.1.

b. If the pellet is too large, resuspend the pellet in 50 µl of Cell Suspension buffer. Pipet off some of the resuspended cells. Pellet the cells and aspirate off supernatant with a pipet. Repeat this step until the pellet size is between A and B in Figure 2.1.

6. Carefully resuspend the cells in 150 µl of Cell Suspension buffer by slowly pipetting up and down. Place the samples at 50 °C to equilibrate (no longer than 15 minutes).

7. In this step each sample should be done individually. To the cell suspension, add 6 µl Lysozyme and 150 µl of Embedding agarose. Mix the solution by gently vortexing or pipetting solution up and down. Immediately pipette 100 µl of the mixture into one well of the plug mold. Pipette slowly so as not to create air bubbles. Fill two wells per sample.

   Note: It is advisable to label the side of the plug mold with the sample number or some type of identification, so as not to mix up samples.

8. Allow the agarose to solidify for 15 to 20 minutes at room temperature. This step can be expedited by placing the molds at 4 °C for 10–15 minutes.

9. For each sample, label a sterile 5 ml culture tube or 2 ml microcentrifuge tube, and add 500 µl of Lysis buffer I and 20 µl Lysozyme. A white precipitate may

occur. This precipitate will go back into solution when incubating at 37 °C.

10. Remove the tape on the plug mold(s) (see plug mold insert for details). From the bottom of the plug mold, push the solidified agarose plugs, using the snap off tool provided on the plug mold, into the tubes containing the Lysis buffer I and Lysozyme. Gently mix by inversion. Incubate the plugs for 1 hour at 37 °C without agitation.
11. Using a disposable transfer pipet, aspirate the Lysis buffer I and rinse the plugs with approximately 1 ml 1x Wash buffer at room temperature (see Section 2.1 for 1x Wash buffer recipe). Remove the 1x Wash buffer (no incubation is needed).
12. Add 500 µl of Proteinase K buffer and 20 µl of Proteinase K to each sample. Gently mix by inversion. Incubate the plugs for 16–20 hours (overnight) at 50 °C without agitation.
13. Aspirate the Proteinase K solution. Wash the plugs in 1 ml of 1x Wash buffer for 30–60 minutes at room temperature with gentle agitation on a rocker.

    Note: If no rocker is available, all wash steps may be done without agitation.
14. Aspirate the 1x Wash buffer. Wash the plugs **three more times** in 1x Wash buffer for 30–60 minutes at room temperature with gentle agitation on a rocker.
15. Aspirate and add 1 ml 1x Wash buffer to the tubes. Store the plugs at 4 °C or continue on to Section 2.3 for restriction enzyme digestion of samples. The plugs are stable for 3–6 months.

Organisms Grown on Solid Media

1. For organisms that do not grow well in broth media, fresh growth of a pure isolate from solid media is suitable. Using a sterile inoculating loop, scrape up an amount of cell mass between the size of the pellets in tubes A and B shown in Figure 2.1, and resuspend in a microcentrifuge tube containing 1 ml sterile saline or broth media. Avoid scraping up the solid media. Prepare one tube per sample.
2. Centrifuge for 1 to 2 minutes in a microcentrifuge (10,000–12,000 x rpm) to pellet the cells. Using a pipette, aspirate off the supernatant. Be careful not to aspirate any of the cell pellet.
3. Compare the pellet size of your samples with the pellet sizes in Figure 2.1. A pellet size between the size range in Figure 2.1 will give sufficient number of cells for sample preparation. It is advisable to keep the pellet size within the size ranges in Figure 2.1.
4. Continue the preparation at Section 2.2 step 6.

2.3 Restriction Enzyme Digestion of Samples

1. Remove one plug from the sample tube (Section 2.2) and place it in a new 1.5 ml microcentrifuge tube. Do the same with a Control plug, which is included at this step to insure that the restriction digestion step worked properly. Add 1 ml of 0.1x Wash buffer to each tube

(see Section 2.1 for 0.1x Wash buffer recipe). Wash for 30–60 minutes with gentle agitation on a rocker at room temperature.

2. Aspirate off the 0.1x Wash buffer and add 500 µl of the *Spe* I buffer to each plug. Incubate for 30–60 minutes with gentle agitation on a rocker at room temperature.
3. Aspirate the *Spe* I buffer and add 300 µl of *Spe* I buffer. Remove the *Spe* I enzyme from the -20 °C freezer and place the vial on ice. Add 8.3 µl (25 units per plug) of the *Spe* I enzyme and mix by gently tapping the tube. Make sure the enzyme is completely mixed with the *Spe* I buffer and the plug is completely immersed in the buffer.

   Note: Immediately store the remaining *Spe* I enzyme at -20 °C as the *Spe* I enzyme will lose activity at temperatures above 0 °C.
4. Incubate the samples for 16–20 hours (overnight) at 37 °C.
5. After digestion, aspirate off the buffer and add 500 µl of 1x Wash buffer. Plugs should be good for 2 to 4 weeks stored at 4 °C.

Section 3
Gel Electrophoresis

These steps should be started following the overnight restriction digestion.

3.1 Preparing the GenePath System for Sample Analysis

1. Prepare an agarose gel according to the instructions included in the GenePath gel kit.
2. Prepare the Electrophoresis Running buffer from the GenePath gel kit according to the instructions included in the kit.
3. Pour the buffer into the GenePath electrophoresis cell. Turn on the GenePath power module. Turn on the pump and adjust the setting to 70–80 (see the GenePath manual for correct set-up of system).
4. Turn on the GenePath cooling module and check that the set temperature is at 14 °C. Allow the buffer to cool to 14 °C before starting the electrophoresis run.

3.2 Sample Loading

1. The sample plug should be placed on a smooth, clean surface, such as a petri dish. When using a 15-well comb, cut the plug into thirds along the narrow axis (2.5 mm high x 5 mm wide). This size will give enough DNA to visualize. The plugs can be cut with a razor blade, metal spatula, or glass cover slip. When using a 10-well comb, cut the plug in half along the long axis (2.5 mm high x 8 mm wide).
2. Save the outside wells for the gel standard plugs and place the sample plug into any of the other wells with the spatula. Gently press the plug to the bottom of the

well. Be very careful not to destroy the plug by poking repeatedly while loading. Repeat this for all other samples and the control plug.

3. The kit comes with one DNA size standard, Lambda ladders (Figure 3.1). Cut the standard plug to the same size as the sample plugs and load the standard plugs into the outermost wells.
4. Melt the low melt agarose solution in a microwave. This will take about 30 seconds. Check the agarose every 15 seconds to make sure the agarose does not boil over. Fill each well with the low melt agarose and allow to harden at room temperature for 15 minutes. This low melt agarose fastens the plug in the well.

Alternative Sample Loading Method

Cast plugs in the gel

1. Melt the agarose solution included in the GenePath gel kit. Equilibrate the solution at 50 °C.
2. Set up the gel casting assembly.
3. Cut sample plugs as described above. Load each sample plug onto a tooth of the gel comb, using a clean spatula. Place a drop of molten agarose (50 °C) onto the plug to hold it in place, and let solidify.
4. Place the comb into the casting stand so that the plugs face the large area of the gel.
5. Carefully pour the molten agarose (50 °C) into the casting assembly. Allow to harden and remove the comb.

3.3 Electrophoresis

1. Place the gel frame into the electrophoresis cell. Remove both end gates of the casting stand and slide the gel and platform out of the stand. Place the gel and platform assembly into the frame so that the bottom of the platform rests on the floor of the cell. Do not remove the gel from the platform. The gel should be oriented with the wells at the top of the gel chamber (DNA will run from top to bottom).
2. Select the appropriate program by using the INCREASE and DECREASE keys on the GenePath power module. Press START to begin the run. Refer to the GenePath manual for details on the operation of the system.

3.4 Staining and Photography of the Gel

1. After the run is completed, lift up one corner of the gel and platform. Carefully slide the gel off the platform into the ethidium bromide solution.
2. Ethidium bromide is used to stain the DNA fragments. The solution is made by adding 5 drops of the 1 mg/ml ethidium bromide stock (GenePath gel kit) to approximately 300 ml deionized water.

   Caution: Ethidium bromide is a possible carcinogen and a known mutagen. Wear gloves and safety glasses at all times and wash contaminated skin with water.

3. Allow the gel to stain for 15 minutes in the ethidium bromide solution. Using gloves, remove the gel from the ethidium bromide solution and place it in a dish containing at least 500 ml dH_2O.
4. Destain for 30–60 minutes in the water. Destaining longer can help reduce the background.
5. Place the gel on the UV Transilluminator. If using Bio-Rad's Polaroid Documentation System, place the camera with shield over the gel. The suggested setting on the camera is: Aperture setting: 5.6 or 8, Shutter speed setting: B, with a 3 to 6 second exposure. Turn on the transilluminator and take a picture of the gel. Refer to the transilluminator and camera manual for a detailed description of the instrument. If using the Gel Doc™ 1000 computerized documentation system, follow the instructions included with the Gel Doc 1000 system.

   Caution: Ultraviolet radiation is dangerous, particularly to the eyes. To minimize exposure, make sure that the UV light source is adequately shielded and wear protective goggles or a full safety mask that efficiently blocks UV light.
6. Refer to Figure 3.1 for the separation of Lambda ladder standards, Culture control, and Control plug. The separation was obtained with the "**Psu**" program which is used for separating *Pseudomonas* DNA. Other programs will give different separation patterns.

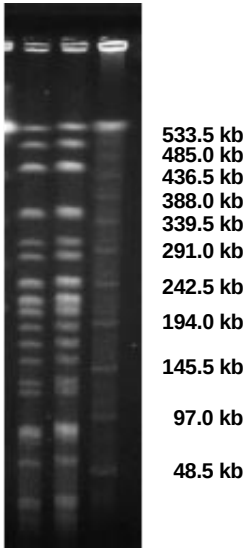

Fig. 3.1. A 1.0% agarose gel was run on the GenePath system using 1x Electrophoresis buffer and the Program "Psu". The samples separated are: Lane 1) Culture Control—Group 3, lane 2), Control Plug—Group 3, and lane 3) Lambda ladders.

Section 4
Interpretation of Results

The DNA fragments generated by PFGE are characteristic of individual strains. In certain cases, fingerprints which are not identical may still be from the same strain of organism. The following guidelines will help in interpreting DNA fingerprints between different samples. Comparative analysis of the banding pattern for the isolates (strains) can be divided into the following categories:[16,17,18]

Class I—Identical: The band patterns between samples are the same and can be given the same designation (*e.g.*, type A).

Class II—Closely Related: Differ by 1 genetic event, a 1–3 band difference. The samples can be designated as subtypes (*e.g.*, type A1 through An).

Class III—Possibly Related: Differ by 2 genetic events, a 4–5 band difference. The samples may be designated as subtypes (*e.g.*, type A1 through An). Non-genetic evidence should be carefully considered in these cases.

Class IV—Different: Differ by ≥ 3 genetic events, a ≥ 6 band difference. The samples are considered different strains (*e.g.*, type B).

Note: A one band difference is defined as the presence or absence of a single band in the sample as compared to the reference sample.

These categories are applicable only to chromosome/restriction-enzyme combinations generating at least 10 distinct restriction fragments.[18]

These categories are only guidelines for distinguishing relatedness between the sample strains. In some situations, it may be difficult to determine interrelationships between strains. In these cases, it is probably best to assume relatedness, especially in a scenario requiring infection control.[17] Each lab should set up its own set of standards for correct interpretation of the results. This may include other microbiological methods that are needed for correct analysis of strain relatedness.

Figure 4.1 illustrates these categories by looking at six isolates. The restriction fragment patterns of isolates A and B are identical and can be considered the same strain (designated strain A). The restriction fragment patterns of isolates E and F differ from each other and from the other isolates by more than six bands. These would represent two unrelated strains of the organism (strain B and C). The restriction fragment patterns of isolates C and D differ from each other by four bands, but differ from isolates A and B by two bands. Isolates C and D could have been derived from isolates A or B by a single deletion or insertion. One can consider isolates C and D to be subtypes of isolates A and B. These strains can then be designated as A1 (samples A and B), A2 (sample C), and A3 (sample D).

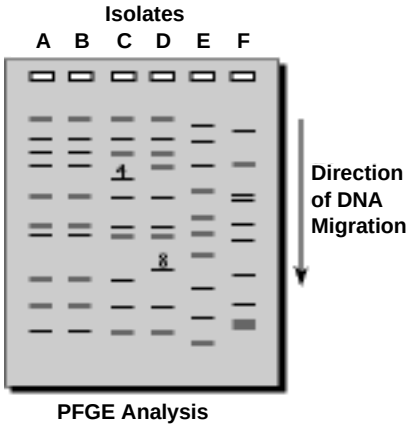

Fig. 4.1. Pulsed field analysis of six pathogenic isolates.

Figure 4.2 is an example of a pulsed field gel run with the GenePath system. The isolates are methicillin resistant *Staphylococcus aureus* that were digested with *Sma* I restriction enzyme. Isolates in lanes 3, 5, 6, 7, 9, 11, 12, 14, 15, and 16 appear to be the same strain (designated strain A1), with lane 8 being a related strain (differ by 1 band, strain A2). Lanes 2 and 4 are possibly related to each other, but not closely related to the other isolates (differ by >3 bands, strains B1 and B2). Lane 17 does not appear related to any of the isolates (strain C). Lanes 10 and 13 are examples of lanes where there is not enough DNA to visualize.

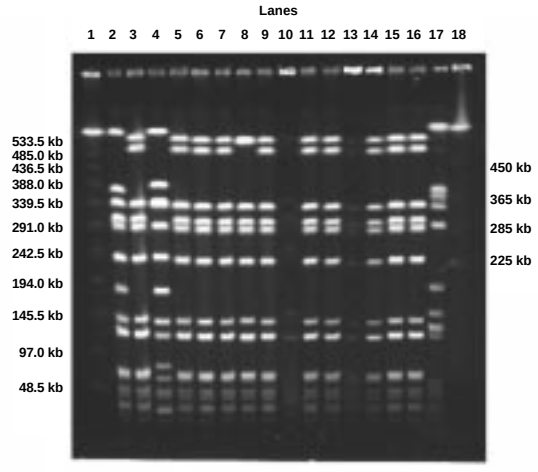

Fig. 4.2. Lanes 2 through 17, methicillin resistant *Staphylococcus aureus* samples (G. Cage, Arizona State Laboratory) that were digested with *Sma* I restriction enzyme. The samples were run using the "**Sta**" program on the GenePath system. The separation size range is from 50 kb to 500 kb. Lane 1 and 18 contains the size standards Lambda ladder and *S. cerevisiae*, respectively.

Section 5
Troubleshooting

Problem	Solution
Distorted bands	1. Wells were distorted or damaged. Recast gel.
	2. Sample plugs were crushed when placed in wells.
	3. Pump flow rate too low. Check for kink along tubing.
	4. Cooling Module not cooling. Check Cooling Module.
Thick bands	1. Too much DNA. Load less sample.
High background in lanes	1. Insufficient washing of samples.
	2. DNA concentration too high.
Bands smeary or fuzzy	1. Cooling Module not cooling. Check Cooling Module.
	2. Sample degraded. Use freshly prepared sample.
	3. DNA concentration too high.
	4. Insufficient washing of samples, resulting in poor restriction digestion.
	5. Restriction enzyme degraded. Use new restriction enzyme.
	6. Too much DNA. Load less sample.
No DNA fragments in samples	1. Poor lysis of cells. Check that correct Lysis buffer and enzyme were used. Lysis enzyme degraded.
	2. Restriction enzyme possibly degraded. Use new restriction enzyme if control plug did not digest.
	3. Strain resistant to restriction enzyme. May need to use a different restriction enzyme. Contact Bio-Rad Laboratories.

Troubleshooting (continued)

Problem	Solution
Control plug did not digest into fragments	1. Check that correct buffer and enzyme was used. 2. Restriction enzyme possibly degraded. Re-digest or use new restriction enzyme.
No DNA fragments, including gel standards	1. Try re-staining the gel with new ethidium bromide solution. Destain for at least 1 hour. 2. Check to make sure the GenePath system is drawing current. If not, check the GenePath manual troubleshooting section.
No DNA fragments, smear in lane	1. DNA degraded during or before preparation. Use cold Cell Suspension buffer and keep samples on ice. 2. Cast plugs immediately after adding Lysozyme and Embedding agarose. 3. Add 5 ml of an overnight culture suspension to 25 ml fresh broth and grow for 4–6 hours to obtain healthy, growing cells.

Check the GenePath manual for other Troubleshooting guides related to instrument problems.

Section 6
References

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13. Chu, G., Vollrath, D. and Davis, R. W., *Science*, **234**, 1582-1585 (1987).

14. CHEF (U.S. Patent 5,165,898 issued to Stanford University) is exclusively licensed to Bio-Rad Laboratories.

15. Dynamic Regulation (U.S. Patent 4,878,008 issued to Bio-Rad Laboratories) is exclusively licensed to Bio-Rad Laboratories.

16. Maslow, J. N., Slutsky, A. and Arbeit, R. D., Applications - Molecular Typing Methods, Chapter 7.1, 563-572 (1993).

17. Goering, R., *Infect. Control Hosp. Epidemiol.*, **14**, 595-600 (1993).

18. Tenover, F., *et al.*, *J. Clin. Microbiol.*, **33**, 2233-2239 (1995).

19. Arbeit, R., The Manual of Clinical Microbiology, Chapter 17 “Laboratory Procedures for the Epidemiologic Analysis of Microorganisms”, 6th edition, 190-208.

Section 7
Appendix

7.1 Additional GenePath Reagents

Catalog Number	Product Description
310-0111	**GenePath Group 1 Reagent Kit**
310-0061	**Enzyme Module Group 1**
310-0060	**Universal Module**
310-0062	**Standard Module Group 1**
310-0162	**Culture Control Group 1**
310-0112	**GenePath Group 2 Reagent Kit**
310-0063	**Enzyme Module Group 2**
310-0060	**Universal Module**
310-0064	**Standard Module Group 2**
310-0164	**Culture Control Group 2**
310-0113	**GenePath Group 3 Reagent Kit**
310-0065	**Enzyme Module Group 3**
310-0060	**Universal Module**
310-0066	**Standard Module Group 3**
310-0166	**Culture Control Group 3**
310-0114	**GenePath Group 4 Reagent Kit**
310-0067	**Enzyme Module Group 4**
310-0060	**Universal Module**
310-0068	**Standard Module Group 4**
310-0168	**Culture Control Group 4**

Catalog Number	Product Description
310-0115	**GenePath Group 5 Reagent Kit**
310-0069	**Enzyme Module Group 5**
310-0060	**Universal Module**
310-0070	**Standard Module Group 5**
310-0170	**Culture Control Group 5**
310-0116	**GenePath Group 6 Reagent Kit**
310-0071	**Enzyme Module Group 6**
310-0060	**Universal Module**
310-0072	**Standard Module Group 6**
310-0172	**Culture Control Group 6**
310-0007	**GenePath Gel Kit**

7.2 GenePath System Components

Catalog Number	Product Description
310-0016	**GenePath Strain Typing System**, 110 V
310-0027	**GenePath Polaroid Documentation System,** 110 V
310-0012	**Gel Doc 1000 Computerized Documentation System,** 110 V
310-0013	**Gel Doc 1000 Computer**, 110 V
310-0022	**Gel Doc 1000 Printer**, 110 V

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4100053 Rev E