طرق الفحص السريعة للكشف عن الممرضات البكتيرية المنتقلة عن طريق الأغذية

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Contents:

طرق الكشف عن الممرضات البكتيرية

- **Detection Methods of Bacterial Pathogens.**

محددات بعض الفحوصات البكتيرية

- **The Limitations of Some Microbial Tests.**

طرق الحرارية

- **Thermal DNA Amplification Methods.**
  - Polymerase Chain Reaction (PCR).
  - Real - time PCR (Quantitative –PCR).
    - Bax Screening System Q7 (Qualicon).
    - IQ Real –time PCR (Bio-Rad).
    - ADIA Food (AES Chemunex).

طرق غير الحرارية

- **Isothermal DNA Amplification Methods.**
  - Molecular Detection Assay (3M).
  - ANSR System (Neogen).

طرق التنميط الجزيئي

- **Molecular Typing Methods.**
  - Ribotyping - Riboprinter System (Qualicon).
  - Pulsed Field Gel Electrophoresis (PFGE).

طرق الكشف عن الممرضات البكتيرية

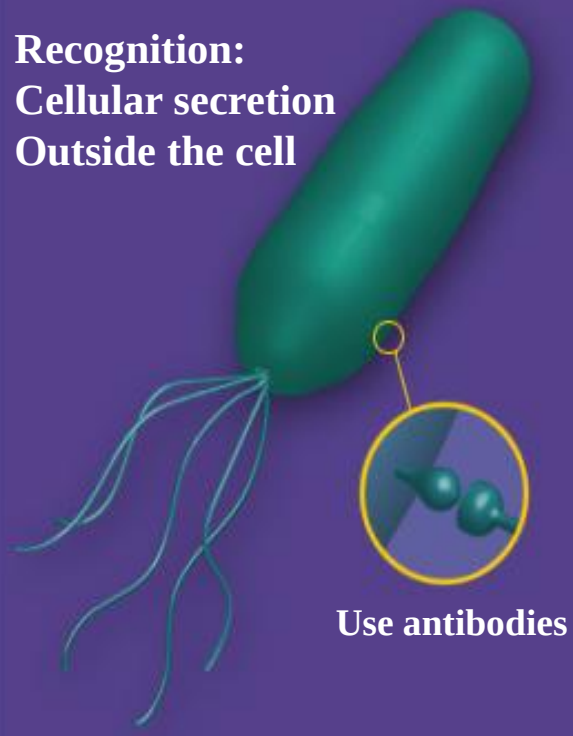
Traditional Microbiology

Recognition: Colonies of cells

Use visual analysis (color, size, etc.)

Serological test Immunoassays

Recognition: Cellular secretion Outside the cell

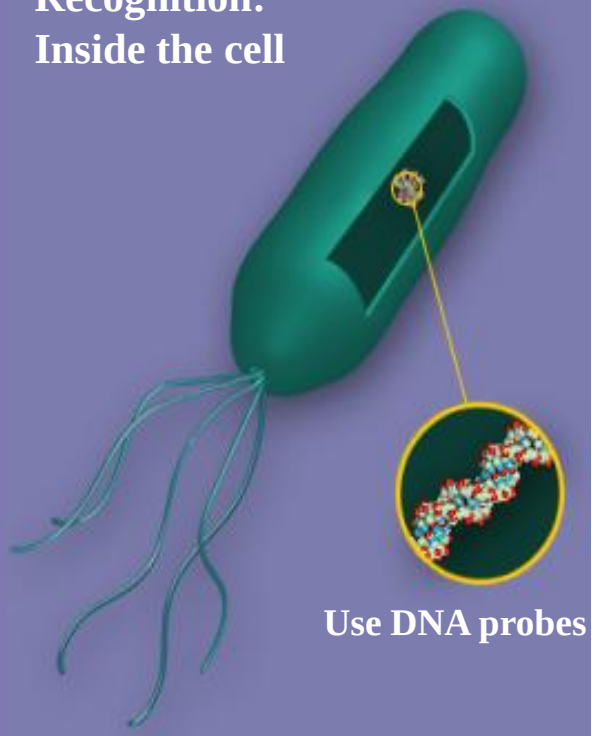


Use antibodies

Use Substrates

DNA Based

Recognition: Inside the cell



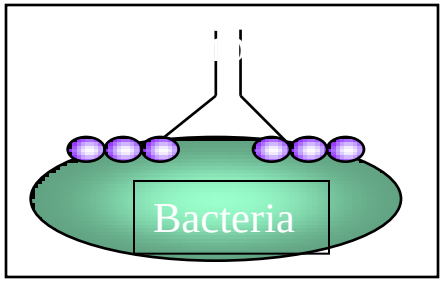
Use DNA probes

تطور تقنيات الكشف عن الممرضات البكتيرية

Traditional Microbiology

Specificity: Low

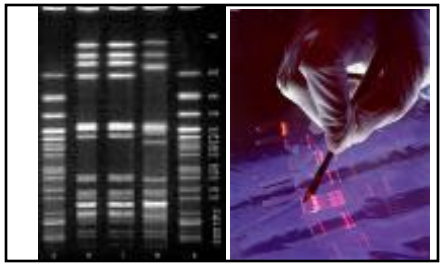
Sensitivity: Low



Immunoassay

Specificity: Hi

Sensitivity: Low

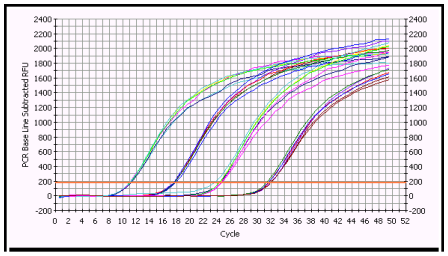


PCR

(Requires post-PCR processing)

Specificity: Hi

Sensitivity: Hi

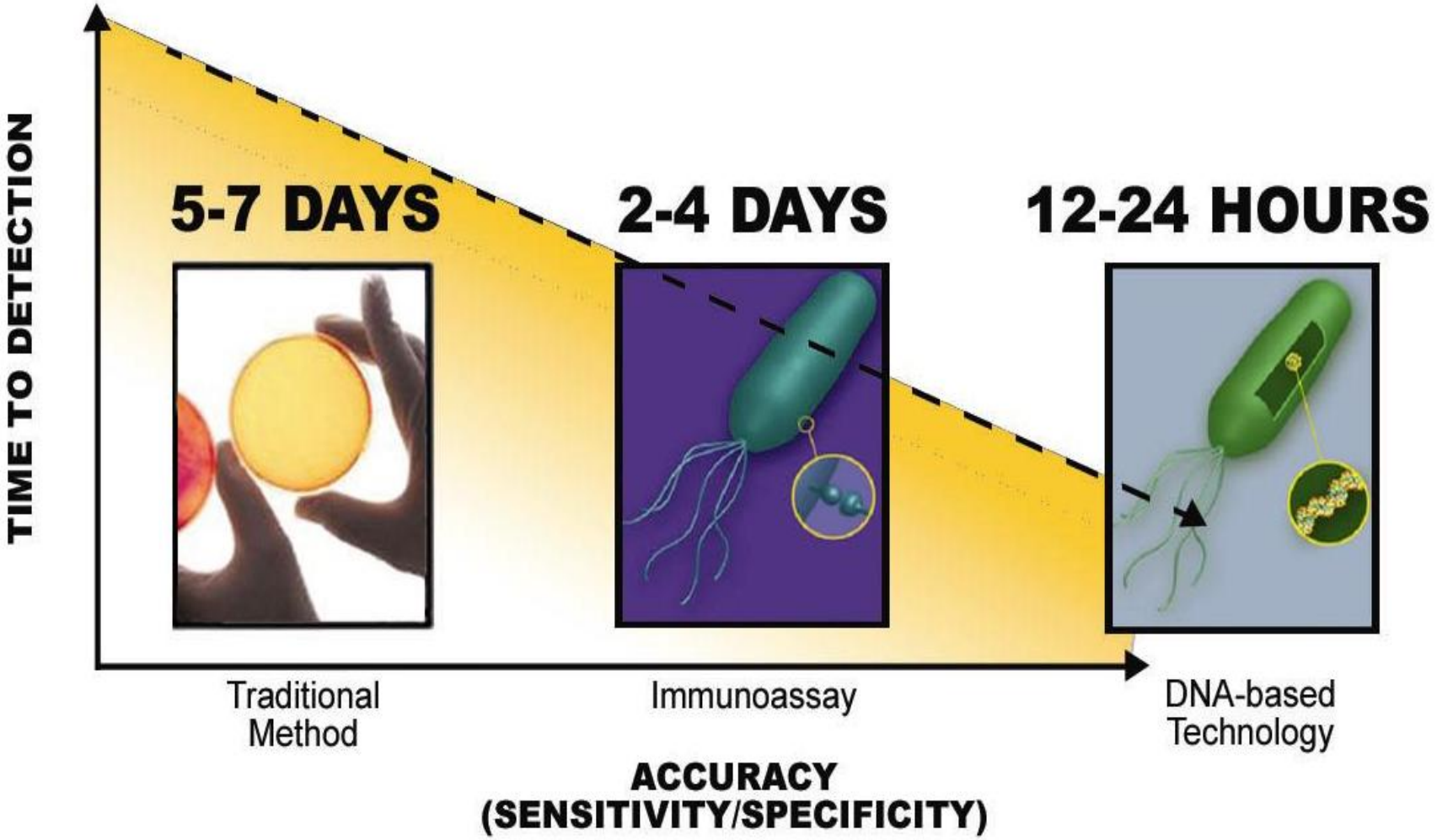


Real-Time PCR

(No post-PCR processing
Results are processed as they happen)

Specificity: Hi

Sensitivity: Hi


TIME TO DETECTION
5-7 DAYS
2-4 DAYS
12-24 HOURS
Traditional Method
Immunoassay
DNA-based Technology
ACCURACY
(SENSITIVITY/SPECIFICITY)

محددات بعض الفحوصات المايكروبية

Biochemical Tests الفحوصات الكيموحيوية

Often **lack discriminatory power** because of **variations gene expression** and **random mutations** that may alter biologic properties of microorganisms.

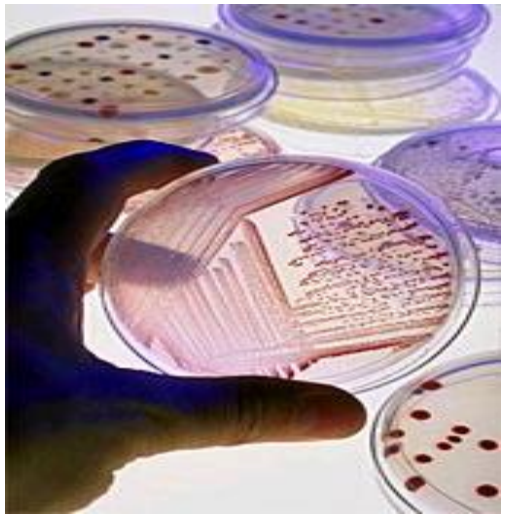
Serological Tests الفحوصات المصلية

Limitations of serotyping include a **lack of availability of certain antisera** and **difficulties with standardization** of different methods.

Bacteriophage Tests الفحوصات المعتمدة على البكتيريوفاج

have some weakness due to a **lack of widespread availability of biologically active phages** and **the technical difficulty** of performing the technique. Moreover, Bacteriophage typing is **labor-intensive** and the method often demonstrates **poor reproducibility and standardization**.

بأستخدام الطرق المعتمدة على الحامض النووي يمكن تغيير طرق الفحص



From Appearances

to DNA

Thermal DNA Amplification Methods:
- Polymerase Chain Reaction (PCR)

is a revolutionary method developed by **Kary Mullis in the 1980s**.

PCR is a biochemical in vitro reaction that permits the synthesis of large quantities of a targeted nucleic acid sequence.

Because DNA polymerase can add a nucleotide only onto a preexisting 3'-OH group, it needs a **primer** to which it can add the first nucleotide.

This requirement makes it possible to define a specific region of template sequence that the researcher wants to amplify.

At the end of the PCR reaction, the specific sequence will be accumulated in billions of copies (amplicons).

Consumables:

-DNA template

-Polymerase

-Nucleotides (d ATP/ d TTP/d CTP/d GTP)

-Primers

-Ions (e.g., Mg^{2+} in soluble complex with dNTPs)

-Buffer

For Rapid Methods (DNA - Based)

Step 1: Enrichment.

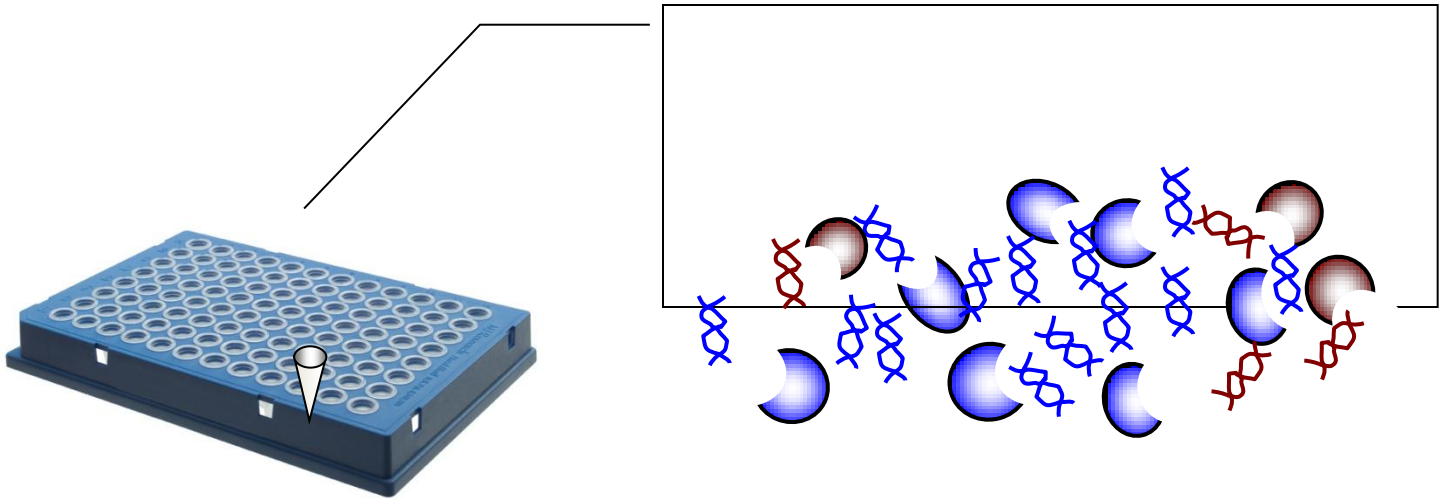
Sample collection

Enrichment medium

Homogenize

Incubate

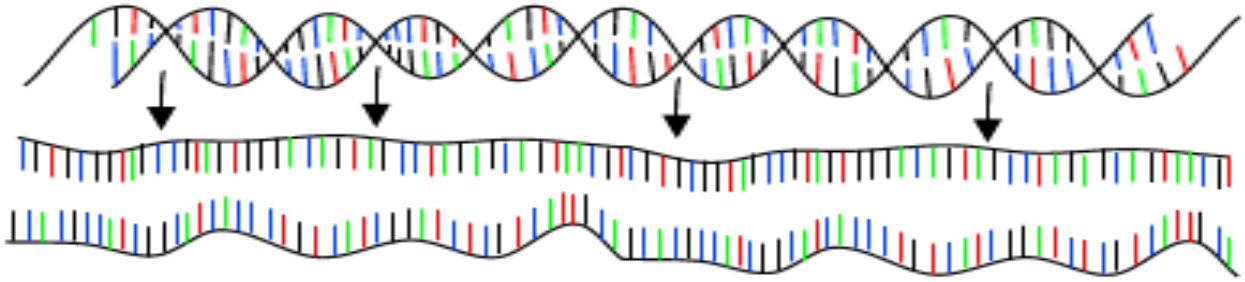
Step 2: Extraction:



Bacterial cells are lysed → DNA released into the solution

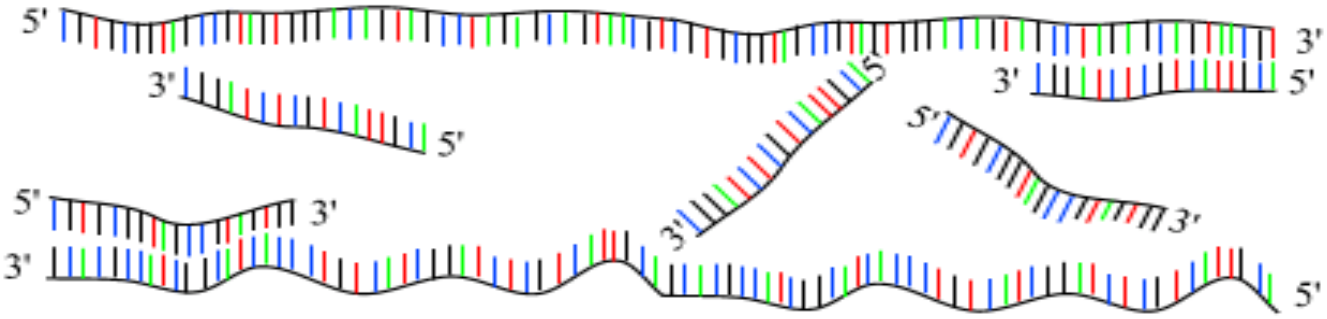
PCR : Polymerase Chain Reaction

30 - 40 cycles of 3 steps :



Step 1 : denaturation

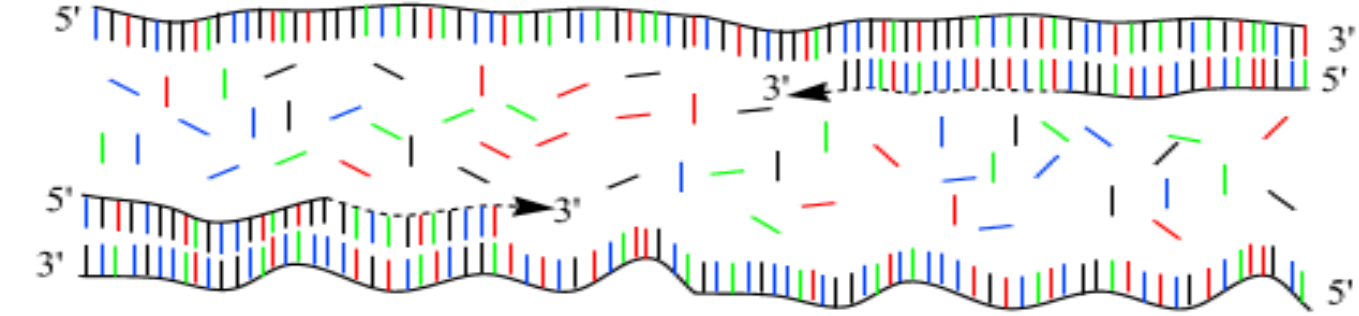
1 minut 94 °C



Step 2 : annealing

45 seconds 54 °C

forward and reverse primers !!!



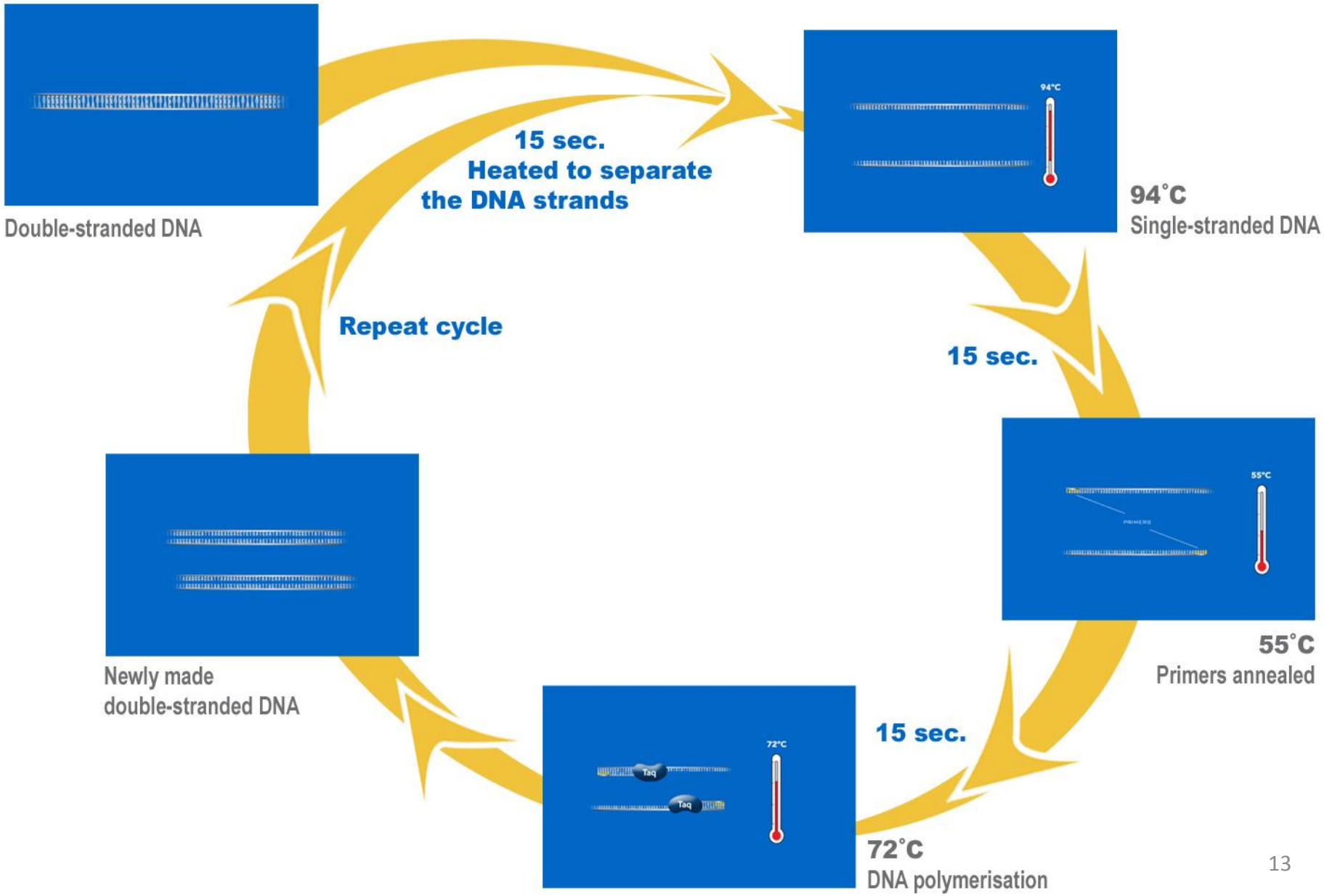
Step 3 : extension

2 minutes 72 °C

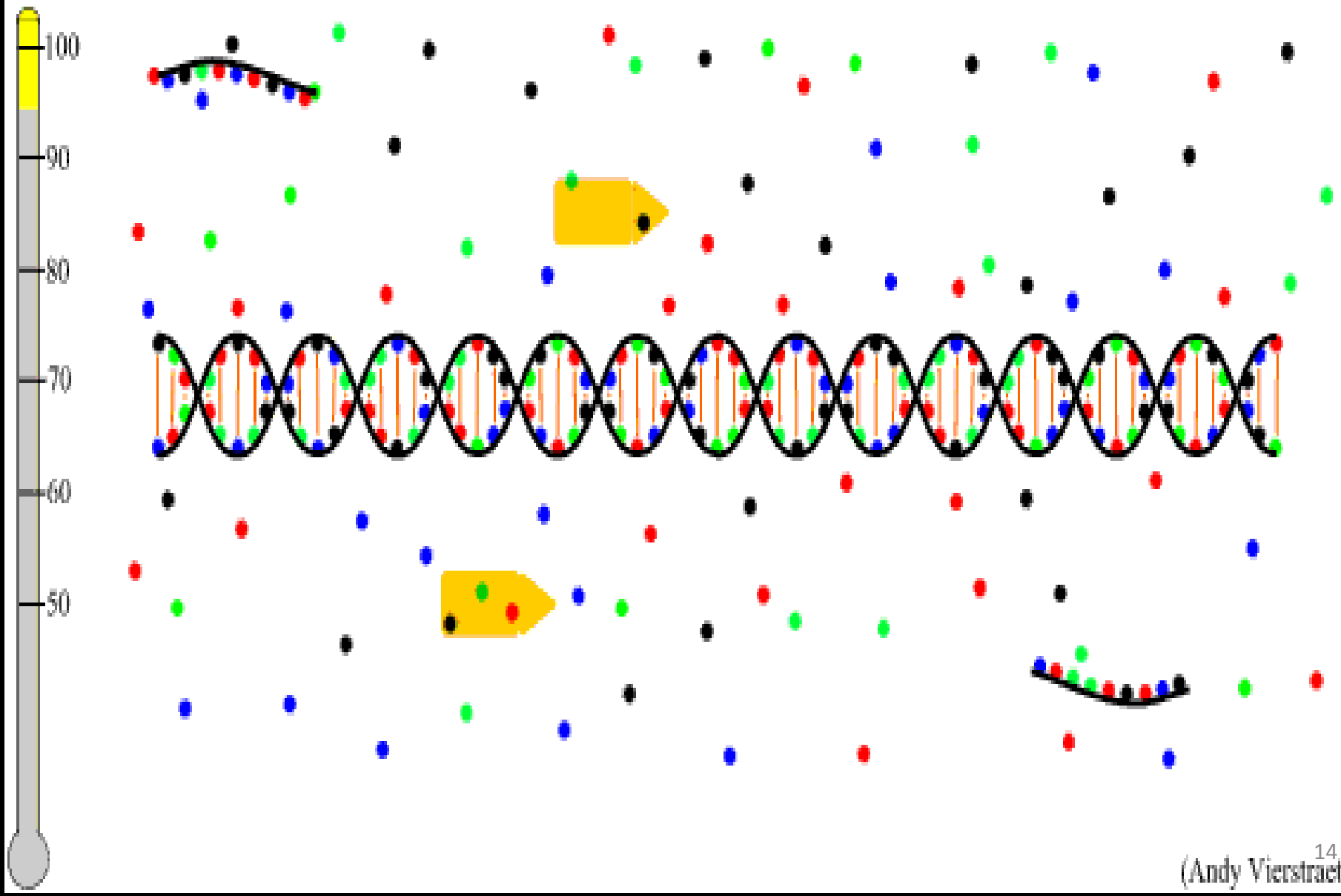
only dNTP's

(Andy Vierstraete 1999)

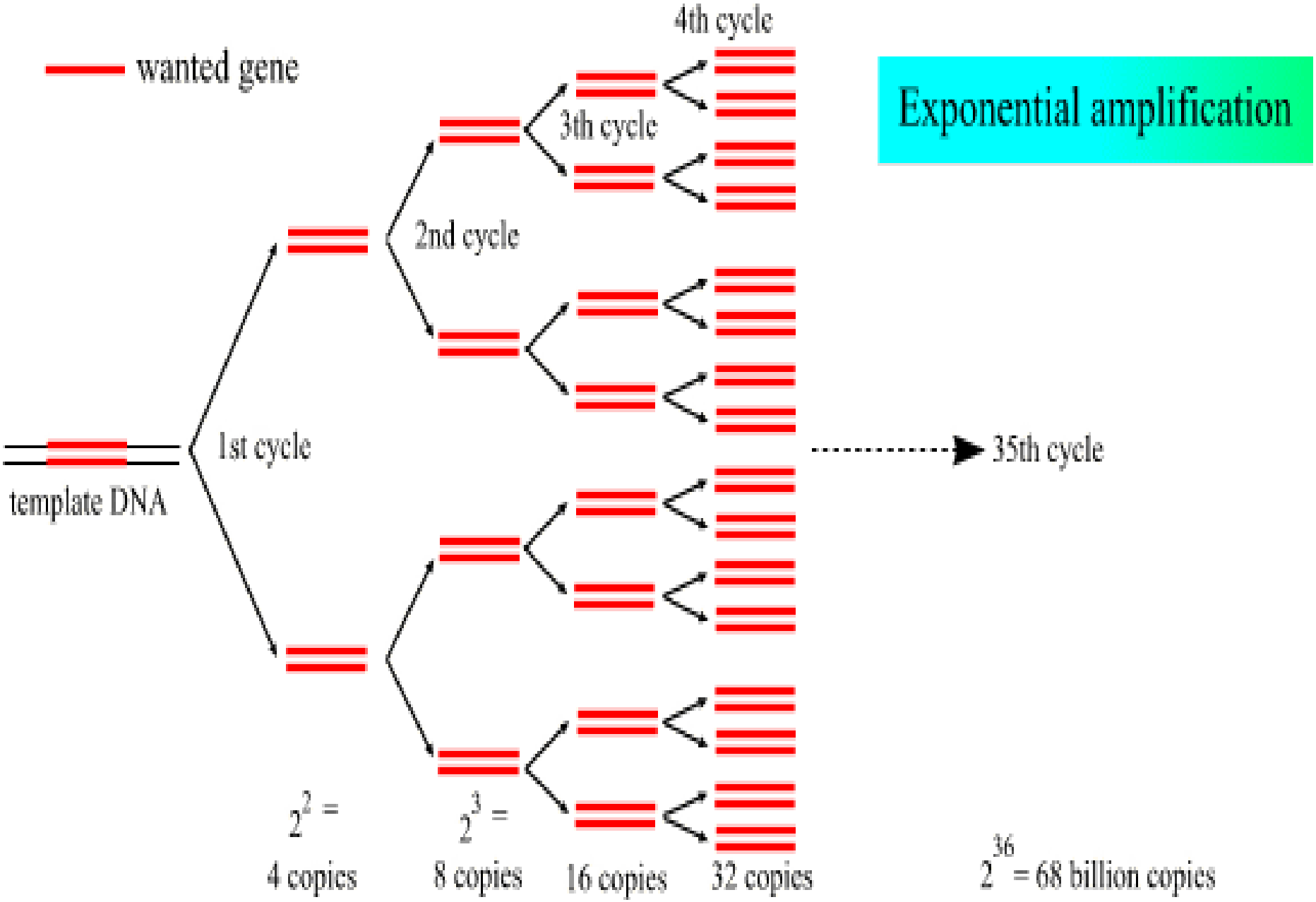
PCR Technique:



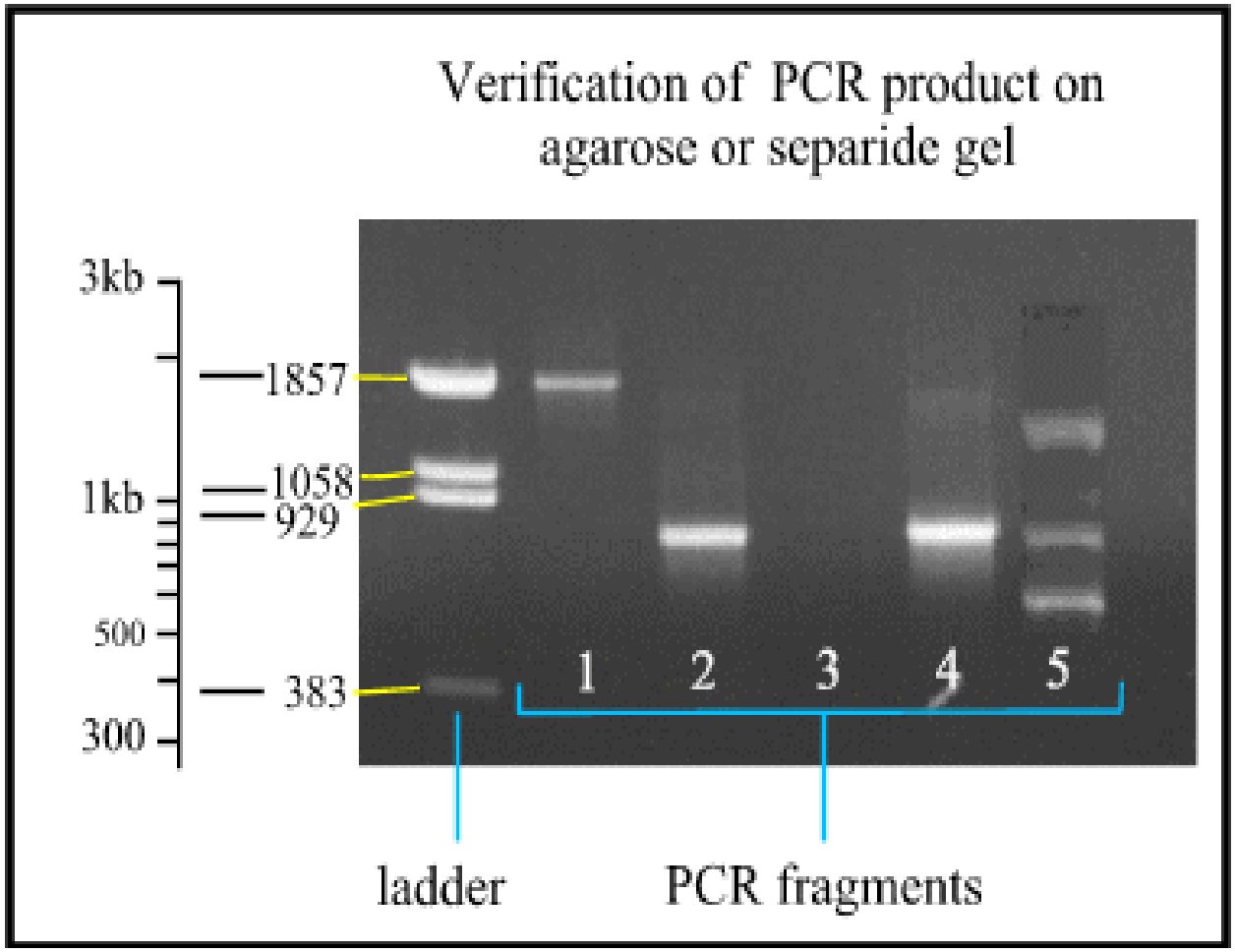
PCR : Denaturation 94°C



(Andy Vierstraete 1999)



(Andy Vierstraete 1999)


Verification of PCR product on
agarose or separide gel
3kb
1kb
500
300
1857
1058
929
383
1
2
3
4
5
ladder
PCR fragments

Multiplex PCR:

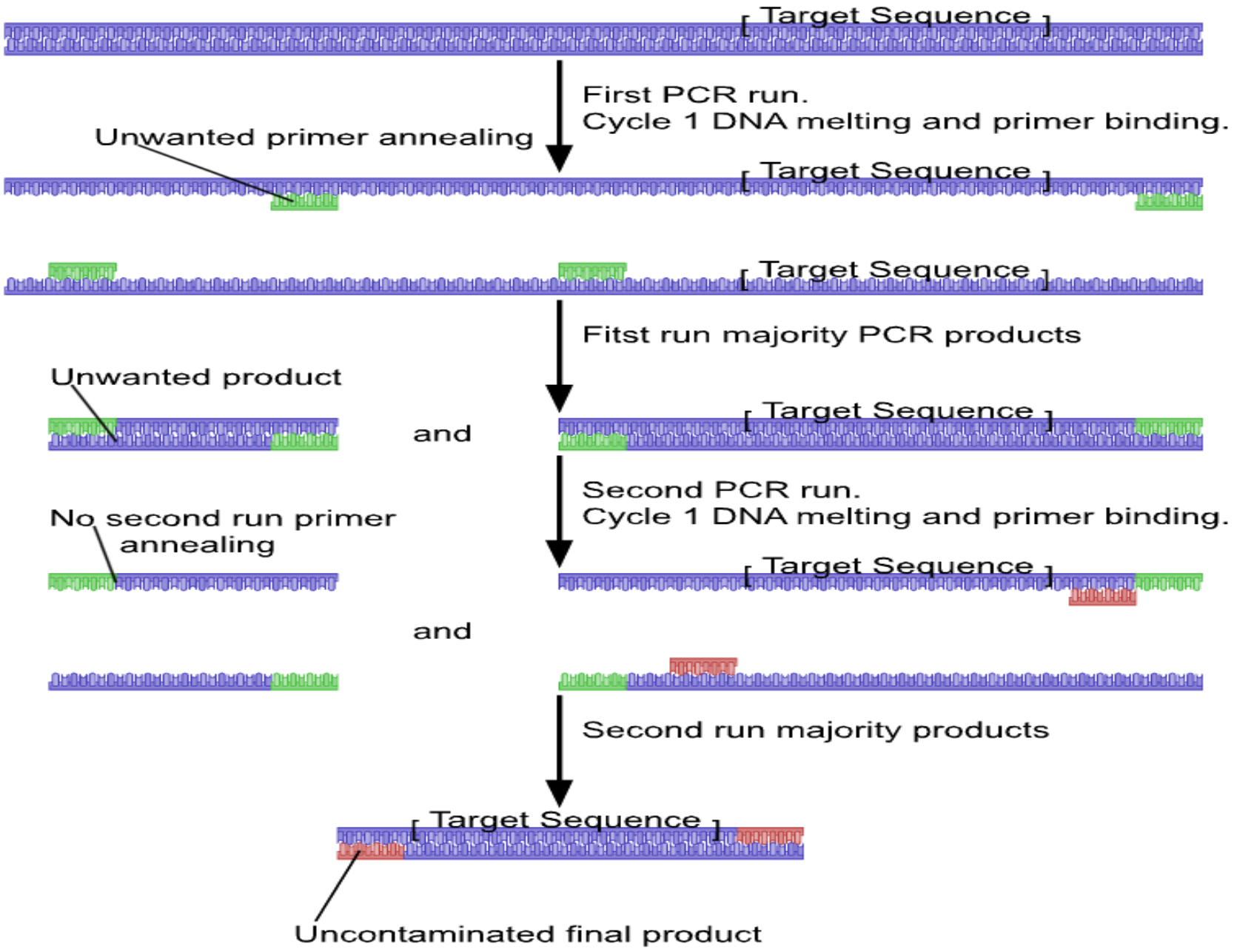
Multiple sets of primers can be included in a single reaction tube in order to increase the efficiency of PCR typing and cut down on reagent costs .

A key strategy in the development of that assay is the design of the primers. Primers must be designed such that **all of the primers have very close annealing temperature** optimums and the amplification products that they produce need to be noticeably different sizes to facilitate interpretation.

Nested PCR :

Intended to **reduce the contaminations in products due to the amplification of unexpected primer binding sites.**

Two sets of primers are used in two successive PCR runs, the **second set intended to amplify a secondary target** within the first run product. This is very successful, but requires more detailed knowledge of the sequences involved.


Target Sequence
First PCR run.
Cycle 1 DNA melting and primer binding.
Unwanted primer annealing
Target Sequence
Target Sequence
Fitst run majority PCR products
Unwanted product
and
Target Sequence
Second PCR run.
Cycle 1 DNA melting and primer binding.
No second run primer annealing
Target Sequence
and
Second run majority products
Target Sequence
Uncontaminated final product

- Quantitative Real-time PCR:

- Real time - PCR is a modification of conventional PCR methodology that **eliminates the need for gel electrophoresis, thus providing results more quickly.**

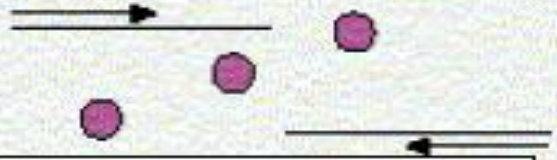
- This method uses **fluorescent dyes and probes** to measure the amount of amplified product in real time by monitoring fluorescence.

SYBR Green Dye Assay

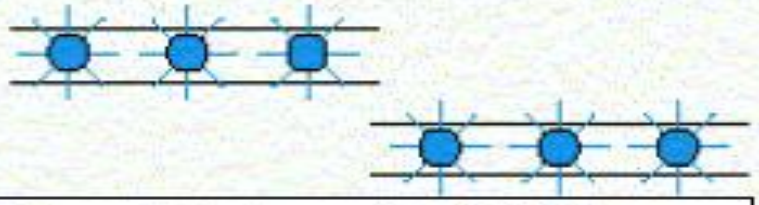
SYBR Green 1 assay

SYBR Green dye attaches when there is double stranded DNA.

When the DNA is denatured the SYBR Green Dye floats free.

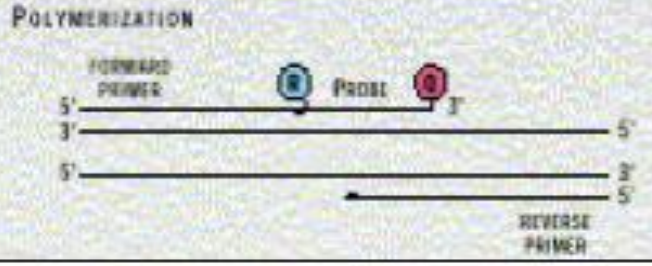


Extension phase begins as primers anneal.

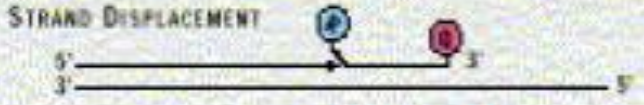


Polymerization is complete. SYBR Green Dye binds to the double stranded product and fluoresces.

5' Nuclease-Taq Man

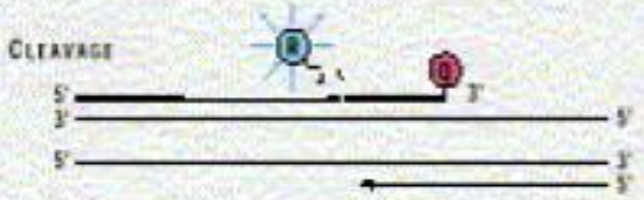


TaqMan Probe and Primers anneal and extension begins.

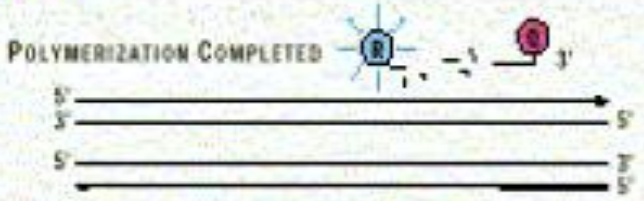


When the enzyme reaches the probe, the 5' nuclease activity of the enzyme begins to displace the probe.

is quenched.



Cleavage of the probe begins and the Reporter starts to fluoresce as it is separated from Quencher.

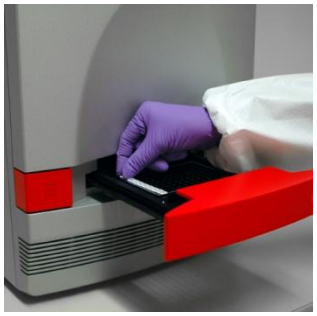


Detection of Food-borne Pathogens Using Bax Screening System Q7

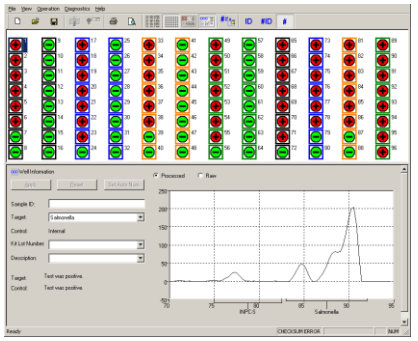
BAX® System Protocol
Requires 3 Major Steps for *Salmonella*

On enriched/re -grown samples…

1. Prepare samples

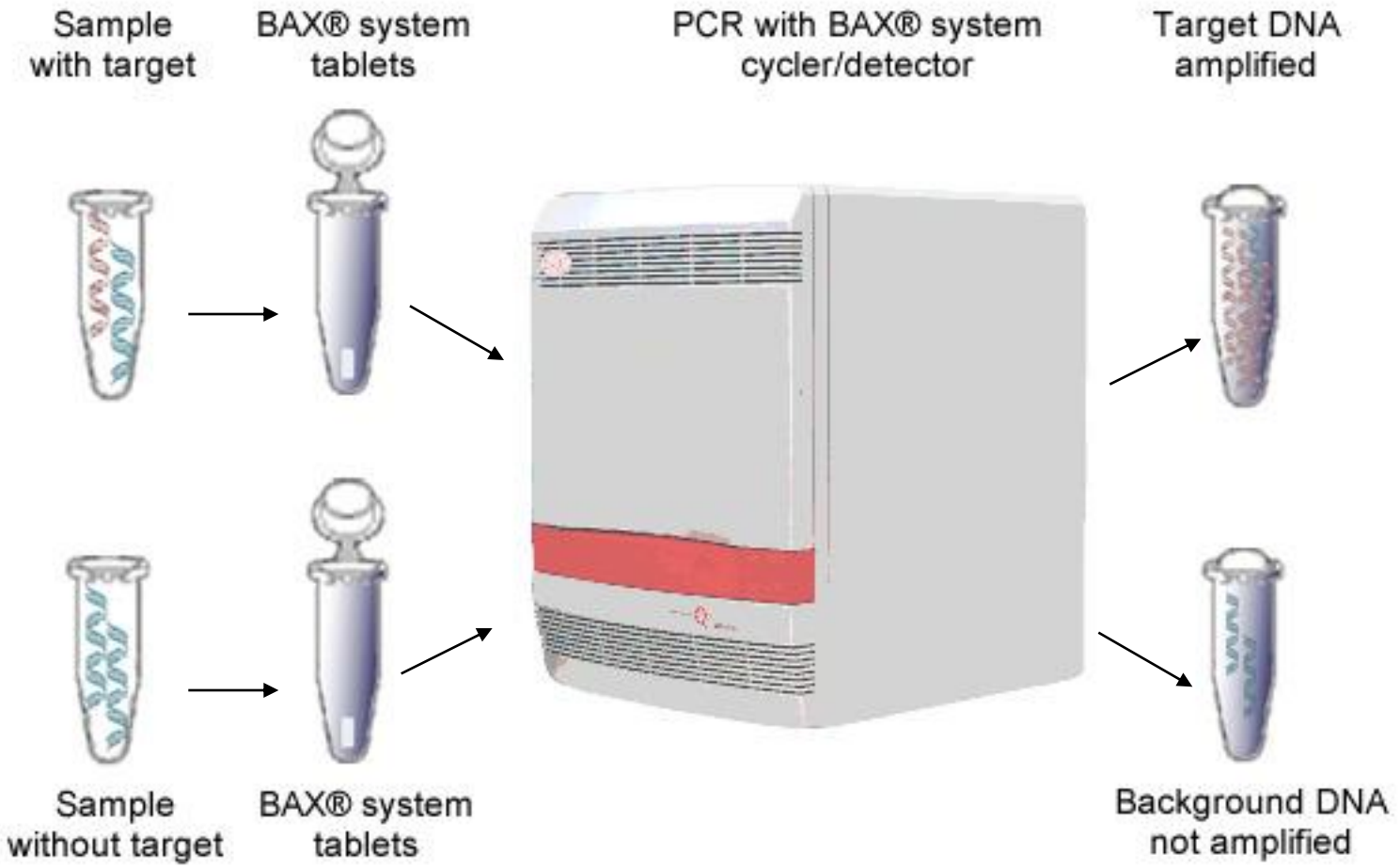


2. Process samples



3. Review results

BAX® System and PCR



BAX® Systems

Next-day results for most assays

Target	Enrichment	Lysis	PCR	Total Time to Result
Salmonella	20-26 hr* (some need extra 3 hr re-growth)	30 min	3.5 hr	24-33 hr
E. coli O157:H7	14-24 hr (beef)	30 min	3.5 hr	18-28 hr
Listeria monocytogenes	30-50 hr	1 hr	3.5 hr	34.5-64.5 hr
Genus Listeria	22-30 hr	1 hr	3.5 hr	26.5-34.5 hr
Enterobacter sakazakii	24 hr	30 min	3.5 hr	28 hr
Campylobacter jejuni/coli	24 hr	30 min	3.5 hr	28 hr

BAX® System PCR Assays for Screening

Available today:

- ✓ ***Salmonella***
- ✓ ***E. coli* O157:H7**
- ✓ ***Genus Listeria***
- ✓ ***Listeria monocytogenes***
- ✓ ***Enterobacter sakazakii***
- ✓ ***Yeast and mold***

Each kit contains 96 tests:

- **PCR tubes with tablets**
- **Optical caps**
- **Lysis buffer**
- **Protease**

Foods Tested with the BAX® System

- ✓ *Dairy*
- ✓ *Meat*
- ✓ *Seafood*
- ✓ *Fruits/Vegetables/Salad*
- ✓ *Grains*
- ✓ *Spices*
- ✓ *Chocolate*
- ✓ *Pet food*
- ✓ *Environmental samples*
- ✓ *Other*

BAX® Systems are Credible

- AOAC International Official Method

  Salmonella #2003.09; L. monocytogenes #2003.12

- AOAC-RI Performance Tested Method

  Salmonella #100201; Listeria monocytogenes #070202; E. Coli O157:H7 #010401; E. coli O157:H7 MP #050501; Genus Listeria #030502

- USDA-FSIS Adoption

  Salmonella #MLG 4C.00; Listeria monocytogenes #MLG 8A.00;
  E. coli O157:H7 and E. coli O157:NM #MLG 5A.00 – MP assay

- **Health Canada Certification**

  Salmonella #MFLP-29; Listeria monocytogenes #MFLP-28; Genus Listeria #MFLP-15e;
  E. coli O157:H7 #MFLP-30; Enterobacter sakazakii #MFLP-27

- AFNOR Certification

  Salmonella #QUA 18/3-11/02

- NordVal Certification

  Salmonella #2003-20-5408

- Brazil MAPA Official Reference Method

  Salmonella MLG-4C.01; Listeria MLG-8A.01

- Japanese Ministry of Health, Labour and Welfare

  Listeria; Listeria monocytogenes

- Swedish National Food Association

  Salmonella; Listeria monocytogenes

Price Quotation:

QTY	PART #	DESCRIPTION	UNIT PRICE
Q7 START-UP PACKAGE (NEW SYSTEMS)			
	41720001 (120v) 41720003 (220v)	**EQUIPMENT** One BAX® system Q7 cycler/detector* with tube holders; computer workstation, monitor, software, and printer** Two BAX® system heating blocks with inserts and thermometers Two capping/decapping tools (cluster tubes, PCR tubes) One multi-channel pipette (8 channels; 5-50 µl); two adjustable pipettes (one 20-200 µl and one 5-50 µl); one repeating pipette Three cooling block assemblies (one PCR and two lysis) **SUPPLIES** Cluster tubes with caps and racks Tips for pipettes Extra PCR tube caps Two fuses Powder-free nitrile gloves User documentation package **TRAINING** (2 days) Lab set-up, equipment validation, procedural instruction **Extended service agreements are available for purchase.* ***Printers and power cables are not shipped with 220V systems, but are sourced locally through regional Qualicon representatives.*	$52,000.00

Price Quotation:

REAGENT KITS			
Real-Time PCR Assays			
	D14203648	BAX® System Real-Time PCR Assay for *E.coli O157:H7* PCR tubes with tablets, extra PCR caps, lysis buffer, protease (96 tests)	$864.00
	D14642964	BAX® System Real-Time PCR Assay S*TEC SCREENING KIT* PCR tubes with tablets, extra PCR caps, lysis buffer, protease (96 tests)	$1,400.00
	D14642970	BAX® System Real-Time PCR Assay STEC PANEL 1 KIT PCR tubes with tablets, extra PCR caps, lysis buffer, protease (48 tests)	$864.00
	D14642987	BAX® System Real-Time PCR Assay STEC PANEL 2 KIT PCR tubes with tablets, extra PCR caps, lysis buffer, protease (48 tests)	$864.00
Standard PCR Assays			
	D11000133	BAX® System PCR Assay for Screening *Salmonella* PCR tubes with tablets, extra PCR caps, lysis buffer, protease (96 tests)	$864.00
	D11000157	BAX® System PCR Assay for Screening *L. monocytogenes* PCR tubes with tablets, extra PCR caps, lysis buffer, protease (96 tests)	$864.00
	D11000147	BAX® System PCR Assay for Screening Genus *Listeria* PCR tubes with tablets, extra PCR caps, lysis buffer, protease (96 tests)	$864.00
	D12404903	BAX® System PCR Assay for Screening *E. coli* O157:H7 MP PCR tubes with tablets, extra PCR caps, lysis buffer, protease (96 tests)	$864.00

Bio-Rad Real-Time PCR Assay

for *Listeria* Receives Health Canada Approval

for detection of *Listeria* spp. in Environmental samples.

The iQ-Check *Listeria monocytogenes* and *Listeria* spp. kits are also being evaluated in the joint AOAC/Health Canada for detection of the organisms from ready to eat (RTE) foods.

The iQ-Check *Listeria* spp. method involves a **single 24 hr primary enrichment.** Due to the sensitivity and specificity of the kit, a secondary enrichment is not necessary. A simple one-step DNA extraction is performed and PCR is run.

Results are available in approximately **2 hr**.

Category Products:

- **iQ-Check *E.coli* O157:H7 Kit**

In **food samples**. Results available in **12 hr.**

- **iQ-Check *Listeria* spp. Kit**

in **food and environmental samples. Next day results.**

- **iQ-Check *Listeria monocytogenes* II Kit**

In **food and environmental samples**. **Next-day results ..**

- **iQ-Check *Salmonella* II Kit**

In **food, animal feed, and environmental samples.**
Next-day results for all samples
or in 12 hr for raw meat samples.

Price Quotation:

Product Description	List Price
CFX96 Real-Time PCR Detection System. Modular thermal cycler platform, includes C1000 thermal cycler chassis and CFX96 optical reaction module.	**$48,930.00**
iQ-Check Salmonella II kit, 96 tests	**$1,236.00**
iQ-Check Listeria spp. II kit , 96 tests	**$1,236.00**
iQ-Check Listeria monocytogenes II kit, 96 tests	**$1,236.00**
iQ-Check E.coli O157:H7, 96 tests	**$1,236.00**
iQ-Check Campylobacter, 96 tests	**$1,500.00**
Total	**$ 55,374.00**

Some of the Common Real Time Cyclers

Rotorgene

Corbett

LightCycler

Roche

Smartcycler

Cepheid

iCycler

BioRad

7500

Applied Biosystems

Main Characteristics of the Equipments

Manufacturer	Equipment	Sample Format	Particularities
Applied Biosystems	ABI 7500 ABI 7700 ABI 7900	Microplate (96) Microplate (96) Microplate (384)	High turnover Speed
BioRad	iCycler	Microplate (96) Tubes (96)	Image of the plate
Cepheid	SmartCycler	Tubes (16)	Individual programmes for each tube possible Speed
Corbett	Rotor-Gene	Tubes (32) Striptubes (72)	No temp. gradient, no need for expensive tubes or capillars
Roche	Lightcycler	Capillaries (32)	Speed Melting curves
Stratagene	Mx 4000	Microplate (96)	Multiplex

Isothermal DNA Amplification Methods

3M™ Molecular Detection Assay *E. coli* O157 (including H7)

3M™ Molecular Detection Assay *Listeria*

3M™ Molecular Detection Assay *Salmonella*

Features

- Detects 1–5 CFU of *Salmonella* per sample size
- Simultaneous amplification and detection process that is complete in 75 minutes
- Positive samples identified as early as 15 minutes

Chemistry Overview

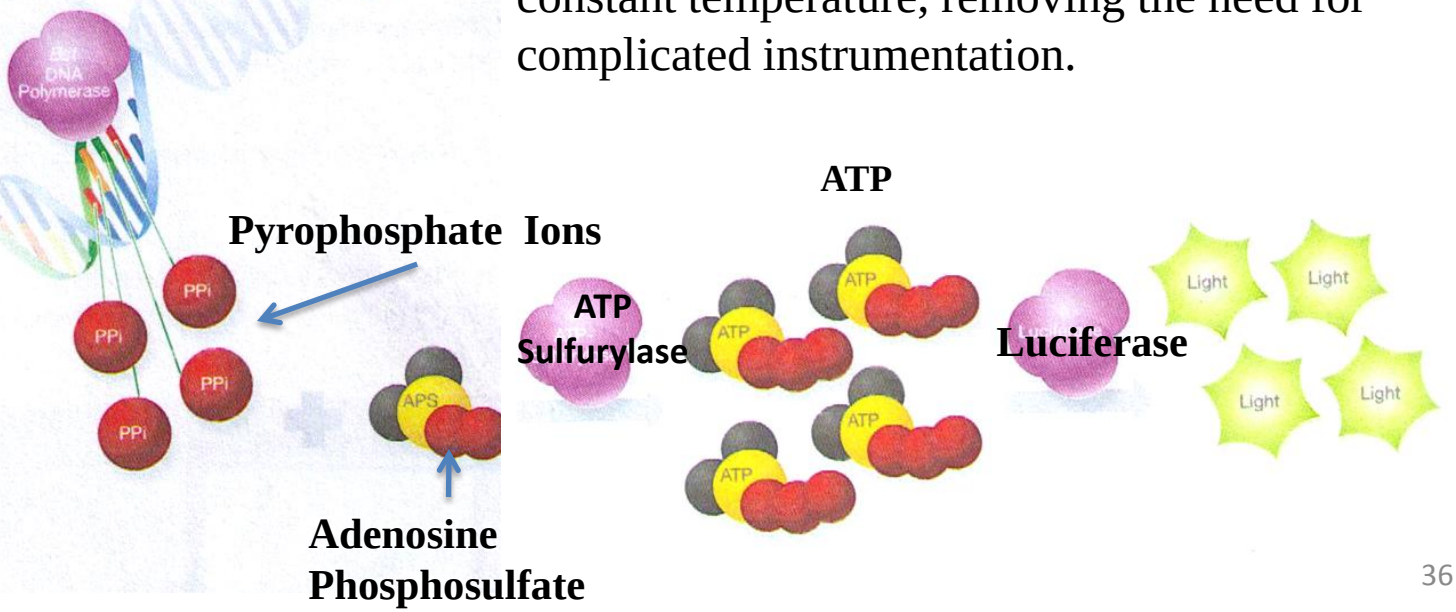
Isothermal DNA Amplification

- Multiple primers recognize distinct regions of the genome
- A DNA polymerase with strand displacement activity
- Efficient, rapid and continuous amplification of target DNA

Bioluminescence Detection

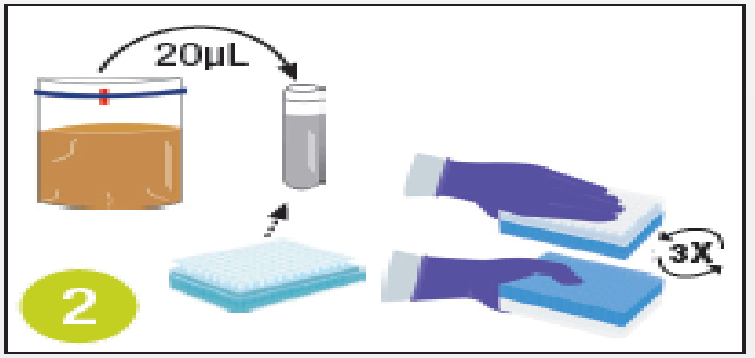
- Exponential generation of pyrophosphate, a by-product of the DNA amplification
- Pyrophosphate conversion to Adenosine Tri Phosphate (ATP)
- Thermostable luciferase uses ATP to generate light

Cost Effective:
Isothermal DNA amplification proceeds at a constant temperature, removing the need for complicated instrumentation.

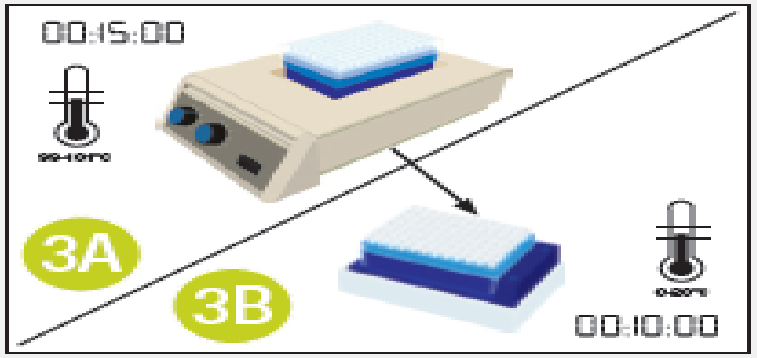


3M™ Molecular Detection Assay Process

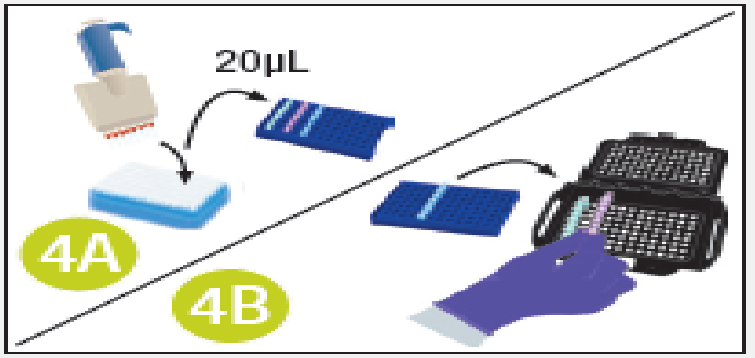
Set up run in software.



Transfer enriched sample to lysis tubes.



Heat lysis tubes, then chill.



Transfer lysate to reagent tubes.

Place speed loader tray into instrument.

Amplification and detection in real time.

Neogen® ANSR™

ANSR's DNA amplification methodology exponentially amplifies the DNA of any target bacteria present in an **enriched food or environmental sample** to detectable levels (10^4 cfu/mL) in only **10 minutes**.

ANSRs unique enrichment and assay results in minimal matrix effects compared to conventional method, in **food matrices and environmental samples.**

What is ANSR?

-Unlike PCR, **ANSR is isothermal, replicating DNA at a <u>constant temperature</u> using a polymerase to exponentially amplify the DNA at 56°C.**

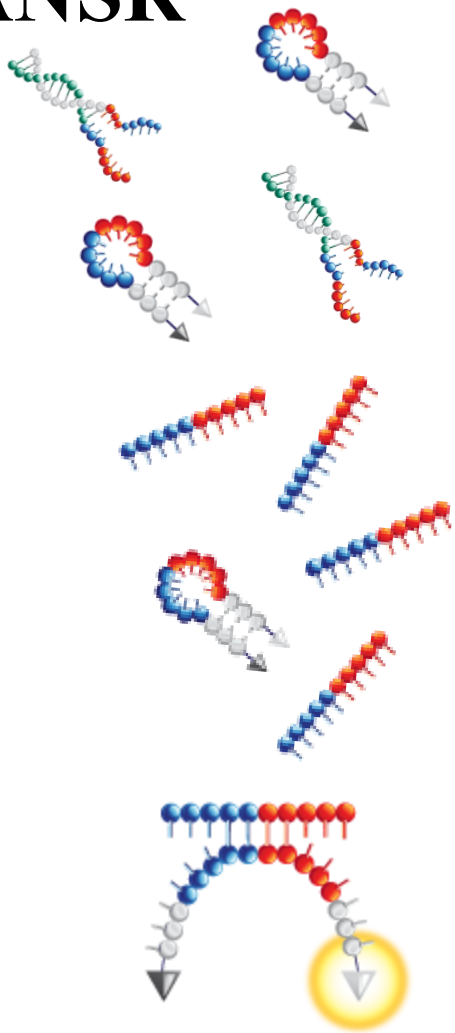
-Target nucleic acid is amplified through a mechanism of polymerization from the ends of nicks created in double-stranded DNA by the action of a specific endonuclease.

-Amplified target sequences are **detected** in real time **using fluorescent molecular beacon probes.**

ANSR *Salmonella* Lysis Protocol

- Step 1. Mix enriched sample with lysis buffer. Heat for 20 minutes (max. 60 minutes).
- Step 2. Mix lysed samples with reagents in the reader.
- Step 3. Seal the tubes, start the reader. Results available in 10 minutes.

Neogen® ANSR™



- **Target pathogen DNA is released** through the lysis of the enriched sample. A special molecular beacon is part of the ANSR reagent mixture.

- When the lysed sample is added to the ANSR reagents, a **special primer targets specific regions** of the pathogen DNA and starts the amplification process.

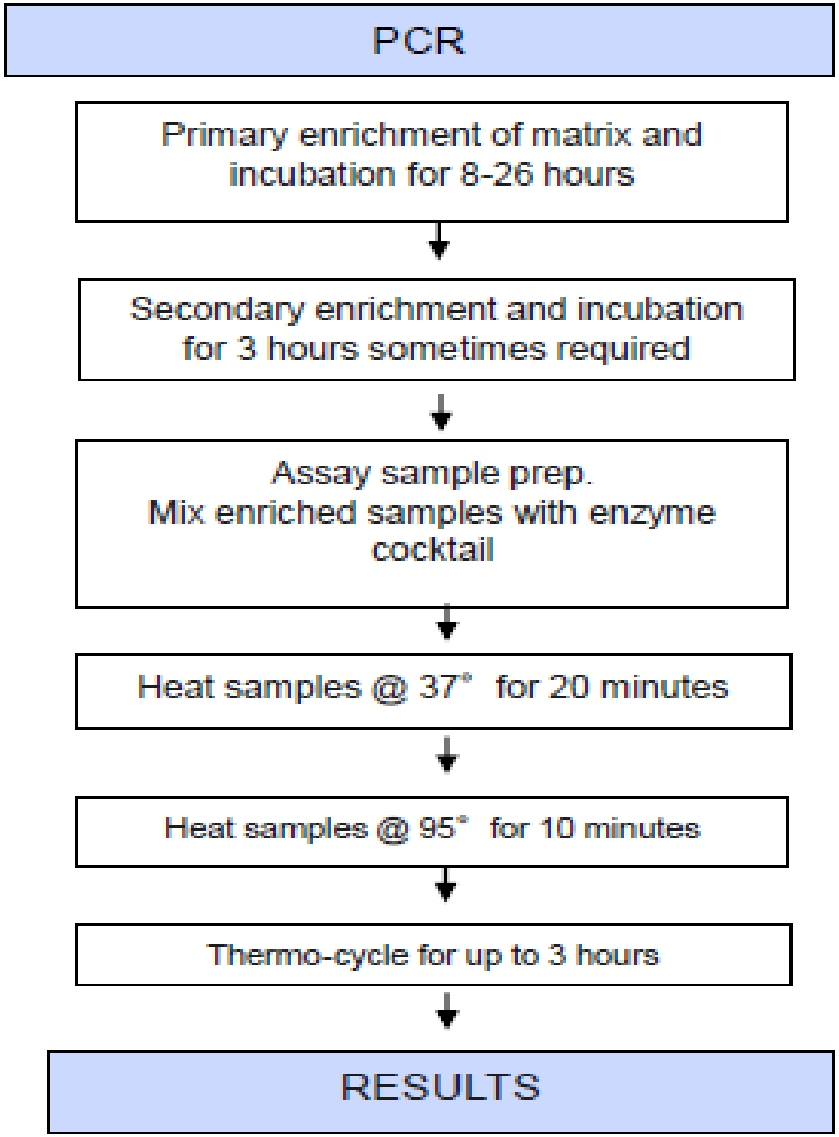
- **Millions of copies** of the target pathogen DNA are **created in minutes**.

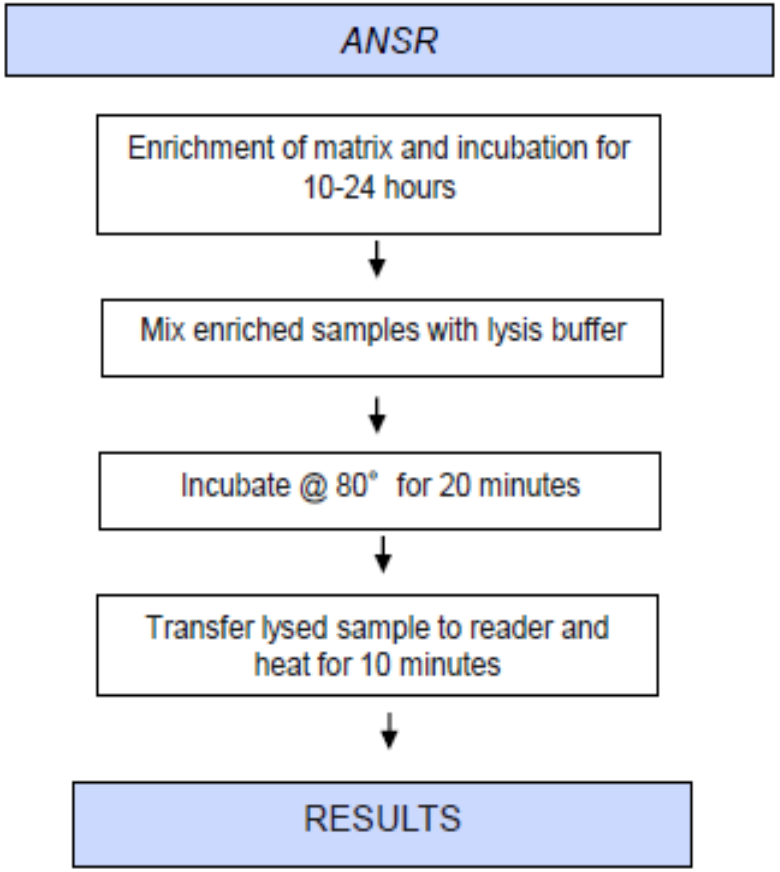
- **Amplified segments** of the pathogen DNA **attach to** special **molecular beacons**.

- The **molecular beacons fluoresce** when bound to the pathogen DNA. This is detected by the ANSR reader.

Amplification and detection are continuous and rapid, resulting in the fastest assay time of only 10 minutes.

Comparative Workflow





Price Quotation:

NEOGEN® CORPORATION

Neogen ANSR™

9820	**ANSR for Salmonella** – 96 tests	$ 900.00
9825	**ANSR Basic System** – contains reader with computer, lysis system and 8-channel pipette	11,630.00
9826	**ANSR Lysis System** – contains lysis system, thermometer and heater block	850.00
9832	**ANSR Computer**	950.00
9811	**ANSR Enrichment Broth 1**	50.00
9812	**ANSR Enrichment Broth 2**	50.00
9813	**ANSR Enrichment Broth 3**	50.00
9829	**ANSR Lysis Block**	225.00
9828	**ANSR Reader**	10,000.00
9828C	**ANSR Reader with Computer** – contains reader with computer	10,500.00
9386	**Heater Block**	650.00
9385	**Pipettor, 50-300 µL, 8-channel**	675.00
9463	**Pipettor, 100-1000 µL**	340.00
9276	**Pipettor, 20-200 µL**	245.00
9282	**Pipettor, 50 µL, fixed**	85.00
9833	**Pipette tip rack, filtered, 50 µL** (96 tips per rack)	6.50
9487	**Pipette tip rack, 100-1000 µL**	10.00

Molecular Typing Methods طرق التنميط الجزيئي

Bacterial Typing Systems

- Tracking sources of microbial contaminants has been a concern since the early days of commercial food processing.

-Molecular methods have provided tools that permit more rapid and highly precise determinations of microbial contamination sources.

Ribotyping التنميط االرايبوسومي

-Ribotyping is a molecular technique that allows for identification and typing of bacteria to the strain level.

- It is based upon differences in rRNA.

Ribotyping:

Ribotyping is a form of restriction fragment length polymorphism (RFLP) analysis that relies on differences in:

- Location,
- Number of ribosomal RNA (rRNA) gene sequences present in the bacterial genome.

It is the analysis of **band pattern differences** obtained through **hybridization**

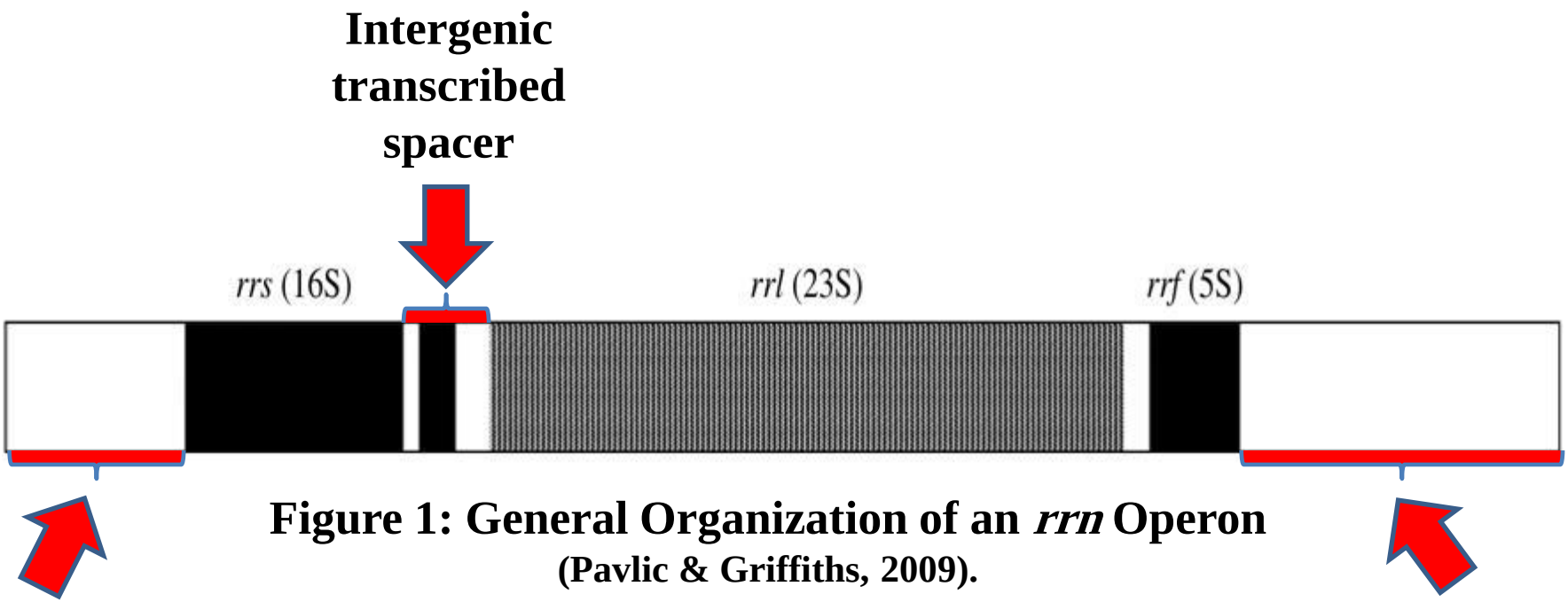


Figure 1: General Organization of an *rrn* Operon (Pavlic & Griffiths, 2009).

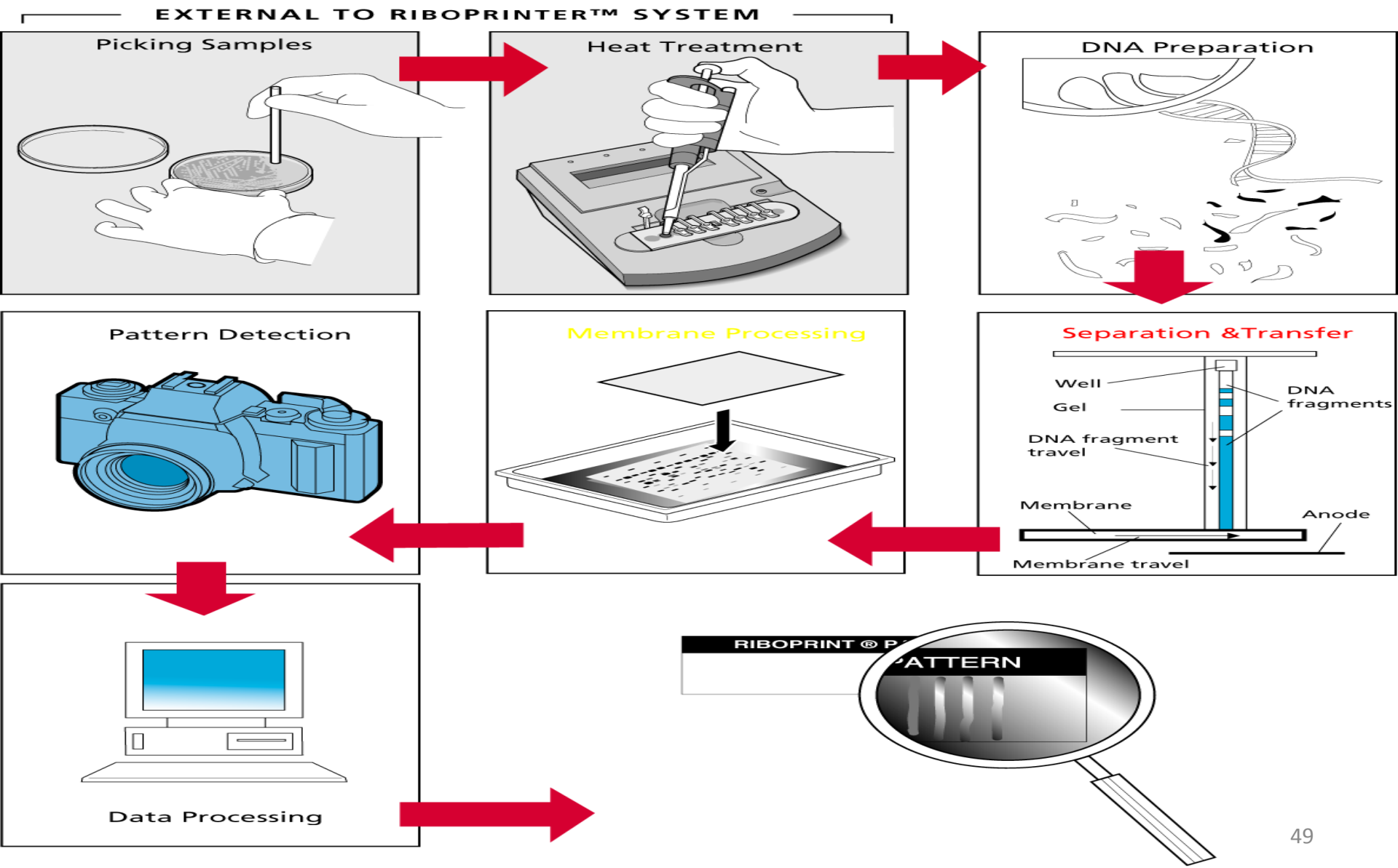
Variations in the **flanking and spacer sequences** give rise to variations in ribopatterns generated by ribotyping.

Automated Ribotyping (AR):

- AR is a genotyping method that can be used to generate genetic fingerprints of bacterial isolates of interest.

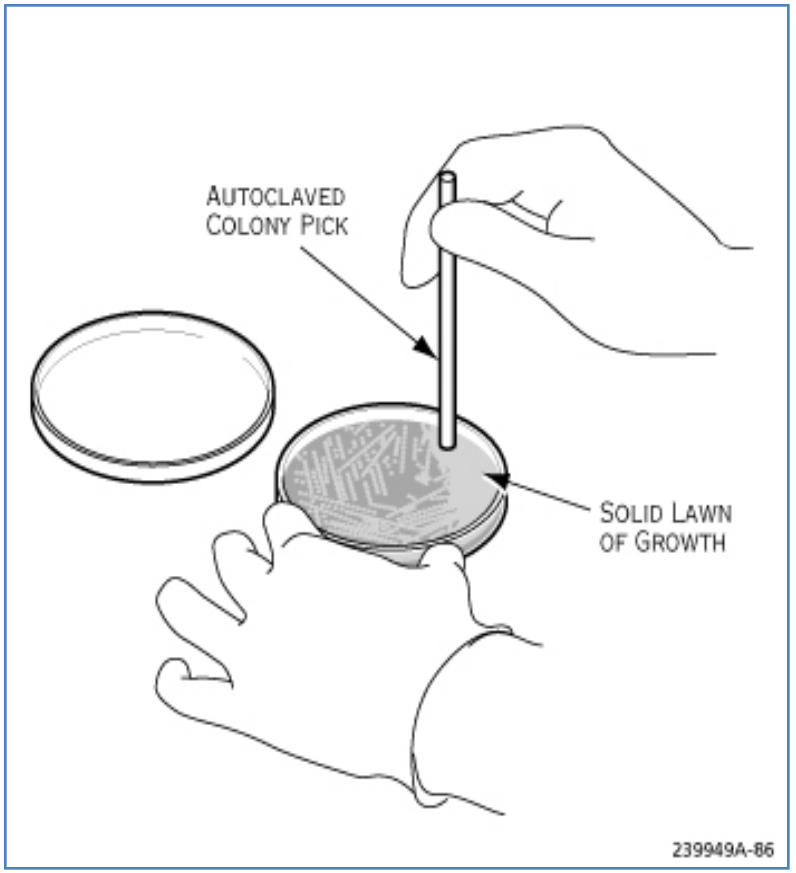
- An entirely automated system, commercially available with a high level of **reproducibility** and **standardization**.

How the RiboPrinter System Works



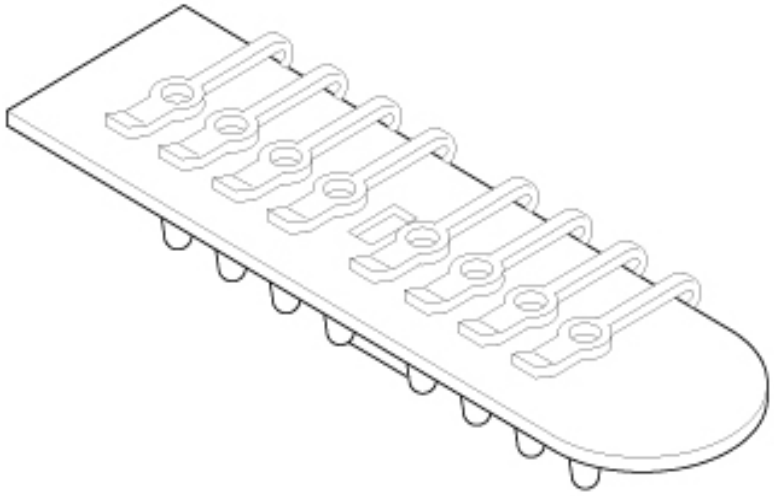
Sample Preparation: Colony Picks:

Start with a pure colony on an agar plate (no selective enrichment required).



Sample Preparation: Sample Carrier:

Dilute sample with buffer
Transfer to one of the eight wells in the sample carrier.

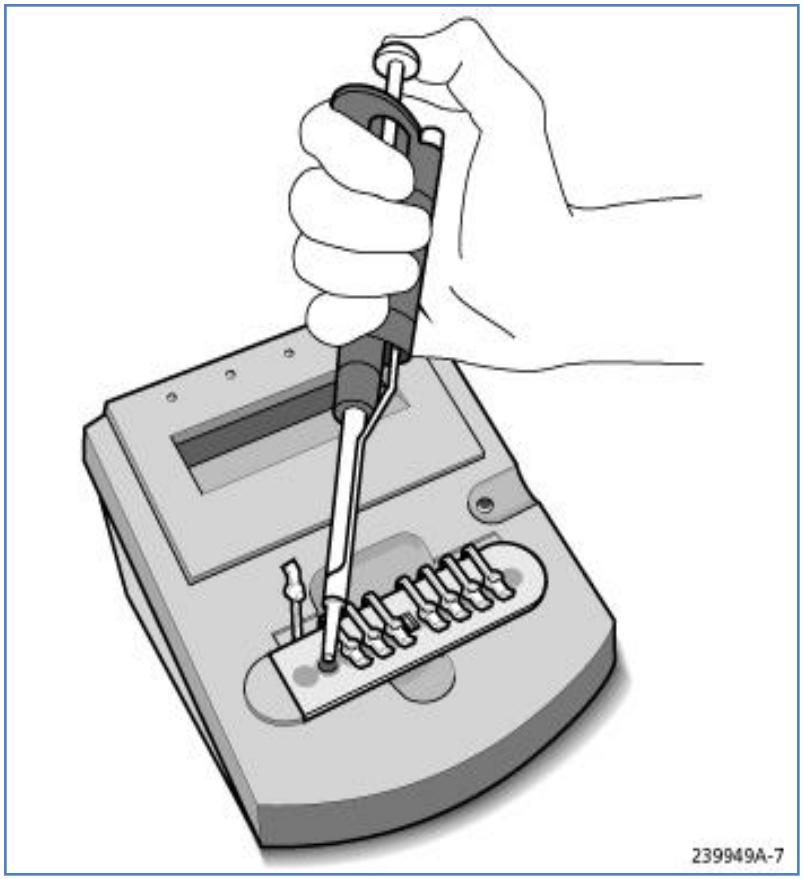


Heat-Treated Cell Suspension,
Plus Lysing Agents

Sample Preparation: Heat Treatment:

- Pre-process samples in the **heat treatment** station **to inactivate viable cells and nucleases.**

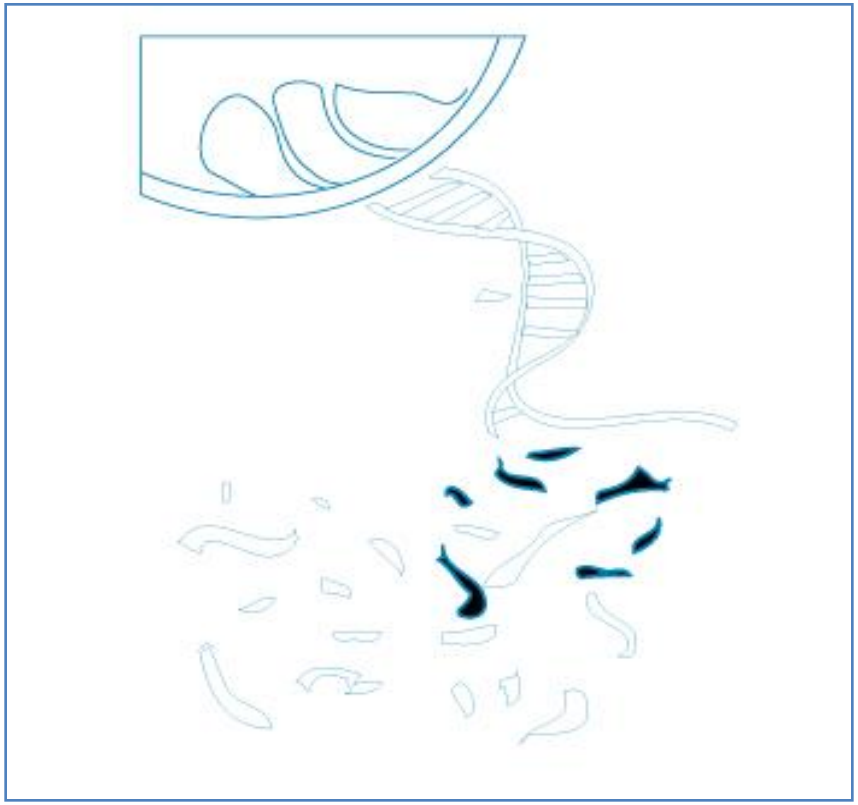
- Add lysing agents.



DNA Preparation (Automated):

- **Lysis**: breaks open the cells.

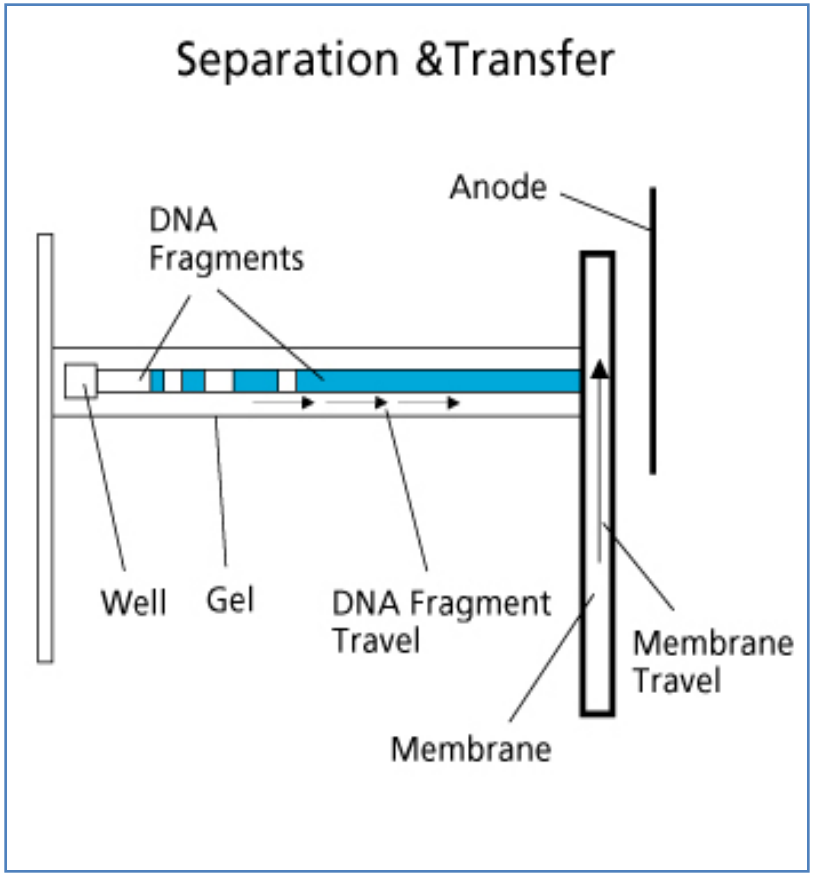
Digestion: restriction enzyme “cuts” DNA into fragments.



Separation and Transfer (Automated):

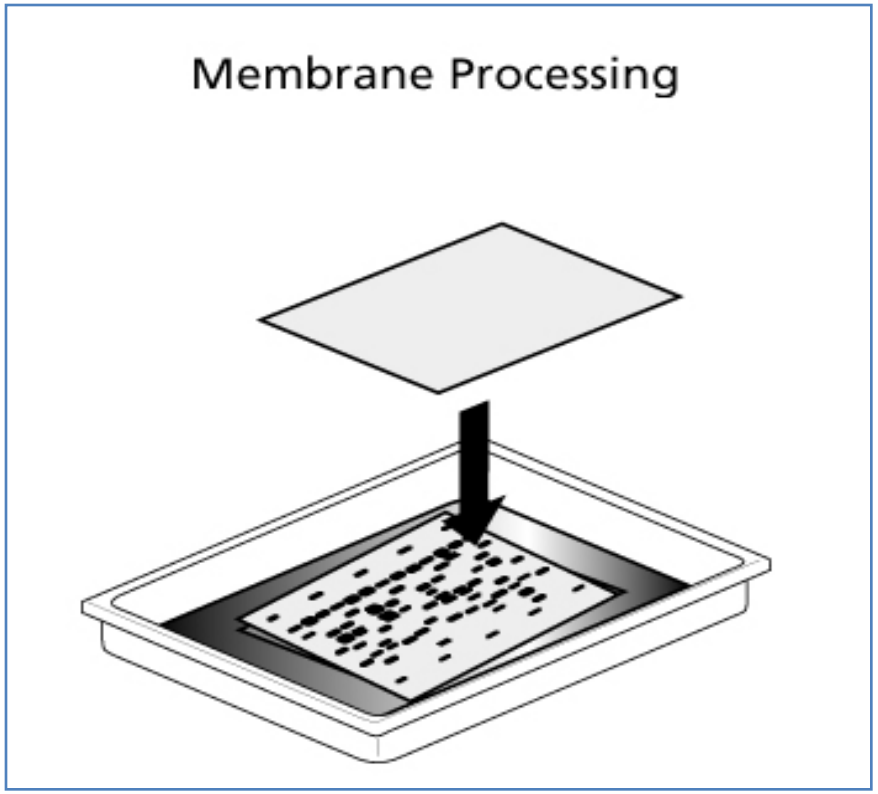
-DNA fragments are **separated** by size using **gel electrophoresis**.

- Fragments are then **transferred** and **immobilized on** a moving **nylon membrane** (direct blotting).

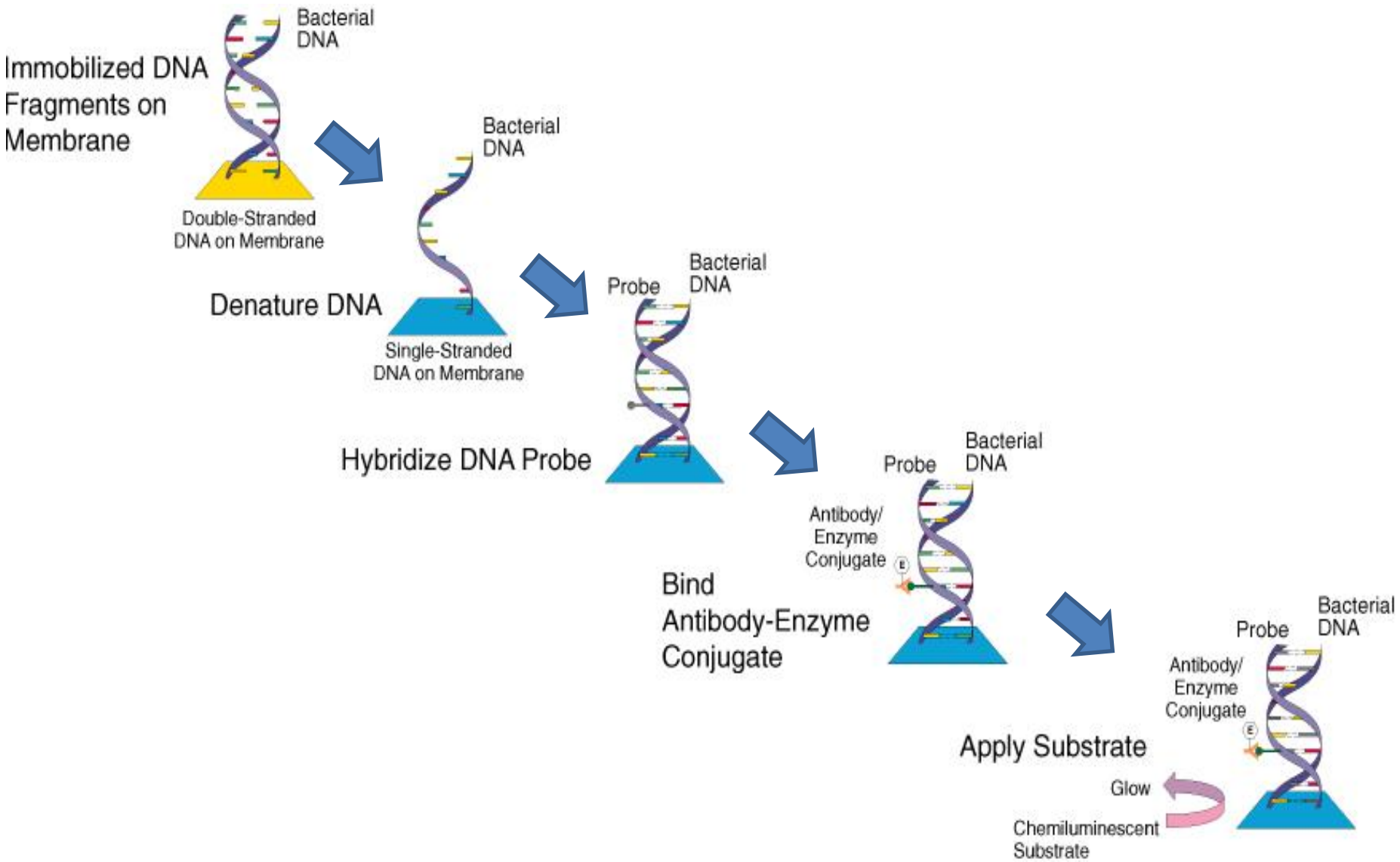


Membrane Processing (Automated):

Membrane is exposed to a series **chemical/enzymatic treatments.**



Membrane Processing (Automated):

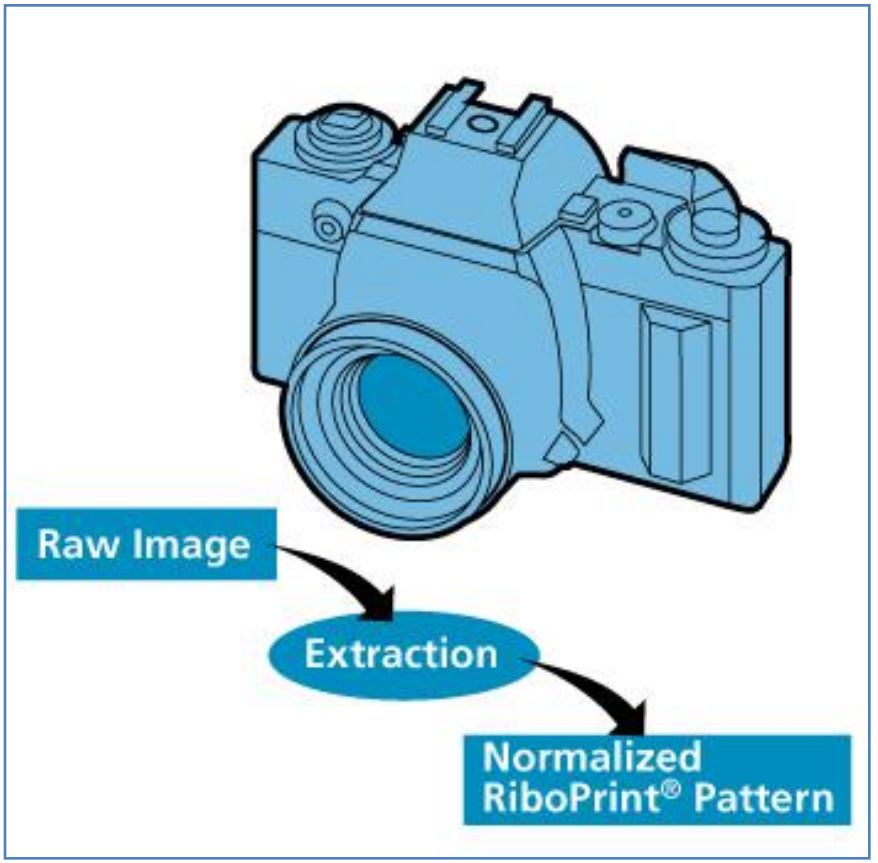


Detection (Automated):

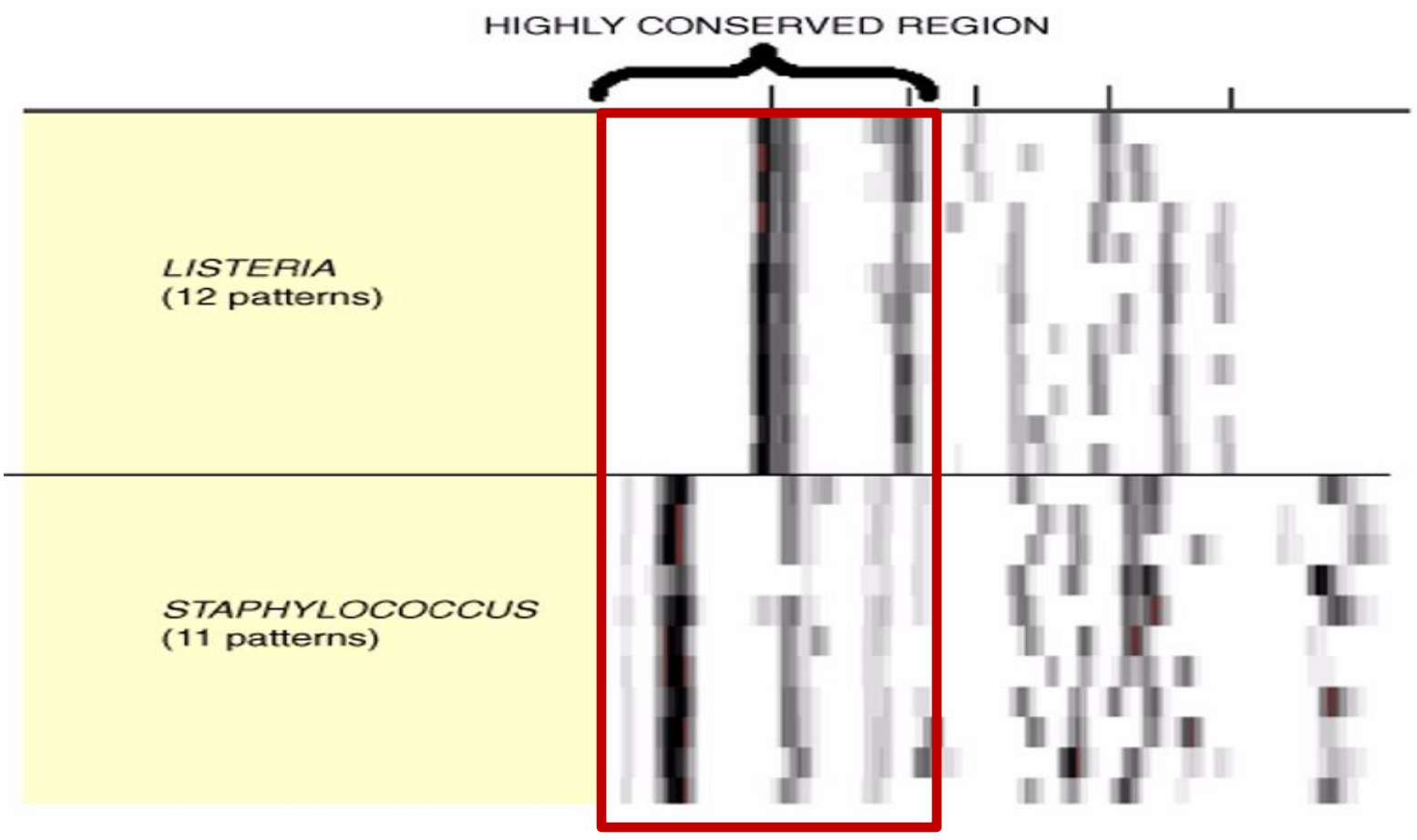
-Band pattern image is **captured by a low-light camera.**

-Image is **stored** in the **computer memory.**

-Software creates RiboPrint® patterns.

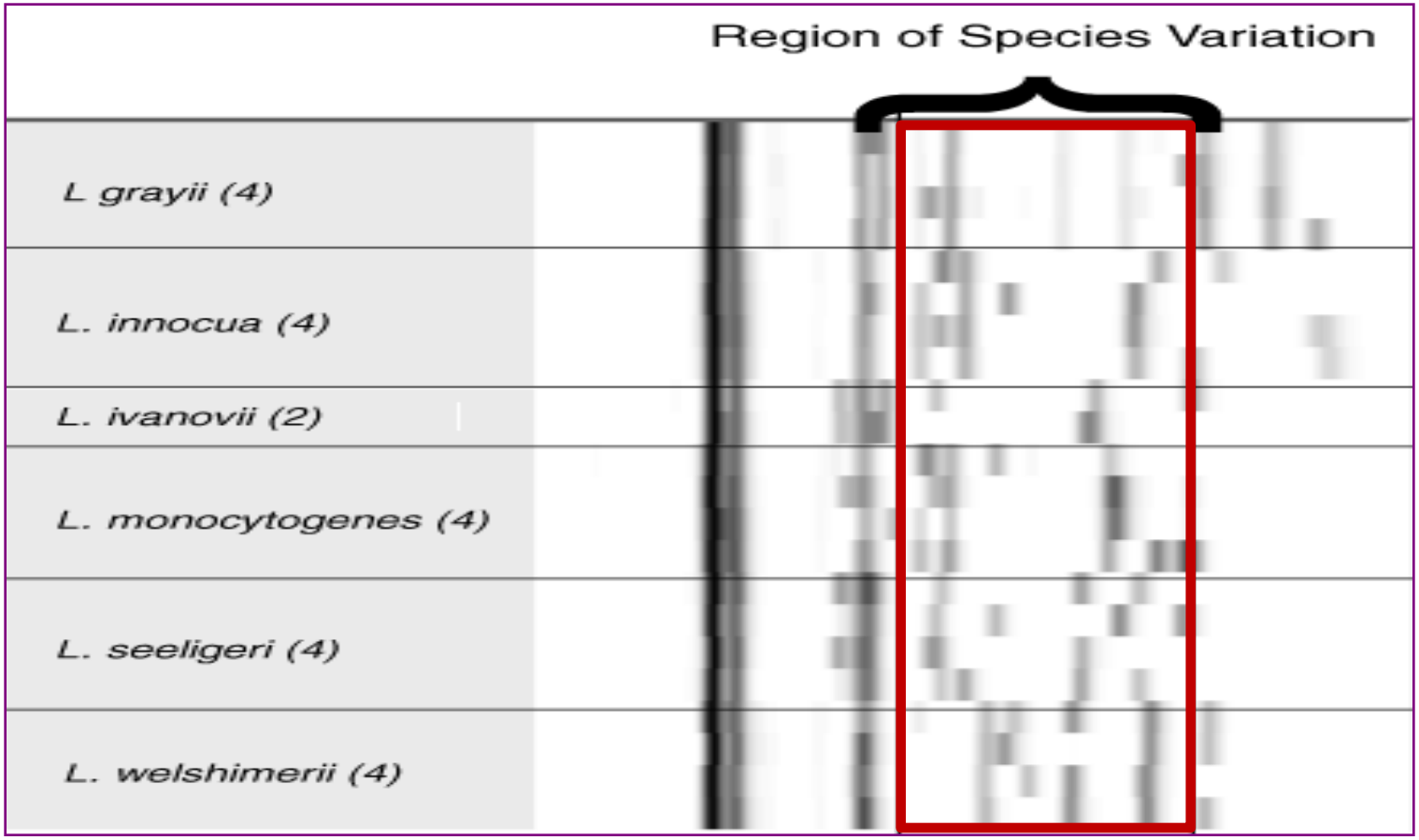


RiboPrint Patterns Clearly Demonstrate Genera Differentiation:

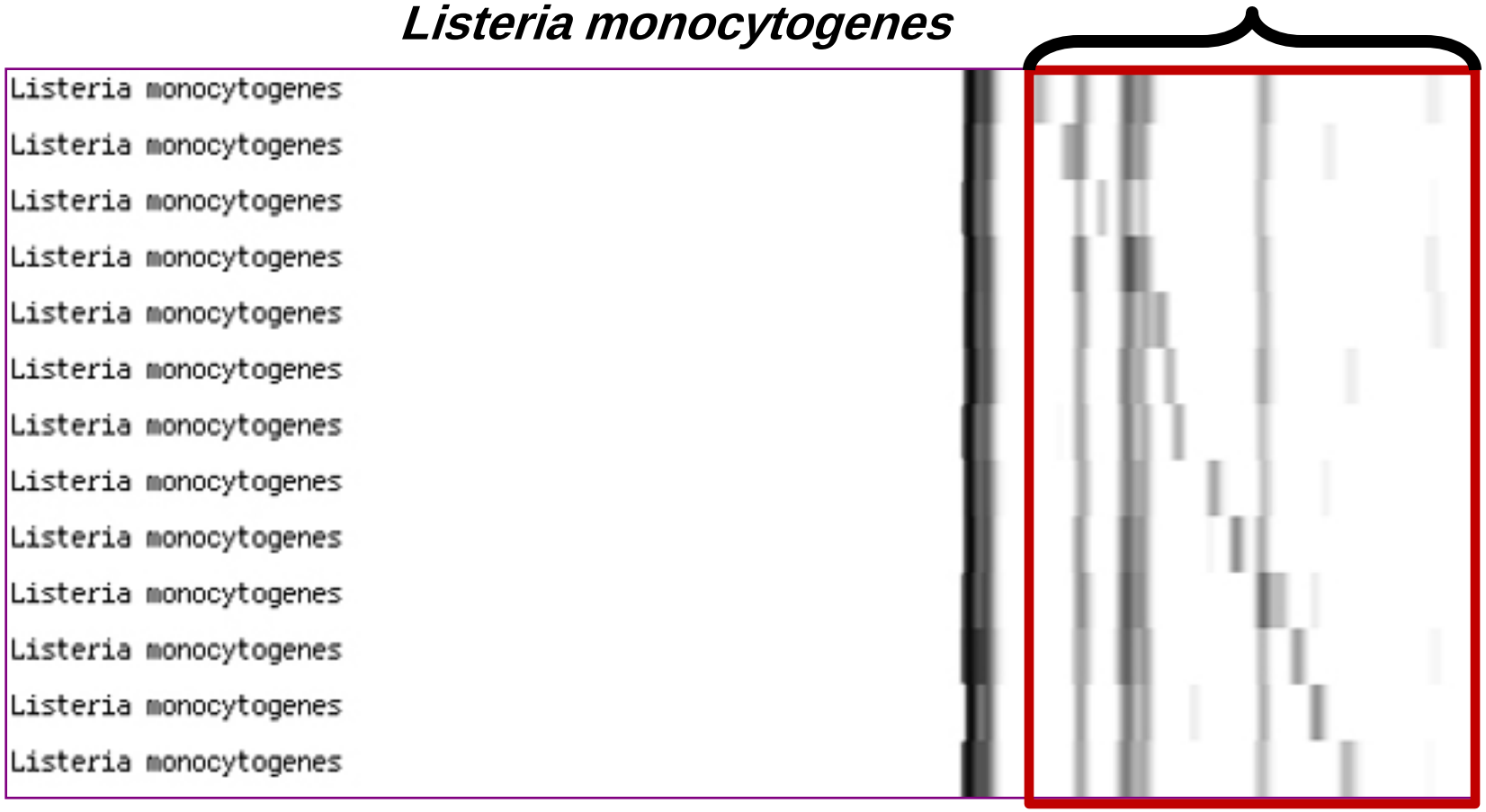


RiboPrint Patterns Provide Conserved Characters that Show Species Variation:

Listeria



RiboPrint Patterns Provide Non-Conserved Characters that Discriminate Below the Species Level:



Price Quotation:

Qty.	Part No.	Description	Unit Price*
		RIBOPRINTER® SYSTEM - WINDOWS PLATFORM	
	20520001 (110 volt) 20520002 (220 volt)**	**RiboPrinter® System*** Characterization unit and integrated software license Computer workstation, monitor, removable storage device, UPS, and printer Windows® operating system, RiboPrinter® system software with online help, backup and antivirus software Heat treatment station and mixer Documentation package Installation and training First year service, including preventive maintenance, field visits, parts and licensed software upgrades ***Extended service agreements are available for purchase.** ****Printers and power cables are not shipped with 220V systems, but are sourced locally through regional Qualicon representatives.**	$175,000.00
		RIBOPRINTER® SYSTEM DISPOSABLES	
	17020464	**Sample Preparation Pack (60 batches, 480 tests)** Sample Carriers, Sample Buffer, Colony Picks	$240.00
	17720533	**RiboPrinter® System *Eco*RI Batch Kit (6 batches, 48 tests)** DNA Preparation Pack, Gel Cassette, Membrane, MP Base Pack, MP Conjugate, MP Probe, MP Substrate, Purified Water, Lysing Agents, Lactic Agent, DNA Prep Enzyme	$2,160.00
	17720632	**RiboPrinter® System *Eco*RI Batch Kit (without water)**	$2,142.00
	17720601	**RiboPrinter® System *Pvu*II Batch Kit (6 batches, 48 tests)** DNA Preparation Pack, Gel Cassette, Membrane, MP Base Pack, MP Conjugate, MP Probe, MP Substrate, Purified Water, Lysing Agents, DNA Prep Enzyme	$2,160.00
	17720634	**RiboPrinter® System *Pvu*II Batch Kit (without water)**	$2,142.00
	17720641	**RiboPrinter® System Batch Kit (without enzyme)** DNA Preparation Pack, Gel Cassette, Membrane, MP Base Pack, MP Conjugate, MP Probe, MP Substrate, Purified Water, Lysing Agents	$2,110.00
	17720642	**RiboPrinter® System Batch Kit (without enzyme or water)** DNA Preparation Pack, Gel Cassette, Membrane, MP Base Pack, MP Conjugate, MP Probe, MP Substrate, Lysing Agents	$2,092.00

Seven samples from the same dairy plant were found to belong to one distinct ribotype pattern (pattern no.8) isolated at different dates (from 1996-2007) using *Eco*RI and *Pvu*II enzymes separately.

Pattern #	Strain #	Source/Site
8	697	Cheese/ Dairy Plant A
	1458	Smear/Dairy Plant A
	2540	Cheese rasp/ Dairy Plant A
	2720	Smear/ Dairy Plant A
	3177	Smear/ Dairy Plant A
	4359	Washing Water/ Dairy Plant A
	4898	Washing Water/ Dairy Plant A

- Among 18 different DuPont ID, 7 isolates in a group containing 13 isolates from the same dairy plant were found to belong to one distinct ribotype pattern no.8, isolated at different dates (from 1996-2007) using *Eco*RI and *Pvu*II enzymes separately.

These results may be a good indicator for the persistence of *L. monocytogenes* belong to pattern no.8 in the same dairy plant during that period.

➢The presence of ribotype DUP-18598 in the finished product (cheese, sample no.697) as well as in the washing water (sample no.4359)

*Eco*RI Pattern #	Strain #	Source/Site	RiboPrinter Pattern	DuPont -ID
8	697	Cheese/ Dairy Plant A		DUP-18598
	4359	Washing Water/ Dairy Plant A		DUP-18598

➢Indicates probable pre-processing contamination of finished products from the washing water source.

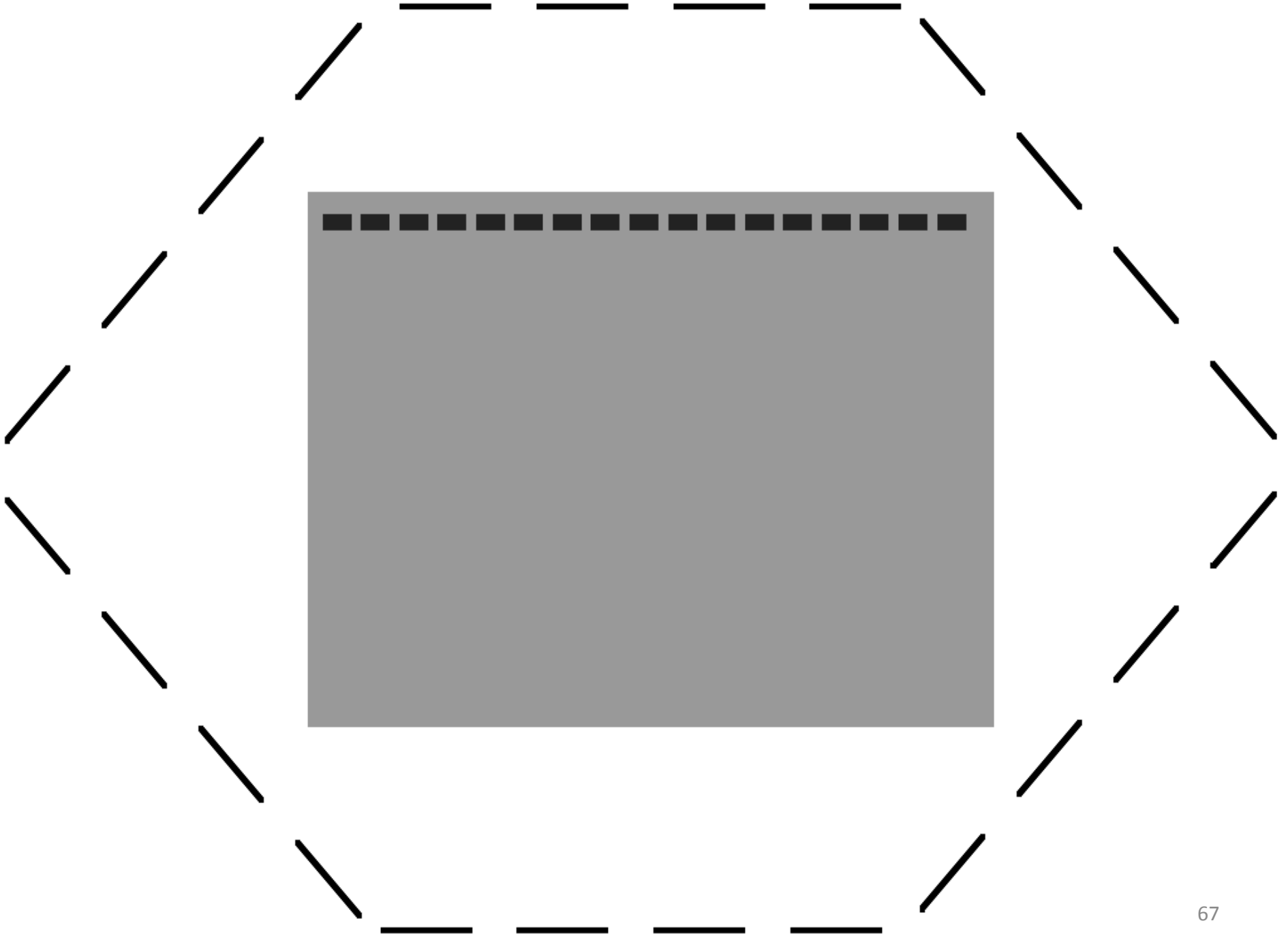
*Eco*RI Pattern #	Strain #	Source/Site	RiboPrinter Pattern	DuPont –ID
8	697	Cheese/ Dairy Plant A		DUP-18598
	4359	Washing Water/ Dairy Plant A		DUP-18598

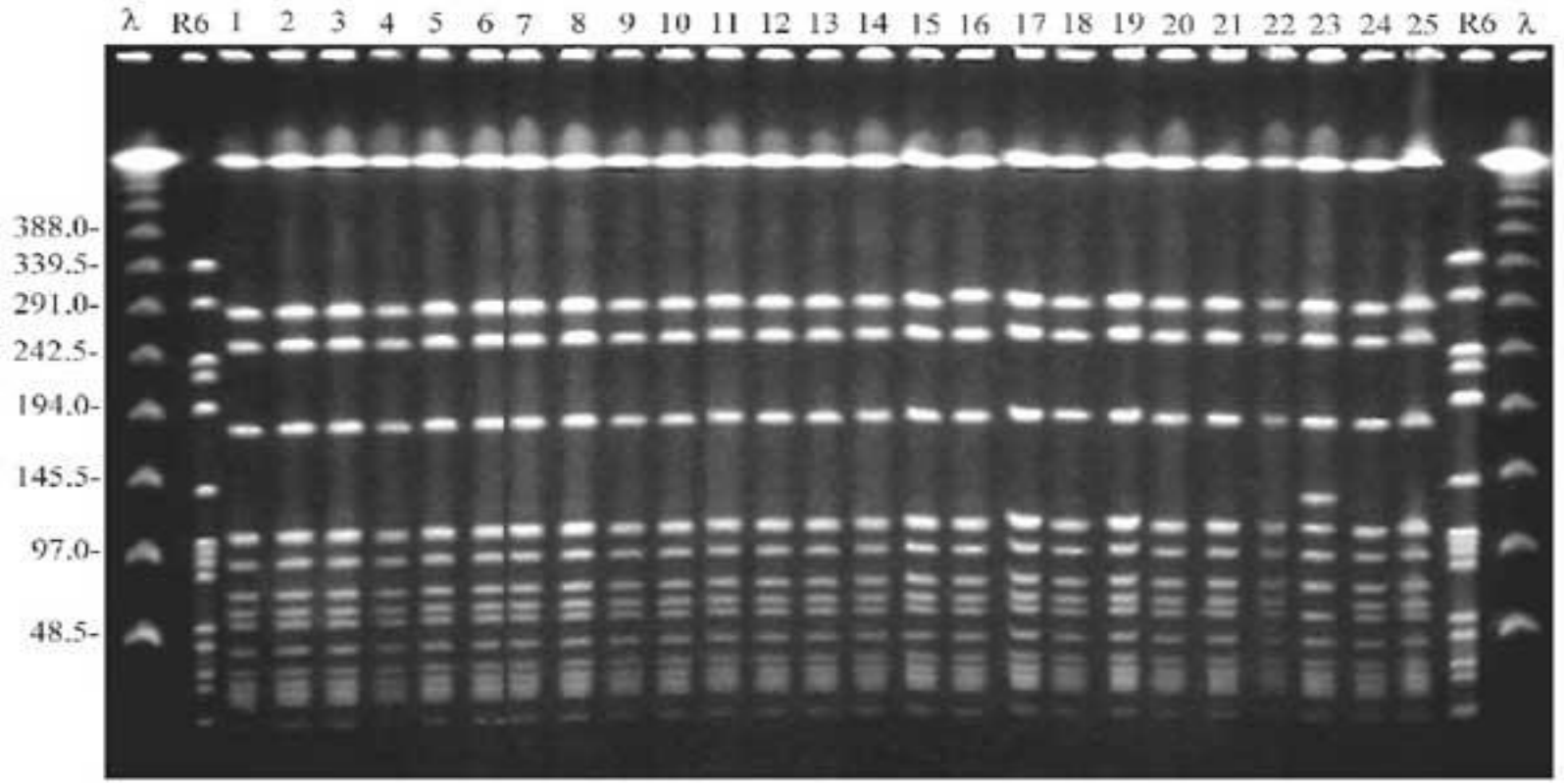
Pulsed Field Gel Electrophoresis (PFGE):

PFGE is the ‘gold standard’ for subtyping of bacterial foodborne pathogens **.**

The concept of subjecting chromosomal DNA of microorganisms to **two alternating electric fields** for separation of large DNA fragments within agarose gels was **introduced in 1984**.

PFGE separate large fragments of 40-1000 kb or even 40-2000 kb in agarose gels **by varying the duration of the electrical pulse** and **shifting the direction of the current frequently.**





Example of a real PFGE; drug resistant *Staphylococcus aureus*. The molecular weight markers are digested lambda phage (λ) and are given in kb.

How PFGE patterns should be interpreted:

- **Same strain**, if **all bands match**.

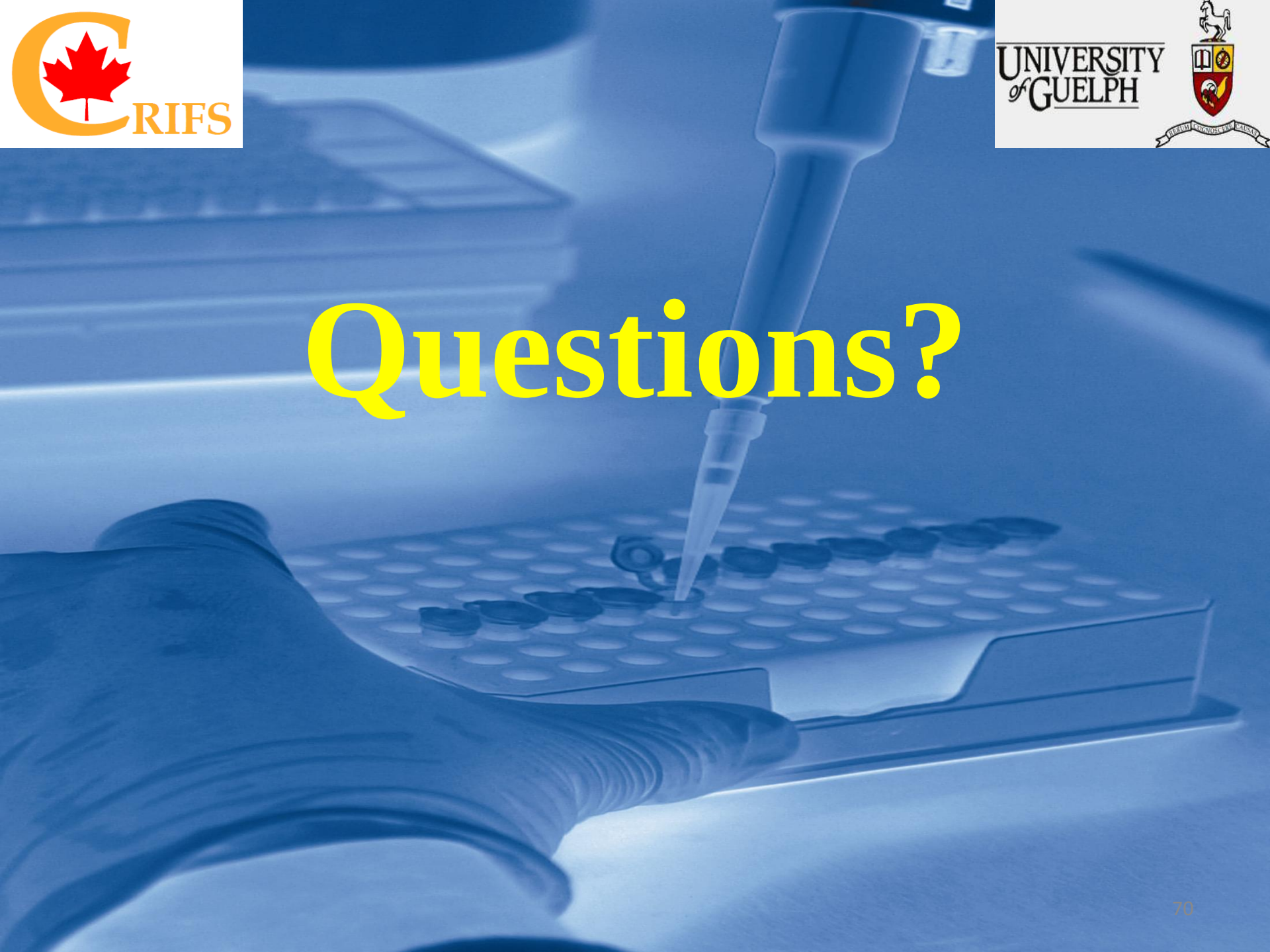
- **Closely related strains**, if the patterns **differ by one to three** bands,

- **Possibly related strains, if** the patterns **differ by four to six** bands,

- **Unrelated strains** if the patterns **differ by more than six** bands

Tenover et al. (1995).

That represented the first challenge to standardize the interpretation of results obtained by a molecular typing technique.


Questions?