RiboPrinter® System

MICROBIAL IDENTIFICATION & CHARACTERIZATION

hygiena

hygiena™

Gain Control of your Environment

Only the **RiboPrinter® System** from Hygiena™ combines automation with the power of DNA to easily and accurately identify and characterize contaminants and track their source at the strain level—helping you gain control of any microbial environment.

Although bacteria are found throughout nature, their presence in certain critical environments such as sterile manufacturing or food processing plants poses a constant threat. And when harmful bacteria enter the population, epidemiologists must work quickly to prevent the spread of disease.

To control unwanted bacteria, you need to know what the organism is and how it entered your process or population. Now you can get the answers to these important questions automatically and simultaneously with the RiboPrinter® System.

RiboPrinter® System Benefits:

- Identify, characterize, and trace contaminants to their source.

- Target precise areas for effective interventions.

- Evaluate effectiveness of sanitation and training.

- Establish a baseline of indigenous florae.

- Monitor for spikes and trends.

- Track emerging trends in antibiotic resistant organisms.

RUN SAMPLES ON THE RIBOPRINTER® SYSTEM

Keep track of samples and easily sort **RiboPrint™** patterns with simplified software.

LEARN SAMPLE IDENTITY

Genus and species are automatically provided by comparing the **RiboPrint™** pattern to the onboard identification database of about 8,500 patterns covering more than 1,700 species.

CREATE A CUSTOM ID DATABASE

Optionally assign genus, species and sub-species of your sample from a separate database of your own custom identifications.

TRACK AND TREND

RiboPrint™ patterns are automatically clustered with similar patterns to form RiboGroups with previously run samples. Strain-level data allow you to track the source of organisms and monitor trends.

SHARE AND BUILD

Remote Client Software

- Perform many **RiboPrinter® System** software tasks from another computer on a local or wide area network.

- Analyze data from an office computer instead of the system computer.

- Run the user interface side-by-side with other applications, making it more convenient to import data from the **RiboPrinter® System** into report documents, spreadsheets or presentations.

Data Merging Workstation

- Automatically integrate data from multiple **RiboPrinter® Systems** into a central database for better data analysis.

- Network multiple **RiboPrinter® Systems** and compare individual samples with those collected at other sites or in other countries.

- Control which data is merged, along with frequency of uploads and processes performed on data.

How it Works

Load up to 8 samples at a time in the automated unit.

Genetic fingerprints (**RiboPrint™** patterns) are generated for each sample in 8 hours.

Designated ID	Label	Similarity to Designated ID QC-101	RiboPrint™ Pattern
DUP_PVUII-2049	Salmonella II ser. Hillbrow	0.38	
DUP-16090	Haemophilus influenzae	0.10	
DUP-16400	Neisseria meningitidis	0.04	
DUP-18390	Aeromonas species	0.09	
DUP_PVUII-3266	Streptomyces aureofaciens	0.17	
DUP-18221	Paracoccus denitrificans	0.35	

ID Label	Sample Comment	Isolated From	Similarity to RiboGroup Environmental 999-281-S-2	RiboPrint™ Pattern
Bacillus thuringiensis	Sterility Failure	Final Product	0.99	
Bacillus thuringiensis	EM Isolate	Fill Line	0.99	
Bacillus thuringiensis	Raw Material	LN# 903	0.81	

IDENTIFICATION OF GENUS AND SPECIES

Taxonomic identification is automatically provided by comparing the unknown isolate against an onboard database of about 8,500 patterns, covering more than 290 genera and 1,700 species.

STRAIN DIFFERENTIATION (CHARACTERIZATION)

Sub-species discrimination along with associated source data helps determine if you've previously seen a particular strain in your environment and if so, where and when.

Meet the RiboPrinter® System

WORKSTATION

The brains of the **RiboPrinter®** System, it controls the characterization unit analyzes and stores data and produces printed reports. The workstation includes a personal computer and printer. The proprietary software provides user-friendly prompts, process monitoring, diagnostics and complete data analysis.

CHARACTERIZATION UNIT

This automated instrument performs DNA restriction digestion, electrophoretic separation, membrane transfer and detection—and automatically records data on the accompanying workstation. Its modular design simplifies troubleshooting and maintenance. Loading and operation are easy and intuitive, requiring little training.

MP BASE AND INSERTS

Holds the DNA probe, conjugate and substrate (which fuel the chemical reaction to create bands from fragments of DNA).

GEL CASSETTE

Holds precast electrophoresis gel. Separates DNA fragments into discrete bands.

DNA PREP PACK

Restriction enzyme and lysis buffer to break open bacteria, release and cut DNA; marker DNA to help normalize patterns.

SAMPLE CARRIER

Holds eight samples.

PURIFIED WATER

For closed-system rehydration of dry disposables and component rinsing.

MEMBRANE

Captures DNA fragments for final processing.

SAMPLE PREPARATION EQUIPMENT

Sample preparation is made rapid, safe and simple with this specialized equipment. The hand-held mixer disperses cells in the buffer solution. The Heat Treatment Station inactivates bacterial samples and renders them harmless.
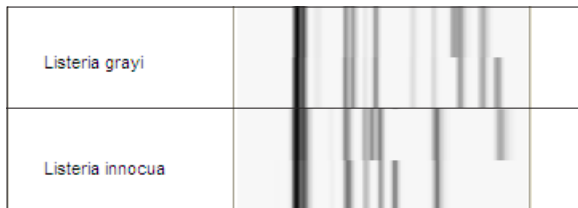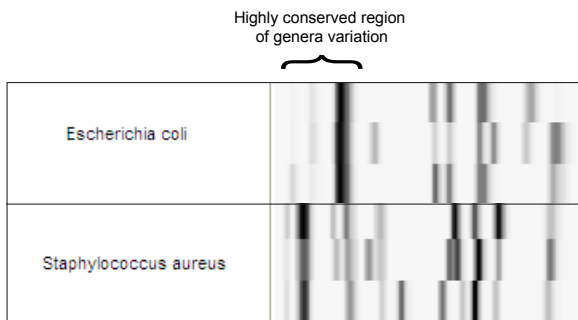
Fast, Automated Answers with Minimal Labor

After colony isolation, the **RiboPrinter®** System automates the rest of the process, reducing the labor required and minimizing errors due to technique. With results in just 8 hours, the **RiboPrinter®** System allows understanding of contamination in a fraction of the time.

SAMPLE PREPARATION
This is the **only** step that takes place outside the **RiboPrinter®** System automated unit. Pure colonies picked from a plate are inactivated by heat treatment.

DNA PREPARATION
DNA is extracted from bacterial cell lysate and cut into fragments by a restriction enzyme.

Well
Gel
DNA fragment travel
Membrane
Membrane travel
DNA fragment
Anode

SEPARATION AND TRANSFER
The DNA fragments are separated according to molecular size by gel electrophoresis and are transferred to a membrane.

MEMBRANE PROCESSING
After hybridization with a labeled DNA probe, a chemiluminescent agent is introduced.

DETECTION
The emission of light from the hybridized fragments is then captured by a digitizing camera and stored as image data.

DATA PROCESSING
Using proprietary algorithms, a **RiboPrint™** pattern for each sample is extracted from the image data. This pattern is compared to other **RiboPrint™** patterns stored in the system to characterize and identify the sample.

PRINTED REPORT
A report that characterizes and identifies the bacterium and includes its **RiboPrint™** pattern is automatically printed for your review. Sophisticated data analysis tools allow you to further process the information and share standardized data among your network of users.

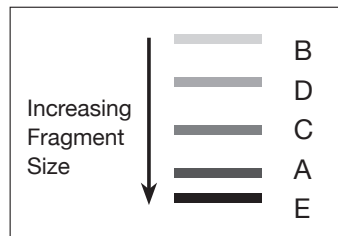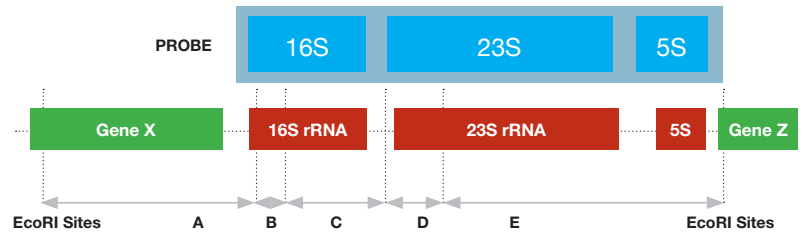# Distinguish More than 290 Genera and Over 1,700 Species

RiboPrint™ patterns characterize environmental isolates, pathogens, spoilage organisms, control strains, beneficial organisms or any bacteria that are important to quality control professionals in the pharmaceutical and food industries, or to epidemiologists and other public health professionals.
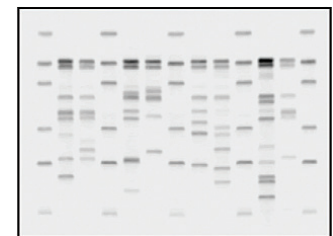
More than 16S Sequencing

The **RiboPrinter® System** automates restriction fragment length polymorphism (RFLP) analysis and targets the RNA-coding region of the bacterial genome. Restriction enzymes, such as *Eco*RI or *Pvu*II, cut bacterial DNA into fragments that are processed to form a characteristic banding pattern or "fingerprint." The system captures an image of the banding pattern and digitizes it as a **RiboPrint™** pattern. This pattern can then be compared to a reference database of patterns from historic samples previously run at a particular location or on a specific instrument.

Rather than just analyzing the region that encodes the 16S rRNA sequence, the **RiboPrinter® System** investigates the regions encoding the 5S, 16S and 23S sequences, as well as the spacer regions and flanking genes on either side. This rich depth of information is what allows highly precise differentiation among strains of the same species, even those with the same 16S sequence.

Highly conserved region of genera variation

Escherichia coli

Staphylococcus aureus

Listeria grayi

Listeria innocua

Region of strain variation

Listeria monocytogenes

Listeria monocytogenes

Listeria monocytogenes

Listeria monocytogenes

Listeria monocytogenes
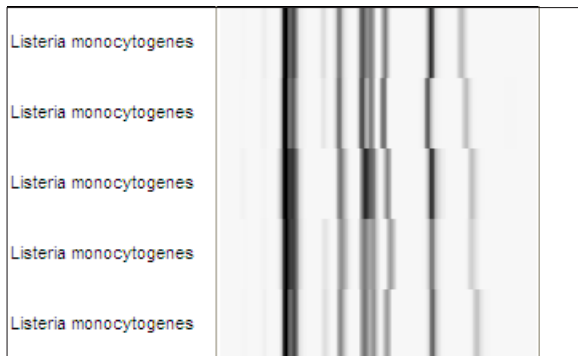
Beyond reliable taxonomic identification, the RiboPrinter® System distinguishes individual strains, providing the granularity of information needed to track, trend and control your environment.

PROBE | 16S | 23S | 5S

Gene X | 16S rRNA | 23S rRNA | 5S | Gene Z

EcoRI Sites | A | B | C | D | E | EcoRI Sites

Increasing Fragment Size

B
D
C
A
E

Fragments from each sample form a banding pattern according to size.

Banding patterns from each sample, along with marker lanes, are captured in a digitized gel image.
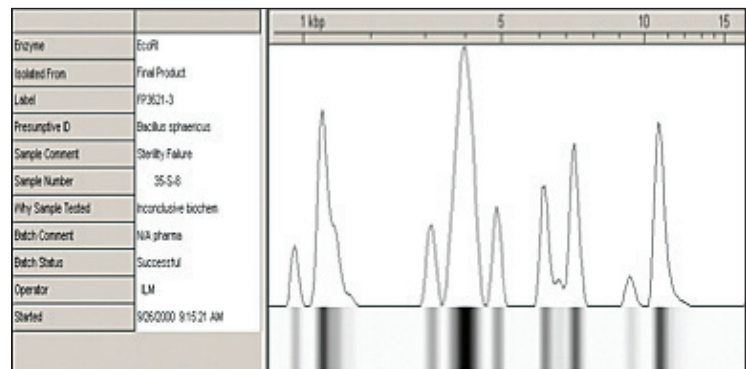
The final report displays the RiboPrint™ pattern along with associated data for each sample.

When Four Popular Tests Show *Salmonella,* the RiboPrinter® System Proves Them Wrong

It was 2 p.m. on a Friday, and after three days of product testing with ELISA, latex agglutination, biochemical screening and serological tests, company microbiologists had determined that their refrigerated, ready-to-eat salads were probably contaminated with *Salmonella*. Company executives feared that recalling the product – a decision that would negatively affect hundreds of vendors in four European countries – was their only option.

Before invoking the recall, the company decided to confirm their internal testing results at an independent laboratory. By early Saturday morning, PCR-based testing with the **BAX® System** had produced startling results: there were no *Salmonella* present in the tested samples.

Isolates were then run on the **RiboPrinter®** System to determine what bacteria was actually present in the samples. Within eight hours, **RiboPrint™** patterns of the isolate showed the bacteria to be *Citrobacter freundii*, a close, but non-pathogenic, cousin of *Salmonella* that has a history of mimicking its harmful relative when tested using biochemical assays.

The near-calamity of a product recall had been narrowly averted, and the company realized a savings of nearly $350,000 in lost product alone. In addition, the company avoided the negative press, loss of consumer trust and brand damage associated with recalls.

A product recall shouldn't depend on anything but the most definitive microbial information available. No biochemical test is as accurate as genetic information for definitively determining if contamination has occurred.

Monitoring Microbes from Your Suppliers

A company selling high-end prepared foods wanted an efficient and accurate due-diligence defense to help ensure product safety against microbial growth. They began using the **RiboPrinter® System** to establish a customized database for monitoring Listeria, Escherichia coli, Salmonella and Staphylococcus aureus.

When the company discovered a trend of non-pathogenic *Listeria welshimeri* in a number of its fancy, ready-to-eat cream cakes, the **RiboPrint™** patterns from their monitoring method provided enough information to determine that this particular strain of *Listeria* was found only in samples from a single supplier. Confronted with this evidence, the supplier immediately began an audit of the cake-making process to determine the contamination source using the **RiboPrinter® System**.

After a microbial analysis of the production area, the results led the supplier to a vat of cream suspended above the production line. While the vat was kept very cold to store the cream, the surrounding warm, humid air was causing condensation to form on the pipe leading from it. When the condensation dripped onto the cakes below, *Listeria welshimeri* found on the pipe went with it to contaminate the final product.

With the source of the contamination identified and targeted cleaning accomplished, the company that sold the cakes was assured that its supplier's problem had been resolved. The supplier's reputation with its customer was restored by using this definitive, fast information.

hygiena.com