



Advantages Of Capillary Electrophoresis-Based Analysis in Molecular Microbiology

Dr. Carola Schade, Assoc. Business Director
Automation, QIAGEN GmbH

Anna Ryberg, Principle Investigator, University
Hospital Linköping, Sweden

The QIAGEN applications presented here are not for use in diagnostic procedures.

Bearing With Them Vs. A Deeper Understanding

From non-molecular to molecular methods



The eras of formal microbiology*

- Speculation (5000 BC – 1675)
- Observation (1675 – 19th century)
- Cultivation (19th century – 20th century)
- Physiological study (early 20th century till)
- Molecular study (1983 – today)
 - DNA as the essential genetic material
 - Invention of PCR
 -

* From *The Development of Microbiology*, P. Collard 1976

Microbes Are Of Interest In Many Fields of Research

Typical application areas



- Basic & evolutionary research
- Clinical microbiology
- Infectious disease
- Immunology
- Vaccine development
- Food testing
- Veterinary

Microorganisms interact with man and his world — everywhere!



Market trends and challenges

- Standardization
- Throughput
- Data output and storage
- Convenience and efficiency
- Cost reduction
- Time-to-result
- Publishing faster

Application-based trends and challenges

- Diverse and complex starting material
 - Large variety of different sample types
 - Limited available sample material
- Accuracy & reliability of results
- What is the best analysis technology?

Microbial Analysis – A Multistep Procedure

Typical workflow

Automation



TissueLyser LT&HT
TissueRuptor



QIAcube

EZ1



QIASymphony



QIAgility

Rotor-Gene Q



QIAxcel



PyroMark 24 & 96

**Sample Collection &
Stabilization,
Sample Disruption**

DNA Purification

Assay Setup

**Detection &
Analysis**

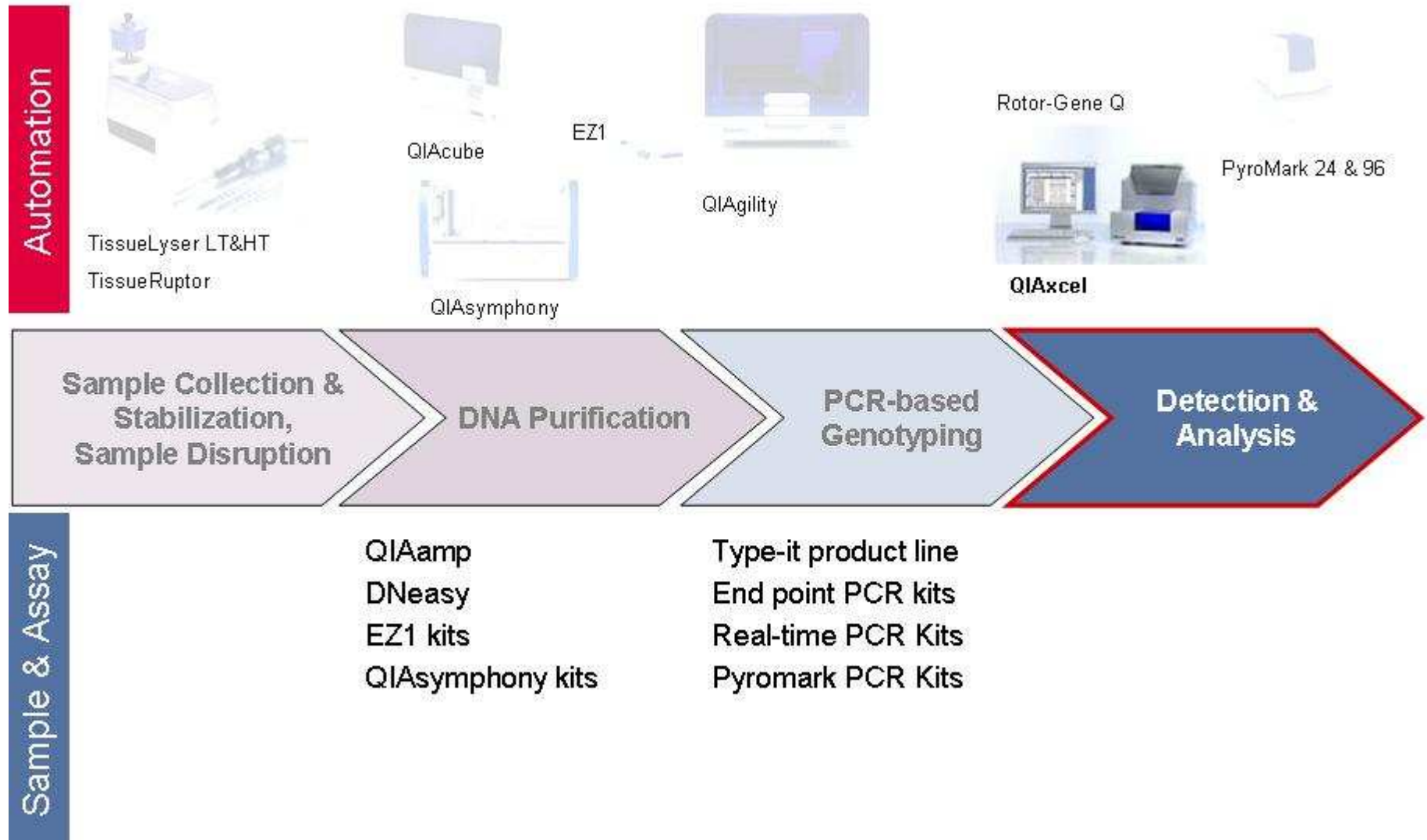
Sample & Assay

QIAamp
DNeasy
EZ1 kits
QIASymphony kits

Type-it product line
End point PCR kits
Real-time PCR Kits
Pyromark PCR Kits

Microbial Analysis – A Multistep Procedure

Typical workflow



The Choice Of Analysis Technologies

Overview



Sequencing

- Whole genome
- Pyrosequencing
- MST/MLST – **M**ulti**L**ocus **S**equencing **T**yping
 - characterization of bacterial species using the DNA sequences of internal fragments of multiple housekeeping genes

PCR-based

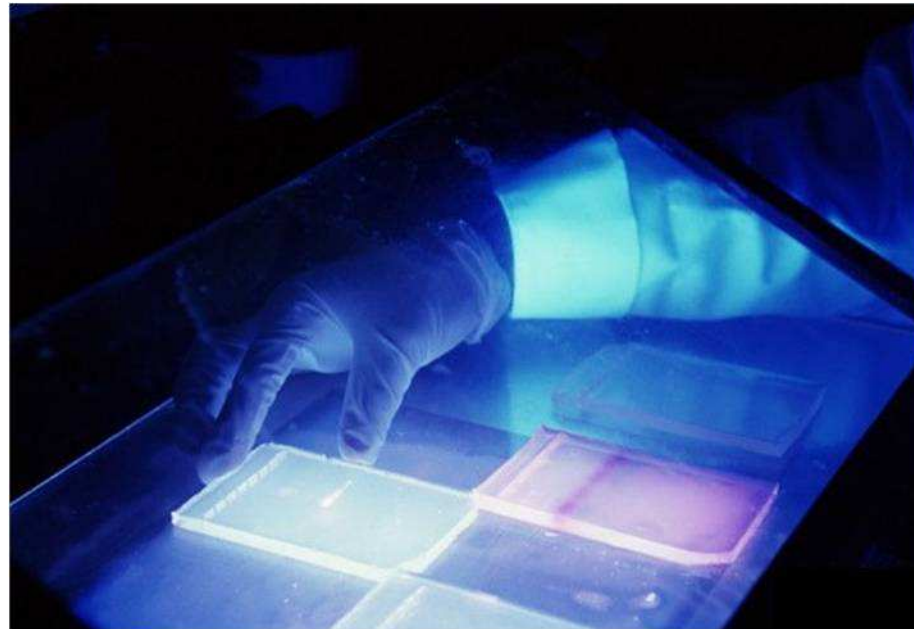
- PCR/RT-PCR
 - Identification of organisms — absence/presence
 - i.e. detection of specific virulence genes
 - Multiplexing capabilities
- Real-time PCR/real-time RT-PCR
 - Additional benefit of quantitative information
- LAMP – **L**oop-mediated isothermal **A**mplification
- VNTR/MLVA – **V**ariable **N**umber of **T**andem **R**epeats/**M**ulti**L**ocus **V**NTR **A**nalysis
- RAPD – **R**andom **A**mplified **P**olymorphic **D**N
 - amplification of random segments of genomic DNA with single primer of arbitrary nucleotide sequence
- AFLP – **A**mplified **F**ragment **L**ength **P**olymorphism
 - Visualization of patterns of amplified DNA restriction fragments

Enzyme Digest

- PFGE – **P**ulse **F**ield **G**el **E**lectrophoresis
 - Separation of large DNA fragments (>30–50 kb)
 - Separation of RFLPs – **R**estriction **F**ragment **L**ength **P**olymorphism

Detection & Analysis of End-point PCR Products

Gel electrophoresis most commonly used method



Is this the best solution?

Have you ever thought about



... how much time you spend on agarose gel electrophoresis?

Process Steps	Time*	
Gel Preparation	20–45	min
Sample Loading	6	min
DNA Separation	30–120	min
Documentation	5	min
Analysis	15	min
TOTAL	76–191	min

Some calculations

- Three gels a week
 - ➔ 190 – 478 hours/year
 - ➔ 1 – 3 months/year

- One gel a day
 - ➔ 317 – 796 hours/year
 - ➔ 2 – 5 months/year

Have you ever thought about



...costs being generated ?

- Materials
 - Agarose or high-resolution agarose
 - Reagents
 - Thermo-printer paper
- Tips, gloves, weighing boats, tape
- Repeat experiments due to manual errors
- Labor time
- Disposal of waste
 - Liquid waste
 - Solid waste

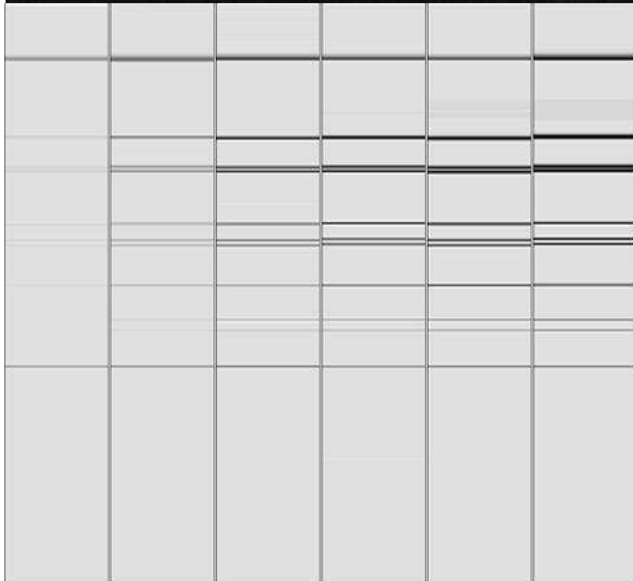
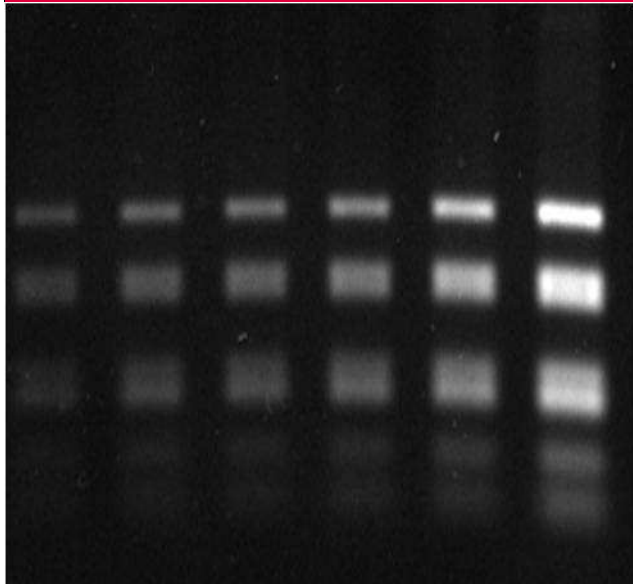
Material	Amount	Unit	Package Price	Price/Unit	Net Price
0.5-10 µl Disposable Tips	13	pc	39.66	960	0.54
1-200 µl Disposable Tips	1	pc	34.02	960	0.04
Adhesive acryl tape	18	cm	6.96	6600	0.02
Weighing boats	1	pc	7.44	100	0.07
Gloves	2	pc	18.96	100	0.38
Tris	1.21	g	78.82	1000	0.10
Na2EDTA • 2H2O	0.19	g	135	1000	0.03
Agarose	1.25	g	188.52	100	2.36
Acetic Acid	0.28	ml	29.64	1000	0.01
100 bp Ladder	0.25	µg	52.8	50	0.26
EtBr	0.005	ml	37.32	10	0.02
Sample No.			12		
SUB TOTAL					3.81
Labor Cost	25	min	20	60	8.33
TOTAL/No. Of Samples					12.15
TOTAL/Sample					1.01

Have you ever thought about



... standardization ?

- Accuracy of results
- bp resolution that can be achieved
- Manual errors
- Automated documentation and analysis





Improved Detection & Analysis of Nucleic Acids

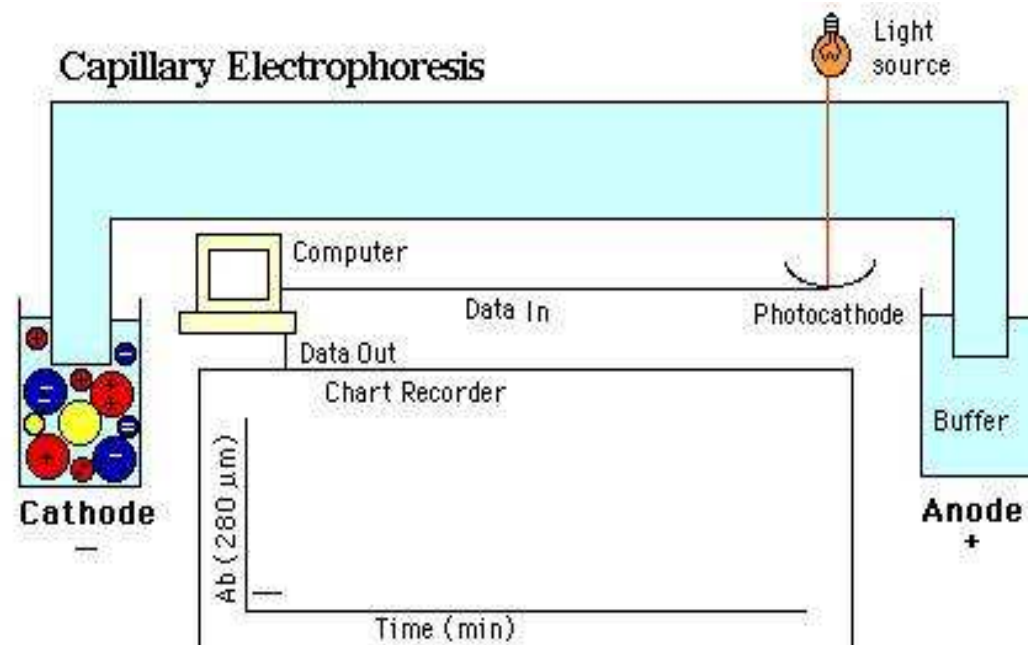
Automated nucleic acid separation systems



The world of nucleic acid separation will change!

The Basic Principle Of Capillary Electrophoresis

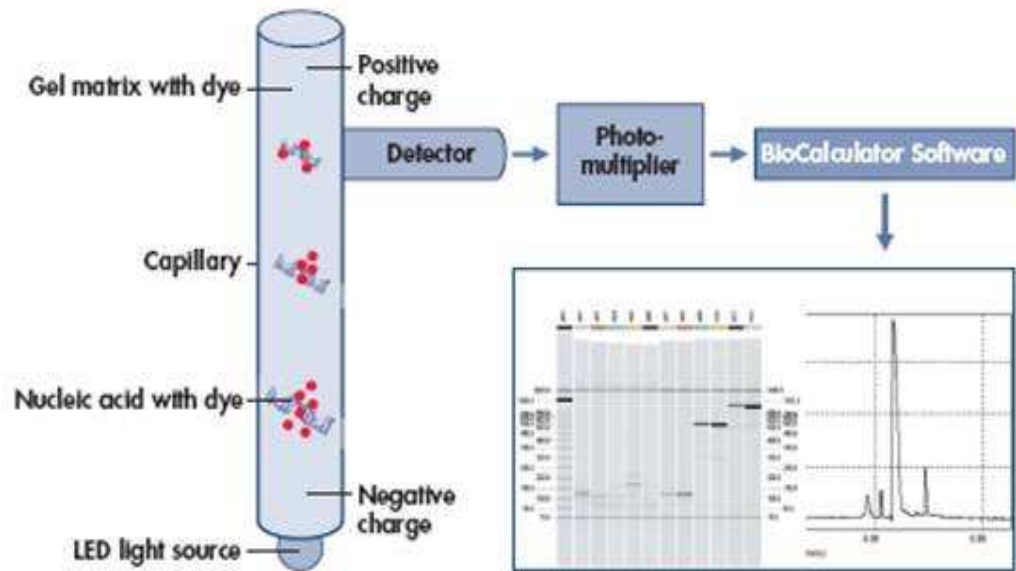
Schematic overview





The QIAxcel Separation Principle

Capillary gel electrophoresis





No More Slab-gel Analysis

Key features and benefits



Key features

1. Fully automated DNA fragment analysis
2. Ready-to-run gel cartridges
3. Fast processing: 12 samples in 5–12 min.
4. Up to 96 samples per run
5. Sample input amounts < 0.1µl
6. Detection limit of 0.1 ng/µl
7. High resolution of 3–5 bp
8. Digital data output



Different Gel Matrices For Different Needs

QIAxcel Gel Cartridges



Gel Cartridge Name	Analyte	Size Range	Best Resolution				Run Time*	Example Applications
			100 bp – 500 bp	500 bp – 1 kb	1 kb – 5 kb	5 kb – 10 kb		
QX DNA High Resolution Cartridge	DNA	15 bp – 5 kb	3 – 5 bp	50 bp	200 bp– 500 bp	–	7 – 12 min	High-resolution genotyping
QX DNA Screening Cartridge	DNA	15 bp – 5 kb	20 bp – 50 bp	50 bp – 100 bp	500 bp	–	5 min	Fast PCR screening
QX DNA Large Fragment Cartridge	DNA	15 bp – 10 kb	3 – 5 bp	50 bp	100 bp	500 bp	22 min	Large and long size range
QX RNA Quality Control Cartridge	RNA	200 bp– 10 kb	200 b	200 b	500 b 1 kb	1 kb	10 min	Checking RNA quality

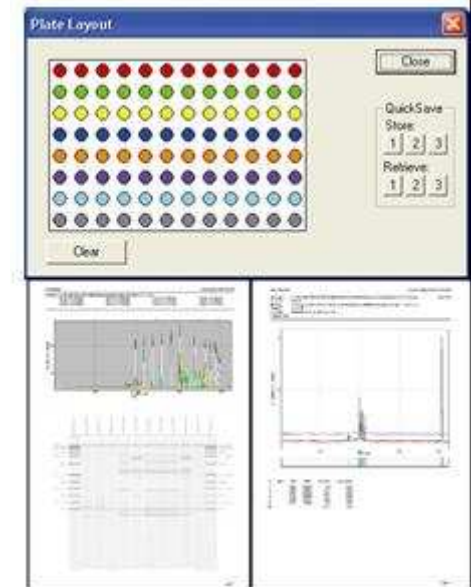
* Run time given for 12 samples; run times will also depend on the software method being chosen to analyze samples

The BioCalculator Software

Operating the system and data analysis



- Operate the instrument using the Instrument Control panel
- Analyze data and determine fragment size and concentration
- Export data and create data reports
 - Automatic saving of gel view as .jpg image
 - Direct printing of
 - Gel image/electropherograms
 - Individual channel information
 - Or generate pdf !
 - Export functionality
 - Gel image as tiff/bmp/jpg
 - Peak properties in .csv format
 - Binary peak table option

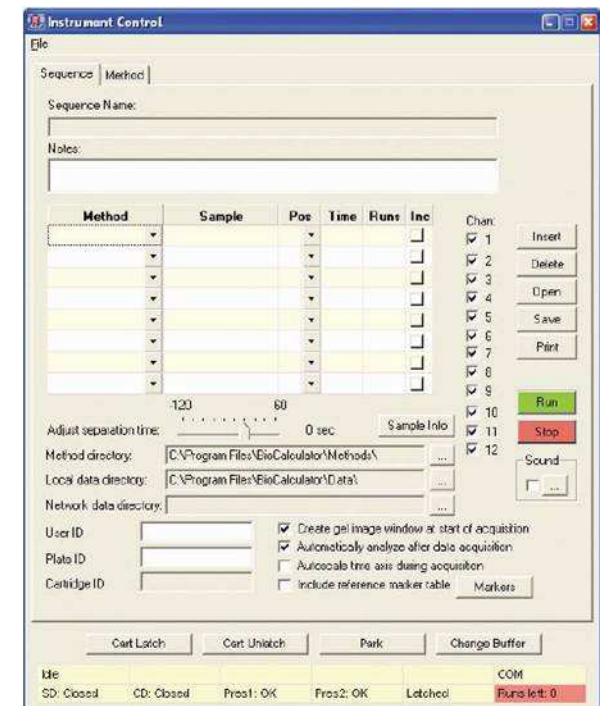


How does it work?

Operating the QIAxcel



- Load the gel cartridge
- Load buffers and reagents
- Load your samples
- Select the method of your choice
- and GO !

The screenshot shows the 'Instrument Control' window with the following fields and controls:

- Sequence Name:** [Text field]
- Notes:** [Text area]
- Method Table:**

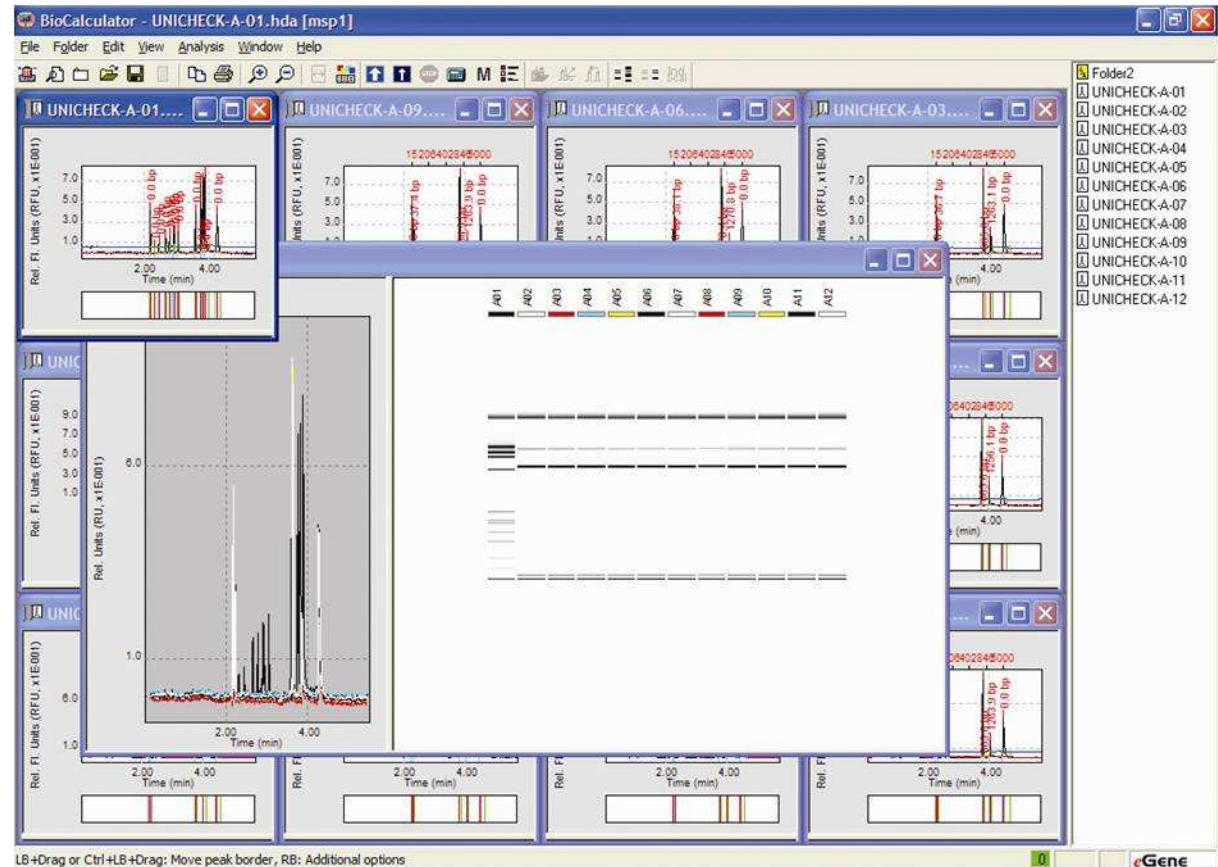
Method	Sample	Pos	Time	Runs	Inc	Chan
						1
						2
						3
						4
						5
						6
						7
						8
						9
						10
						11
						12
- Adjust separation time:** [Slider from 1.20 to 60] [0 sec] [Sample Info]
- Method directory:** [C:\Program Files\EleCalculator\Methods\]
- Local data directory:** [C:\Program Files\EleCalculator\Data\]
- Network data directory:** []
- User ID:** []
- Plate ID:** []
- Cartridge ID:** []
- Checkboxes:**
 - ☒ Create gel image window at start of acquisition
 - ☒ Automatically analyze after data acquisition
 - ☐ Autocalibrate time axis during acquisition
 - ☐ Include reference marker table
- Buttons:** Insert, Delete, Open, Save, Print, Run, Stop, Sound
- Bottom Controls:** Cart Latch, Cart Unlatch, Park, Change Buffer
- Status Bar:**

Idle	SD: Closed	CD: Closed	Press1: OK	Press2: OK	Latched	COM
						Runs left: 0

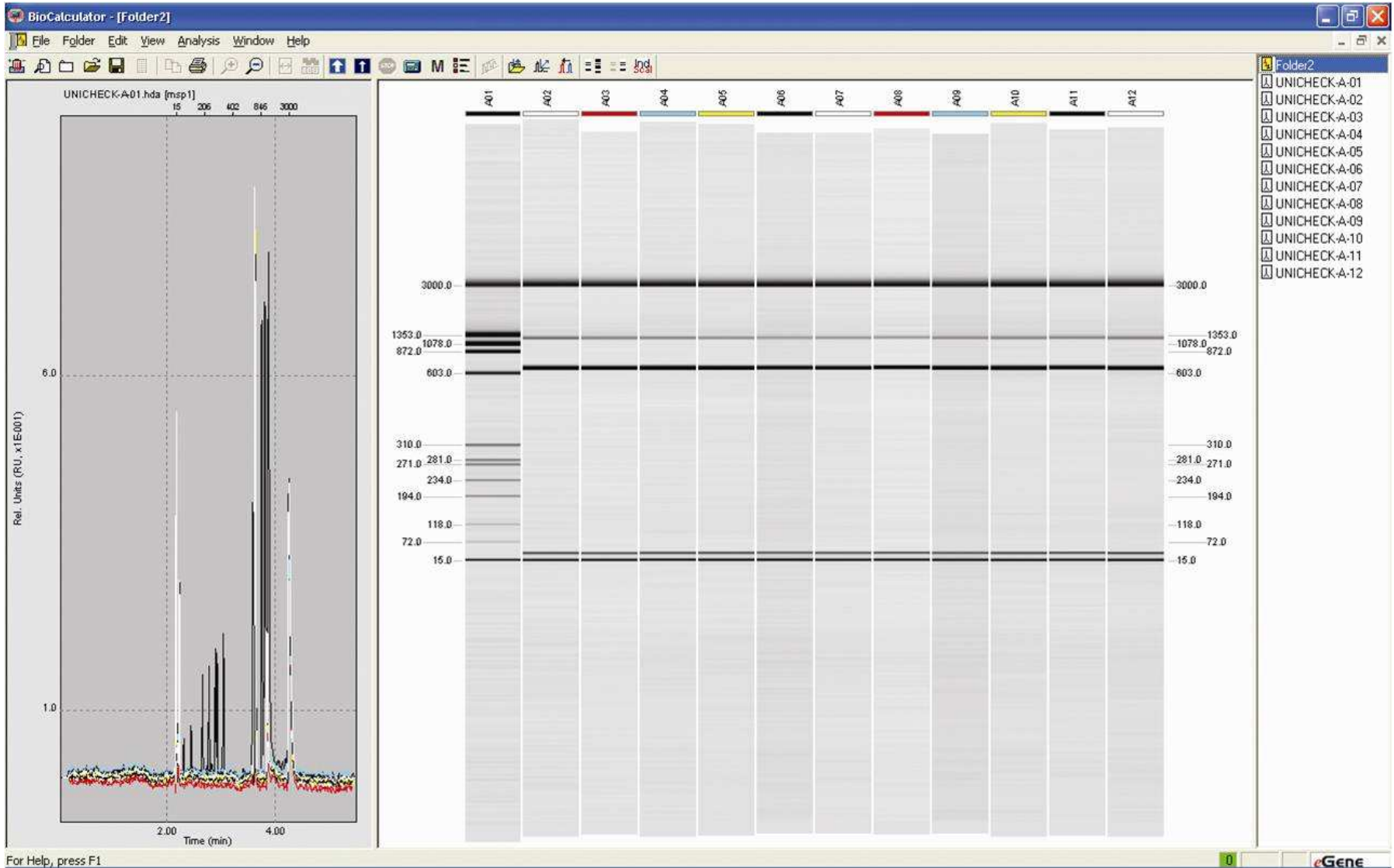


Views

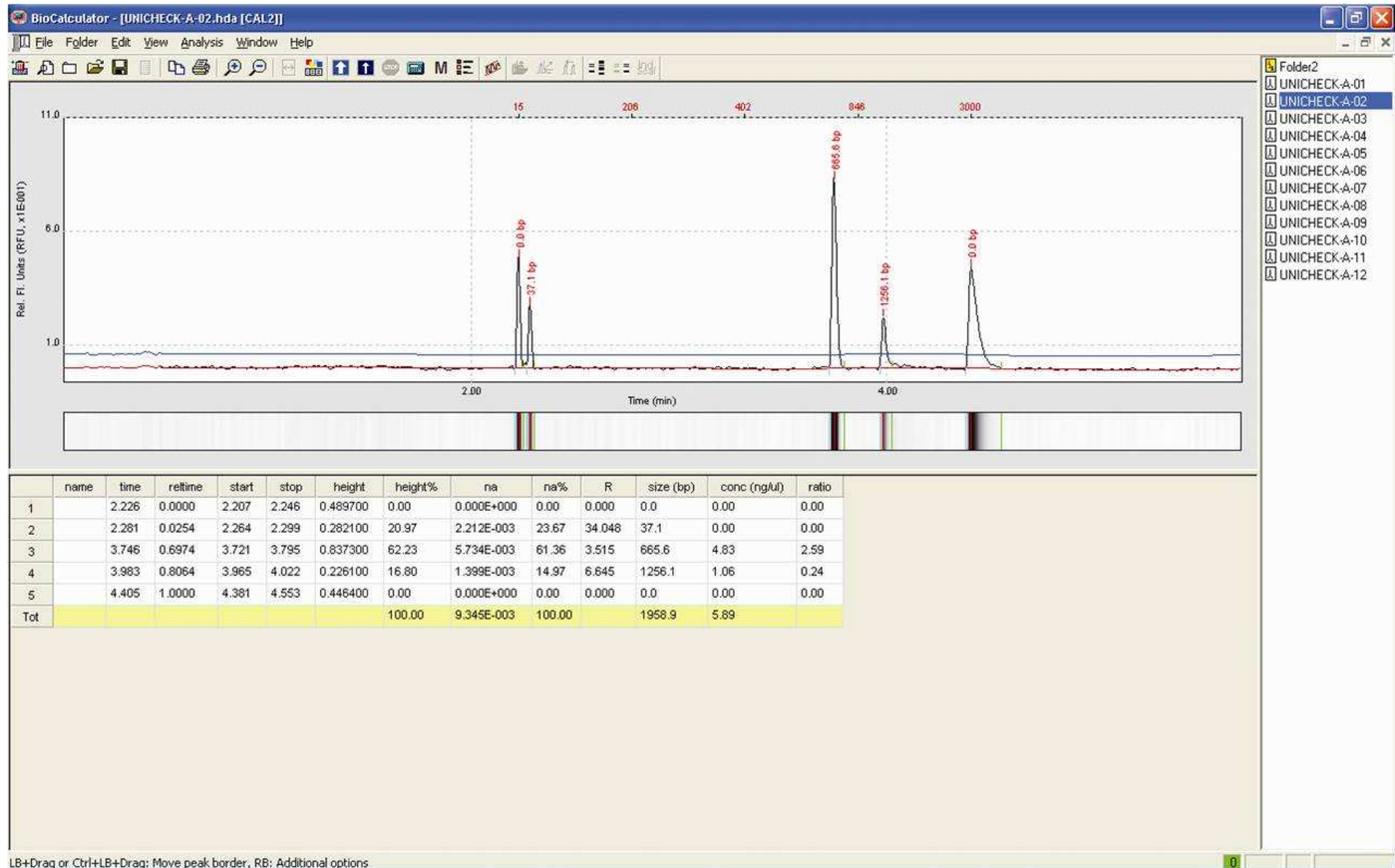
- Gel Image
- Electropherogram
- Folder View
- Individual Channels



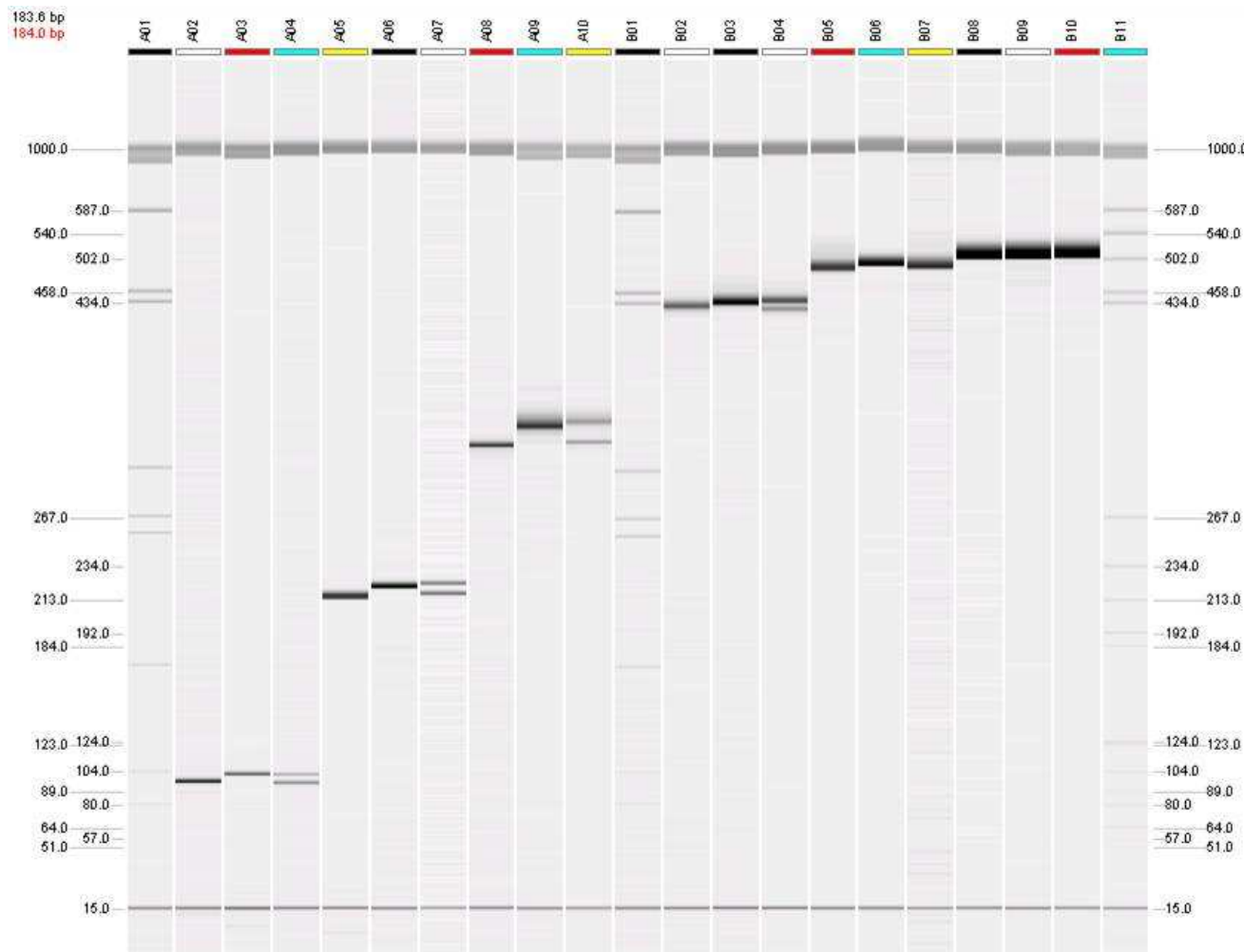
Size Determination Results



Size Determination Results



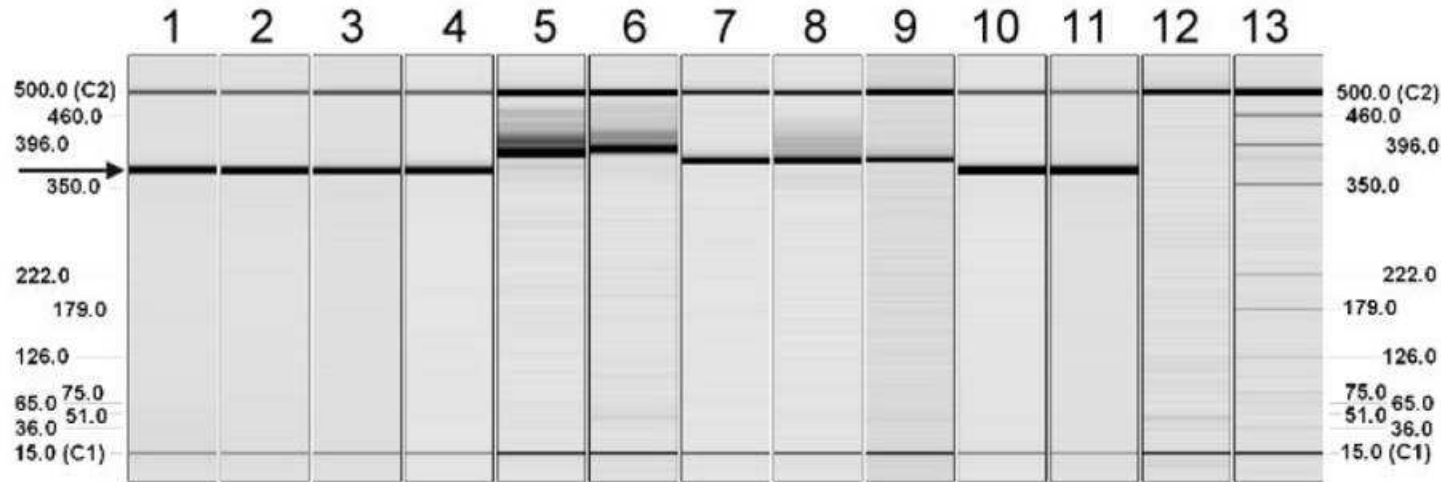
High-resolution Nucleic Acid Separation



A02	96 bp
A03	99 bp
A04	96/99 bp
A05	222 bp
A06	225 bp
A07	222/225 bp
A08	332 bp
A09	334 bp
A10	332/334 bp
B02	441 bp
B03	444 bp
B04	441/444 bp
B05	496 bp
B06	499 bp
B07	496/499 bp
B08	518 bp
B09	523 bp
B10	518/523 bp

Distinguish Between *wt* & Lapinized CSFV Strains*

Example from the veterinary field

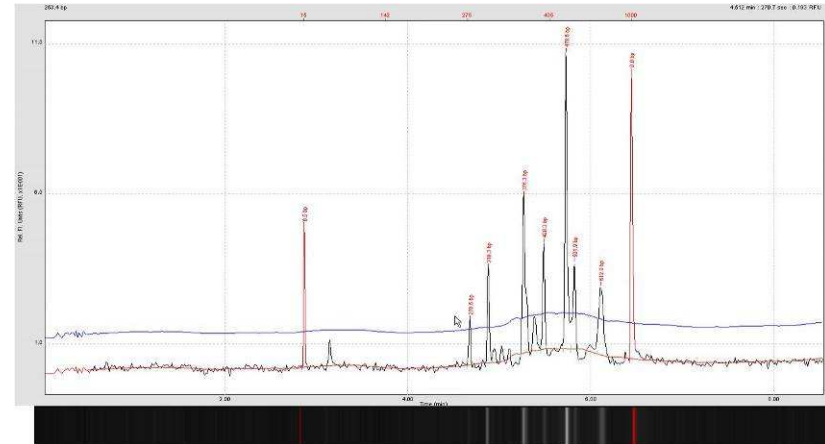
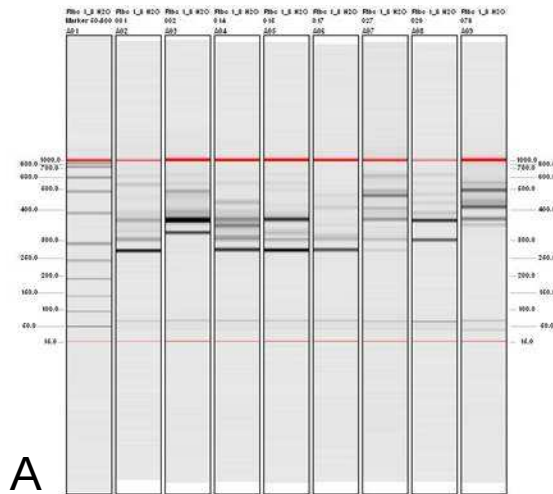


Multi-capillary electrophoresis analysis of T-rich insertions in wild-type and lapinized vaccine strains of classical swine fever virus (CSFV). Lane 1: 85-12A (367 bp); lane 2: 84-KS1 (367 bp); lane 3: 92-TC1 (367 bp); lane 4: 90-YL1 (367 bp); lane 5: LPC (lapinized Philippines Coronel)/TS (Tam-Sui; 403 bp); lane 6: LPC/PRK (primary rabbit kidney; 409 bp); lane 7: LPC/AHRI (Animal Health Research Institute; 379 bp); lane 8: HCLV (hog cholera lapinized virus; 379 bp); lane 9: vaccine-type virus in clinical samples (379 bp); lane 10: wild-type virus in clinical samples (367 bp); lane 11: ALD virulent strain (367 bp); lane 12: negative control; lane 13: pGEM DNA marker. The detected amplified 367-bp fragment from the wild-type CSFV is indicated by an arrow on the left of the gel.

* From Lai S-S et al. (2008), J Vet Diag Invest 20:448-456; Rapid detection and differentiation of wild-type and attenuated lapinized vaccine strains of classical swine fever virus by reverse-transcription polymerase chain reaction

C. difficile Ribotyping

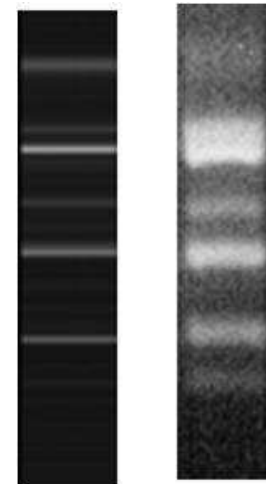
Ribotype patterns of various *C. difficile* strains



A

A Ribotype pattern of various *C. difficile* strains (left). Electropherogram view of the 027 ribotype (right) – the Alignment Marker Fragments are marked in red. **B** Ribotype pattern of the 027 ribotype obtained by classic agarose gel-based electrophoresis (on the right), compared with those obtained using the capillary gel electrophoresis-based method (left)

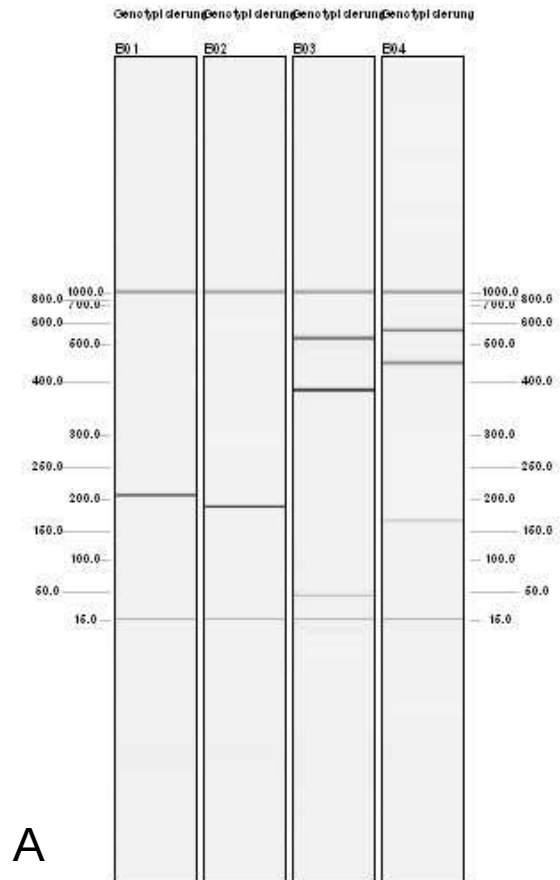
B



Data kindly provided by V. Braun, tgc biomids

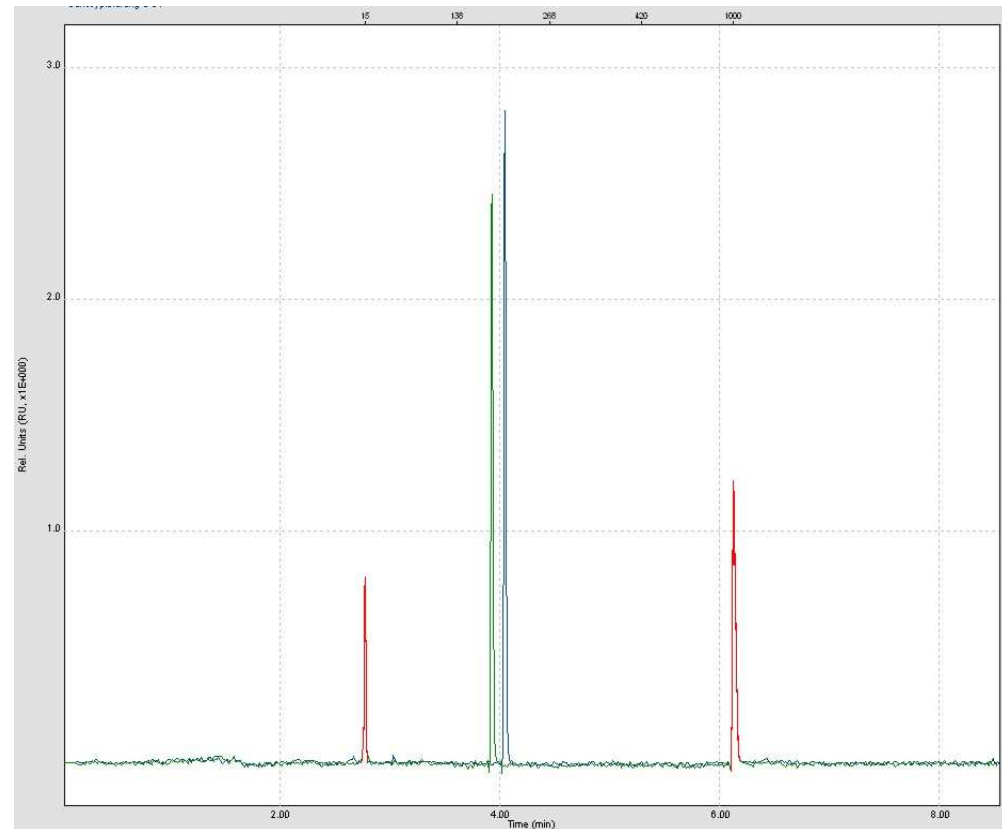
Analysis Of *C. diff* Toxin Genes

Detection of *tcdC* deletions



A

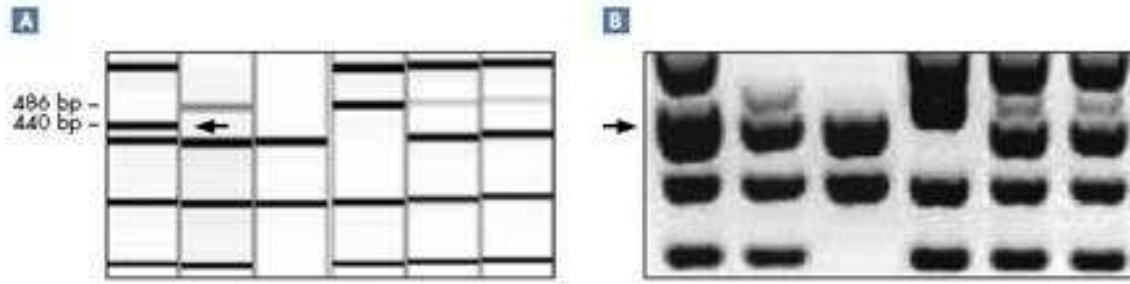
B



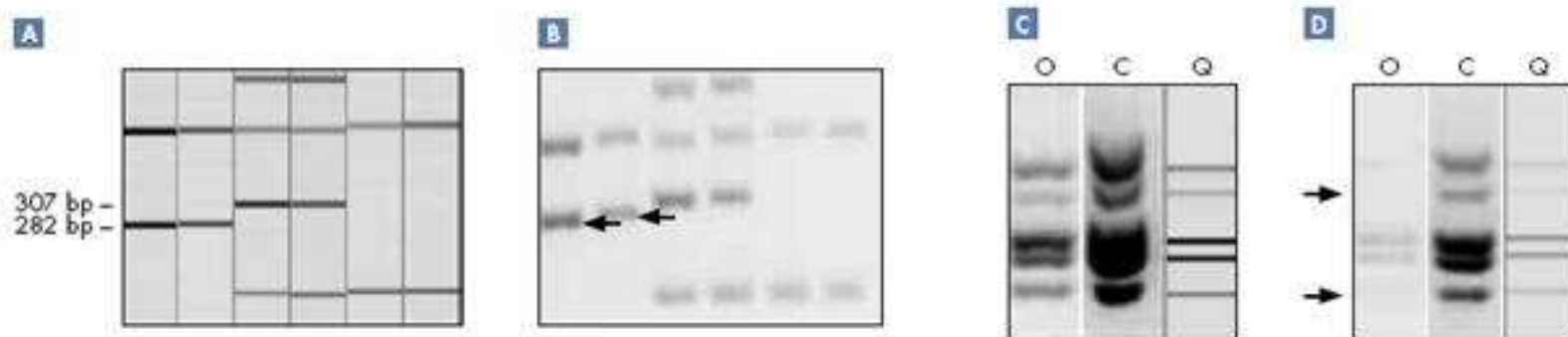
C. difficile 027 PaLoc analysis ; B01: *tcdC* control, B02: *tcdC* deleted, B03: *cdt A/B*, B04: *tcdA/B*. **B** Overlay of electropherogram views of lane B01 (206.8 bp, blue) and B02 (188.4 bp, green) demonstrating the 18bp *tcdC* deletion; peaks for the upper and lower alignment marker are shown in red.

Circumventing Gel Artifacts

Examples from CGF of *Campylobacter jejuni**



False positive error calls due to poor resolution on agarose gels



Misclassification due to poor resolution and/or gel anomalies (A & B)

False negative error calls due to weak amplicons (C & D)

Data kindly provided by Steven Mutschall¹, Susan Ross², Cody Buchanan¹, and Eduardo N. Taboada¹

¹ Laboratory for Foodborne Zoonoses, Public Health Agency of Canada, Lethbridge, Alberta, Canada

² Animal Diseases Research Institute, Canadian Food Inspection Agency, Lethbridge, Alberta, Canada



Application of capillary electrophoresis in a clinical microbiology department

Anna Ryberg, molecular biologist (MSc)
Clinical Microbiology
University Hospital, Linköping, Sweden





Clinical Microbiology, Linköping, Sweden

- Diagnostics and analysis of bacteria, virus, parasites and fungi
- Biosafety level 3 laboratory for Tuberculosis diagnostics
- 300 000 samples per year (2009)
 - Bacteriology 174876
 - Serology 12782
 - Viruses 75461
 - Parasites 3027





VRE – background

- Vancomycin resistant enterococci
- Natural inhabitants of the human intestinal microflora
- Tough, survivalists
- Spread of *E. faecalis* and *E. faecium* with acquired *vanA* or *vanB* is a serious threat in hospital environment.
- Aim: to develop a screening method for VRE detection.





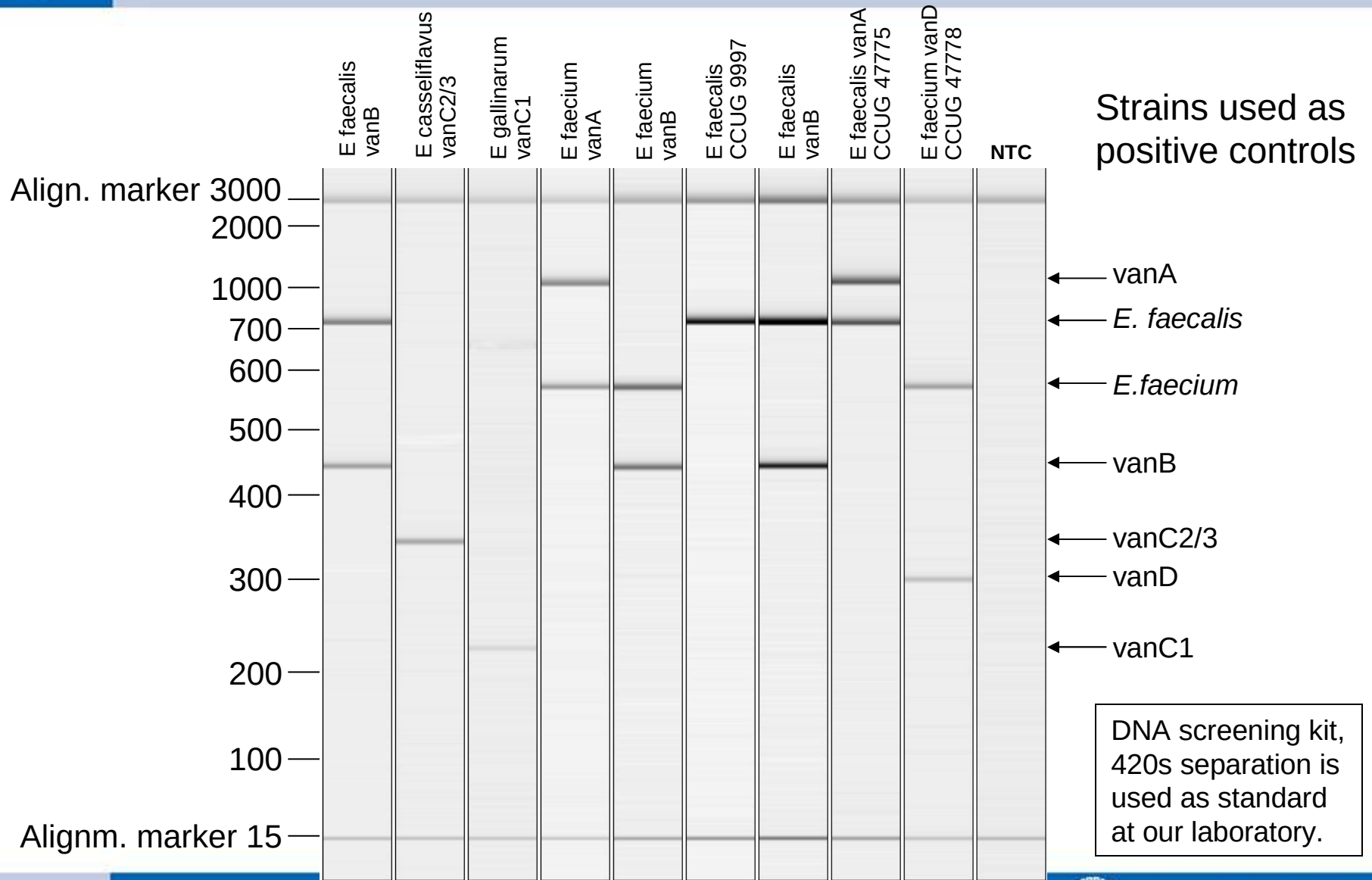
VRE – method

- Screening procedure
 - Bile Esculin Azide broth, Vancomycin 4mg/L
 - Min 18h incubation with shaking
 - 1mL broth is pipetted to an 1,5mL microcentrifuge tube and centrifugated
 - Pelleted bacteria is resuspended in StafLys buffer:
 - 20 mM Tris-HCl (pH 7.6)
 - 0.5% Tween20
 - 0.5 % Igepal CA-630
 - Lysozyme (2.6mg/mL) and Proteinase K (1mg/mL)
- PCR detection¹ of
 - *vanA*, *vanB*, *vanC1*, *vanC2/3*, *vanD*
 - *ddl* genes for *E. faecium* and *E. faecalis*
- Automated gel analysis
- Detection limit:
 - 0-4 CFU/mL of pure *E. faecalis* or *E. faecium* cultures or 1-35 CFU/mL mixed with *E. gallinarum* or *E. casseliflavus*.
 - 5pg DNA





VRE – method results





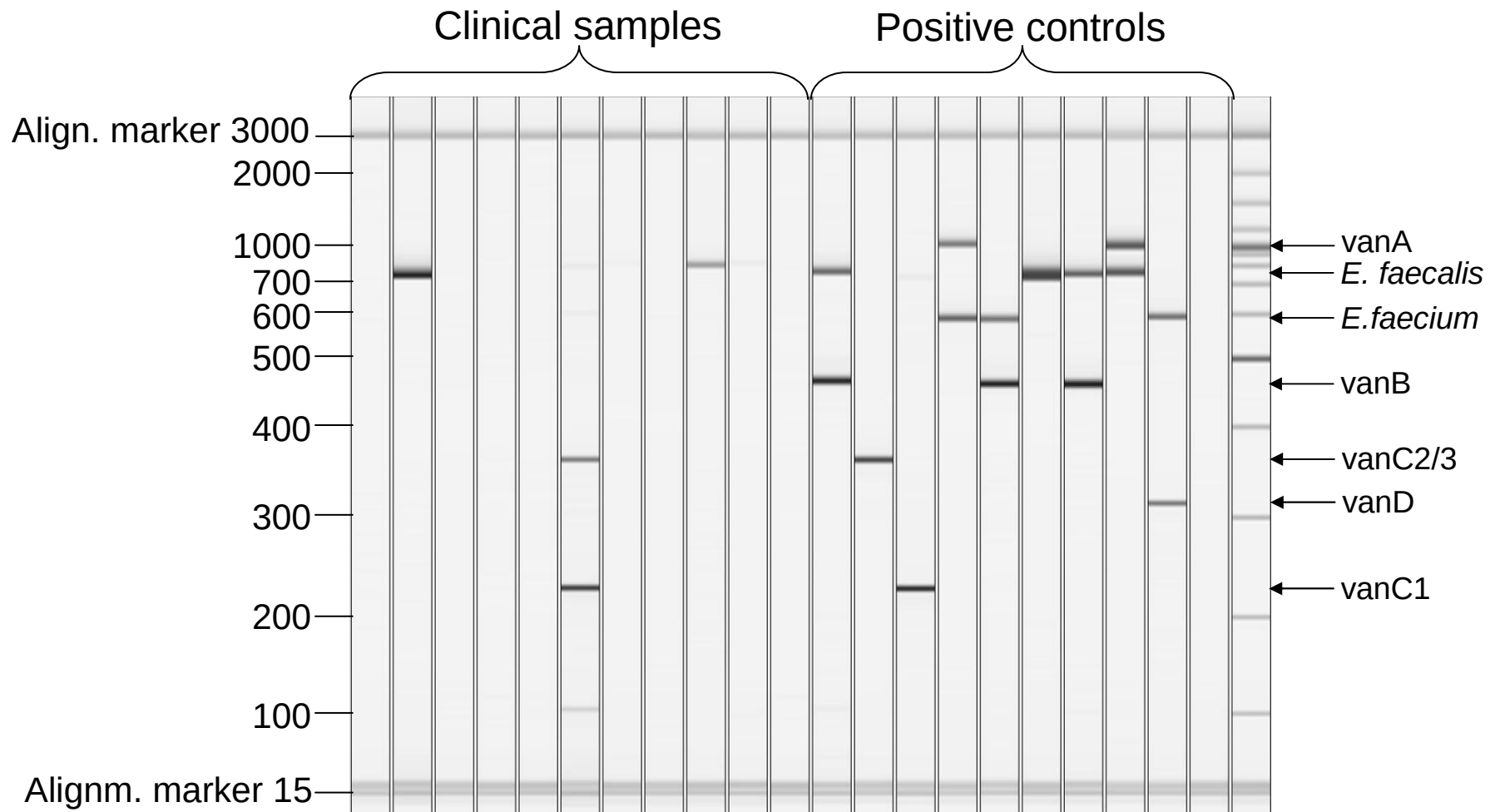
VRE – situation in Linköping

- 1 identified carrier of VRE in 2010
 - *E. faecium*, *vanB*
- 244 screening samples followed
 - No VRE detected
 - 3 *vanB* genes (prob from anaerobic flora)
 - 16 *vanD* genes (prob from anaerobic flora)





VRE – method results





AmpC - background

- AmpC β -lactamases hydrolyses a broad and extended spectrum of cephalosporins.
- ESBL definition includes plasmid mediated class C β -lactamase genes (*ampC*).
 - Need to differentiate from natural chromosomally located *ampC* in Enterobacteriaceae, which usually are inducible or due to mutations in *ampC* promoter region.
 - Not possible using phenotypic typing methods.
- Spread of plasmid mediated *ampC* is a growing problem in hospitals.
- Isolates are tested for *ampC* using synergy test with cefoxitin/cefoxitin+cloxacillin strips (Etest)
 - 1/3 of all phenotypically *ampC*-positive isolates are plasmid mediated.
- Species dependent analysis
 - Chromosomal OR plasmid mediated $\rightarrow$ *E. coli* and *Shigella* spp.
 - Always plasmid mediated $\rightarrow$ *K. pneumoniae*, *P. mirabilis* and *Salmonella* spp.
 - Always chromosomal $\rightarrow$ *Enterobacter* spp., *C. freundii*, *Providentia* spp., *Morganella morganii*, *Serratia* spp., *Hafnia alvei*





AmpC - method

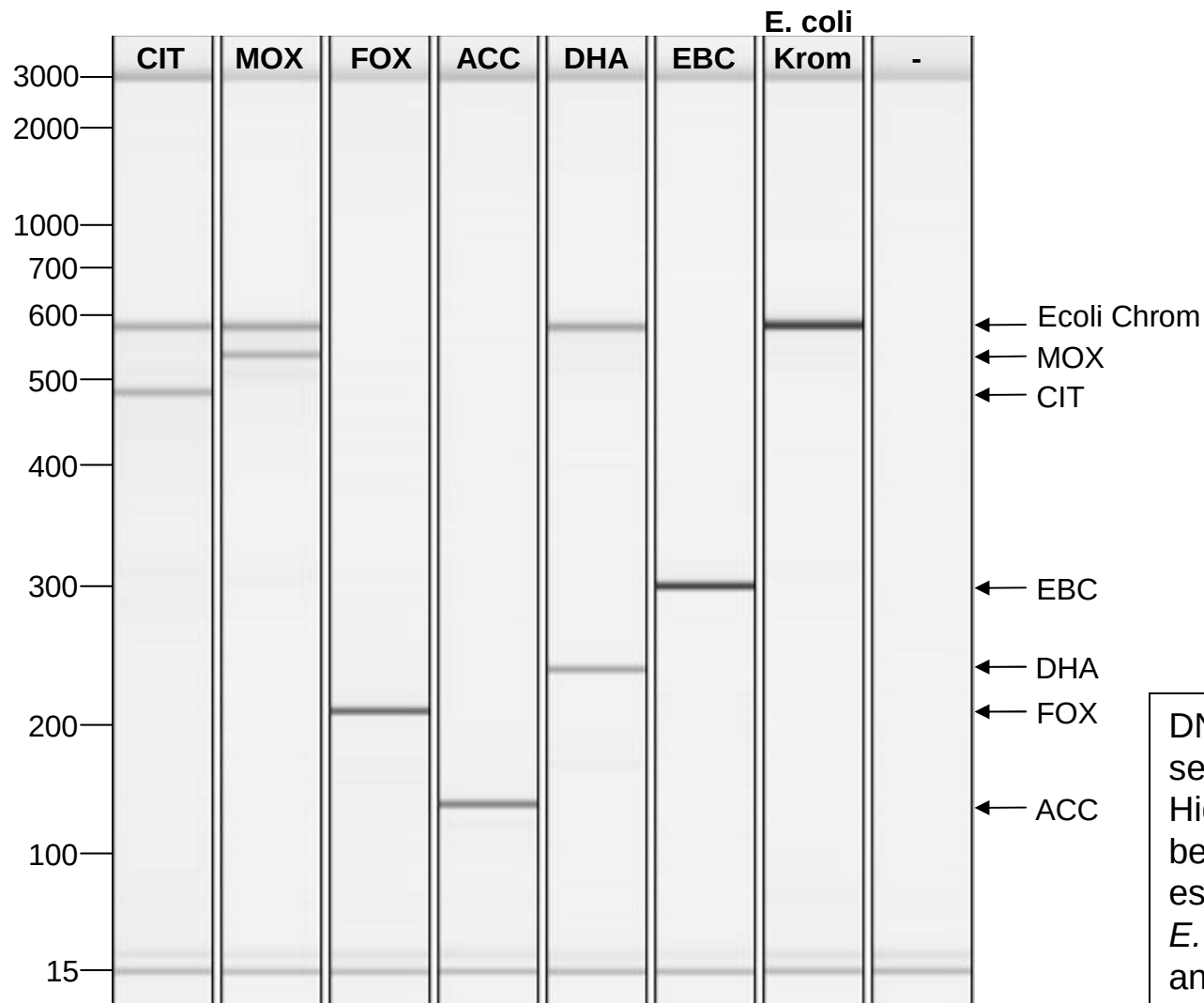
- Aim: To adapt a multiplex PCR analysis for detection of *ampC* groups using capillary electrophoresis and to include identification of *E. coli* chromosomal gene as control.
- DNA extracted from colonies using automated extraction robots.
- Modified multiplex PCR² to detect *ampC* gene groups:
 - CIT (*Citrobacter freundii*)
 - MOX (*Aeromonas hydrophila*)
 - FOX (*Aeromonas caviae*)
 - ACC (*Hafnia alvei*)
 - DHA (*Morganella morganii*)
 - EBC* (*Enterobacter cloacae* / *E. asburiae*)
- *E. coli* chromosomal *ampC* gene*.

* Newly designed primers





AmpC – method results



DNA screening kit, 420s separation.
High resolution kit gives better resolution, especially between the *E. coli* chromosomal and the MOX gene.





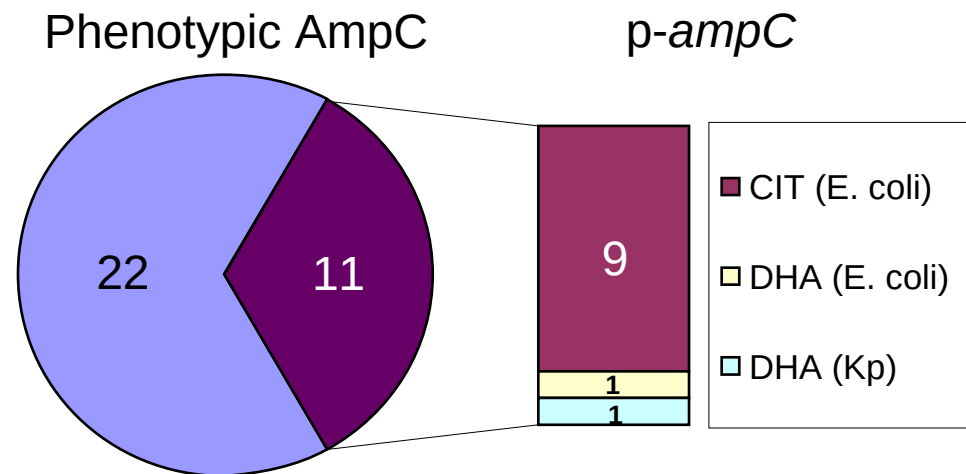
AmpC – situation in Östergötland

- 2010

16 clinical isolates analysed (*E. coli*)

- All isolates has chromosomal *ampC* gene.
- 5 isolates positive for CIT group

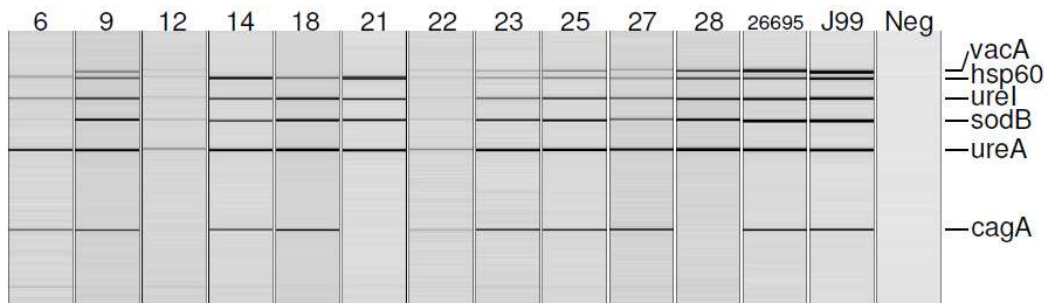
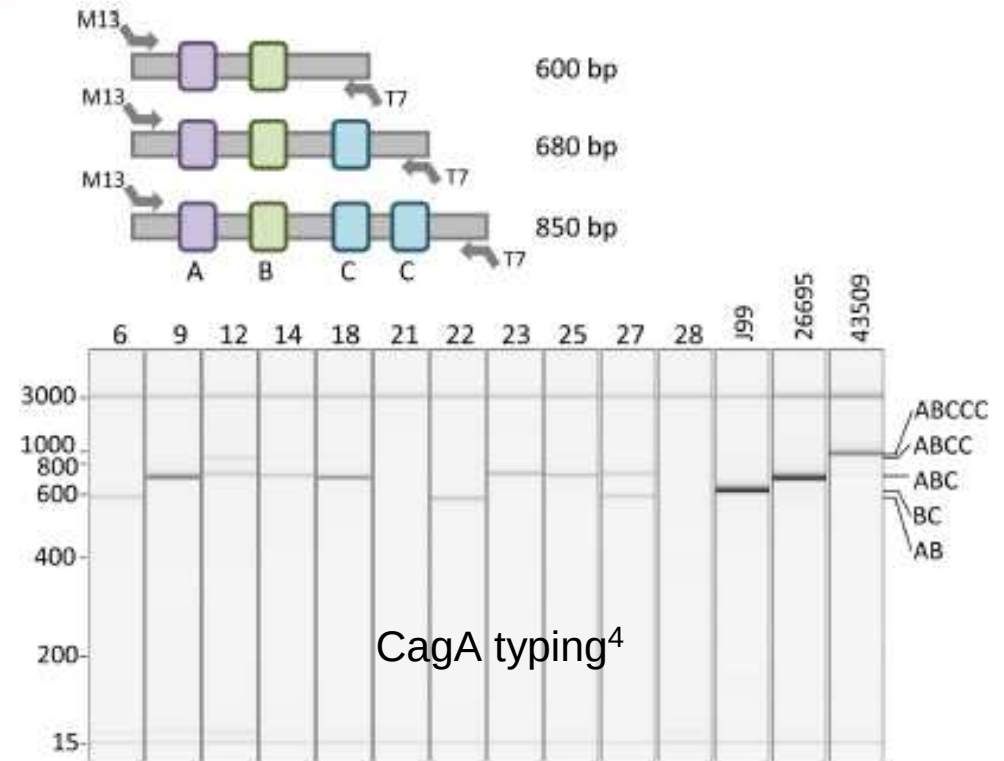
- 2008-2009:





Research application

- Detection of virulence genes in *H. pylori* directly from total DNA isolated from human gastric biopsy specimens³.



DNA screening kit, 420s separation, but High resolution kit gives better separation.

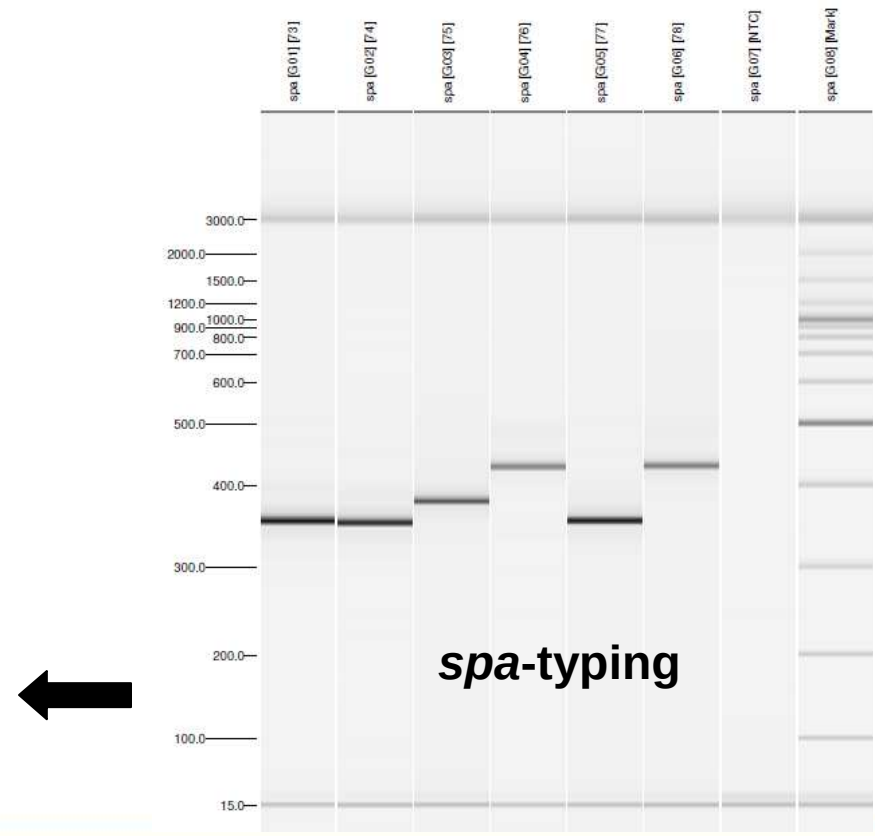
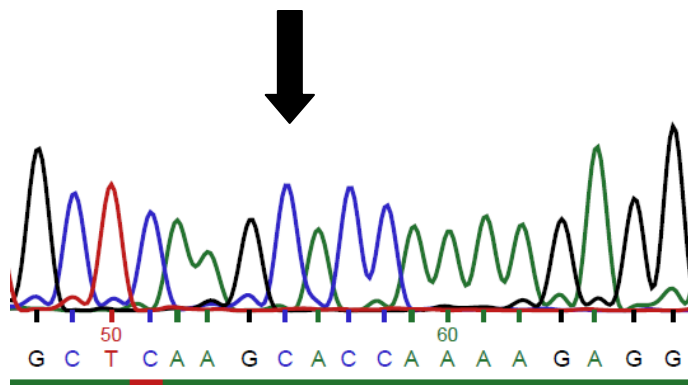
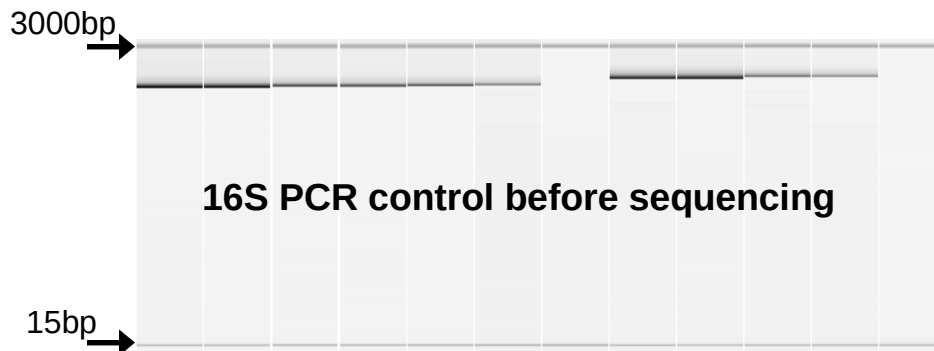




Research application

DNA screening kit,
420s separation.

- Identification of PCR products before downstream applications.

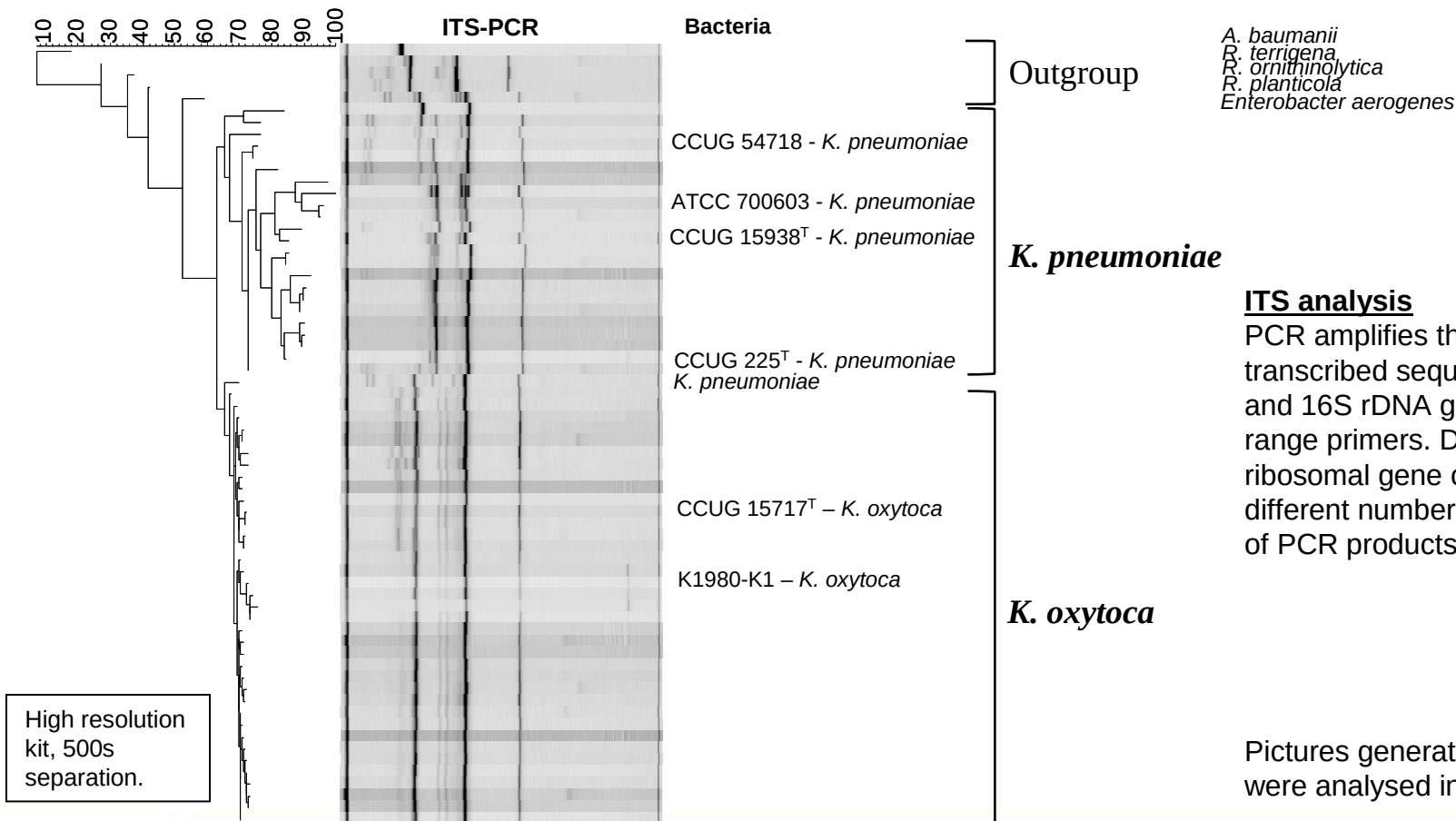




Research application

- Klebsiella species identification

Cosine coefficient (Opt:3.00%) (Tol 1.0%-1.0%) (H>0.0% S>0.0%) [4.6%-72.2%] –Neighbor joining





Acknowledgment

Prof. Hans-Jürg Monstein, Linköping

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Dr. Anders G Johansson, Linköping

Dr. Anneli Karlsson, Linköping University.

Staff at Clinical Microbiology department,
University Hospital, Linköping

Thank you!

