Solutions for non-O157 STEC detection and characterization

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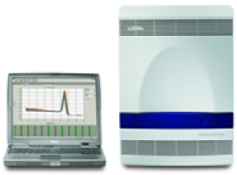
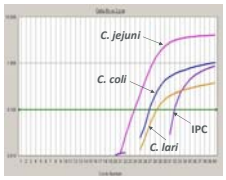
Leaders in real-time PCR

Life Technologies was formed in 2009 by the merger of Applied Biosystems and Invitrogen to form the global leader in genetic innovation for life science research and validated markets. A leader in qPCR innovation, Life Technologies offers the gold standard in real-time instrumentation. Designed for compatibility with TaqMan® assays and the flexibility of unlimited real-time chemistry choices, we make it easy for you to get started and get the results you need. Features include ease of use, rapid cycling, and assay multiplexing with up to six dyes in a single reaction, and further discrimination with melt-curve analysis.



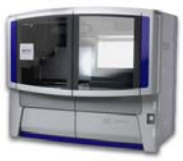
Applied Biosystems 7500 Fast qPCR System



Multiplex detection of three *Campylobacter* species in a single tube

Identification of signature sequences by whole genome sequencing

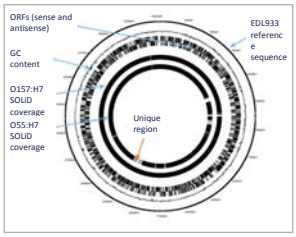
Life Technologies has been a pioneer in the field of genetic analysis. The Life Technologies sequencing portfolio includes the 3500 Series Genetic Analyzer capillary electrophoresis instruments, as well as the SOLiD™ System and ION Torrent Personal Genome Machine™.



Applied Biosystems 3500 SOLiD™ System

ION Torrent Personal Genome Machine (PGM)™

The SOLiD™ system has been put to use in our internal assay design program in order to obtain genome sequences for microbes of interest, in cases where such sequences are not adequately represented in public databases. By deciphering these genome sequences in-house, we are able to identify conserved, specific signatures that discriminate closely related pathogens. For example, many assays targeting E. coli O157:H7 also detect its near-neighbor, O55:H7. In order to develop assays specific for O157:H7, the genome of O55:H7 was sequenced on the SOLiD™ system, which allowed the identification of assays that could discriminate between the two serotypes.



Currently available solutions for *E. coli* detection

Life Technologies currently offers integrated end-to-end workflows for food safety testing.

- The Applied Biosystems Pathogen Detection System includes complete and validated workflows for the rapid and accurate detection of many harmful pathogen targets, such as *E. coli* O157:H7, in a qPCR format.
- Invitrogen Dynabeads® immunomagnetic separation (IMS) products simplify culture detection methods and enable consistent and rapid isolation of many common pathogens, including EHEC and STEC targets : O157, O26, O103, O111 and O145.
- Custom TaqMan® assays are available for detection of *stx1* and *stx2*, encoding the Shiga toxin proteins

Elements of an effective STEC detection strategy

An effective strategy for molecular STEC detection requires an integrated workflow that can deliver accurate results quickly and reliably. The Applied Biosystems Pathogen Detection System delivers solutions at every step of the workflow.

Workflow step	Needs	Life Technologies solution
Enrichment	Facilitate the detection of scarce organisms, allow retrieval of viable organisms for validation	Dynabeads® immunomagnetic separation (IMS) reagents
Sample prep	Automated extraction of high quality nucleic acid at high yields, automation	PrepSEQ™, MagMAX™, and AutoMate Express™ Systems
Detection assays	Sensitive, specific, and accurate detection of the pathogen	TaqMan® and MicroSEQ® Pathogen Detection Kits
Real-Time PCR instrument	Rapid, automated, multiplexed runs in flexible plate formats	7500 Fast, ViiA 7™, and OpenArray® Real-Time PCR Systems
Data analysis	Unambiguous interpretation of instrument data	RapidFinder™Express software

The relative complexity of the non-O157 STEC workflow may also impose some additional requirements.

- Multiplexing to keep number of assays low: the ViiA™ real-time PCR system allows four or more assays to be combined in a single reaction.
- Tracing multiple tests back to single cell: existing tools in the Life Technologies portfolio such as the Attune® Acoustic Focusing Cytometer and the OpenArray® Real-Time PCR platform, offer potential solutions to this problem, and single cell measurements of RNA by TaqMan® assays have been demonstrated.

Molecular targets for characterization of STEC

In response to the growing needs by both regulatory agencies and the food industry, Life Technologies is developing a qPCR based workflow for the rapid and highly specific detection of the Top 6 non-O157 STECs.

Shiga toxin (*stx1* and *stx2*)

- All *stx1* sequences are predicted to de detected by a single assay
- Detection of all *stx2* alleles is achieved by a multiplex reaction to include the variant *stx2f* and *stx2g* alleles

Intimin (*eae*)

- A single assay is capable of detecting all known *eae* alleles

O-antigen biosynthesis loci

Serotype	Library type
O26:H11	Fragment
O26:H11	Fragment
O26:H11	Fragment
O26:H32	Mate-pair
O45:H10	Fragment
O45:H2	Mate-pair
O103:H2	Fragment
O103:H2	Fragment
O103:H2/H35	Fragment
O103:H21	Mate-pair
O103:H8	Mate-pair
O111:H10	Mate-pair
O111:H8	Fragment
O111:H8	Mate-pair
O111:NM	Fragment
O113:H21	Fragment
O121:H19	Mate-pair
O121:H7	Fragment
O145:H28	Fragment
O145:NM	Mate-pair

The genomes of 20 *E. coli* strains with Top 6 O-serotypes were sequenced on the SOLiD™ system. Comparative analysis of these genomes, together with publicly available genomes facilitated signature identification and design of highly specific assays.

Assays have been developed against multiple genes within the *rfb* clusters of the Top 6 STEC serotypes: O26, O45, O103, O111, O121, and O145. Laboratory validation of these assays is underway through collaborations with the USDA. Assays will be screened against a panel consisting of over 400 bacterial strains including all known *E. coli* O-types.

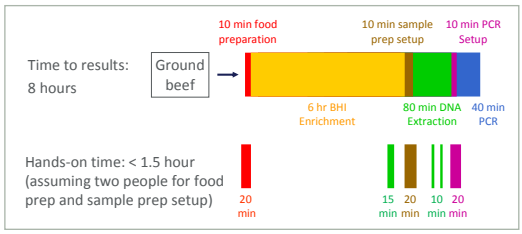
Adapting to changes in the STEC population

Although detection assays are designed against conserved signature regions, the rapid mutation rates of bacteria may lead to the emergence of strains that are not efficiently detected by a given assay. To minimize the risk of failing to detect variant bacteria, pathogen detection assays are routinely mapped against a database of public and in-house microbial sequences. In the event a mismatch is detected, assay oligos can be modified or redesigned, using proprietary bioinformatics tools, to ensure ongoing inclusivity of the assay.

Over time, regulatory requirements may change to include or exclude specific *E. coli* subtypes. Using in-house sequencing and assay multiplex design tools, existing assays can be quickly modified to meet the new requirements. Life Technologies has a proven track record of rapidly responding to public health needs, having developed assays for the CDC in response to the 2009 Influenza H1N1 crisis, and the TaqMan® *Salmonella* Enteritidis Detection Kit in response to SE outbreak in eggs in 2010.

Advantages of the Applied Biosystems Pathogen Detection system

The Applied Biosystems Pathogen Detection System is faster and more sensitive than other methods, detecting cell concentrations as low as 10^3 –10^4 cfu/mL after enrichment. This level of sensitivity ensures the detection of many pathogens in less than 24 hours, as compared to several days with conventional methods. Further increases in speed and throughput can be achieved using sample prep automation solutions. Validated workflows have been developed for a wide variety of food matrices including ground beef and beef trim, chicken rinses, smoked fish, cheese, milk, produce, and pet food.



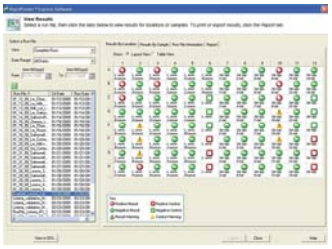
An eight-hour workflow for detection of *E. coli* O157:H7 from ground beef using the MicroSEQ® *E. coli* O157:H7 Detection Kit.

AB MagMax Express ™ Automated Magnetic Particle Processing Instrument

AB AutoMate *Express* ™ DNA Extraction System

Automated data collection and analysis

RapidFinder™ Express Software is easy to use and guides you through each step of the assay, from run file set up to final results. Amplification, detection, data collection, and analysis are fully automated.



For more information go to www.appliedbiosystems.com/foodsafety

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