

Advances in Molecular Techniques for Food Safety

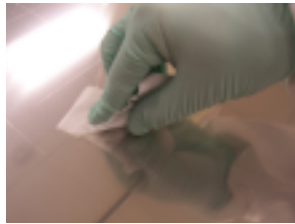
George Tice

Director, Research and Development
DuPont Qualicon



The miracles of science™

Food testing – process flow



Collect
samples



Enrich



Prepare
samples

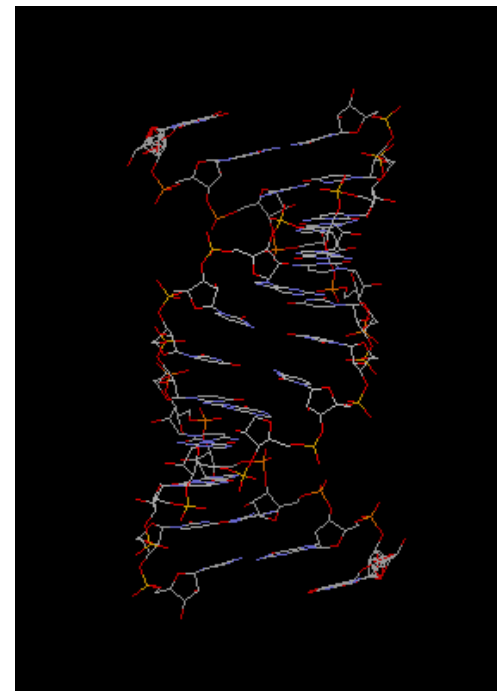
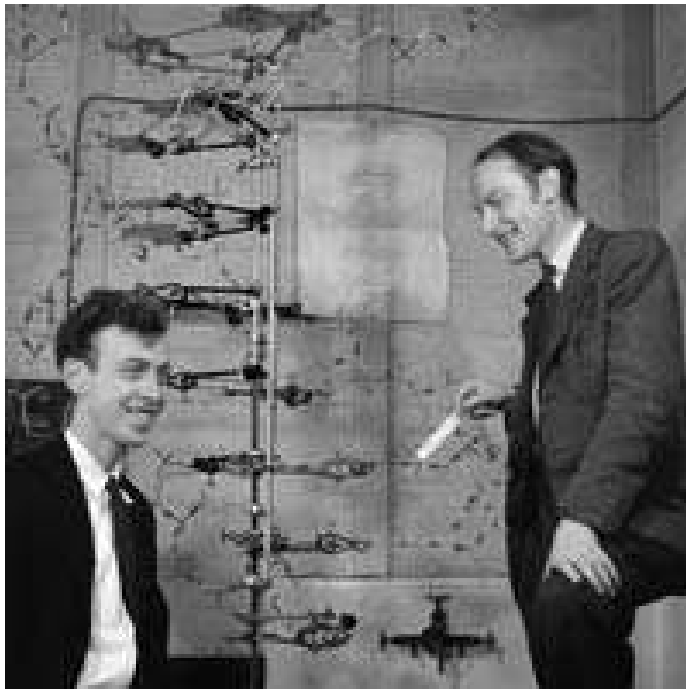


Detect
target



Isolate &
Characterize

Discovery of DNA



1953 J. Watson, USA, and F. Crick, UK, show that the DNA molecule consists of a double helix, thus making one of the most important discoveries of this century

DNA Structure



Deoxyribonucleic acid:

A linear polymer that consists of four nucleotides:

Adenine

Cytosine

Guanine

Thymine

Primer binding

A - T

C - G

Primers are specific to DNA fragment

primer

5' ACGTACGTAAAGGG3' →

|||||

3' AAAAAAAAAATGCATGCATTTCCCAAAAAAAAAA5'

template

primer

5' ACGTACGTAAAGGG3' → ~~XXXXX~~

|||||

template

Amplification techniques

Nucleic Acid Sequence based Amplification (NASBA)

Transcription Mediated Amplification (TMA)

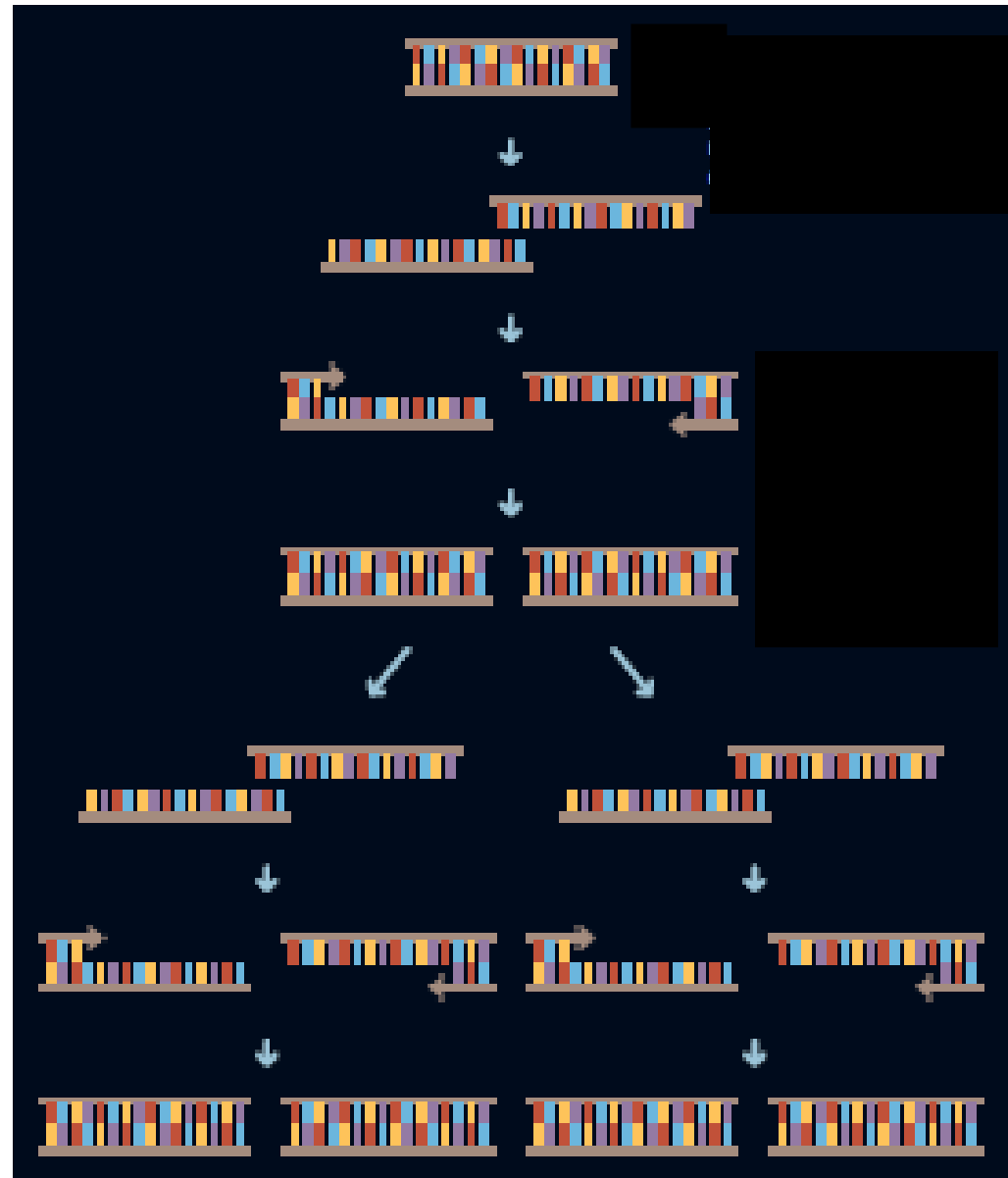
Strand Displacement Reaction (SDA)

Ligase Chain Reaction (LCR)

Reverse Transcriptase

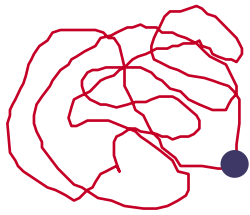
Polymerase Chain Reaction (PCR)

Polymerase Chain Reaction (PCR)



PCR amplifies specific fragments only

Target organism DNA



+

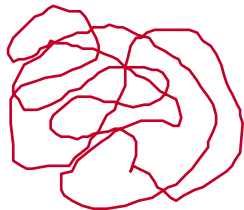
Primer specific
amplification

=

PCR



Background DNA



+

Primer specific
amplification

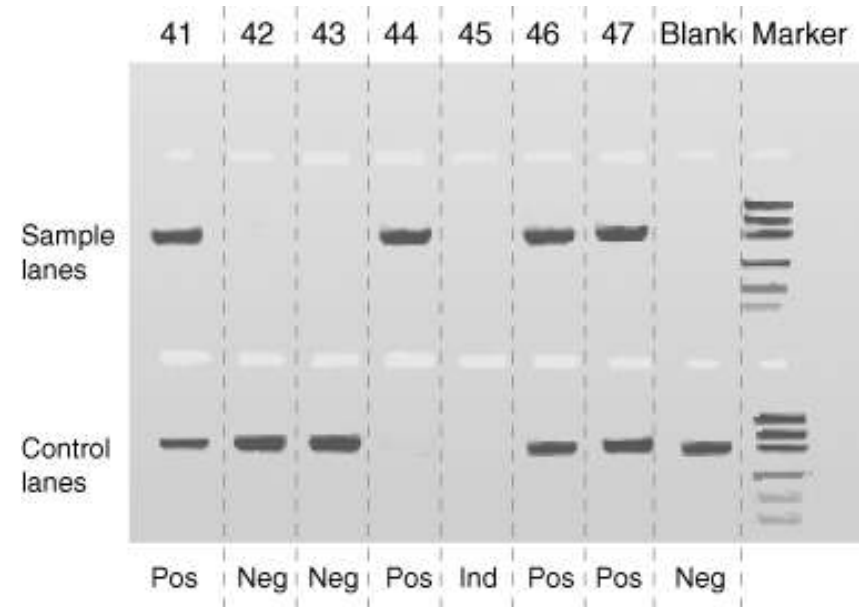
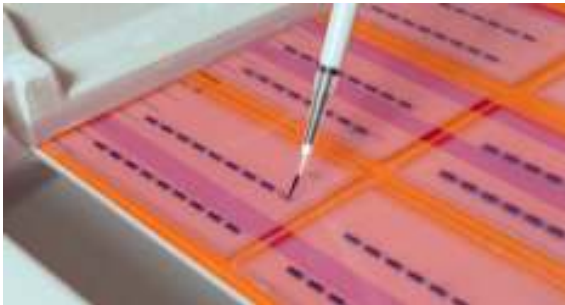
=

PCR

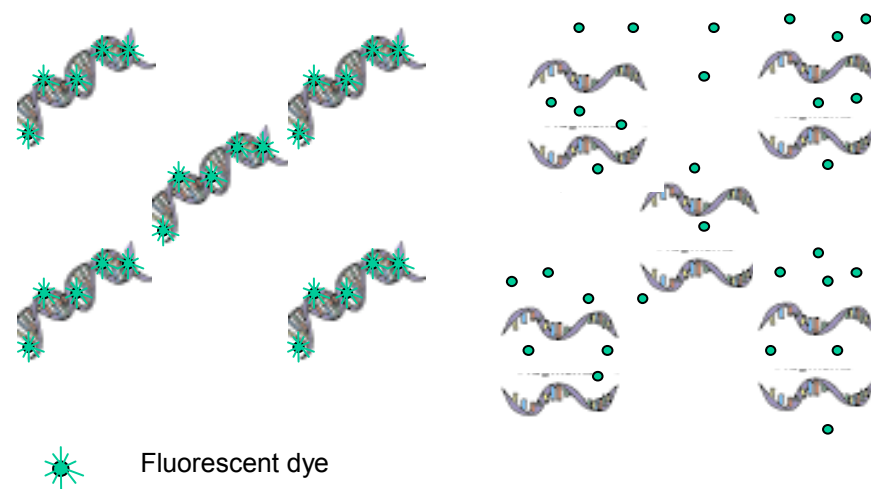


*no detectable
amplification*

PCR gel detection

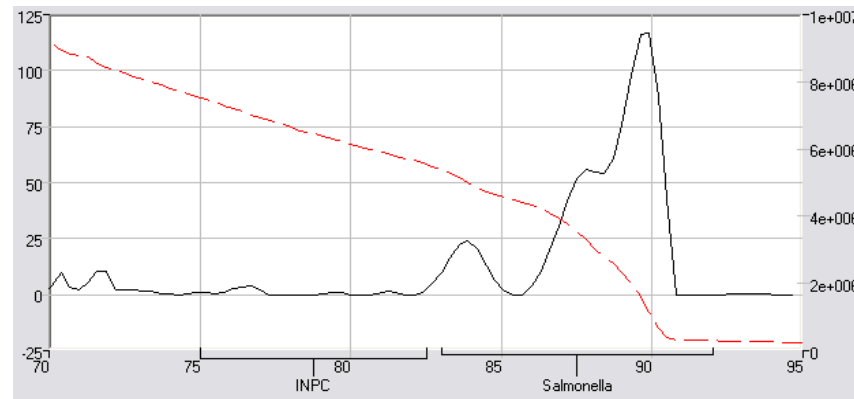


PCR automated detection- melting curve analysis

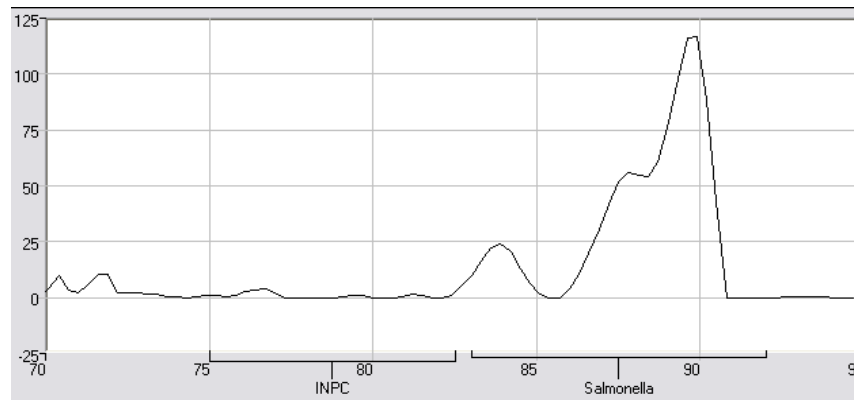


Algorithms convert raw melt curve to processed peaks

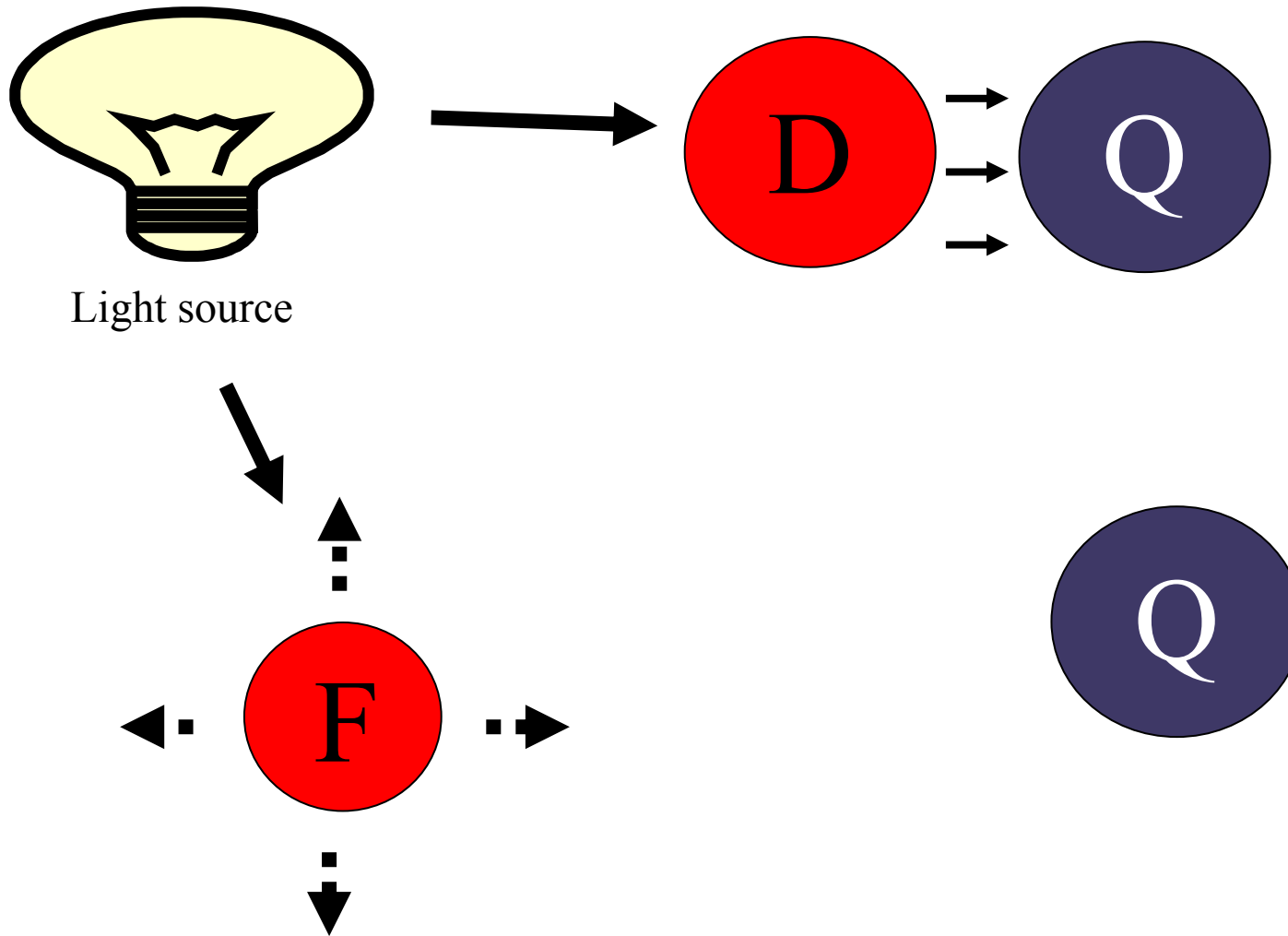
**Raw
Data**



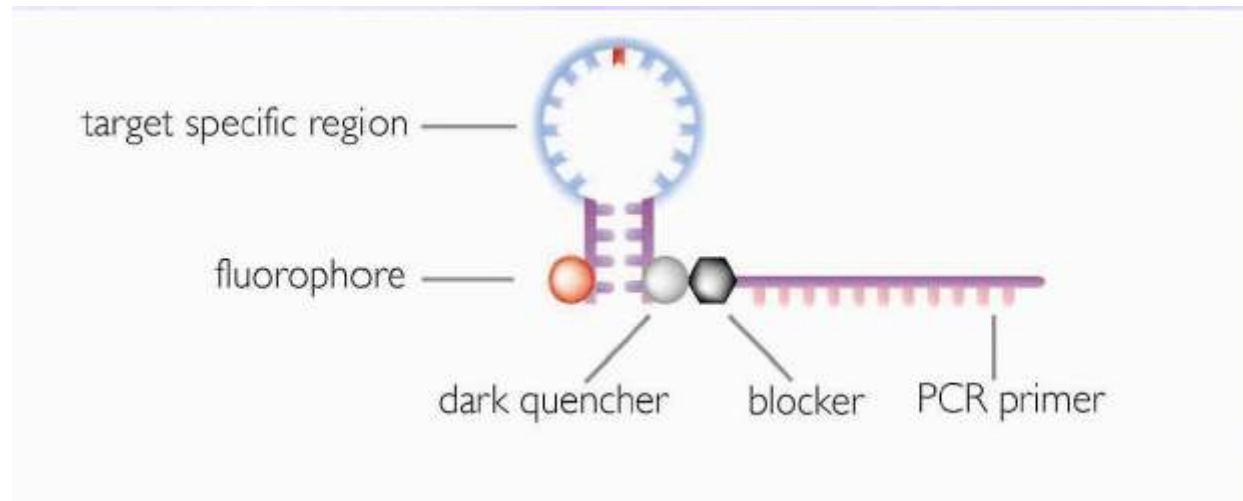
**Processed
Data**



Fluorescent Resonate Energy Transfer (FRET)

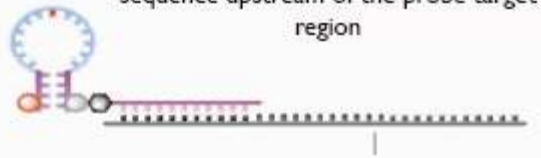


Scorpion Primer

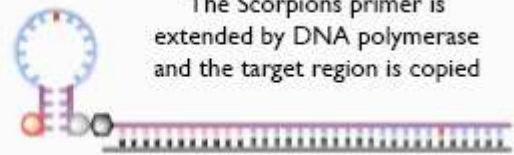


The Scorpions Reaction

Step 1



Step 2



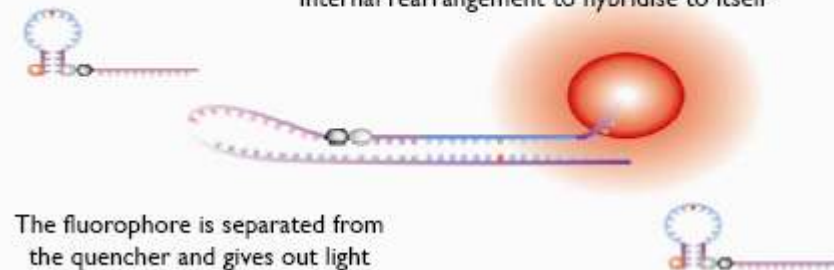
Step 3

The temperature increases and the newly extended Scorpions primer denatures

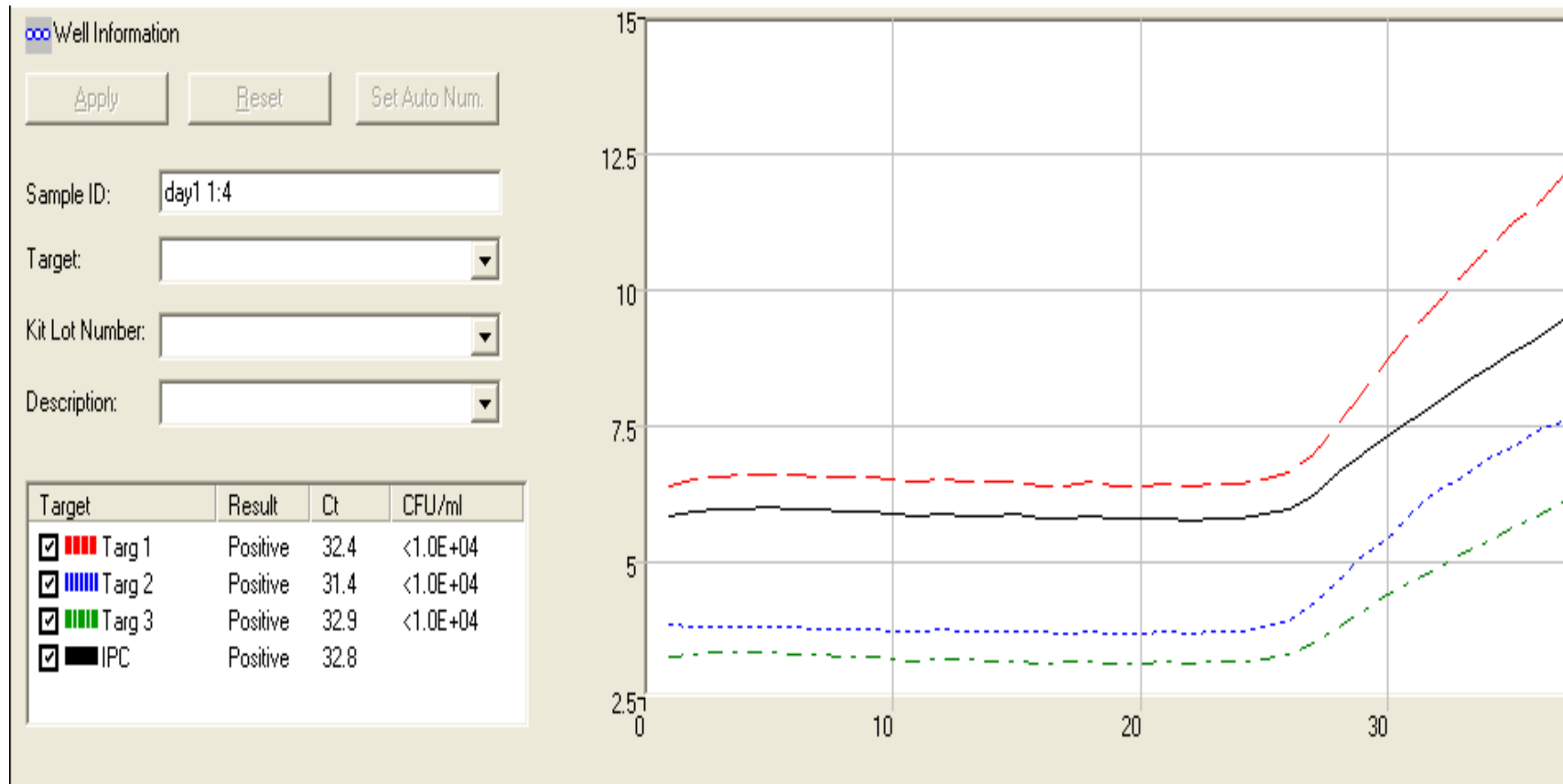


Step 4

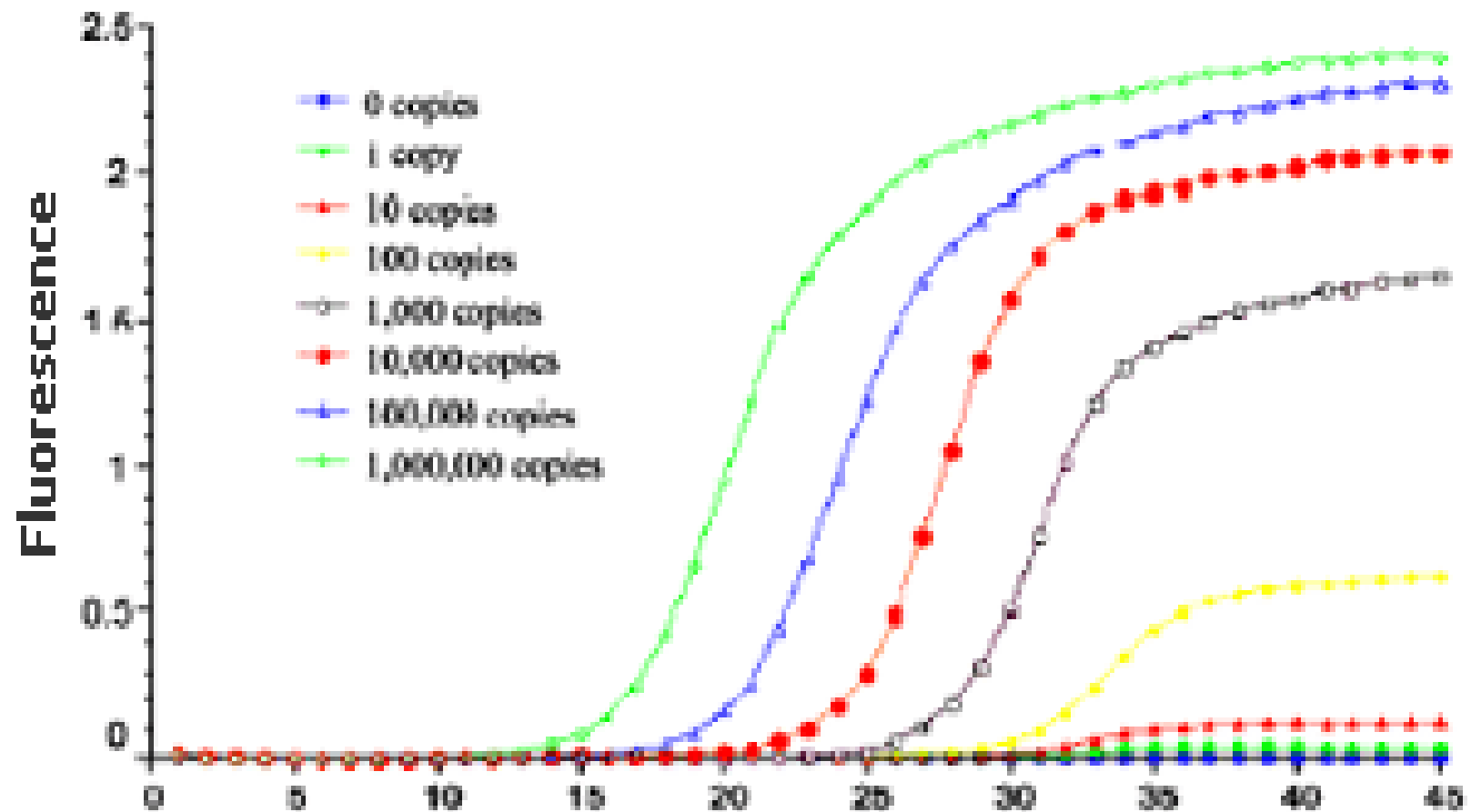
On cooling the extended Scorpion undergoes an internal rearrangement to hybridise to itself



Amplification plots



Quantitative PCR



Advantages of Amplification Techniques

- Detects specific microbial DNA sequences within a background of high levels of non-target DNA.
- Rapidly produces sufficient copies of specific DNA for easy detection
- More sensitive than conventional techniques
- Highly specific
- Does not rely on phenotypic expression of antigens

Disadvantages of Amplification Techniques

- The process requires instrumentation
- Sample preparation
- Amplicon contamination of work environment
- PCR inhibitors from the sample matrix
- Culture confirmation could be difficult

Current applications

Pathogen detection

- *Salmonella*
- *E. coli* O157:H7
- *Listeria monocytogenes*
- *Campylobacter*
- *Cronobacter spp*
- *Vibrio*

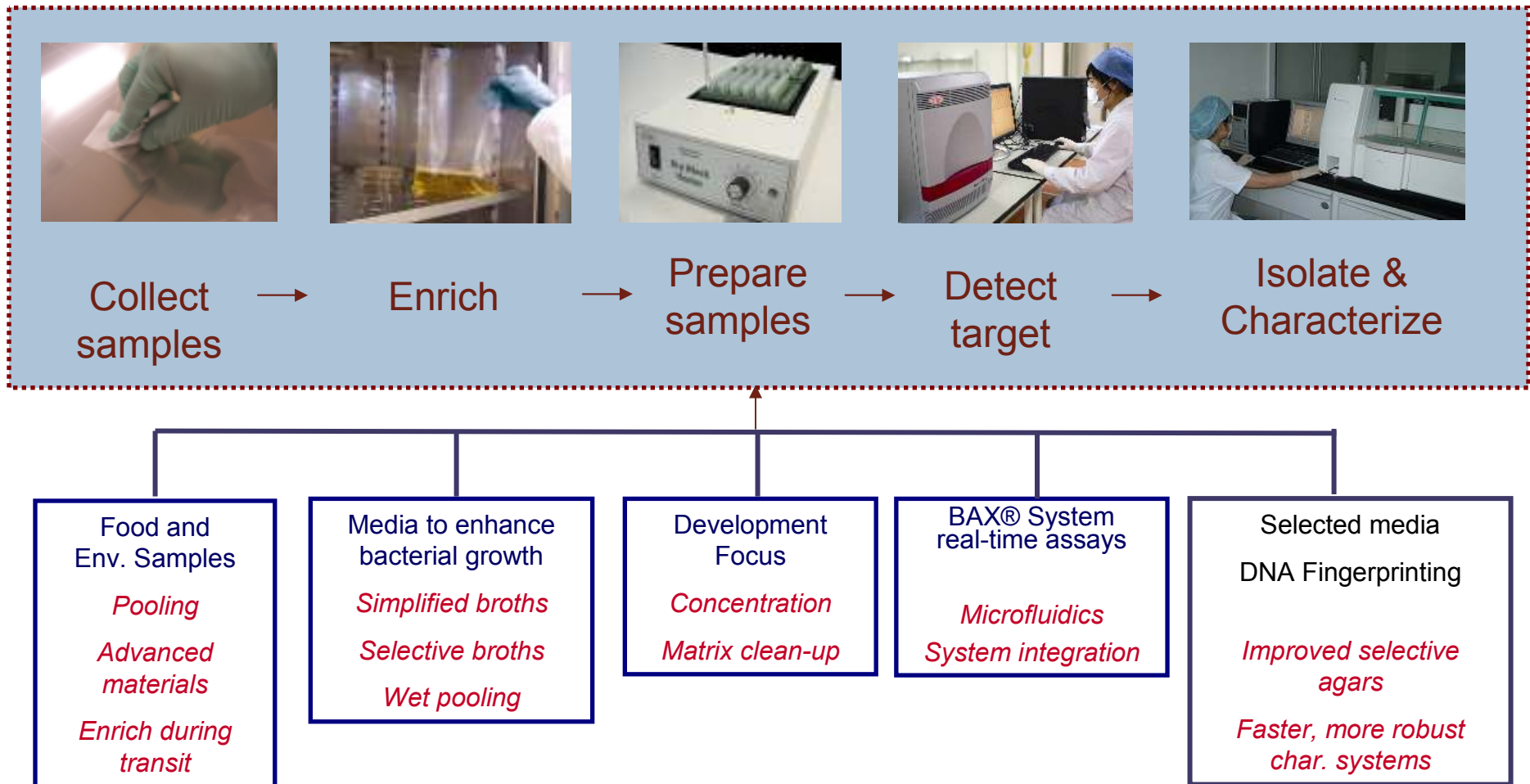
Indicator tests

- *Listeria spp*
- *Enterobacteriaceae*

Quality tests

- Y&M
- *Staph aureus*

Food testing – future process – integrated





The miracles of science™