Veterinary Laboratories Agency®

Rapid Genotyping of VTEC Isolates Using a Miniaturised Microarray Chip

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working for public and animal health

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Bacterial Genotyping Kits

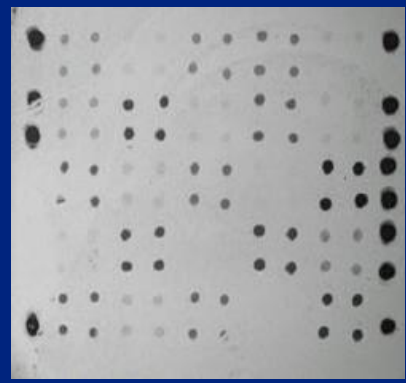
The Idenitbac miniaturised microarrays: What is it?

- An eppendorf tube with oligonucleotide gene probes at the bottom.

Array Tube - Microarrays

low density arrays

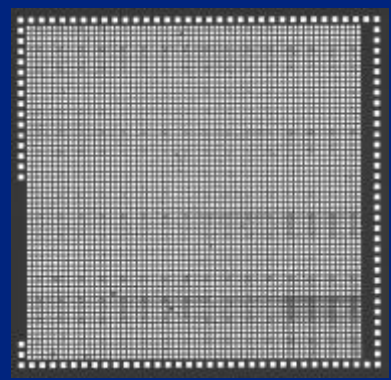
- up to 144 probes/array



array with 100 probes

high density arrays

- >10.000 probes/array



arrays with 4096 features

- various substance may be used as probes (oligos, PCR-products, peptides, proteins)

What is it's purpose?

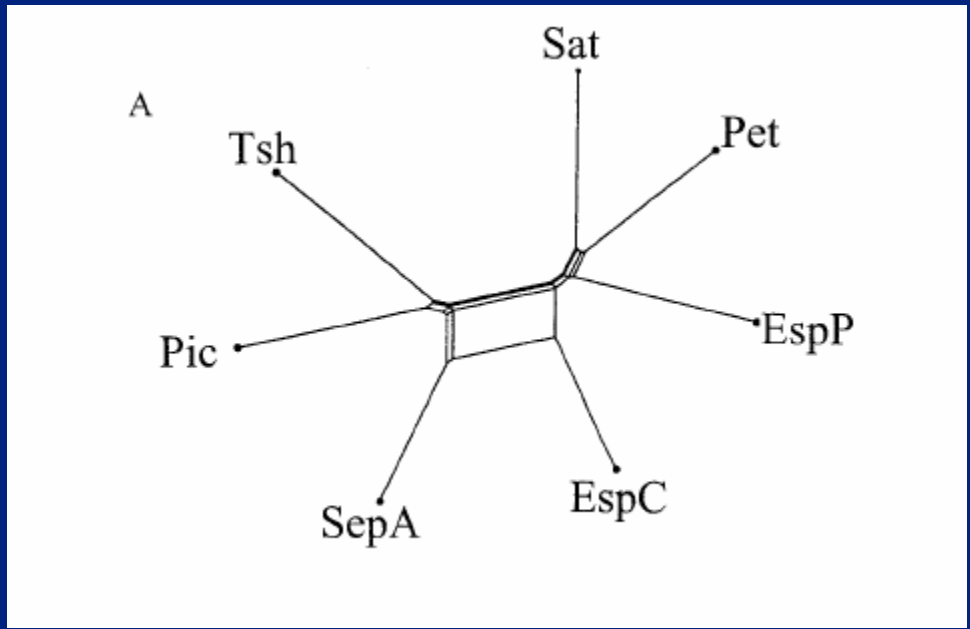
- To detect presence/absence of marker genes/proteins in microbial (or other biological) samples.

Method for probe/primer design

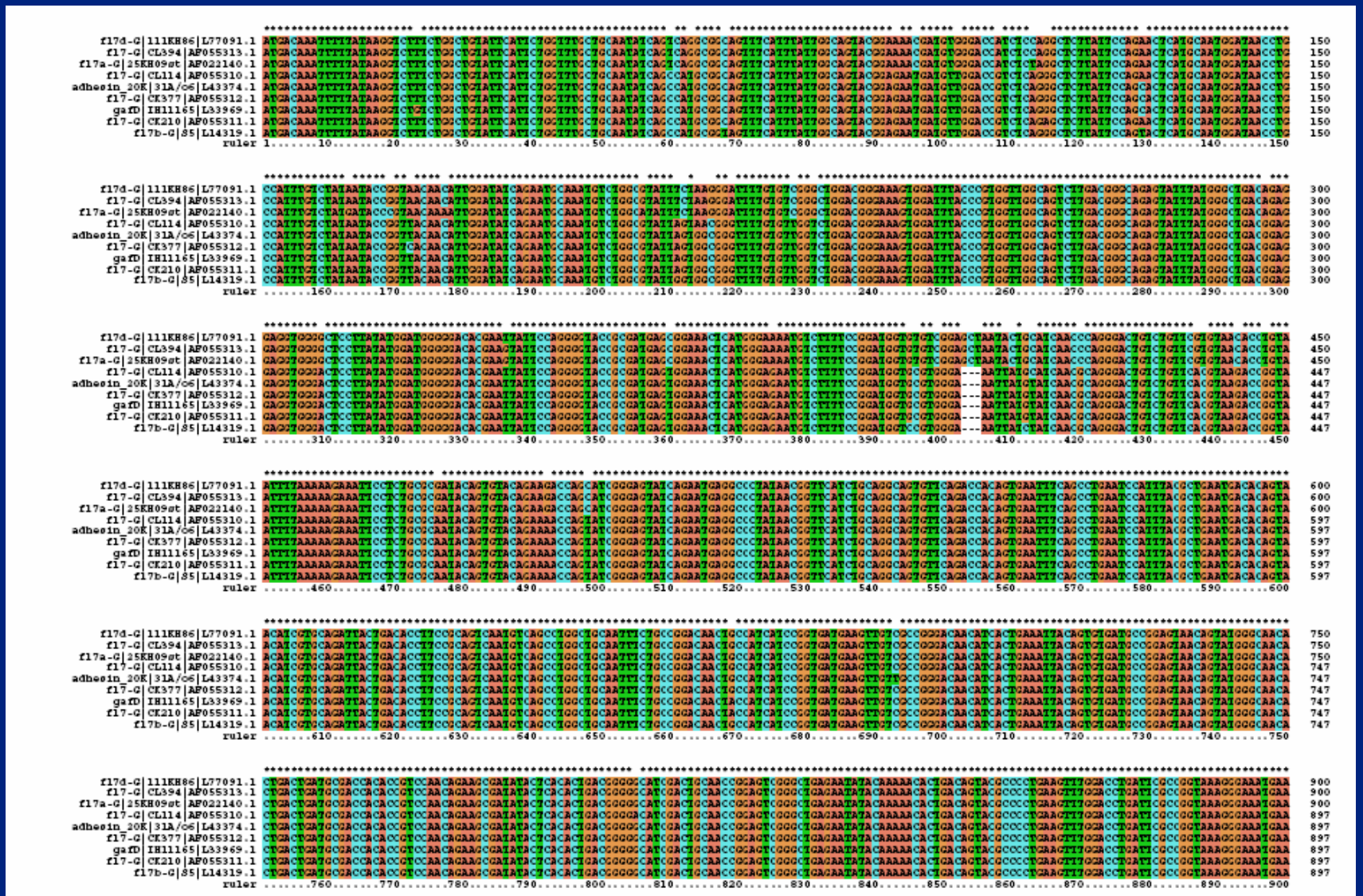
Gene name and accession no.

BLAST to find homologues

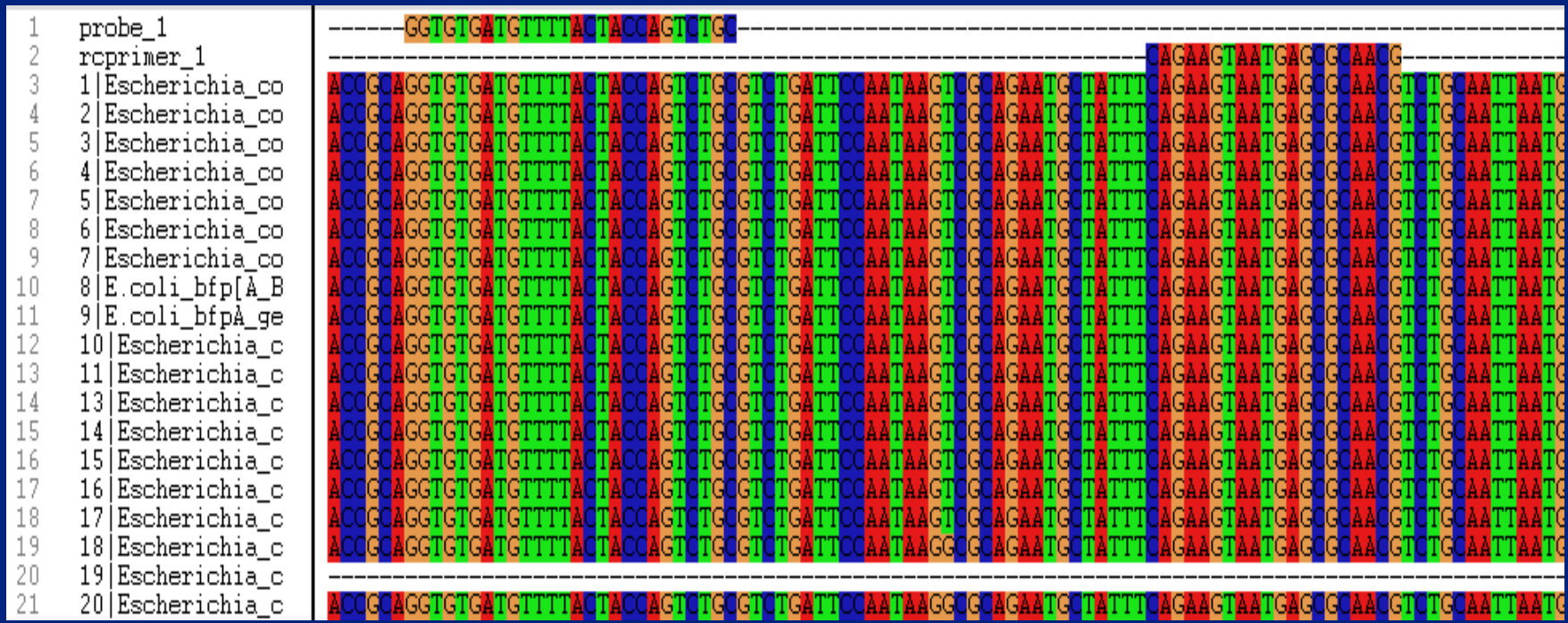
Make phylogram in ClustalX and align genes in each subgroup



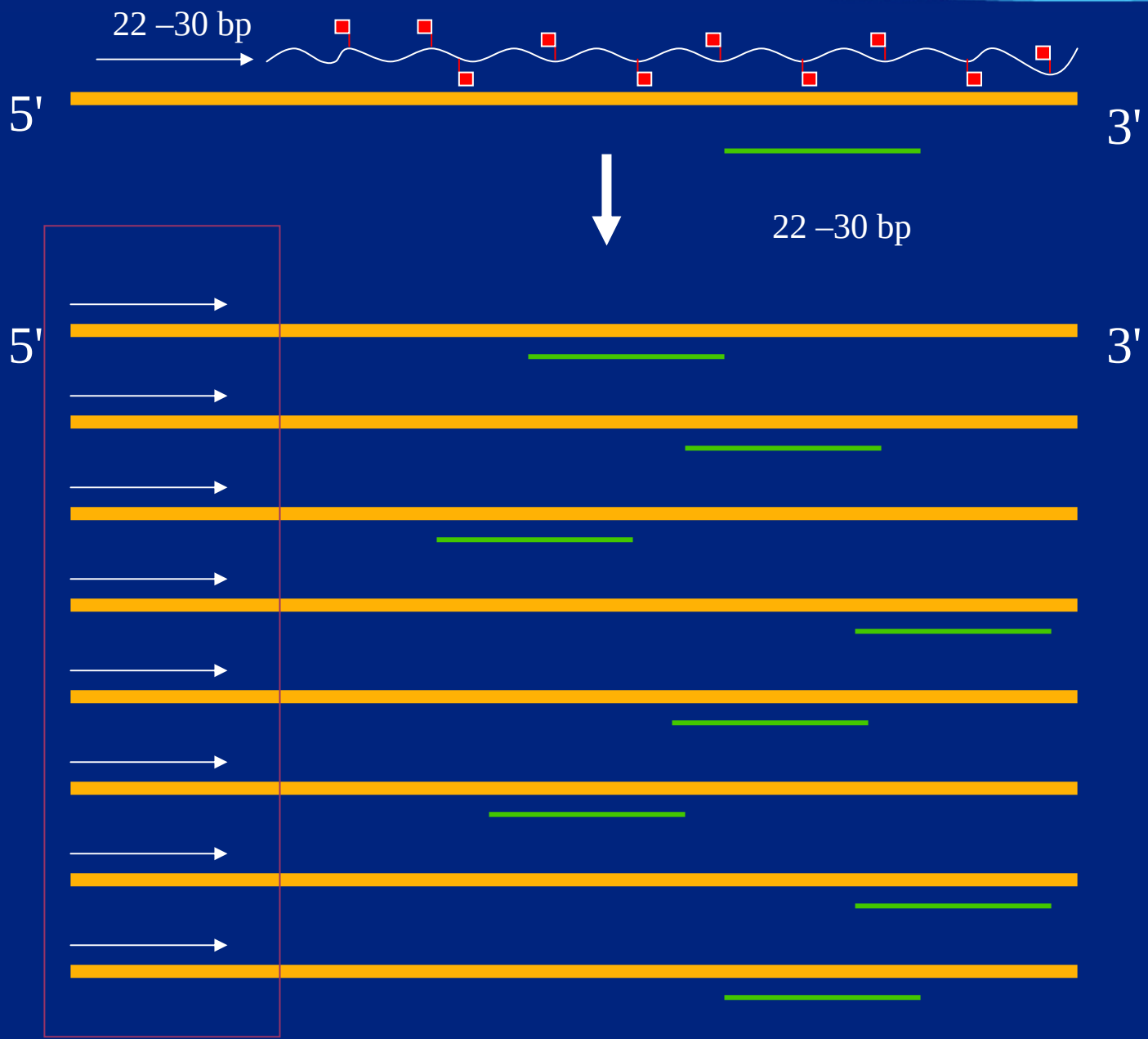
Multiple alignment of genes / subgroups of interest to find consensus region



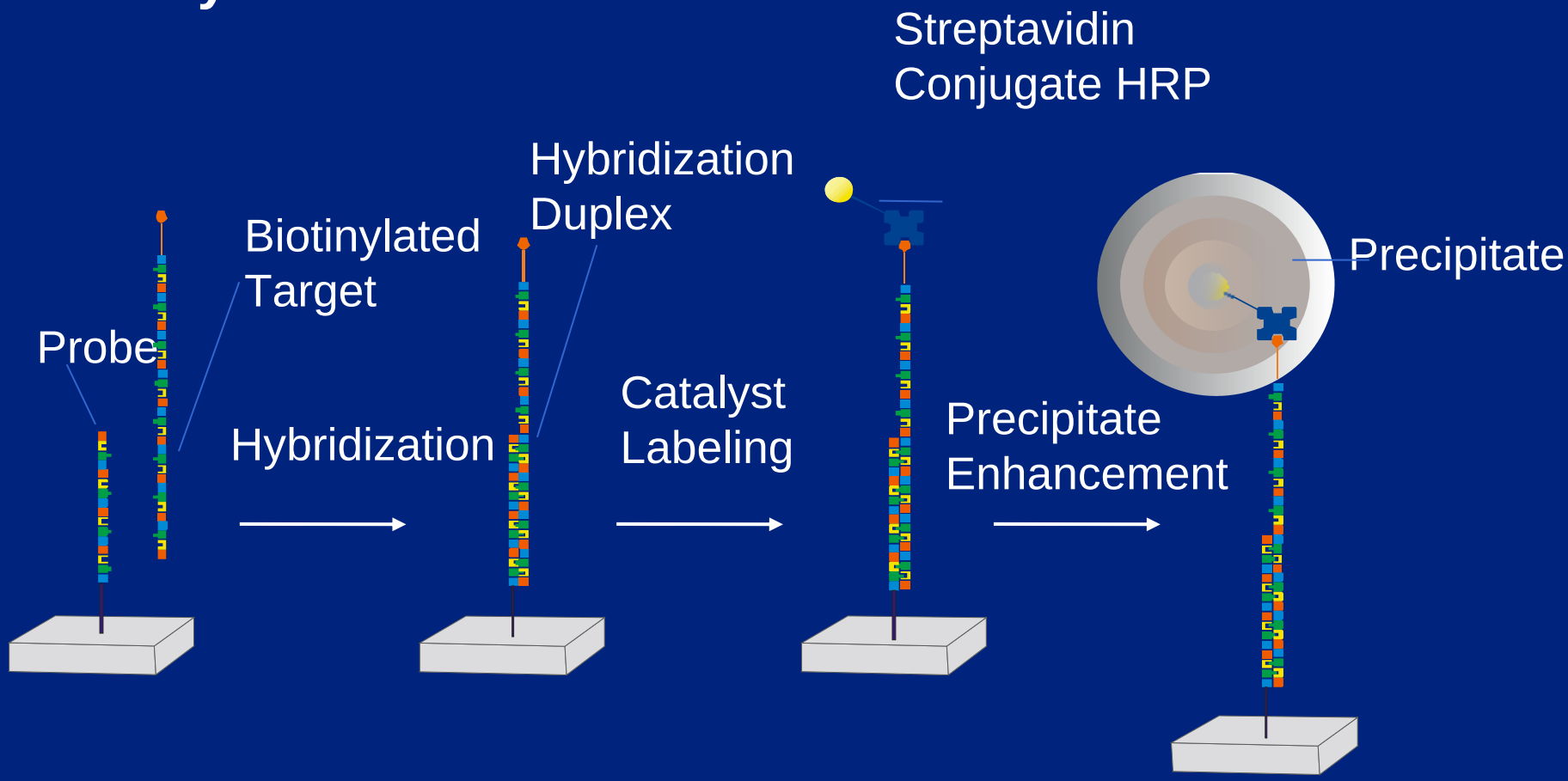
Probe and primer design from multiple alignment of sequence

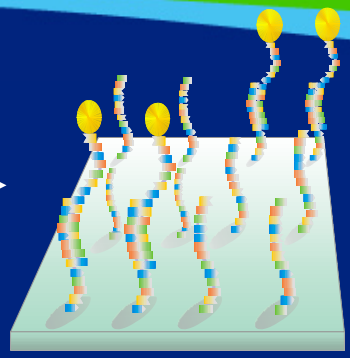


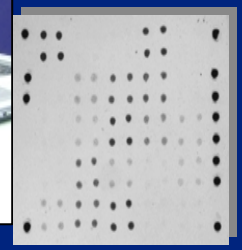
Genes with many allelic variants multiple probes/primers are designed e.g *eae*, *dfr*


22 –30 bp
5'
3'
22 –30 bp
5'
3'

The ArrayTube® Workflow







1 ArrayTube

Add biotynalted labelled gDNA fragment

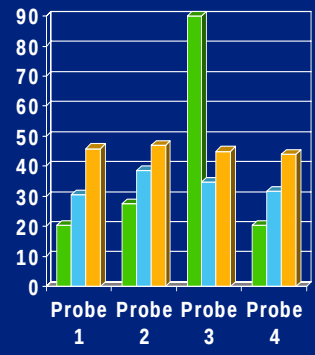
2 Precipitation

Hybridisation occurs and add strep-HRP for visualisation

3 ArrayTube Reader

used to record image

- Takes ~5 h from labelling to process results.
- Can process 24 samples simultaneously.
- High through-put and cheap system in development.



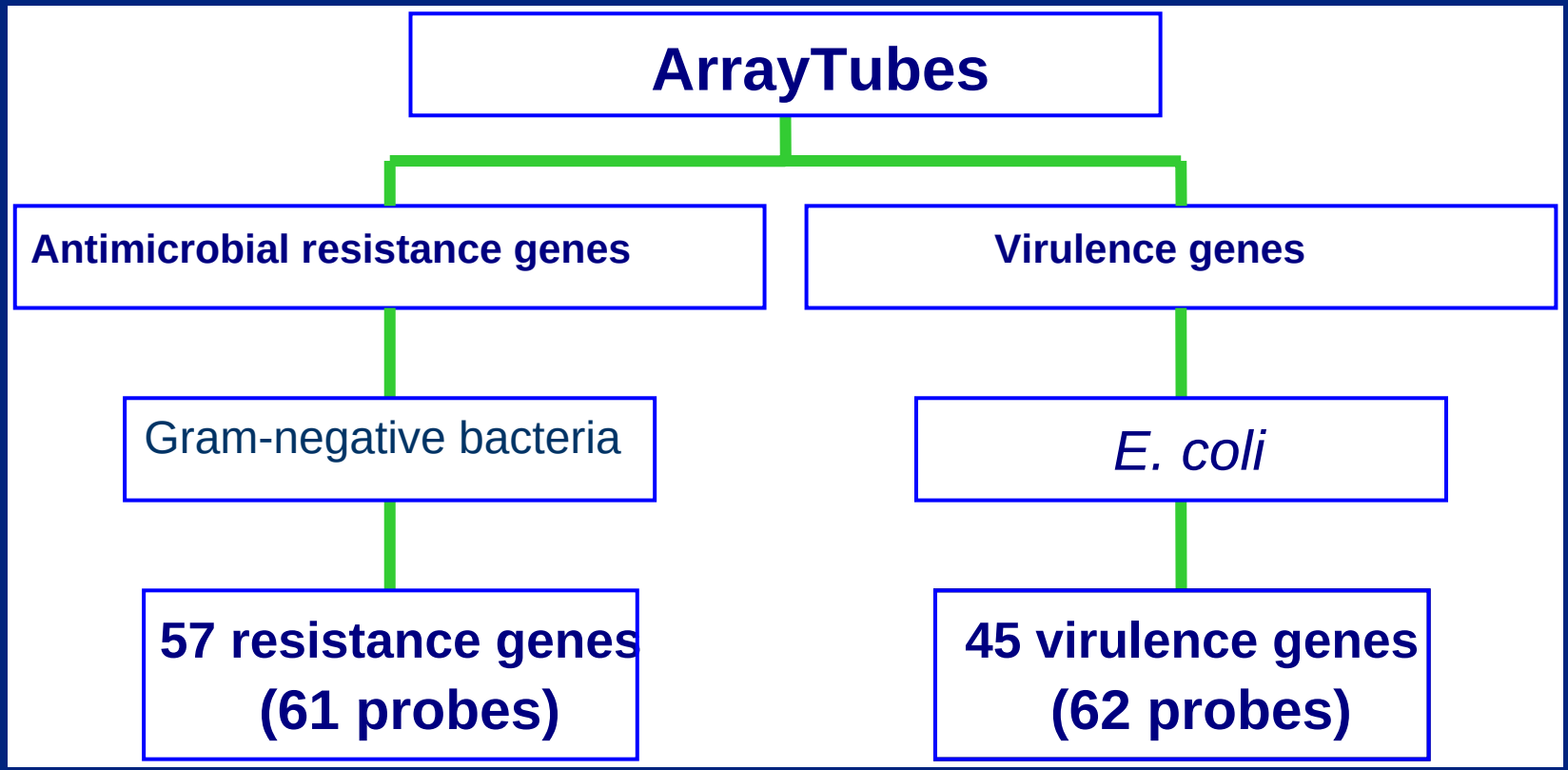
4 IconoClust

image analysis software to detect spots present

Schematic showing the procedure for using ArrayTubes

What is it's advantage?

- It can detect multiple marker genes rapidly, due to its simplicity


ArrayTubes
Antimicrobial resistance genes
Virulence genes
Gram-negative bacteria
E. coli
57 resistance genes
(61 probes)
45 virulence genes
(62 probes)

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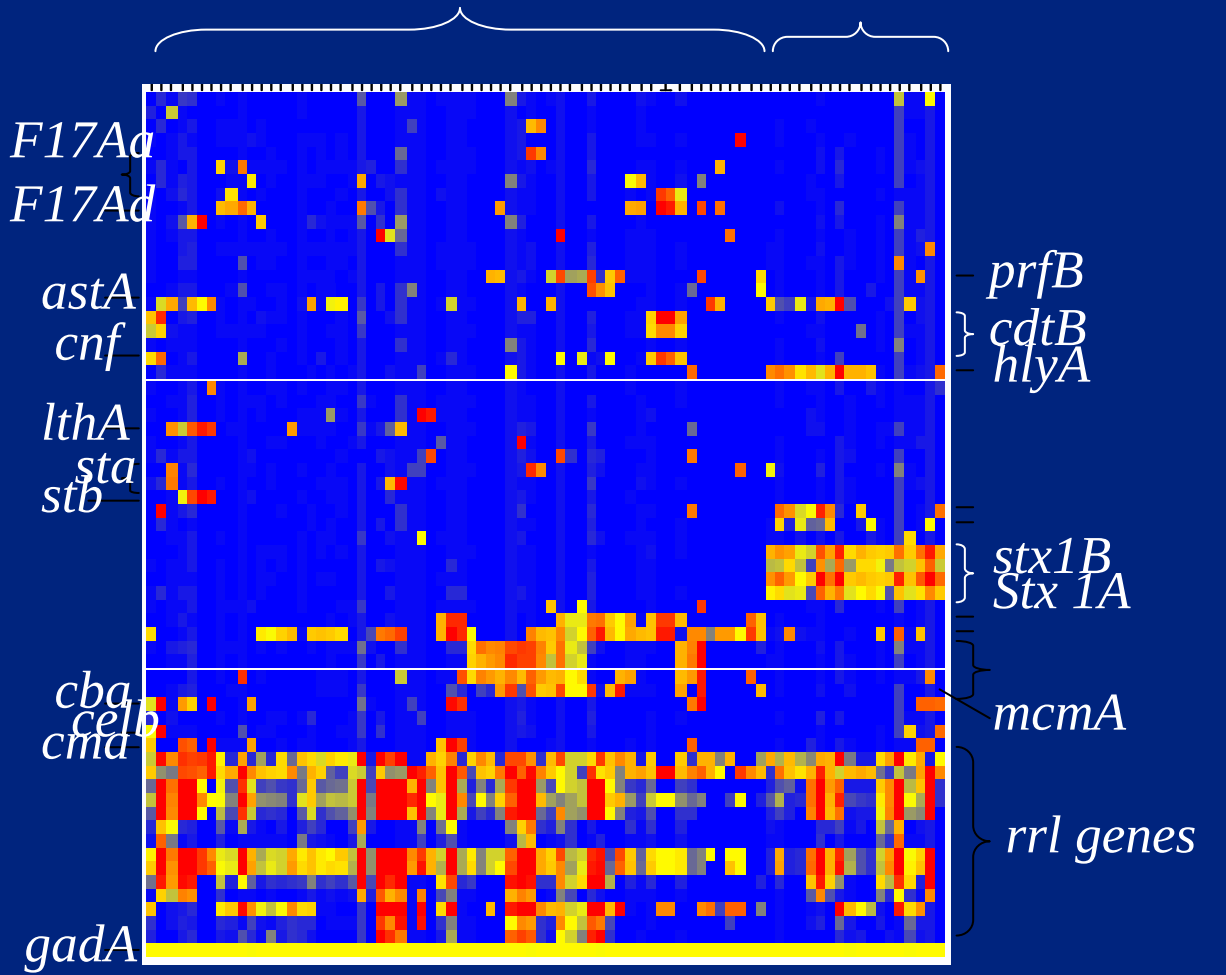
E. coli virulence gene array

- Genes coding for : toxins, fimbriae, adhesins, siderophores, bacteriocins.
- Published literature, database search for marker genes.
- Multiple sequence alignment of homologous genes.
- Clustal X to design oligonucleotide probes/primers (22-30mers)

- **45 different virulent genes + control sequences**

Pathotyping of *E. coli* isolates



63 *E. coli* isolates

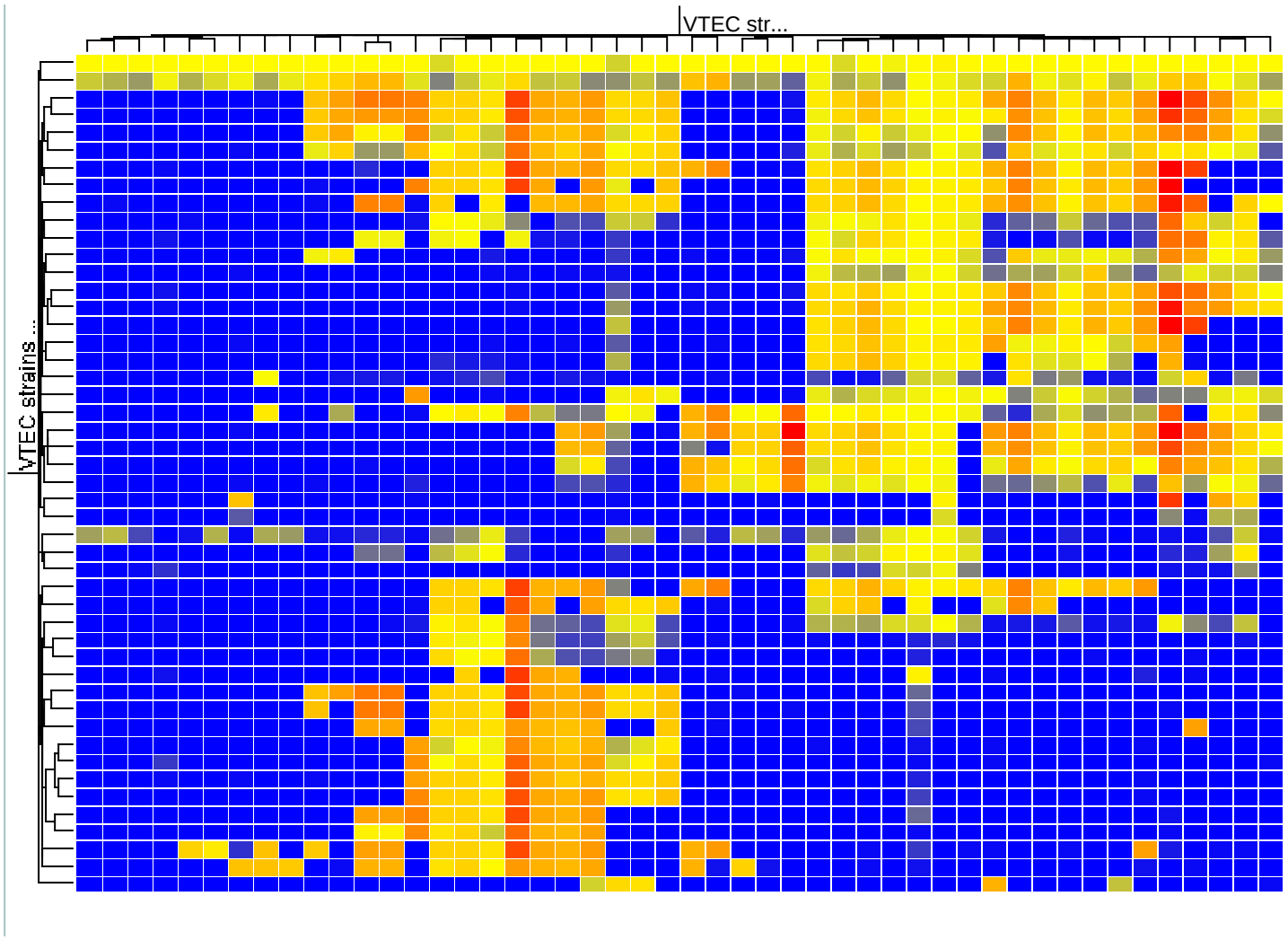
19 – VTEC
8 - EPEC
18 – UPEC
15 – ETEC
1 – EIEC
3 – EAEC
3 –virulence genes undetected

blue – gene absent; yellow / red – gene present

Anjum *et al* 2007, Appl & Env Micro 73(17).

New Iteration of the *E. coli* virulence gene chip

- Have added 71 new genes (see list of genes)
- Genes are EPEC/VTEC virulence factors from O157 and other VTEC strains
- Will be able to subtype some of the major *vt2* subtypes (*2d*, *2e*, *2f*, *2g*)
- Will be able to distinguish between subtypes found in some of the major virulence gene families *e.g. espA*, *nleB, tir*)
- Addition of genes from the SPATE (serine protease autotransporters in *Enterobacteriaceae*) family.
- Chip is currently being validated


VTEC str...
VTEC strains ...
Stx genes
Strains

Antimicrobial oligonucleotide TubyArray

- Genes coding for resistance to:
  sulphonamides, trimethoprim, tetracyclines, streptomycin, carbenicillinases, chloramphenicol, florphenicol, aminoglycosides, ß-lactamases, integrase, quinolones.

- Published literature, database search for marker genes.

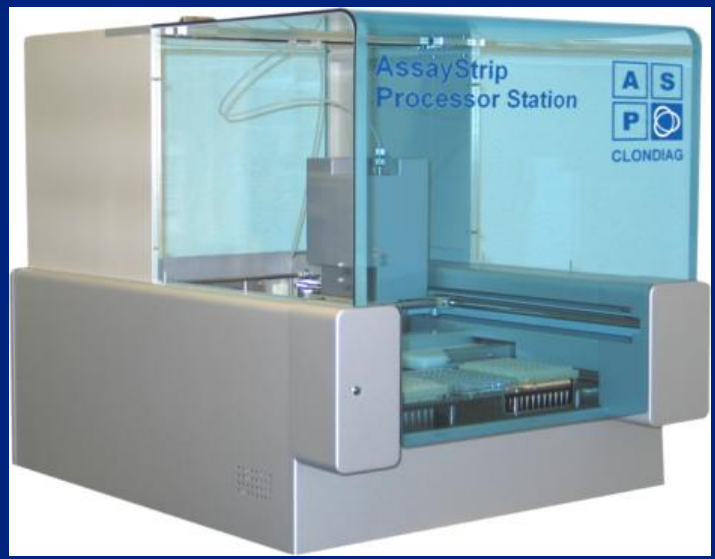
- Multiple sequence alignment for subgroups within antimicrobial gene family.

- Clustal X to design oligonucleotides (22-30mers)

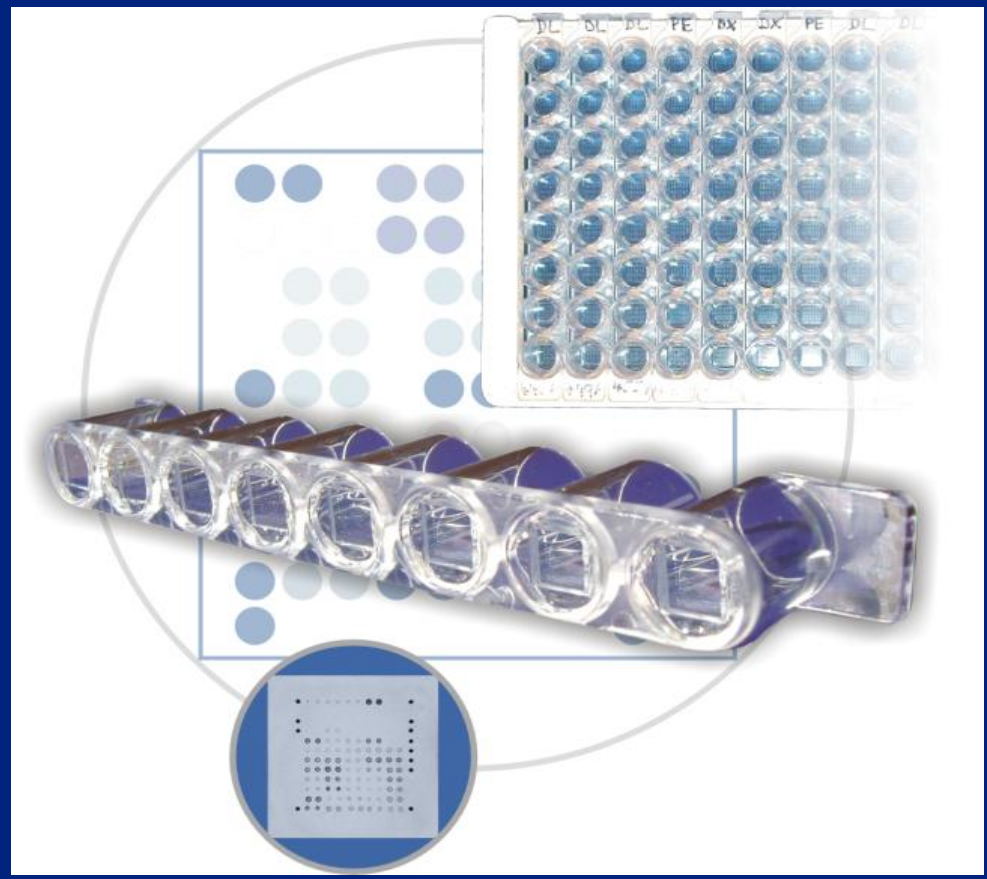
  57 different target genes and positive controls (*ihfA* and *gapA*)

ArrayStrip Platform

parallel multiplex testing



Array Strip Processor (ASP)
for automatic test processing



Array Strips (AS)
probe integrated in 8 well-strips, up to 12 strips in standard microplate format

Combined chip

- A combined chip containing *E. coli* virulence genes, O- and H- antigen genes and Gram-ve antimicrobial resistance genes
- Capacity for approximately 600 probes
- Assay performed using a similar protocol as the single chips
- Currently the chip and method is being validated using control strains.

Identibac family

www.identibac.com

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Array gene list and details available at:
www.idenitibac.com