

**M16sS Bacterial Ribotyping Kit**  
*Instruction Manual*

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## Warranty and Liability

This product is not for diagnostic purposes and is to be used solely for life science research.

BioVentures, Inc. is dedicated to exploring cutting edge concepts in genetic diversity as a leading developer and manufacturer of DNA standards and pharmacogenomic tools.

Products for PCR do not include any rights to perform PCR. This product is optimized for use in the Polymerase Chain Reaction (PCR) covered by patents owned by Roche Molecular Systems, Inc. and F. Hoffmann-La Roche, Ltd. ("Roche"). No license under these patents to use the PCR process is conveyed expressly or by implication to the purchaser by the purchase of this product.

# I. Introduction

## A. Background

Terminal Restriction Fragment Length Polymorphism (T-RFLP) profiling allows quick and accurate determination of bacterial populations in soil, water, tissue and blood. The use of the 16s rRNA gene in T-RFLP profiling is a proven means of analyzing microbial communities and identifying specific members within bacterial populations (Kitts, 2001; Kent *et al.*, 2003; Dunbar *et al.*, 2000). This process combined with an appropriate analysis program provides the ability to answer numerous questions regarding the bacterial composition within a sample, its structure, diversity and dynamics.

Researchers using the M16sS Bacterial Ribotyping kit will find the process straightforward and that the work flows without difficulty. The first step is DNA extraction from the sample of interest. This step is made quick and simple by the inclusion of GeneReleaser<sup>®</sup>. In the second step PCR amplification is easily achieved with optimized PCR primers and master mix. The third step includes PCR product purification and helps to rid the products of potential contaminants minimizing downstream problems. The fourth step, consisting of restriction enzyme digestion will deliver DNA fragments that can be analyzed using practically any fluorescence detector instrument. Also included in this kit is MapMarker<sup>®</sup>1000, an accurate sizing ladder that will facilitate accurate size determination of the fragments present after the digest. Once the sample fragment sizes are determined, this kit includes references to a computer program that can help the end user determine what population of bacteria species may be present in the sample. The BioVenture's Inc. website ([www.bioventures.com](http://www.bioventures.com)) also includes step by step formatting instructions to more easily use the available program.

## B. Intended Applications

The M16sS Bacterial Ribotyping kit provides researchers with many of the components necessary to perform T-RFLP in a single package, including a complete and detailed protocol.

## C. Susceptibility to Contamination

It should be kept in mind that the PCR primers included in this kit are **universal bacterial** primers that amplify a highly conserved sequence. Many TAQ DNA polymerases are made from recombinant organisms; therefore, these solutions could contain fragments of DNA that could be amplified resulting in negative controls turning up positive. Also any reagents used could become contaminated and alter results. No matter whether the contamination is alive or not, it could cause complications due to the presence of bacterial DNA. It is **very important** to keep reagents and the work area as clean as possible. Please follow all warnings.

## D. Other Important Considerations

The PCR primers included in this kit were designed specifically for the highly conserved 16s rRNA region of bacteria. Therefore, if a detectable amount of bacteria is present in the sample, it will be amplified. Knowing this, it must be remembered that it is possible for many different forward

and reverse peaks to be present in one sample. Organisms in the same family can have similar cut sites and this same cut site may be shared across several organisms. Noting these possibilities, the results from the FragSort 5 will likely be an extensive list of organisms. Many of these organisms may belong to the same family or different strains of the same organism. In most cases the FragSort can be used to determine a profile of the families of bacteria present in the sample.

It is possible to determine the relative population of the organisms present in the sample. This may be determined by examining peak heights and peak areas of the forward or reverse fragments. It is important to note at this time how enrichment could alter this analysis. It is possible that a sample may contain organisms that grow at vastly different rates. Slower growing organisms, or those with specialized nutrient, oxygen, temperature or time requirements, may be overshadowed by faster growing, less fastidious organisms. Thus, the relative population of an organism may be falsely over or under estimated due to enrichment procedures.

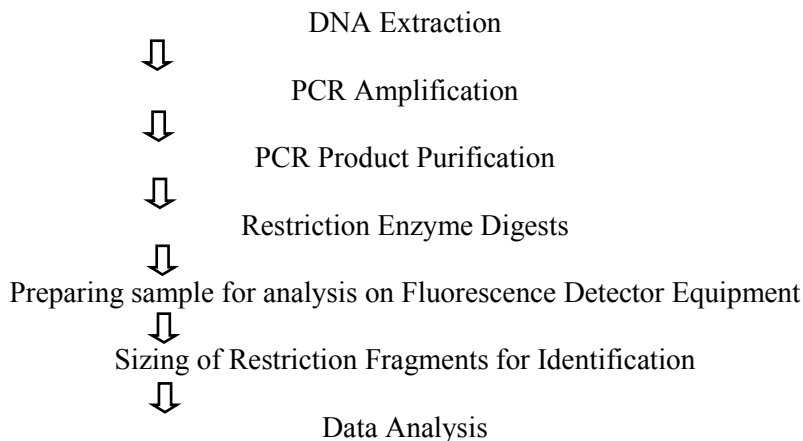
### E. Components Included with the Kit

- GeneReleaser<sup>®</sup> (2 tubes)-4.0mls (Store at 4°C)
- MapMarker<sup>®</sup> 1000 X-Rhodamine Labeled (1 tube)-150µl (Store at 4°C)
- Amplification Primers (1 tube each) (Store at -20°C) (**Protect from light**)
  1. 27F FAM (50pmol/µl)-50µl
  2. 1492R HEX (50pmol/µl)-50µl
- 2X PCR Buffer (1 tube)-2.5mls (Store at -20°C)
- Restriction Enzymes (1 tube each) (Store at -20°C) (**Keep on ice while in use**)
  1. Hha I (10u/µl)-50µl
  2. Msp I (10u/µl)-50µl
  3. Rsa I (10u/µl)-50µl
- Restriction Enzyme Buffers (1 tube each) (Store at -20°C)
  1. Buffer C (10X) (used for Hha I, Rsa I)-1.0ml
  2. Buffer B (10X) (used for Msp I)-500µl
  3. BSA (10µg/µl) (used in all restriction enzyme reactions)-50µl
- Glycogen (20ng/µl) (1 tube)- 400µl (Store at -20°C)
- 3M Sodium Acetate (2 tubes)-2.0mls (Store at -20°C)
- BioMarker<sup>®</sup> EXT (1 tube)- 600µl (Store at 4°C)

### F. Required components not included with the kit

- |   |   |
|---|---|
| <ul style="list-style-type: none"> <li>• Template DNA</li> <li>• Taq</li> <li>• Sterile DI H<sub>2</sub>O</li> <li>• Ethanol (95% and 80%)</li> <li>• Isotonic saline (0.9% NaCl)*</li> <li>• Nutrient Media(if starting with low bacterial count)</li> </ul> | <ul style="list-style-type: none"> <li>• 10% Tween 20*</li> <li>• 1X TE</li> </ul> <p>* For soil only</p> |
|---|---|

### Work Flow Chart



## !!!PRECAUTIONS!!!

**\*\*ALL WORK SHOULD BE PERFORMED UNDER HOOD; ANY AMOUNT OF BACTERIAL CONTAMINATION FROM THE ATMOSPHERE COULD CAUSE NEGATIVE CONTROLS TO COME UP POSITIVE.\*\***

**\*\*UV TREAT WATER AND GENELEASER® ONLY FOR ~30 MINUTES PRIOR TO STARTING (DO NOT EXPOSE THE SAMPLE TEMPLATES OR PRIMERS TO UV LIGHT!)\*\***

**\*\*PRE-PCR AND POST-PCR SAMPLES SHOULD BE PROCESSED IN SEPARATE AREAS TO PREVENT CROSS-CONTAMINATION OF UNAMPLIFIED SAMPLES WITH PCR PRODUCTS FROM AMPLIFIED SAMPLES.\*\***

**\*\*MAKE SURE GENELEASER® IS MIXED THOROUGHLY; IT MAY NEED TO BE SHAKEN BETWEEN SAMPLES TO PREVENT CLOGGING OF PIPET TIPS.\*\***

**\*\*KEEP FLUORESCENTLY LABELED AMPLIFICATION PRIMERS PROTECTED FROM LIGHT AT ALL TIMES.\*\***

**\*\*KEEP RESTRICTION ENZYMES ON ICE WHILE IN USE\*\***

## II. M16sS Bacterial Ribotyping Protocol

### A. DNA Extraction

**GeneReleaser® Setup:** Perform this step before addition of sample to PCR reagents.

GeneReleaser® is a proprietary reagent which releases DNA from whole blood, tissue cultures, bacterial colonies or other samples intended for T-RFLP analyses. Lysis is accomplished directly in the amplification tube on a thermocycler. GeneReleaser® sequesters cell lysis products which might inhibit polymerases and improves amplification yield and specificity. GeneReleaser® greatly simplifies the amplification of genomic DNA by avoiding the requirement of more complex methods to purify DNA. Thus, lengthy protocols and excessive sample manipulations which may introduce contamination are unnecessary.

It is important to note that this kit may also be used to extract bacterial DNA from water. The sample may need to be concentrated and/or enriched in order to produce a bacteria sample that is a manageable size for GeneReleaser®.

#### **BEFORE BEGINNING:**

It is essential to note that if the sample to be used may contain a low number of bacteria, please follow enrichment procedure (Trouble Shooting Section, pg. 24) before proceeding to GeneReleaser® protocol. If sample is soil, it is only necessary to follow the soil procedure before proceeding to GeneReleaser® protocol; it is **NOT** necessary to perform the enrichment procedure.

It is necessary to understand that the enrichment procedure will most likely alter the relative composition of the bacteria in the sample. Only those bacteria present that are capable of growth in nutrient broth will grow. For those species that do grow, some species will grow faster than others. The resultant population densities will not represent all of the species originally present, nor will the relative population sizes be maintained. You should be aware that enrichment should be used only for detection purposes and not determining relative population sizes. If it is important to determine the relative amounts of the bacterial species present in the sample, enrichment is not recommended.

It is also important to note the necessity of using some type of DNA fragment analysis instrument that employs fluorescence detection equipment. The PCR primers included in this kit are fluorescently labeled on the 5' end. The restriction enzyme will cut at the first recognition site after the 5' labeled end. This will then become the labeled fragment to be sized. An agarose gel **MAY NOT** be used for sizing because all fragments, labeled and unlabeled, would be visualized. The fragments produced through the use of this kit may be detected on any model of fluorescence detection equipment as long as the equipment can detect at least the ROX dye (MapMarker®1000), FAM dye (forward fragments) and HEX dye (reverse fragments).

This kit has been tested with different types of soil samples and the protocols have been written to be as robust as possible. However, some samples may require adjustments to the protocols to improve their success. The user should be aware of and make use of the Trouble Shooting Section on page 19 or visit the company's website at [www.bioventures.com](http://www.bioventures.com) or email for help at [support@bioventures.com](mailto:support@bioventures.com).

### **i. Soil Procedure:**

**Note: It is very important to complete steps 1-4 to help remove PCR inhibitors that may be present in the soil.**

1. Prepare a 10% slurry of soil sample in isotonic (0.9%) saline. We recommend 0.25g of sample with 2.25ml of 0.9% saline solution.
2. Gently invert 20-30 times.
3. Centrifuge sample at 500xg for 1 minute.
4. Decant supernatant.
5. For every 0.25g of soil used, add 1 ml of a solution of 0.1% Tween 20 in isotonic saline (990µl 0.9% saline solution + 10µl of 10% Tween 20).
6. Mix gently to resuspend slurry.
7. Remove 10µl of the suspended slurry and place into a 0.5ml PCR tube.
8. Thoroughly resuspend the contents of the GeneReleaser<sup>®</sup> tube by inverting 10-20 times.
9. Add 40µl of the resuspended GeneReleaser<sup>®</sup> to the tube containing the 10µl of soil sample slurry.
10. Perform either the thermocycler program (II.A.ii, pg. 11) or the microwave protocol (Trouble Shooting Section, pg. 23).
11. Perform the amplification reaction according to amplification protocol (II.B, pg. 12).

## **GeneReleaser<sup>®</sup> Processing**

### **ii. Thermocycler Procedure for GeneReleaser<sup>®</sup> Treatment**

**NOTE:** When using the Perkin Elmer **GeneAmp<sup>®</sup> PCR system 9600** or **2400** all times listed below should be reduced by 50%.

1. Mix the appropriate amount of GeneReleaser<sup>®</sup> and sample together.
2. Place samples onto thermocycler, with a heated lid, with the following program:

| <b>Step</b> | <b>Temperature</b> | <b>Heating Rate</b> |
|-------------|--------------------|---------------------|
| 1           | 65°C               | Hold 30 sec         |
| 2           | 8°C                | Hold 30 sec         |
| 3           | 65°C               | Hold 90 sec         |
| 4           | 97°C               | Hold 5 minutes      |
| 5           | 8°C                | Hold 60 sec         |
| 6           | 65°C               | Hold 180 sec        |
| 7           | 97°C               | Hold 2 minutes      |

|    |      |              |
|----|------|--------------|
| 8  | 65°C | Hold 60 sec  |
| 9  | 4°C  | Hold forever |
| 10 |      | End          |

3. Once program has finished, centrifuge tubes at 5,000xg for 5 minutes.

4. Remove supernatant and use as DNA template.

5. Proceed to amplification protocol (II. B, pg. 12).

\*For an additional microwave protocol, see the Microwave procedure in Trouble Shooting Section (pg. 23).

## B. PCR Amplification Protocol

After you have processed your sample for amplification, use the following protocol for amplifying each sample:

1. For each 50µl reaction, mix the following reagents: (We suggest preparing enough master mix to accommodate all samples.) (**Remember to protect labeled primers from light**)

| Reagent                              | Amount   |
|--------------------------------------|----------|
| 2X Common Master                     | 25µl     |
| 27F Fam Forward Primer (50pmol/µl)   | 0.5 µl   |
| 1492R Hex Reverse Primer (50pmol/µl) | 0.5 µl   |
| Sterile DI H <sub>2</sub> O          | 21.25 µl |
| Taq DNA Polymerase (5u/µl)           | 0.25 µl  |

2. Aliquot 47.5µl of the above master mix into the wells of a 96 well plate.

3. Add 2.5µl of template to the appropriate well. Do not use mineral oil overlay.

4. Perform the amplification with the following program using a thermocycler with a heated lid:

| Step | Temperature | Heating Rate                 |
|------|-------------|------------------------------|
| 1(a) | 95°C        | Hold 2 min                   |
| 2    | 95°C        | Hold 30 sec                  |
| 3    | 50°C        | Hold 20 sec                  |
| 4    | 72°C        | Hold 1min 30 sec             |
| 5    |             | Go to Step 2, X times (b, c) |
| 6    | 72°C        | Hold 6 min                   |
| 7    | 4°C         | Hold forever                 |
| 8    |             | End                          |

a. If using a hot start Taq DNA polymerase, be sure to check the product recommendations for initial denaturation temperature and time.

b. If amplifying what might be a low copy number, it is recommended to complete 35 cycles.

c. If amplifying what might be a high copy number, it is recommended to complete 25 cycles.

5. Load 2µl on a 1% gel. Load 6µl of BioMarker<sup>®</sup> EXT ladder onto gel. BioMarker<sup>®</sup> EXT\* contains linear double-stranded DNA bands of 2000, 1500, 1000, 700, 525, 500, 400, 300, 200, 100 and 50 base pairs. The concentration of each band is @ 50ng/5µl of BioMarker<sup>®</sup> EXT applied. PCR should produce a band of ~1500bp. If PCR product is visible, proceed onto PCR Product Purification (II.C, pg. 14). If PCR product is absent, refer to the trouble shooting section (pg. 19).

\* See Reference section for photo (pg. 26).

## C. PCR Product Purification\*

This kit has been validated using the following method of PCR product purification.

1. Precipitate the PCR product by adding the following reagents to each amplified sample:

| Reagent            | Amount   |
|--------------------|----------|
| Glycogen (20ng/µl) | 1 µl/Rxn |

|                           |                   |
|---------------------------|-------------------|
| 3M Sodium Acetate (5.2pH) | 1/10 total volume |
| 95% EtOH                  | 2X total volume   |

- Place in a -20°C freezer for a minimum of 1 hour.
- Remove from -20°C freezer and centrifuge at 10,000xg for 30 minutes.
- Carefully decant the 95% EtOH.
- Wash the pellet with 2X total volume of 80% EtOH.
- Centrifuge for 45 minutes at 10,000xg.
- Carefully decant 80% EtOH and allow the pellet to air dry.
- Dissolve the dried pellet in 50µl of 0.1X TE to set up the restriction enzyme digests. See II. D (pg. 15).

\*Desalting columns are also an option for PCR product purification at this step and may be used **instead of** the described method.

#### D. Restriction Enzyme Digest

Included in this kit are the restriction enzymes Hha I, Msp I, Rsa I and their buffers. Be sure to use the correct buffer for each enzyme since this can affect the efficacy of the enzyme. Set up a 50µl reaction for **each** of the three included enzymes.

- For **each** enzyme prepare a 50µl reaction.
- Add the following reagents to each sample: **(Remember to keep Restriction Enzymes on ice while in use)**

| Reagent                               | Amount     |
|---------------------------------------|------------|
| Purified PCR product (from Section C) | 10 µl      |
| Restriction Enzyme Buffer (10X)**     | 5 µl       |
| BSA (10µg/µl)                         | 0.5 µl     |
| Restriction Enzyme (10u/µl)           | 0.5µl (5u) |
| Sterile DI H <sub>2</sub> O           | 34µl       |

\*\* Use Buffer C with Hha I **and** Rsa I; Buffer B with Msp I.\*\*

- Gently invert, pulse centrifuge and place all samples on a thermocycler programmed with the following program:

| Step | Temperature | Heating Rate    |
|------|-------------|-----------------|
| 1    | 37°C        | Hold 3 hours    |
| 2    | 65°C        | Hold 20 minutes |
| 3    | 4°C         | Hold forever    |
| 4    |             | End             |

- Precipitate each digest by adding the following reagents to each sample and place all samples into a -20°C freezer for a minimum of 1 hour. Desalting columns are also an option for PCR product purification at this step:

| Reagent                   | Amount            |
|---------------------------|-------------------|
| Glycogen (20ng/µl)        | 1µl/Rxn           |
| 3M Sodium Acetate (5.2pH) | 1/10 total volume |
| 95% EtOH                  | 2X total volume   |

- Remove from -20°C freezer and centrifuge at 10,000xg for 30 minutes.
- Carefully decant the 95% EtOH.
- Wash the pellet with 2X total volume of 80% EtOH.
- Centrifuge for 45 minutes at 10,000xg.
- Carefully decant 80% EtOH and allow the pellet to air dry.

## **E. Preparing sample for analysis on Fluorescence Detector Equipment\***

\*This protocol has been verified using the ABI 310® and the ABI 3100®. However, any analyzer capable of high resolution DNA fragment separation and detection of FAM and HEX can be used.

1. Using the pellet from step D. 9 above, suspend the pellet with 13µl 1X TE. At this time, this is considered full strength.
2. Multiple dilutions may need to be performed in order to determine the optimal amount to load on the instrument. Typically a 1:50 dilution is a good starting point.
3. Make dilutions in 1X TE. For example: A 1:50 dilution would be 1µl of sample + 49µl of 1XTE.
4. Use 1µl of diluted sample + 11.5µl deionized Formamide + 0.5µl MapMarker® 1000.
5. Based on initial results, scale dilutions up, down or keep at 1:50 in order to analyze sample.
6. Mix completely and pulse centrifuge sample to ensure complete sample recovery and load onto instrument.

## **F. Sizing of Restriction Fragments for Identification**

MapMarker® 1000 is included in this kit for use in sizing the DNA samples for easy identification. MapMarker® is a set of high quality fluorescently labeled DNA fragments designed to provide consistent intensities and migration patterns for DNA sizing standards and are compatible with all fluorescent-based separation instrument systems. MapMarker® DNA fragments are uniformly spaced to provide accurate base calling and precision sizing of samples. MapMarker® 1000 contains 23 discrete DNA fragments ranging in size from 50 to 1000 base pairs. The fragments are 50, 75, 100, 125, 150, 200, 250, 300, 350, 400, 450, 475, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950 and 1000 base pairs.

## **G. Data Analysis**

We have found that the best method to analyze TRFLP fragments to be the Ohio State University program called FragSort 5\*. The program can be downloaded from Ohio State University at the following address: <http://www.oardc.ohio-state.edu/trflpfragsort/downloads.php>. This program comes preloaded with a few forward and reverse primers to begin your search. The primer choice of 11F-907R forward and reverse is compatible with the primers provided in this kit. Data can be taken directly from the GeneScan software, sent through the website normalizer and then through the program. The program then gives you a list of all the possible organisms that could correspond with your fragment information. This program is unique in that if you decide to use alternate primers that are not included with this kit, the program can be customized to include those primers. The OSU FragSort website gives information on how to make your own customized database that can be used with the FragSort 5 interface.

\*Developed by Professor Frederick Michel and Stephen Sciarini of Ohio State University, Department of Food, Agricultural and Biological Engineering.

### III. Trouble Shooting

#### Preparation and Amplifying of Samples

| Observation   | Possible Causes  | Questions (?)   | Recommended Actions   |
|---|--|---|---|
| There was either weak PCR product or none at all.                 | An incorrect amount of one or more reagents was used or a reagent was omitted. | Were all reagents included and was the correct amount used?   | Set up the reactions again using the correct amount of each reagent.  |
|   | Incorrect cycling conditions were used.  | Has the thermal cycler program been checked?  | If the program is incorrect, re-program the cycler and set up the reactions again.  |
|   |  | Were the recommended conditions followed?   | Follow the recommended conditions.<br>It may be necessary to run gradients in order to find a temperature that is suitable for bacteria in your sample. |
|   | No sample DNA was used.  | Was sample DNA loaded onto gel?   | Re-run gel to ensure that sample DNA was loaded onto gel.   |
| Possible inhibitors in sample that could be interfering with PCR. | Is this a soil sample or sample that could contain inhibitors to PCR?          | It may be necessary to filter your sample through a 0.45µm spin filter after the GeneReleaser <sup>®</sup> protocol. You can centrifuge the spin filters 500xg for 5 minutes. |   |

| Observation                                   | Possible Causes  | Questions (?)   | Recommended Actions  |
|---|--|---|--|
| A DNA band is seen in the negative control.   | The band may be spillover from the loading of adjacent wells on the gel.       | Is the band relatively weak?  | Run the negative control again on a mini-gel with an empty well on either side. Run marker beside one of the empty wells.  |
|   | There is contamination in the negative control or one or more of the reagents. | Have you re-run the product on a gel to see if it is merely spillover? Have you seen evidence of contamination in other PCR products? | Make sure to UV treat all non-DNA material for ~30 minutes prior to setting up reactions. Be sure to set up reaction under hood. Carefully make up a new negative control and do a trial PCR. If that product is positive, it may be necessary to take steps to find and eliminate the lab of the contamination. |
| Trouble pipetting GeneReleaser <sup>®</sup> . | Did not shake GeneReleaser <sup>®</sup> well prior to pipetting.               | Did you shake GeneReleaser <sup>®</sup> well before pipetting?  | Make sure to thoroughly shake GeneReleaser <sup>®</sup> before pipetting. It may be necessary to shake the GeneReleaser <sup>®</sup> before removing an aliquot each time.   |

#### Restriction Enzyme Digests

| Observation  | Possible Causes                               | Questions (?)   | Recommended Actions                                    |
|--|---|---|--|
| No peaks observed after capillary electrophoresis        | An incorrect amount of sample was loaded.     | Was there enough of the correct dilution added or was any sample added? | Set up reactions again using the correct amount.       |
|  | Pellet was lost during precipitation/cleanup. | Was a pellet observed during precipitation/cleanup?                     | Check waste collection tubes for pellet.               |
| Only one peak seen, but it appears at the end of marker. | Incomplete digestion.                         | Was recommended program followed?                                       | Set up reactions again and follow recommended program. |

|  |                              |                                       |  |
|--|------------------------------|---------------------------------------|--|
| Peaks are seen but are either too high (saturate detector) or are very low and not called. | Incorrect dilution.          | Were dilutions done correctly?        | If peaks are too high, try doing a higher dilution. If peaks are low or very little, try running full strength or at a smaller dilution. |
|  | Incorrect threshold setting. | Have threshold settings been checked? | A majority of literature states using a threshold of 100. Set threshold at 100 and use all labeled peaks that are called over 100.       |

| Observation   | Possible Causes                      | Questions (?)  | Recommended Actions   |
|---|--------------------------------------|--|---|
| Unable to differentiate organisms by restriction fragment profiles. | Closely related strains or families. | Are there any other restriction enzymes that may produce a more distinguishing fragment profile? | Perform a virtual restriction enzyme digest with various other restriction enzymes to see if a more distinguishing profile is produced. Incorporate the new restriction enzyme(s) into the restriction enzyme protocol. |

## **MICROWAVE PROCEDURE**

**Note: Make sure the racks used in this procedure are MICROWAVE SAFE!**

We have found that the microwave treatment of specimens affords a rapid sample preparation and facilitates the amplification of the more intractable types of specimens.

1. Place 1µl of specimen (10µl of soil slurry) with 20µl (40µl for soil sample) of GeneReleaser<sup>®</sup> into either a 0.5ml PCR tube or 1.5ml tube.
2. Vortex the tubes containing specimen and GeneReleaser<sup>®</sup> for ~10-30 seconds.
3. An oil overlay is optional.

4. Place the closed tubes in polyethylene or propylene racks. **Make sure that the lids are loosely closed. If lids are closed too tightly tubes could rupture.**
5. Place the rack in a microwave oven and heat at maximum power setting for 5-7 minutes (5 minutes if wattage is 900 or higher, 7 minutes if wattage is 500). A total of 4500 watt-minutes is the optimum.
6. Remove rack from microwave and centrifuge the tubes at 5000xg for 5 minutes. After centrifuging samples, remove supernatant and use as DNA template.
7. Perform the amplification reaction according to the amplification protocol described in II.B (pg.12).

### **ENRICHMENT PROCEDURE**

This procedure may be used for samples that may contain a very low number of bacteria. Be aware that enrichment will most likely alter the relative population sizes.

1. Add between 100µl-500µl of sample to nutrient enrichment broth or plate organism onto specific/nonspecific media.
2. Follow instructions for media of choice. For nutrient broth, requirements are typically 37°C for 6-8 hours.
3. Once growth is observed in media, transfer contents of broth to 15ml tube and centrifuge at 5,000xg for 5 minutes. For plated organisms choose 1 isolated colony and move forward to the Thermocycler procedure (II. A. ii, pg. 11) or the microwave procedure (Trouble Shooting Section, pg. 23).
4. Carefully decant supernatant and resuspend pellet with 50µl of 0.1XTE.
5. Use 1µl of the resuspended pellet to perform DNA extraction according to the Thermocycler Procedure (II. A. ii, pg. 11) or the microwave procedure (Trouble Shooting Section, pg. 23).

## IV. Technical Assistance

For questions related to this product call: 1-800-235-8938 8:30AM to 5:00PM CST Monday-Friday. You can email any questions to support@bioventures.com or visit our website at <http://www.bioventures.com>.

## References

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Kitts, C.L. 2001. Terminal restriction fragment patterns: a tool for comparing microbial communities and assessing community dynamics. *Curr. Issues Intest. Microbiol.* **2**:17-25.

Web Sites:

<http://www.bioventures.com>

<http://www.oardc.ohio-state.edu/trflpfragsort/downloads.php>

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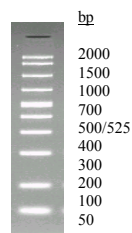
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MapMarker 1000<sup>®</sup> is a registered trademark of BioVentures, Inc.

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GeneAmp<sup>®</sup> PCR system 9600 and GeneAmp<sup>®</sup> PCR system 2400 are registered trademarks of PerkinElmer.

### **BioMarker<sup>®</sup>EXT**



BioMarker<sup>®</sup> EXT contains linear double stranded DNA bands of 2000, 1500, 1000, 700, 525, 500, 400, 300, 200, 100, and 50 base pairs. The concentration of each band is @ 50ng/5µl of BioMarker<sup>®</sup> EXT applied.

BioMarker<sup>®</sup> EXT is evaluated for resolution, intensity and background on 10% acrylamide and 3:1 NuSieve<sup>®</sup> Agarose gels with 1X TAE buffer and ethidium bromide staining.