

Welcome to Idaho Technology's (ITI) Autumn *Amplitimes*. In this issue, we feature the progress the R.A.P.I.D.® LT Food Security System is making in the food industry. We also highlight another useful tool using Hi-Res Melting®

and LunaProbes™ using a new system we will discuss in the Winter *Amplitimes*—the LightScanner® 32. Our RAZOR® EX salespeople and FilmArray™ highlights are also featured.

R.A.P.I.D. LT Food Security System Highlights

The R.A.P.I.D. LT Food Security System (FSS) is gaining momentum. We attended multiple meetings this year, including the Food Safety Summit and International Association of Food Protection, where the system was demonstrated and well received (go to <http://www.idahotech.com/Support/posters.html> to view posters).



We are now on the way to providing a full suite of food pathogen assays to be used with the RAPID LT FSS. *Salmonella* LT has been officially launched and gained AOAC-RI approval earlier this year. *Listeria* LT and *E. coli* LT are on the schedule for AOAC-RI approval in early 2009.

Built upon LightCycler® technology, the R.A.P.I.D. LT FSS reliably identifies test samples in 35 minutes. Because of its sensitivity, accuracy, and high speed, customers are finding that the R.A.P.I.D. LT FSS is the ideal instrument for rapid pathogen detection and genotyping.

Inside this Issue

- 1 R.A.P.I.D. LT FSS
- 2 Mutant Allele Amplification Bias
- 2 RAZOR EX Troubleshooting Tips
- 3 Chrissie Flowers and Tom Sawyer
- 3 Fall Photo of the Quarter
- 4 Dates to Remember
- 4 R.A.P.I.D. and RAZOR Training
- 4 FilmArray Corner

The advantages of the R.A.P.I.D. FSS system include:

Speed—With some of the shortest enrichment times and PCR time on the market, the R.A.P.I.D. LT is the fastest real-time pathogen detection system available today. A comparison of the R.A.P.I.D. LT with DuPont's BAX® system (also a PCR test) is shown in the following table.

Comparison of R.A.P.I.D. LT and DuPont BAX		
Function	R.A.P.I.D. LT	BAX
PCR	35 min.	3 1/2 hours
Enrichment— <i>Listeria</i> spp.	24 hours no transfers	24–28 hours
Enrichment— <i>Salmonella</i>	16 hours no transfers	20–24 hours

Easy-to-Use Reagents—Reagents are freeze-dried combining all necessary components in one tube: primers, probes, enzymes and buffers. This minimizes user errors, saves time, reduces reagent waste, and minimizes cross contamination. Each test ensures reagent quality by incorporating an amplification control into every reaction.

Accuracy—Idaho Technology uses FRET (fluorescence resonance energy transfer) probe chemistry because it has higher specificity over conventional real-time PCR detection methods that use SYBR® Green or TaqMan® probes.

Cost Effective—The R.A.P.I.D. LT FSS is the only AOAC approved pooling solution on the market. This validated pooling technique allows food producers to test more samples, enabling faster and better decisions at a lower cost. The pooling protocol uses post-enrichment pooling as opposed to conventional pre-enrichment sample pooling, maintaining sample integrity and traceability.

For more information about the R.A.P.I.D. LT FSS, please contact Melissa Tucker at 801-230-9238 or at melissa_tucker@idahotech.com

Mutant Allele Amplification Bias using Rapid Cycle-Real Time PCR and Hi-Res Melting® with LunaProbes™ on the LightScanner 32

Introduction

High resolution melting (Hi-Res Melting) was introduced as a homogeneous method of scanning PCR amplicons for heterozygous sequence variants. Based on the use of dsDNA saturating dyes, Hi-Res Melting is capable of detecting SNPs and insertion/deletions in amplicons up to 400 bp at a sensitivity >99%. Since its introduction in 2003, additional applications for Hi-Res Melting have been developed, including genotyping for known sequence variants using small amplicons or unlabeled probes (LunaProbes). LunaProbes are blocked on the 3' end to prevent extension during PCR and use the dsDNA saturation dye LCGreen® Plus to discriminate the genotype of the allele based on probe melting temperature (T_m). The probe sequence can be designed to match either allele and is based on maximizing the ΔT_m between the perfect match and mismatched probe.

Methods

We investigated the use of LunaProbes to discriminate the mutant allele at $\leq 5\%$ in a background of the wild-type allele. Because LunaProbes are inherently large (25–30 bp) to generate sufficient fluorescent signal, they present a unique opportunity to preferentially bias amplification of the mismatch allele. Mutant allele amplification bias (MAAB) is achieved by setting the annealing temperature of PCR such that it is approximately halfway between the T_m of the perfectly matched probe and the T_m of the mismatched probe. At this mid- T_m annealing temperature, the perfectly matched probe (wild-type allele) is bound to its target and is stable enough to partially retard amplification. Rapid cycle PCR performed on the LightScanner 32 was required to maintain the stringency of the target annealing temperature and hinder amplification of the wild-type allele. In contrast, the slower temperature transition rates of a conventional thermal block cycling instrument were not practical for MAAB.

Results

Detection sensitivity of the mutant allele without MAAB was determined to be 5%. Based on the observed T_m 's of the LunaProbes (62°C for the wild-type allele and 54°C for the mutant allele), an annealing temperature of 58°C was

used to induce MAAB in a dilution series of mixed samples. MAAB was not observed when the same approach to setting the annealing temperature of PCR was performed on a standard thermal block cycler, presumably due to the slower transition rates between annealing and denaturation temperatures.

Conclusions

Rapid cycle PCR was critical for inducing MAAB over the wild-type allele in samples that were mixed at 50% of each allele. A MAAB factor of approximately 10X allowed discrimination of the mutant allele down to 0.7–1.5%. Applications of this method include detection or confirmation of common somatic mutations (p53, EGFR, BRAF) and early identification of mutant bacterial infections (malaria) where standard therapies are contraindicated. The ability to use a combination of real-time PCR data and Hi-Res Melting on the LightScanner 32 instrument, coupled with the rapid-cycle PCR approach, appear to be critical to performing MAAB using LunaProbes. For more information regarding MAAB or the LightScanner 32, please contact Jason McKinney at jasonm@idahotech.com or at 801-736-6354 x. 411.

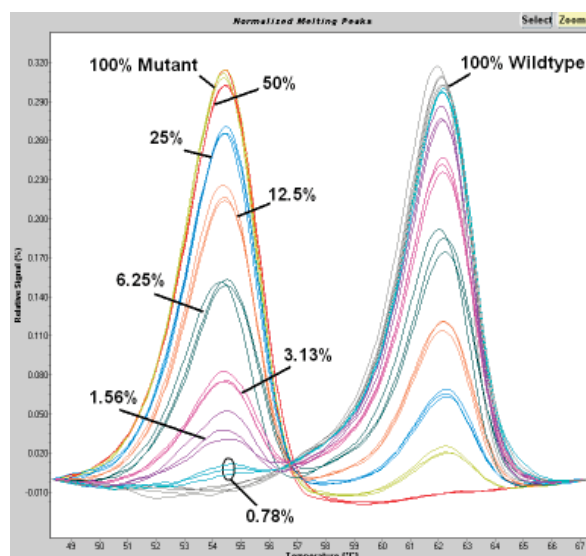


Figure 1. Normalized derivative peaks from LunaProbes. Wild-type allele (gray) = 62°C. Mutant allele (red) = 54°C. An annealing temperature of 58°C was used to bias the amplification of the mutant allele in the 50–50 mixed samples (yellow). This amplification bias allows greater resolution of the mutant allele by a factor of ~10X and sensitivity down to 0.7–1.5%.

RAZOR EX Troubleshooting Tips

Always run the RAZOR instruments with the battery pack installed. This will guard against poorly regulated power conditions and unexpected power failures. Think of it as free uninterruptible power supply.

When you are twisting the plungers, make sure you rotate each of the plungers 90° toward the center of the pouch; this prevents leaking and cross-contamination of neighboring wells.

Chrissie Flowers



Chrissie Flowers is ITI's new East Coast Regional Sales Manager for Biodefense, with responsibility for the sale of the RAZOR EX, R.A.P.I.D., and R.A.P.I.D. LT, and associated consumables.

Chrissie has a bachelor's degree in Biology from Virginia Tech and received her master's in Public Health from Eastern Virginia Medical School in Norfolk, Virginia. She is also certified in Tropical Medicine and Vector-borne Emerging Infectious Diseases from Johns Hopkins University.

Her undergraduate education was complimented by four years of research in the epidemiology and surveillance of West Nile virus and investigating the neurological manipulation of La Crosse encephalitis virus on disease competent vectors. She also worked with Quest Diagnostics Laboratories in northern Virginia during the 2002 anthrax attacks and is a certified Level 1 HazMat Instructor.

Chrissie came to ITI from being an emergency planner with the Virginia Department of Health. She has a wealth of experience in grants management and emergency preparedness and response in Norfolk, Virginia and the Western Tidewater region of Hampton Roads. She can be contacted via e-mail at Christina_Flowers@idahotech.com or by phone at 801-244-3087.

Tom Sawyer

Tom Sawyer is ITI's new Western Regional Sales Manager for BioDefense with responsibility for the sale of the RAZOR EX, R.A.P.I.D., R.A.P.I.D. LT, and associated consumables.

Tom attained a bachelor's degree in Zoology (with emphasis on Wildlife Ecology) and master's degree in Microbiology and Parasitology (with emphasis on Public Health), both from Brigham Young University. His academic experience is complemented by 10 years in pharmaceutical sales followed by 21 years of technical selling in the clinical and molecular diagnostic market. Over the past 4 years, his major emphasis has been in the public health, first responder, and military segments for biothreat.

Tom has held the position of senior sales representative, field trainer, national accounts manager, and business development manager. He has sold capital equipment, reagents, and kits to a wide variety of customers (i.e., hospitals, clinical reference labs, veterinary labs, academic centers, biotech companies, forensic labs, public health labs, and various government agencies). Tom can be contacted via e-mail at tom_sawyer@idahotech.com or by phone at 801-556-3234.



Photo of the Quarter

La Caille
(photographer: Kathy Jedrzejczyk,
Research Associate)

Dates to Remember

November

- 10–13** qPCR Symposium
Millbrae, California
<http://www.qpcrsymposium.com>
- 12–14** American Society of Human Genetics (ASHG)
Philadelphia, Pennsylvania
<http://www.ashg.org/2008meeting>

December

- 2–4** 3rd National Conference on Environmental Sampling and Detection for Bio-Threat Agents
Las Vegas, Nevada
<http://www.sampling-conference.com/info.htm>

January

- 10–14** Plant and Animal Genome XIV
San Diego, California
<http://www.intl-pag.org>

February

- 22–25** ASM Biodefense and Emerging Diseases
Baltimore, Maryland
<http://www.asmbiodefense.org/>

Editor's Note: If you have comments or suggestions for articles, please e-mail the editor at loretta_orgill@idahotech.com.

Department of State Note: The JBAIDS System, R.A.P.I.D. System, RAZOR Instrument, and RAZOR EX Instrument are controlled for export under the International Traffic in Arms Regulations (ITAR), administered by the U.S. Department of State, Directorate of Defense Trade Controls (DDTC) and may not be exported or transferred to any foreign national without prior approval of the DDTC.

R.A.P.I.D.[®] and RAZOR[®] Systems Training

ITI offers training courses for the R.A.P.I.D. and RAZOR systems. Training for two people is included with the purchase of the R.A.P.I.D. or RAZOR instruments, and more can attend for an additional cost. The training courses are three days for the R.A.P.I.D. and one day for the RAZOR. Courses focus on concepts of operation, sample preparation, reagent setup, and software. If you would like to attend or schedule a training course, please contact our training staff at 1-800-735-6544 x. 439.



FilmArray Corner

ITI's FilmArray is the latest in user-friendly automated multiplex PCR. The system integrates sample preparation, amplification, detection, and analysis into one easy-to-use system capable of massively multiplexed PCR. It is also fully automated and turns a sample into results in less than 1 hour.

The FilmArray Respiratory Panel* can simultaneously detect the following viral and bacterial respiratory pathogens in one sample:

Viral: Adenovirus, Bocavirus, Coronavirus (4 strains), Influenza A (with H1/H3 subtyping), Influenza B, Metapneumovirus, Parainfluenza 1–4, RSV, and Rhinovirus.

Bacterial: *Bordetella pertussis*, *Bordetella parapertussis*, *Chlamydomphila pneumoniae*, *Legionella pneumophila*, and *Mycoplasma pneumoniae*.

For more information about the FilmArray, please contact Wade Stevenson at wade_stevenson@idahotech.com or at (801) 736-6354 x. 463.

*Clinical trials for FDA clearance are scheduled to begin November 2009.

Great things are about to happen.



390 Wakara Way, Salt Lake City, UT 84108 USA
1-800-735-6544 / www.idahotech.com

