ABI Complete Solution for Sequencing

– From CE to Semi-Conduction Technology

陳俊銘 (Jimmy Chen)
應用技術專家 (Application Support Specialist)
美商應用生命系統(股)公司 (Applied Biosystems)

DNA Sequencing Methods

Two methods for the large-scale sequencing of DNA were published in the same year (1977) in the same journal.

1. Maxam-Gilbert sequencing :
   chemical cleavage method using double-stranded (ds) DNA.
2. Sanger-Coulson sequencing :
   chain termination method using single-stranded (ss) DNA.

Nowadays Sanger-Coulson is the more popular method. Various modifications have been developed and it has been automated for very large-scale sequencing.

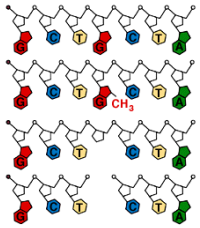
Maxam-Gilbert Sequencing

(a)
DNA labeled at one end with ^{32}p

Base modification

Release or displacement of reacted bases

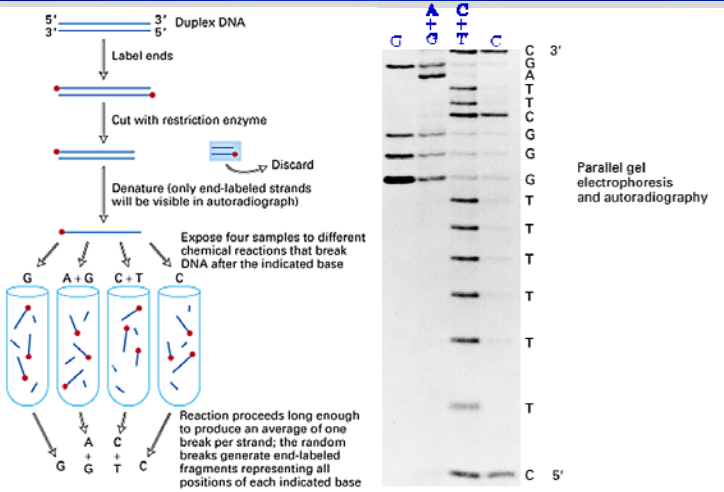
Strand scission



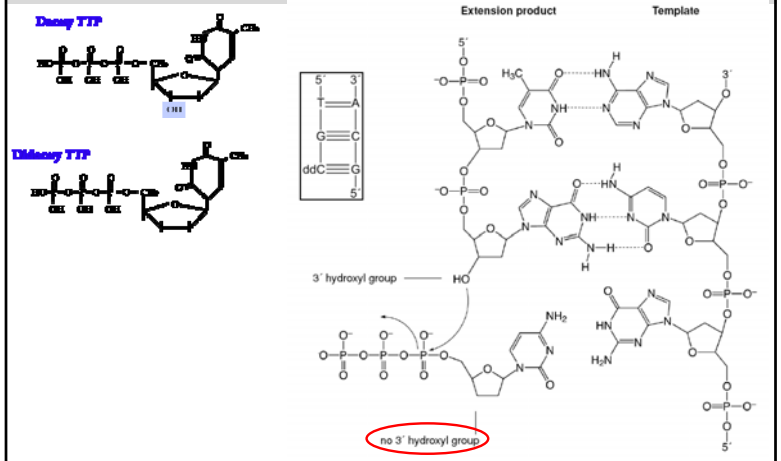
(b)

^{32}pGpCpTpGpCpTpApGpGpTpGpCpCpGpApGpC
G G G G G G G
^{32}p
^{32}pGpCpTp
^{32}pGpCpTpGpCpTpAp
^{32}pGpCpTpGpCpTpApGp
^{32}pGpCpTpGpCpTpApGpGpTp
^{32}pGpCpTpGpCpTpApGpGpTpGpCpCp
^{32}pGpCpTpGpCpTpApGpGpTpGpCpCpGpAp
^{32}pGpCpTpGpCpTpApGpGpTpGpCpCpGpApGpC

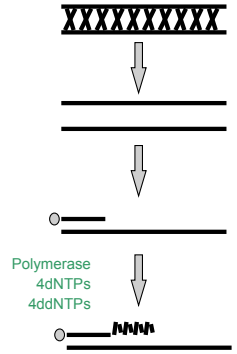
Maxam-Gilbert Sequencing



Sanger-Coulson Sequencing



Sanger Sequencing



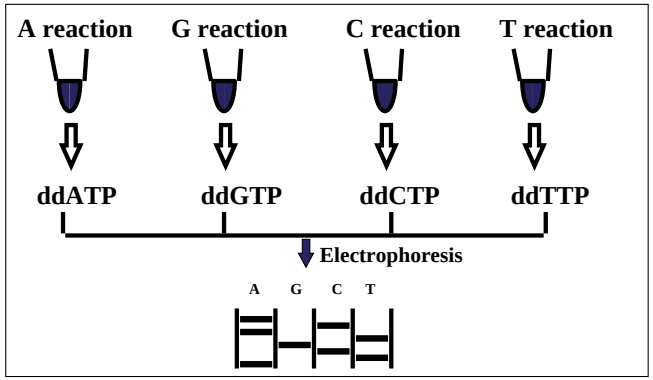
Double-stranded DNA template

Denature to separate two strands

Anneal end-labeled oligonucleotide to one strand

Carry out DNA synthesis primed by the oligonucleotide

Sanger DNA Sequencing



DNA定序 - 傳統使用放射性元素定序

製作電泳膠片

人工控制定序反應作用時間

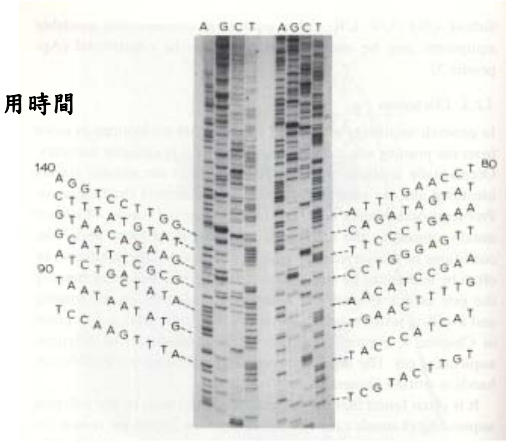
人工上樣

人工控制電泳時間

電泳膠烘乾壓片

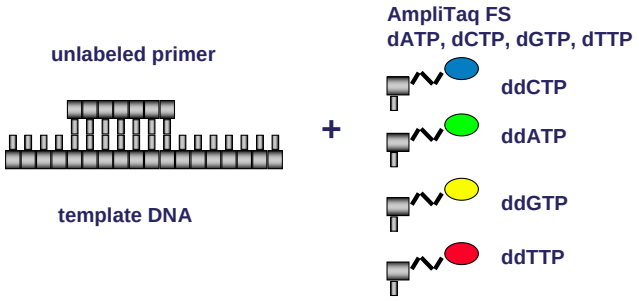
顯影沖片

人工判讀, 輸入電腦



Terminator Sequencing Sample Prep

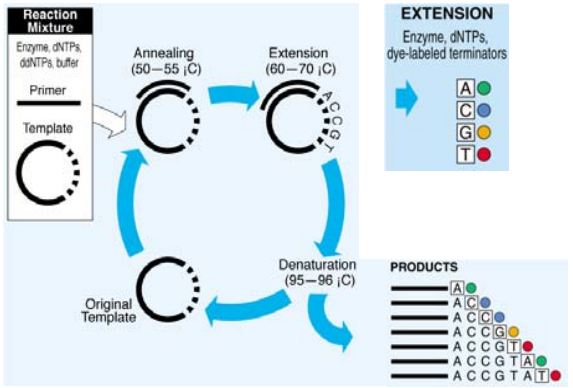
1 reaction within which each terminator is labeled with a different dye



Sample is purified by ethanol precipitation to remove unincorporated ddNTP's before loading

Sanger Dideoxy Cycle Sequencing

Performed in a thermocycler with a thermostable Taq polymerase

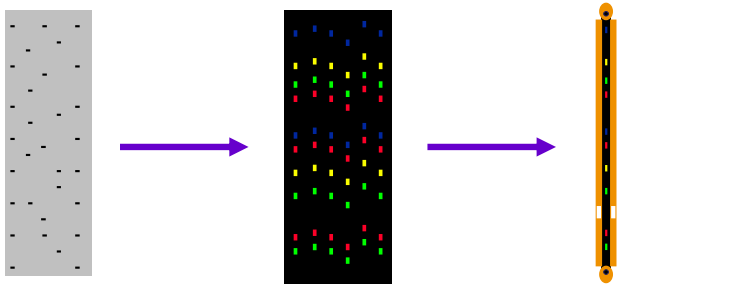


Evolution of Automated* DNA Analysis

Radioactive or EtBr bands analyzed with a phosphorimager, digitizing tablet, or CCD camera

Multi-fluorescent fragments detected real-time during electrophoresis, or bands analyzed with a scanner after electrophoresis

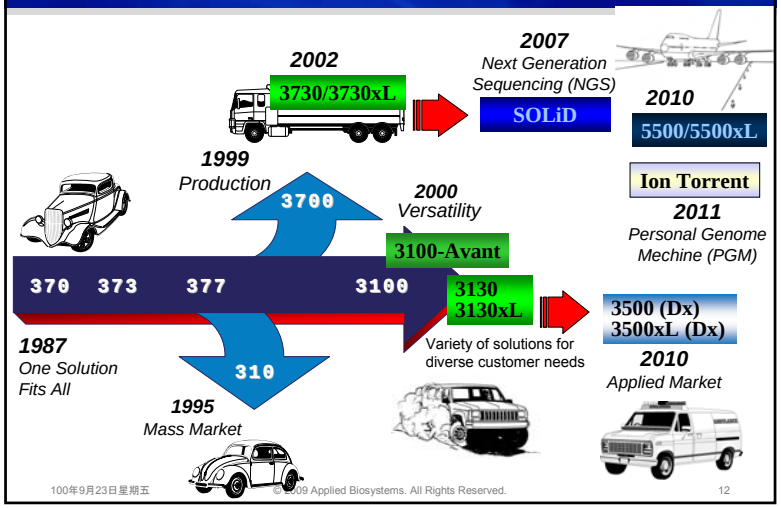
Multi-fluorescent fragments detected real-time



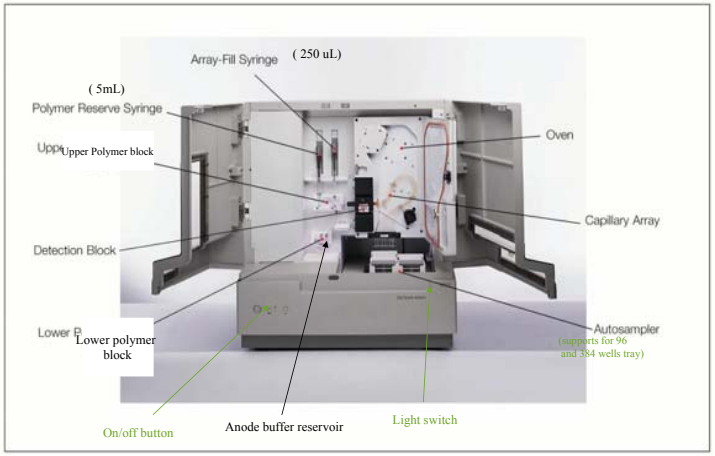
Slab Gel | Slab Gel | Capillary with Polymer

* Automation has historically referred to data analysis only, but capillary electrophoresis automates the electrophoresis as well

Evolution of DNA Analyzers

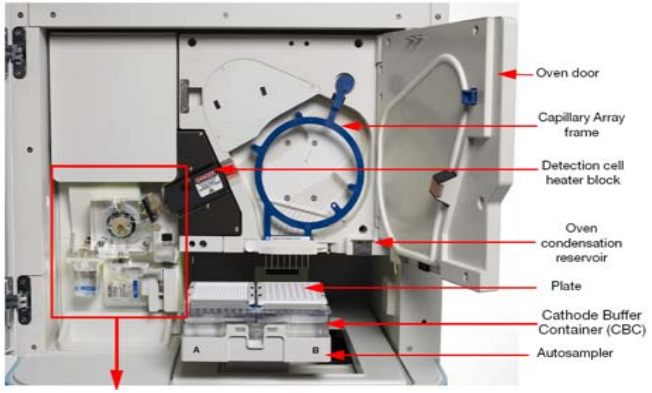


ABI PRISM® 3100 Genetic Analyzer: Doors Open



3500 Instrument Interior

Instrument interior components



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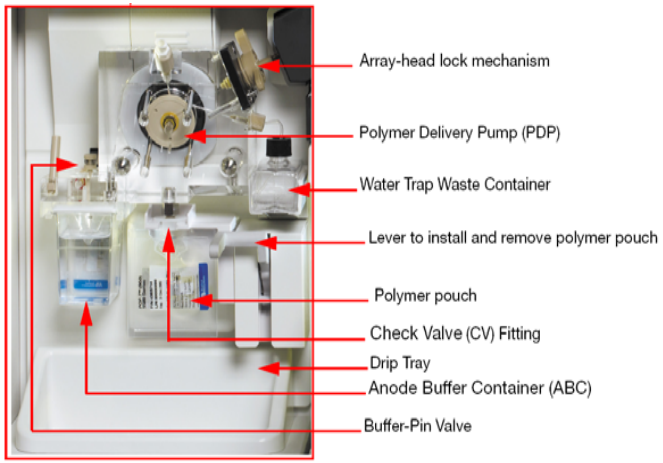
Zoom in of Polymer Pump and buffer area



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Life Technologies Sequencing Portfolio:

Ion Complements LIFE Portfolio

CE

One to Hundreds of long reads per sample, Mbp of data per day

- Targeted (amplicon. medical) resequencing
- Bacterial, fungal, and viral identification
- Multiple fragment analysis apps (microsatellite and multiplexed SNP detection)

PGM

Thousands to Millions of moderate reads per sample, Hundreds of Mbp of data per day

- Microbial genome sequencing
- Preliminary library assessment and QC
- Targeted resequencing (larger scale)

SOLiD

Millions to Billions of short reads per sample, Gbp of data per day

- Whole human genome sequencing
- Whole human exome sequencing
- Whole transcriptome analysis

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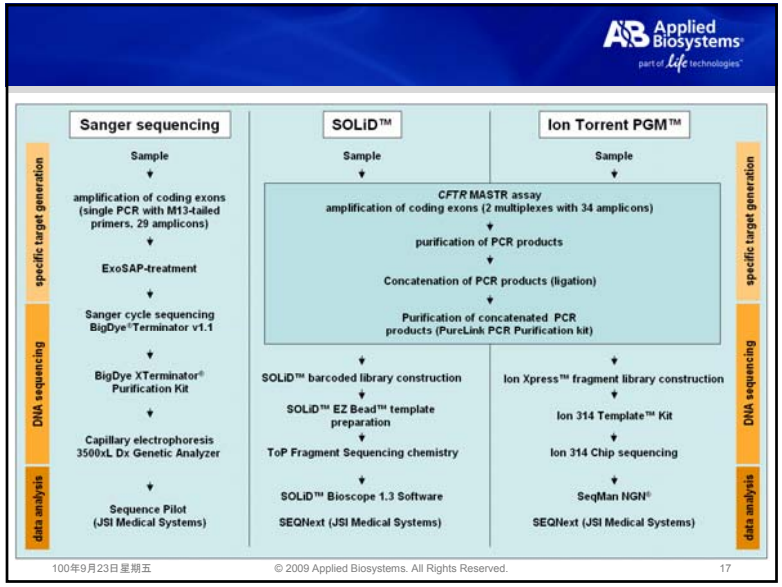
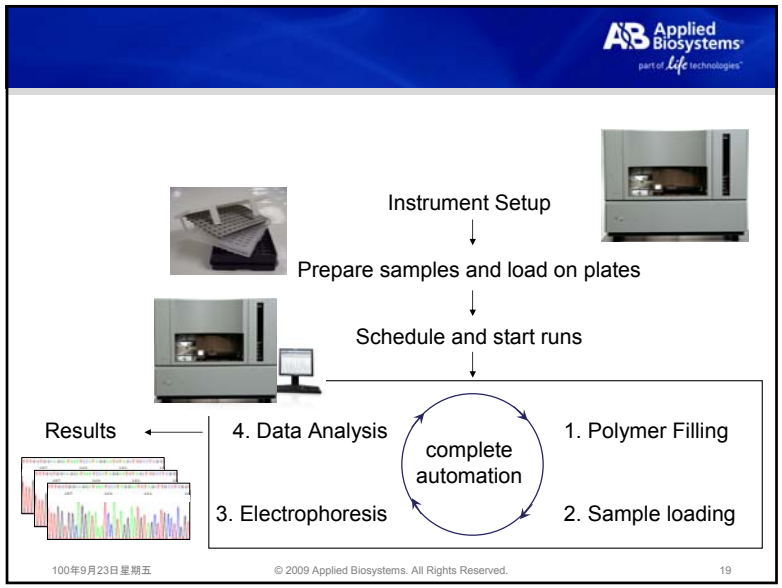
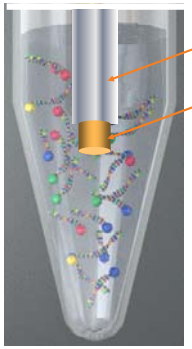


ABI PRISM® 3730 0verview

- **Improved Production Capacity**
  - **48 or 96 capillary array**
  - **Simultaneous Injection and Analysis of 48/96 samples**
  - **Improved Data Quality**
- **Walkaway Automation**
  - **Automated Plate Loading from a Stacker that Accommodates up to 16 Plates (96- or 384 well plate)**
  - **Automated Polymer Replenishment**
  - **Internal Barcode Reader**
- **Minimized Operational Cost**
  - **In- Capillary Detection**
- **Active Temperature Control : 18 - 70 degree**



Electrokinetic Injection

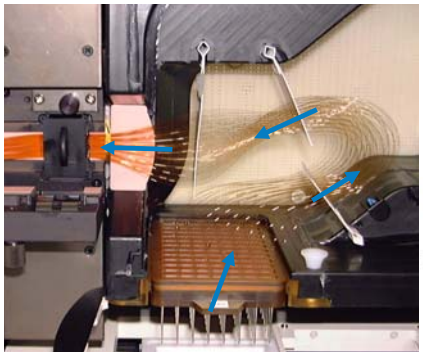


Electrode (Cathode)

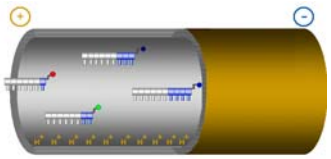
Capillary

- Capillary and electrode (cathode) are placed into the sample
- Voltage is applied
- Negatively-charged DNA enters the capillary as it migrates toward the postively-charged electrode (anode) at the other end of the capillary

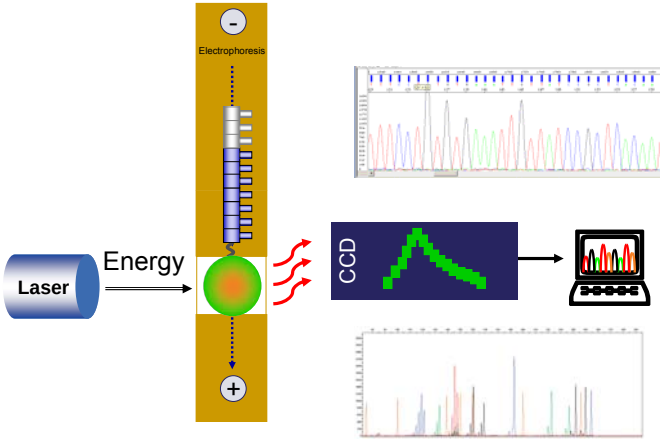
Capillary Electrophoresis



Migration of anionic DNA fragments



Fluorescent Signal Detection

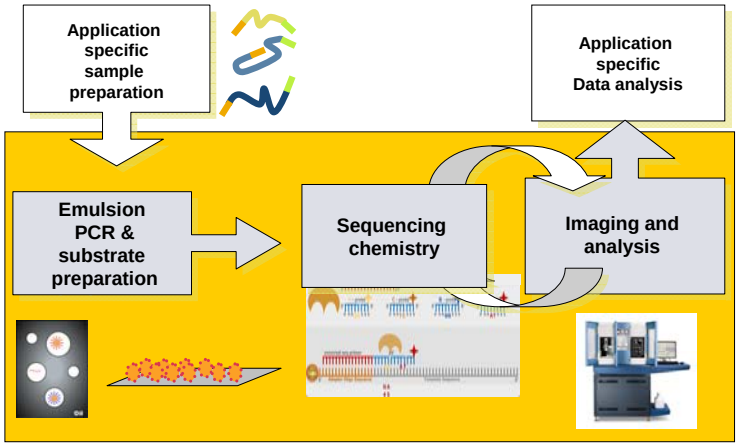


SOLiD™ System

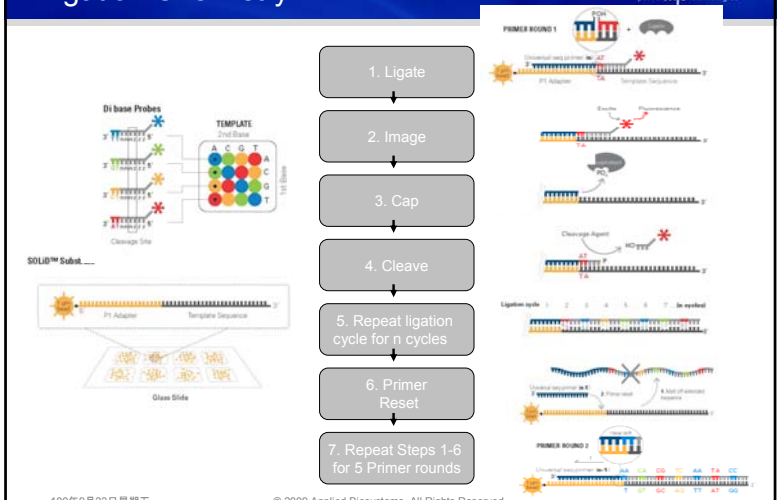
Next Generation Sequencing

- Next Generation Sequencing is massively parallel !
- 1 to 2 Billions of sequencing reactions run in parallel !
- SOLiD Sequencing sequences up to 2 x 10^{11} bases per run

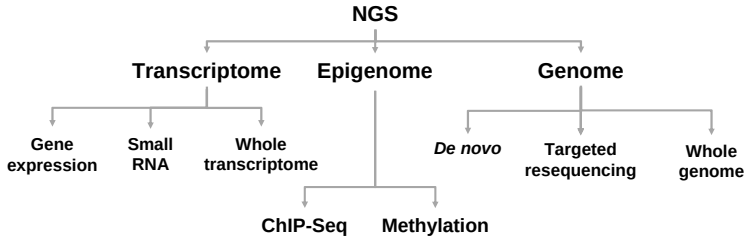
SOLiD Workflow



Ligation Chemistry



Next generation sequencing on SOLiD™



Targeted resequencing

- *Focus on specific regions of interest rather than whole genome*
- *Leverage throughput and accuracy to sequence at higher coverage while multiplexing samples*

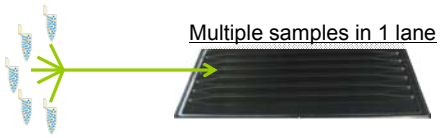
Multiplexing with barcodes

Flexibility on 5500 Series FlowChip

Applied Biosystems — part of life technologies

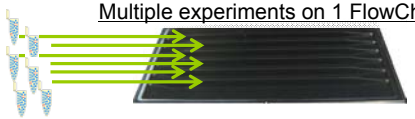
Targeted resequencing
Small RNA
ChIP

Multiple samples in 1 lane



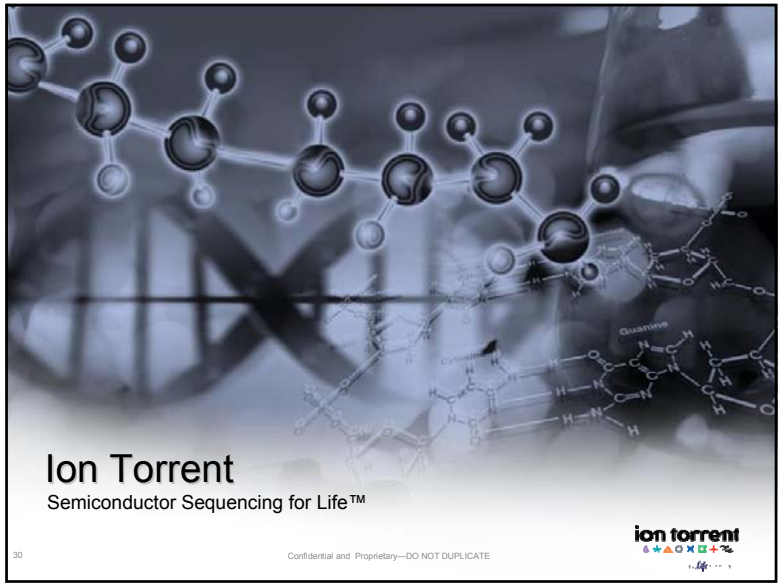
Whole Transcriptome
Whole Exome

Multiple experiments on 1 FlowChip



Whole Genome
Sequencing

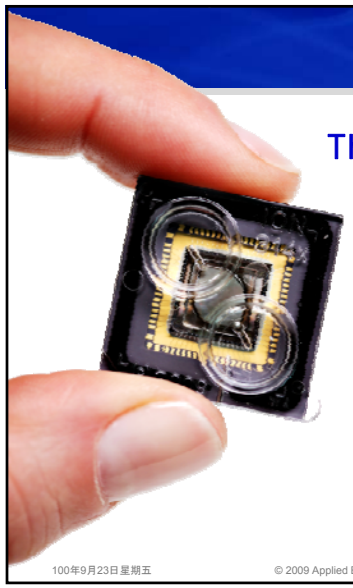
1 sample across multiple lanes



Ion Torrent

Semiconductor Sequencing for Life™

ion torrent



The Chip is the Machine™

Scalability

Simplicity

Speed

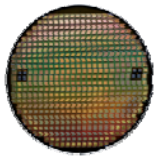
Bench Top Convenience

Applied Biosystems — part of life technologies

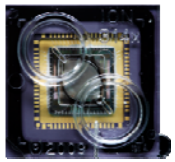
Low cost, convenient,
single use device.

Easy, automatic fluid connections.
Match the size of the Ion chip to your application.

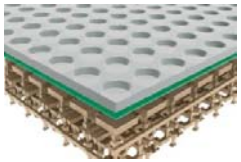
Scalable Semiconductor Technology



Wafer
Semiconductor Manufacturing



Chip
Semiconductor Packaging

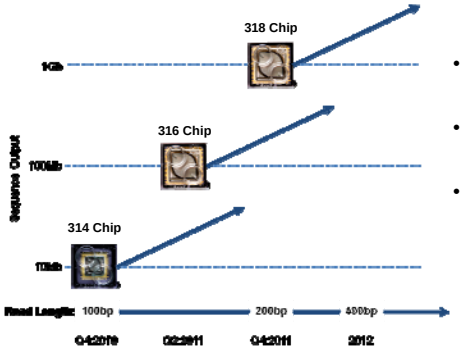


Chip Cross Section
Semiconductor Design

Leverages trillion dollar investment and $50 billion annual spend on semiconductor manufacturing, packaging, and design technology.

Scalability – The Chip is the Machine™

10Mb to 1Gb in one year (100x)

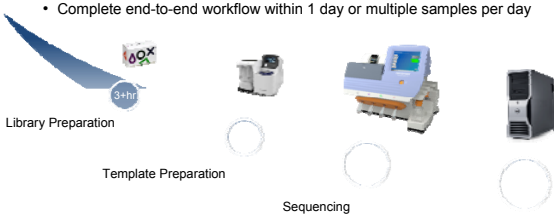


- Series of chips that build on 40 years of Moore's law
- More reads and longer read lengths within each chip
- All chips on single platform with ~2 hour sequencing runs

Speed

Single Day Workflow

- ~2 hour sequencing runs – enabled by PostLight™ Sequencing
- Innovative automated template preparation for PGM sequencer matches the speed of semiconductor sequencing
- Complete end-to-end workflow within 1 day or multiple samples per day



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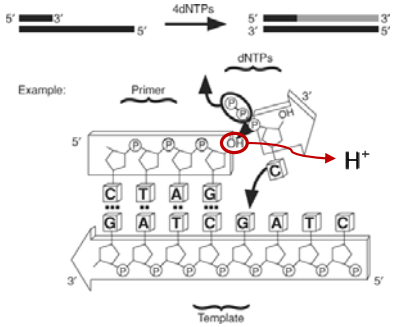
Some products have not yet been officially released and information about those products is subject to change without notice

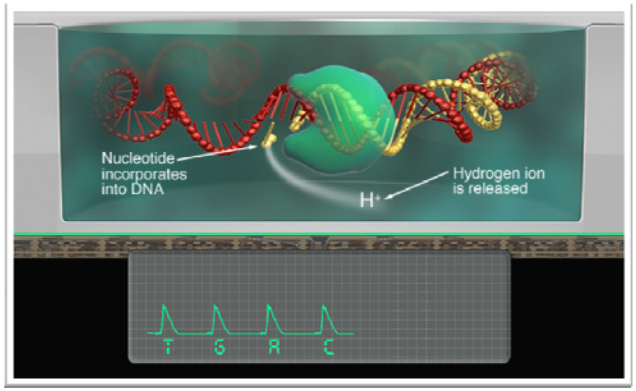
Simple Natural Chemistry



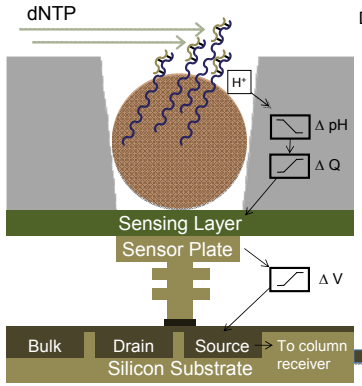
Eliminate source of sequencing errors:
- Modified bases
- Fluorescent bases
- Laser detection
- Enzymatic amplification cascades

Eliminate source of read length limitations:
- Unnatural bases
- Faulty synthesis
- Slow cycle time

Simple Chemistry

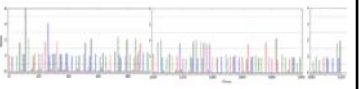


Fast Direct Detection



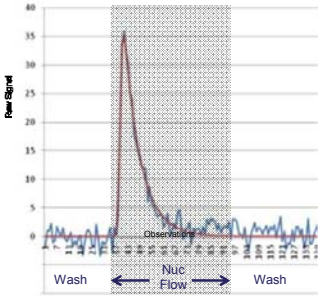
DNA → Ions → Sequence

- Nucleotides flow sequentially over Ion semiconductor chip
- One sensor per well per sequencing reaction
- Direct detection of natural DNA extension
- Millions of sequencing reactions per chip
- Fast cycle time, real time detection



Precise Measurement of Each Incorporation

Single Well Incorporation Trace



Frames (15 frames = 1 second)

- Fast sequencing
  A few seconds per incorporation
- High signal to noise
  Many data points per incorporation trace
- Enables high raw accuracy

Supported Applications

Microbial sequencing
- Accurate, fast bacteria and virus de-novo & resequencing

Mitochondrial sequencing
- Highly multiplexed mitochondrial sequencing for research, clinical, and forensic applications

Amplicon sequencing
- Multiplexed amplicon sequencing for rapid detection of germline and somatic mutations
- Fully compatible workflows for CE designed primers
- Genotyping by sequencing
- Bacterial and viral typing
- Plasmid sequencing

Custom targeted resequencing by target enrichment
- Fast and simple workflows optimized for all major target enrichment providers

Validation of whole genome and whole exome mutation
- Orthogonal technology to validate SOLiD® System/Illumina whole genome/whole exome results

Library Assessment
- Rapid library complexity validation/QC prior to run on high throughput sequencing platforms

RNA-Seq
- Affordable, fast and simple RNA-Seq solution *(Initially focused on small RNAs & low complexity transcriptomes)*
- RNA-Seq kit developed by Ambion featuring low input material and fast and streamlined workflow

Supported Applications

Coming Soon

Whole-transcriptome human RNA-Seq
- New RNA-Seq kits featuring faster workflow and lower RNA input for human whole transcriptome analysis
- Simplified and intuitive data analysis tools to make seamless transition from microarrays

Chip-Seq
- Fast and affordable analysis of DNA binding proteins target sequences

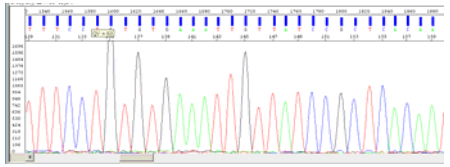
Copy number detection
- Accurate Targeted copy-number detection for basic and clinical research application

Sequencing Application

陳俊銘 (Jimmy Chen)
應用技術專家 (Application Support Specialist)
美商應用生命系統(股)公司 (Applied Biosystems)

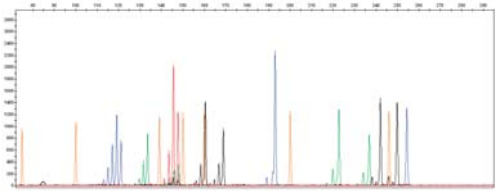
Sequencing vs. Fragment Analysis

Sequencing = determining the base pair sequence of a DNA fragment



1. Base Calling

Fragment Analysis = separate fluorescently labeled fragments and determine their relative size



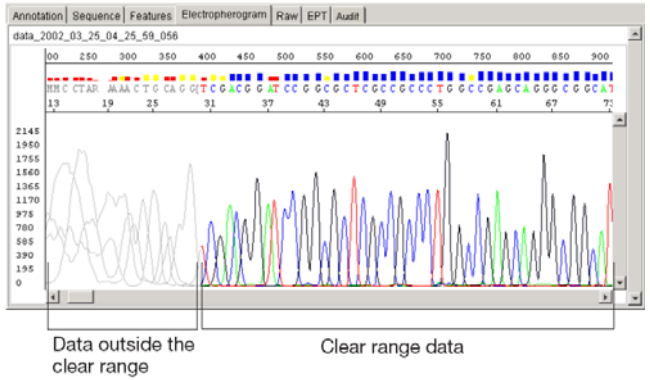
1. Size Calling (Size Curve)
2. Allele Calling

Sequencing Applications

- *De* Novo Sequencing
- Resequencing
  - Mutation detection, SNPs, insertions, deletions
  - Checking Clone Constructs
  - Comparative genomics
- Typing
  - BAC End-Sequencing
  - Microbial and Fungal Identification
  - MLST (Multi-Locus Sequence Typing
  - HLA Typing
  - Viral Genotyping
- Others
  - Methylation (Bisulfite Sequencing)
  - SAGE (Serial Analysis of Gene Expression) Method

Color Legend:
AB Supported
AB Demonstrated
Customer Demonstrated

Sequence Data Analysis



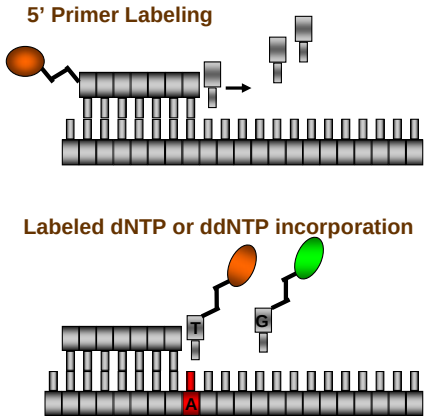
Fragment Analysis Applications

- Microsatellite/STR
  - Linkage Mapping
  - Animal breeding
  - Human Identification
  - Chimerism study
  - Custom Microsatellites
  - Instability/RER
- B and T cell Clonality
- Relative Fluorescence Quantitation
  - LOH
  - MLPA
  - QF-PCR/QMPSF
- SNP Genotyping
  - SNaPshot
  - OLA
- Fingerprinting
  - AFLP
  - T-RFLP
  - BAC
- Conformation
  - SSCP
  - HMA
- TILLING

Color Legend:
AB Supported
AB Demonstrated
Customer Demonstrated

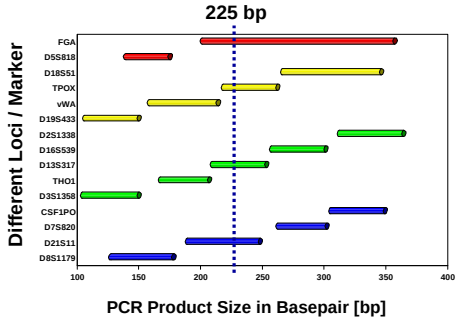
Fragment Analysis Workflow

Generation of labeled DNA Fragment
↓
Electrophoresis
↓
Data Analysis (sizing)
↓
Data Analysis (Genotyping)

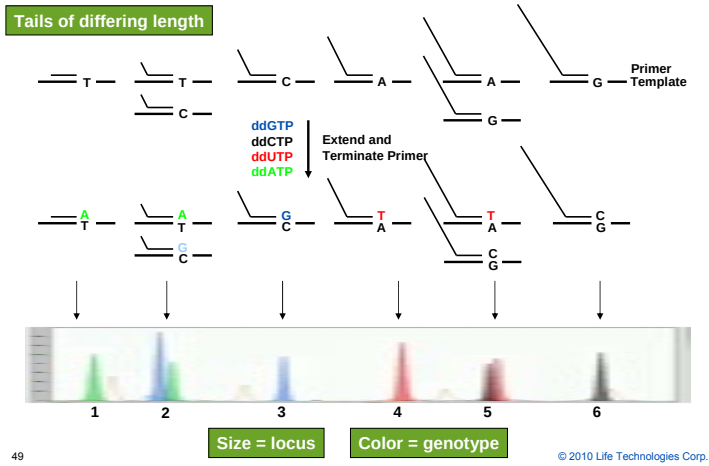


Fragment Analysis Workflow

Generation of labeled DNA Fragment
↓
Electrophoresis
↓
Data Analysis (sizing)
↓
Data Analysis (Genotyping)



SNaPshot® Multiplex System



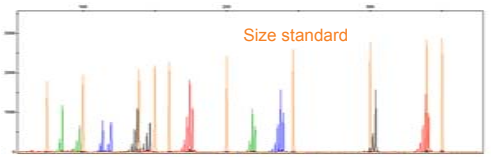
Fragment Analysis Workflow

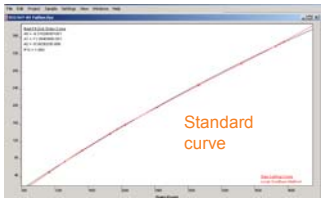
Generation of labeled DNA Fragment

Electrophoresis

Data Analysis (sizing)

Data Analysis (Genotyping)





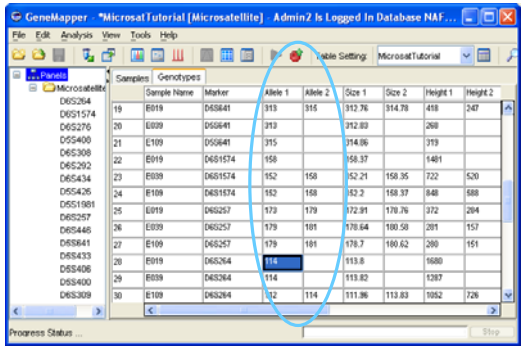
Fragment Analysis Workflow

Generation of labeled DNA Fragment

Electrophoresis

Data Analysis (sizing)

Data Analysis (Genotyping)



Goal: Extensive menu that exploits unique capabilities of CE

Partner or build to develop extensive menu of assays in select areas to drive sales

Oncology	**Infectious Disease**
KRAS/BRAF	Respiratory Panel
Leukemia/Lymphoma Panel	Bacterial and Fungal ID
BRCA1/2 Resequencing Panel	Tuberculosis Genotyping
p53 Resequencing	HPV Genotyping
Microsatellite Instability/LOH	**Genetic Disease**
Lung Cancer Panel	Aneuploidy
Immunology	Pregnancy Loss
HLA (SeCore)	Fragile X

- Menu exploits CE advantages over other platforms
  - Fragment analysis
  - Variable mutant detection
  - Large panel multiplexing
- Menu development primarily driven by partnerships
- Efficient partnership process and infrastructure key to success

Practical Concern: Interrogation of common and rare KRAS and BRAF mutations

KRAS: 12 mutations, 2 tubes

DNA

Codon 12	Codon 13
Gly12Ser (GGT>AGT)	Gly13Ser (GGC>AGC)
Gly12Arg (GGT>CGT)	Gly13Arg (GGC>CGC)
Gly12Cys (GGT>TGT)	Gly13Cys (GGC>TGC)
Gly12Asp (GGT>GAT)	Gly13Asp (GGC>GAC)
Gly12Ala (GGT>GCT)	Gly13Ala (GGC>GCC)
Gly12Val (GGT>GTT)	Gly13Val (GGC>GTC)

Codon 12

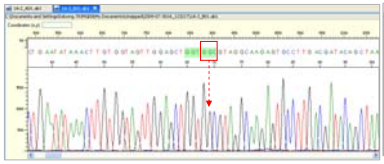
Codon 13

BRAF: 3 mutations, 1 tube

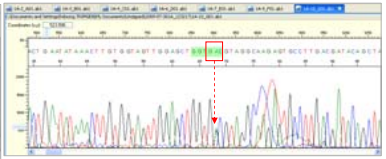
DNA

Codon 600
V600E (GTG>GAG)
V600A (GTG>GCG)
V600G (GTG>GGG)

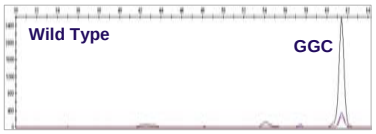
1A) Sequencing Result - KRAS Wild Type, codon 13: GGC



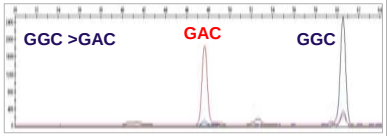
2A) Sequencing Result - Mutation in KRAS codon 13: G(G/A)C



1B) Fragment Analysis Result (codon13)



2B) Fragment Analysis Result (codon13)



Practical Concern: Detecting and Identifying Respiratory Pathogens

- Respiratory tract infections (**RTI**) are the leading cause of morbidity and mortalit both the adult and pediatric patient populations
  - Significant economic impact ($ 10 B in US)
  - Upper and lower RTI: URTI and LRTI
  - RTIs can be caused by viruses and bacteria

Rhinovirus – common cold

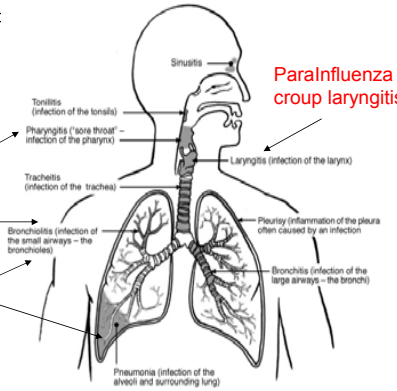
ParaInfluenza croup laryngitis

Adenovirus – pharyngitis

Influenza virus – influenza

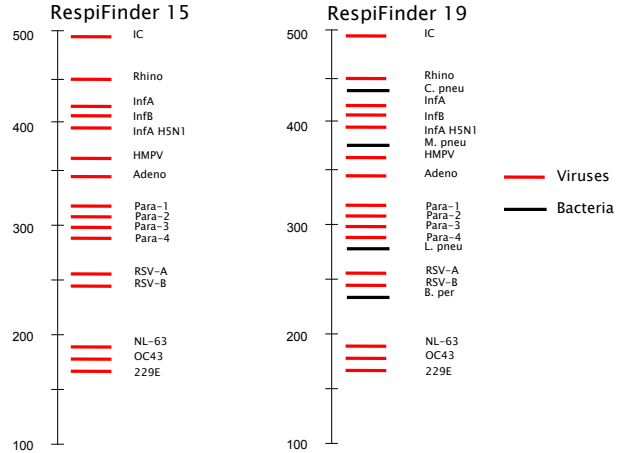
Human metapneumovirus

Respiratory syncytial virus (RSV)

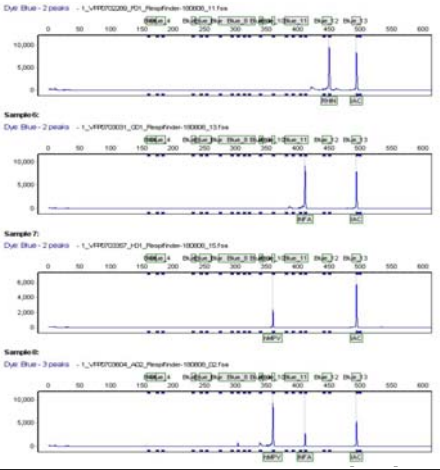


Infections of the respiratory tract

TrueScience™ RespiFinder® Panels: composition



RespiFinder®: Pathogen Detection by Peak Migration/Location in Capillary Electropherogram



Rhino virus
+ Amp control (IAC)

Influenza A virus
+ Amp control (IAC)

Human metapneumovirus
+ Amp control (IAC)

Mixed infection
Influenza A virus
Human metapneumovirus
+ Amp control (IAC)

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German E. coli Outbreak Control Strategy

陳俊銘 (Jimmy Chen)
產品應用專員 (Application Support Specialist)
美商應用生命系統(股)公司 (Applied Biosystems)

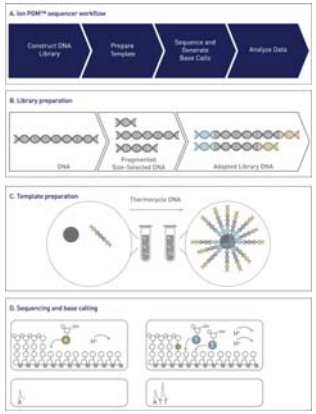
- **Characterization**
  - **What is it ?** (Phenotypes / Genotypes)
- **Identification**
  - **What's the different ?**
- **Screening**
  - **How to prevent ?** (Rapid detection / Screening methods)

Life Technologies Provides a Suite of Tools for Rapid Identification and Screening of Food Borne Pathogens

Initial Characterization – MLST using Sanger (CE) Sequencing

- Multi-Locus Sequence Typing (MLST) using Applied Biosystems® 3130 Genetic Analyzer to characterize isolates using internal fragment sequences derived from several bacterial housekeeping genes.
- For the target isolate, the alleles identified at these loci define the allelic profile or sequence type (ST) - a putative identification can be made by comparing to a database of previously identified strains.
- The outbreak O104:H4 strain was identified as belonging to ST678, the same type as *E. coli strain HUSEC41, an Stx2-positive O104:H4 strain isolated in 2001.*

Workflow for Ion PGM™ will only get faster

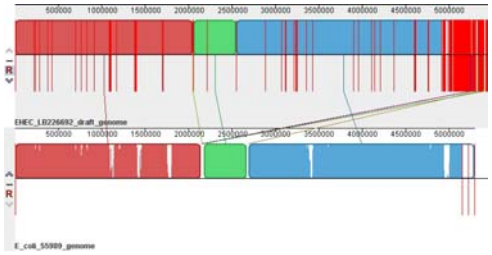


- Today
  - Library Prep ~6 hours
  - Template Prep ~5.25 hours
  - Sequencing ~ 2 hours
- Q3 Workflow
  - Library Prep ~3.5 hours
  - Automated Template Prep ~3 hours
  - Sequencing ~2 hours

Genome alignment between EHEC LB226692 draft assembly and EAEC 55989 complete genome

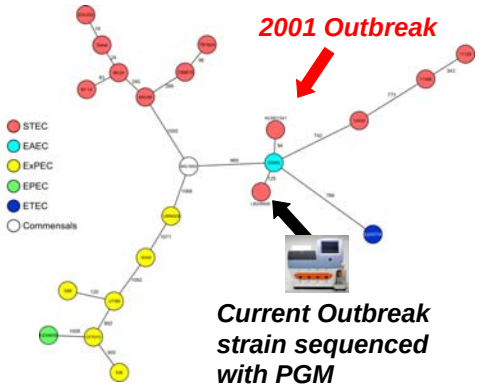
E. coli LB226692 draft assembly

E. coli 55989 complete genome



	perc A	perc C	perc G	perc T	Sum contig length	Num contigs	Mean contig length	Median contig length	N50 value	N90 value	Max contig length
Life	24.7	25.3	25.3	24.7	5,450,264	364	14,973	762	181,540	14,537	475,662

How the Current Outbreak Strain Compares to Previous Outbreak Strains



Current Outbreak strain sequenced with PGM

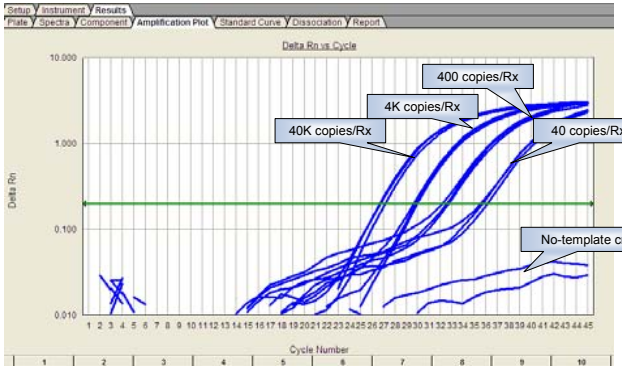
Phylogenetic relationship of the current STEC HUSEC041 (O104:H4) outbreak strain with the historical STEC HUSEC041 from 2001 (www.ehec.org) and additional *E. coli* strains

Figure from: Alexander Mellmann, Universitätsklinikum Münster

Assay Design Process

Analyzed outbreak *E. coli* strain sequence	• Looked for unique features to strain, subgroup, and isolate
Designed candidate assays to uniquely detect outbreak strain	• Designed candidate assays to selected target areas including $fliC_{H4}$, wzx_{O104}, agg, and unique signature regions
Tested candidate assays vs panel of *E. coli* strains	• Tested vs. target and other *E. coli* strains such as O157:H7, O55:H7, O26:H11, O111:NM, O145:NM, as well as other bacteria
Custom TaqMan® *E. coli* O104 and O104:H4 Assays	• Manufactured and ready for shipment in ~1 week

Sensitivity of TaqMan® *E. coli* O104 Detection Assay



Food Testing Workflow

6 – 18 hr	0.5 – 1 hr	2 hr	2 hr
Enrich	Prepare Sample	Test for *stx1* and *stx2*	Test for *E. coli* O104:H4
• 25 g food • 225 ml media	• PrepSEQ Rapid Spin Kit - or - • PrepSEQ Nucleic Acid Extraction Kit	• Custom TaqMan® VT1 (*stx1*) Assay • Custom TaqMan® VT2 (*stx2*) Assay	• Custom TaqMan® *E. coli* O104:H4 Assays

stx1⁻ *stx2*⁺



Questions ?

www.appliedbiosystems.com.tw
support.tw@lifetech.com
0800-251326 ; 02-23582838 ext. 3