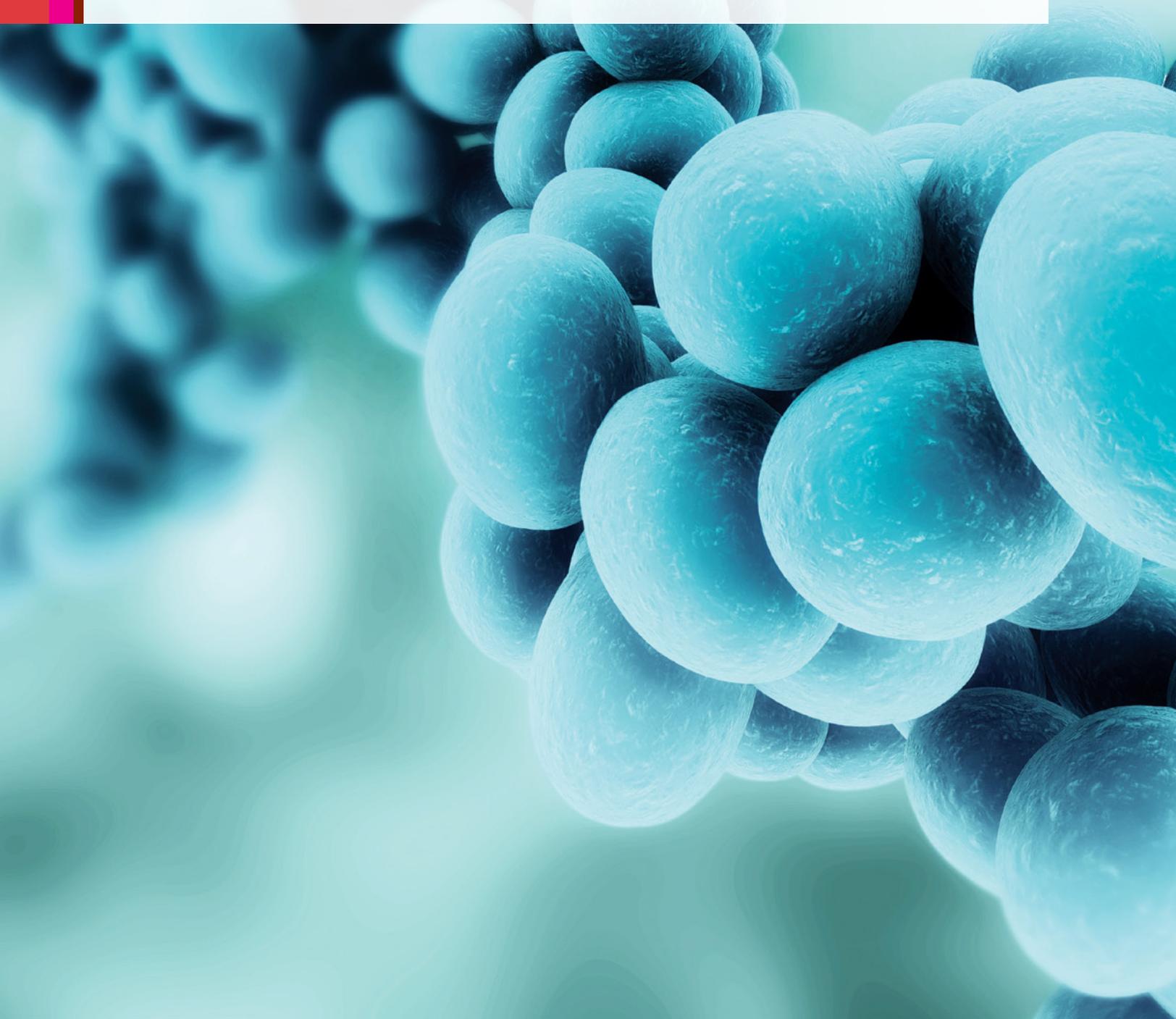




The miracles of science™

DUPONT™ RIBOPRINTER® SYSTEM
POWERFUL MICROBIAL MONITORING
NAME IT. TRACE IT. CONTROL IT.



GO BEYOND IDENTIFYING AND DOCUMENTING A PROBLEM—GAIN CONTROL OF YOUR PROCESS AND ENVIRONMENT WITH THE DUPONT™ RIBOPRINTER® SYSTEM



Only the RiboPrinter® System from DuPont Nutrition and Health combines automation with the power of DNA to accurately identify 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 HELPS HOSPITAL FIND SOURCE OF DANGEROUS BACTERIA

When several babies in a hospital's neonatology unit were diagnosed with septicemia, epidemiologists focused on finding the source quickly and accurately.

Samples taken from the babies and from items in the nursery—including the new incubators that had recently been added—were examined by hospital staff microbiologists using traditional biochemical methods. Although bacteria such as *Pseudomonas fluorescens* were found in a number of locations in the nursery, the biochemical tests indicated that the babies were all infected with *Ralstonia pickettii*.

The hospital sought assistance from an external expert lab that used the RiboPrinter® System. These genomic scientists quickly found that the organism making the babies sick did not match *Pseudomonas fluorescens* or *Ralstonia pickettii* identification patterns. Its genetic fingerprint was identical to one from the humidifying reservoir of the new incubators. However, the water supply coming into the incubator did not contain an organism with that fingerprint.

Further DNA sequencing identified the bacteria as MC5, an organism that is very closely related to *Ralstonia solanacearum* and was formerly a member of the *Pseudomonas* family. MC5, a strain so new that it didn't have a name, is known to degrade plastic. This led investigators to conclude that the bacteria was most probably leaching from the incubator's plastic reservoir into the water that was being used to humidify the nursery.

The RiboPrinter® System helped to accurately determine the source of infections, enabling the hospital to take quick action in replacing the incubators and preventing additional cases of septicemia among these fragile patients.

AN AUTOMATIC SOLUTION FOR IDENTIFYING AND CHARACTERIZING BACTERIA IN ANY ENVIRONMENT



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



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



DuPont ID	Label	Similarity to DuPont ID QC-101	RiboPrint® Pattern			
			1 kbp	5	10	15 50
DUP_FVUII-2049	Salmonella ser. Hilbrow	0.38				
DUP-16090	Haemophilus influenzae	0.10				
DUP-16400	Neisseria meningitidis	0.04				
DUP-18390	Aeromonas species	0.09				
DUP_FVUII-3266	Streptomyces aureofaciens	0.17				
DUP-18221	Paracoccus denitrificans	0.35				

IDENTIFICATION OF GENUS AND SPECIES

Taxonomic identification is automatically provided by comparing the unknown isolate against an onboard database of about 7,000 patterns, covering more than 180 genera and 1,400 species.

Source	Number	Label	RiboPrint™ Pattern			
			1 kbp	5	10	15 50
End Product	DUP-16836	Bacillus subtilis				
Hands	DUP-12545	Bacillus subtilis				
Discharge Line	DUP-18405	Bacillus subtilis				
ATCC 6051	DUP-9501	Bacillus subtilis				
Raw material 473	DUP-18116	Bacillus subtilis				
Raw material 811	DUP-16836	Bacillus subtilis				
Bench surface 92	DUP-18513	Bacillus subtilis				

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.

RIBOPRINTER® SYSTEM HELPS PHARMACEUTICAL COMPANY RULE OUT WORKER AS SOURCE OF CONTAMINATION

A manufacturer of high-quality eye care products compared traditional biochemical methods to the RiboPrinter® System to demonstrate the difference in dependable microbial information.

First, the company examined two *Staphylococcus* isolates, one from a sterility test failure and the other from the garment of a line worker.

Conventional testing with both a fatty-acid profile and a colorimetric result indicated that the isolates were *S. haemolyticus*.

The RiboPrinter® System was able to further discriminate and automatically establish that they were indeed different strains of *S. haemolyticus*.

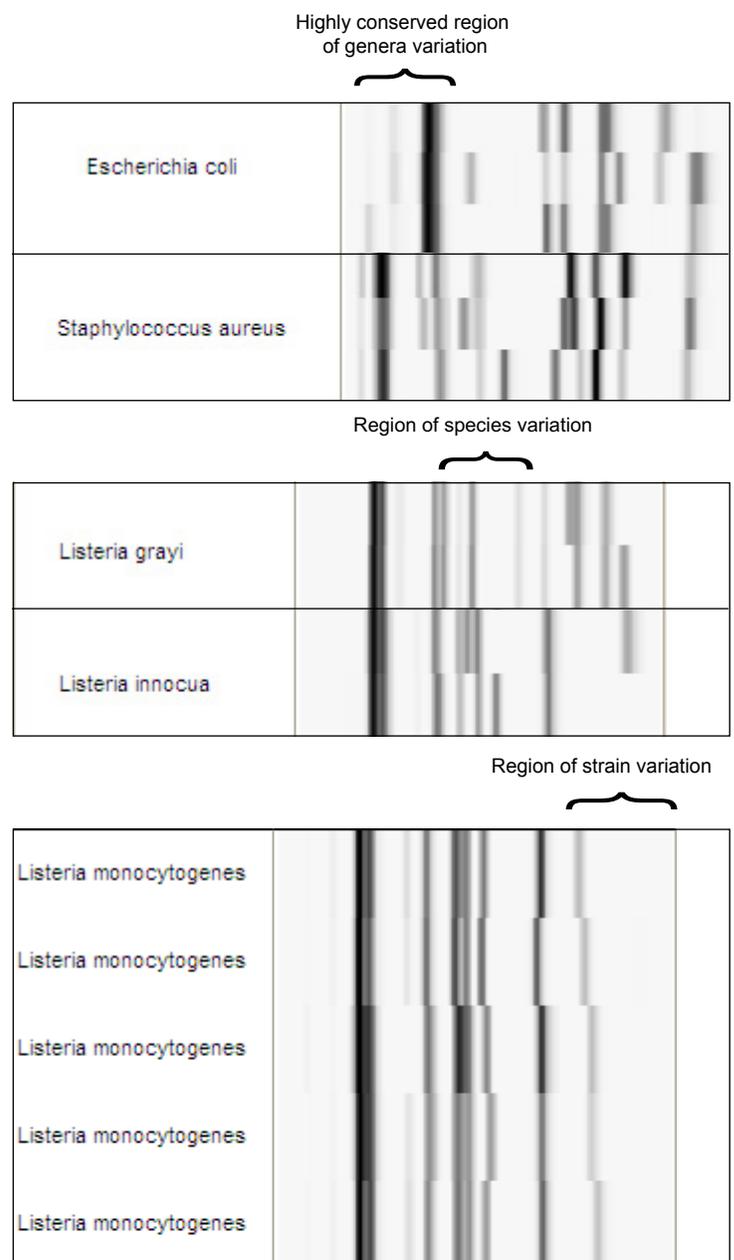
These results showed that the sterility failure was not the result of contamination by the line worker. The company needed to look elsewhere for its culprit.

Using the RiboPrinter® System proved to be more helpful than relying on information from other tests not capable of giving as much information, especially beyond the species level.

The system allows this manufacturer to have greater confidence in the definitiveness of their microbial information.

THE DUPONT™ RIBOPRINTER® SYSTEM COMPARES HIGHLY CONSERVED REGIONS OF THE GENOME TO DISTINGUISH MORE THAN 180 GENERA AND OVER 1,400 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.

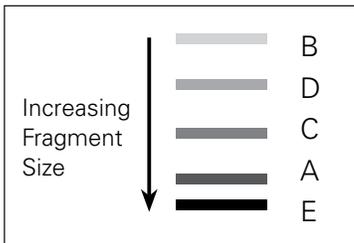
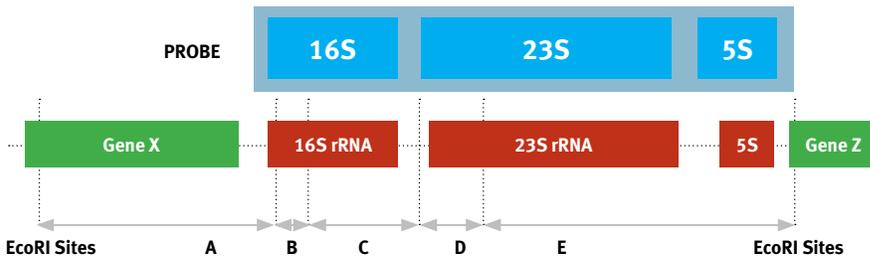


Beyond reliable taxonomic identification, the RiboPrinter® System distinguishes individual strains, providing the granularity of information needed to track, trend and control your environment.

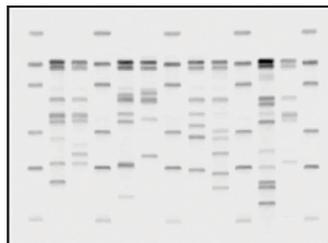
MORE THAN 16S SEQUENCING

The DuPont™ RiboPrinter® System automates restriction fragment length polymorphism (RFLP) analysis and targets the rRNA-coding region of the bacterial genome. Restriction enzymes, such as EcoRI or PvuII, 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.

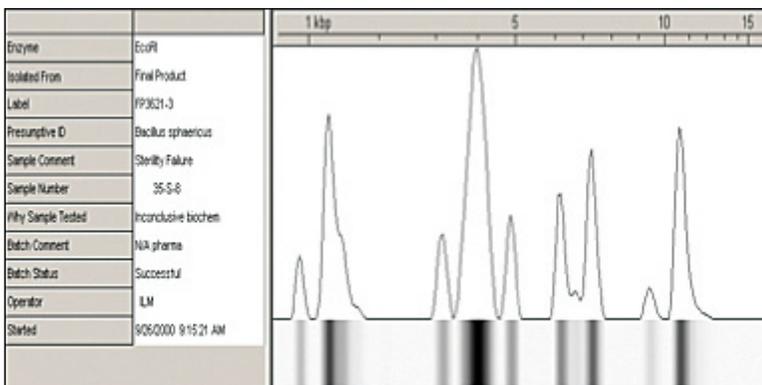
The RiboPrinter® System technology investigates a much larger portion of the bacterial genome than other bacterial identification systems can. Rather than just analyzing the region that encodes the 16S rRNA sequence, the RiboPrinter® System investigates the regions encoding the 5S, 16S and 23S sequences, 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.



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.

RIBOPRINTER® SYSTEM HELPS BREWER MONITOR NOVEL BEER SPOILAGE SPECIES

Researchers at a large brewing company conducted a survival study of environmental organisms using several molecular methodologies, including the RiboPrinter® System.

For the study, they spiked beer with 170 strains of *Lactobacillus*. Of these 170 strains, 55 were able to survive, including 45 strains of *L. brevis*, 3 strains of *L. lindneri*, 1 strain of *L. casei* and 6 unknown strains that were not identified by either PCR or a fluorescent staining assay.

The RiboPrint™ patterns of the six unknown strains did not fall within the RiboGroups for other *Lactobacillus*. Instead, they formed two new RiboGroups of three strains each, which the brewer called LA-2 and LA-6.

Analysis with 16S sequencing indicated that LA-2 had a >99% similarity to *L. collinoides*. Although results from the 16S analysis suggested a high level of relatedness, the RiboPrint™ patterns for LA-2 were distinctively different from those for *L. collinoides*.

In the case of LA-6, the 16S sequencing could not match the isolates to any organism, and therefore provided no additional information.

Encouraged by the much finer discrimination of RiboPrint™ patterns, the researchers felt they could now monitor these two environmental spoilage organisms through routine use of the RiboPrinter® System.

DUPONT™ RIBOPRINTER® SYSTEM HELPS PRODUCER OF INFANT FORMULA KEEP PRODUCT SAFE

A producer of infant formula found that its finished product was consistently contaminated with low levels of *Enterobacter sakazakii*.^{*} The company realized that the bacteria could grow rapidly in reconstituted milk and they wanted to understand how the product became contaminated.

Quality control personnel were able to isolate *E. sakazakii* frequently in products from two factories and infrequently in products from a third. Although the production processes were essentially the same in each of the three factories, the operations and layout varied considerably.

A microbial survey of raw materials and environmental swabs at each site yielded several hundred samples of *E. sakazakii*. Preliminary screening with biochemical methods was not helpful in tracking the contamination, but the RiboPrinter® System was able to discriminate at the strain level, allowing investigators to see which strains were confined to a single site and which ones were common to all three factories.

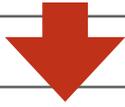
Important trends also became evident. For example, a significant source of contamination was discovered when investigators determined that resident flora of a specific type of *E. sakazakii* had been able to evade standard cleaning and sanitation regimens.

The company changed its cleaning practices to address this issue and built the information into the database for this organism. By monitoring for changes in the flora at the strain level, the team can recognize a problem in process or hygiene practices and quickly direct cleaning crews and engineers to the area of concern.

The RiboPrinter® System was invaluable in helping this company quickly, definitively address the source and route of contamination throughout its complex manufacturing operations.

^{*} Note: The organism *Enterobacter sakazakii* was re-classified in June 2008 into the new genus *Cronobacter*. The genus *Cronobacter* is synonymous with *Enterobacter sakazakii*.

MAP YOUR FACILITY AND RUN SAMPLES ON THE RIBOPRINTER® SYSTEM
Keep track of samples and easily sort RiboPrint™ patterns with simplified software.



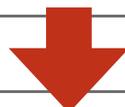
LEARN SAMPLE IDENTITY
Genus and species are automatically provided by comparing the RiboPrint™ pattern to the onboard identification database of about 7,000 patterns covering more than 1,400 species.



CREATE A CUSTOM ID DATABASE
Optionally assign genus and 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 using 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 the frequency of uploads and the processes that are performed on the data.

THE DUPONT™ RIBOPRINTER® SYSTEM IS SELF-CONTAINED, RELIABLE AND EASY TO USE

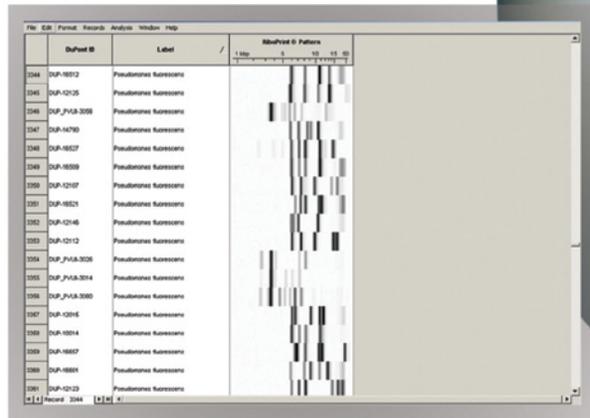
CHARACTERIZATION UNIT

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.



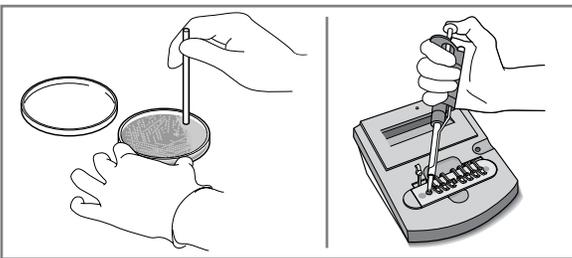
WORKSTATION

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 keyboard, mouse, monitor, CD R/W, laser printer and uninterruptible power supply. The proprietary software provides user-friendly prompts, process monitoring, diagnostics and complete data analysis.



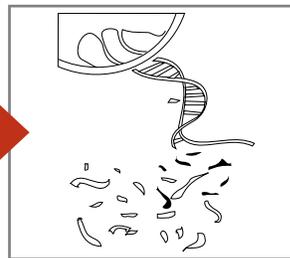
FAST, AUTOMATED ANSWERS WITH MINIMAL LABOR REQUIRED

After 30 minutes of simple preparation, the RiboPrinter® System automates the rest of the process, reducing the labor required and minimizing errors due to technique. And, with results in just 8 hours, the RiboPrinter® System allows understanding of contamination in a fraction of the time it would normally take.



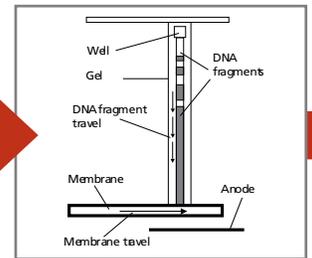
SAMPLE PREPARATION

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.



SEPARATION AND TRANSFER

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

MEMBRANE

Captures the DNA fragments for final processing.

PURIFIED WATER

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

SAMPLE CARRIER

Holds eight samples.

DNA PREP PACK

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

GEL CASSETTE

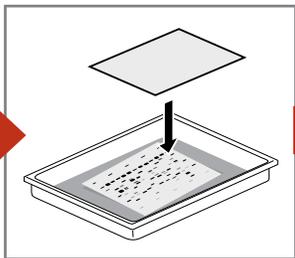
Holds precast electrophoresis gel. Separates DNA fragments into discrete bands.

MP BASE AND INSERTS

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

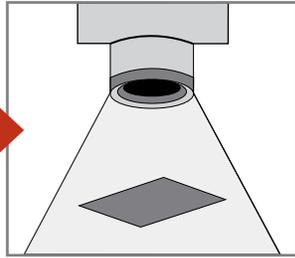
SAMPLE PREPARATION EQUIPMENT

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.



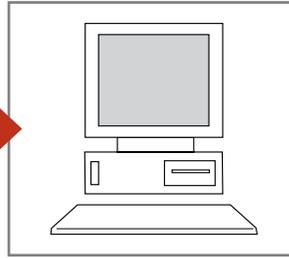
MEMBRANE PROCESSING

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.

Sample Number	Accession Point	Label	Default Label	RiboPrint™ Pattern
00000001.0	Micro Array 1	00001	Bacterium 1	[Pattern]
00000002.0	Micro Array 2	00002	Bacterium 2	[Pattern]
00000003.0	Micro Array 3	00003	Bacterium 3	[Pattern]
00000004.0	Micro Array 4	00004	Bacterium 4	[Pattern]
00000005.0	Micro Array 5	00005	Bacterium 5	[Pattern]
00000006.0	Micro Array 6	00006	Bacterium 6	[Pattern]
00000007.0	Micro Array 7	00007	Bacterium 7	[Pattern]
00000008.0	Micro Array 8	00008	Bacterium 8	[Pattern]

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.

EVERYTHING YOU NEED FOR FAST, AUTOMATED ANSWERS

SYSTEM COMPONENTS

Characterization unit

Computer workstation

Printer

Heat treatment station

SYSTEM DISPOSABLES

Sample carriers and buffer

Colony picks

Gel cassettes and membranes

Restriction enzymes—choose EcoRI, PvuII or your own custom enzyme

Lysing agents

Probes, including IS6110

Conjugate, base and substrate

DNA prep pack

VALIDATION PACKAGES

For regulated industries

Detailed protocols already established

DATA MERGING WORKSTATION

Computer with enhanced software

Centralize data from multiple systems into one database

REMOTE CLIENT SYSTEM

Computer with analysis software only

For use outside the lab



ABOUT DUPONT NUTRITION & HEALTH

DuPont Nutrition & Health is a business dedicated to delivering a wide range of sustainable, bio-based ingredients and advanced molecular diagnostics, formerly available under the Qualicon name, to provide safer, healthier and more nutritious food.

Pharmaceutical, cosmetics and personal care companies can also use innovative diagnostics, such as the DuPont™ RiboPrinter® System, to help gain control of their manufacturing environment. Beyond identifying and documenting contamination problems, this automated method generates strain-level “fingerprints” that quickly help trace the source and track the spread of unwanted bacteria.

For more information on microbial monitoring systems from DuPont, please see www.microbialmonitoring.dupont.com



The miracles of science™