

PCR and Hi-Res Melting[®] Using the LightScanner[®] 32

If you are interested in Hi-Res Melting but don't require the throughput of a 96- or 384-well platform (LightScanner) or simply desire qPCR results on the same samples before Hi-Res Melting, you will be interested in Idaho Technology's newest instrument: the LightScanner 32. The LS32 combines the rapid, real-time thermocycling technology of a R.A.P.I.D.[®] and the gold-standard Hi-Res Melting capability of an HR-1[™] on a single instrument.

The LS32 offers a versatile application suite without sacrificing performance. Some of its advantages include:

- Analyzes qPCR and Hi-Res Melting results on 32 samples per run in less than 60 min.
- Configures with three-color detection for real-time PCR multiplex capability and optimized single-color detection of LCGreen[®] Plus dye for Hi-Res Melting.
- Rapidly generates high quality gene expression data.
- Accurately discriminates even the most subtle DNA mutations using one of several Hi-Res Melting applications supported by the most comprehensive software suite in the industry.
- Offers multiple genotyping applications offering greater flexibility and specificity than TaqMan[®].



Basic genotyping or real-time PCR with genotyping results can be generated on the LS32 using a variety of labeled probe chemistry including SimpleProbe[®] and HybProbe[®]. Optimal Hi-Res Melting results for mutation discovery are generated using LCGreen Plus dye.

PCR on the LS32 works just the same as on a R.A.P.I.D. thermocycler. The added capability of the LS32 allows those same samples to be individually melted immediately after PCR using a temperature-controlled melter similar in design and concept to the HR-1. The HR-1 was the first instrument on the market and introduced the concept of Hi-Res Melting to the world.

Software for the LS32 supports not only Hi-Res Melting scanning applications (mutation discovery), but also a suite of genotyping applications, including small amplicon genotyping (SAG) with internal reaction calibration and LunaProbes[™] genotyping with unlabeled, 3' blocked oligos. Both of these genotyping applications take advantage of the dsDNA saturation dye LCGreen Plus, eliminating the need for expensive fluorescent labeled probes. The flexibility of SAG and LunaProbes allows for easy assay development and the ability to genotype SNP targets that are not possible to genotype with traditional TaqMan probes.

The LS32 comes with the only complete software package in the industry, evidenced by its ability to perform scanning as well as the genotyping applications. Idaho Technology is continuously adding functionality through collaborative research and development efforts with some of our most innovative customers.

For more information about the LightScanner 32, please contact Cameron Gundry at Idaho Technology (cameron_gundry@idahotech.com, +1-801-736-6354 x. 444, or 800-735-6544 x. 444).

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LightScanner System FAQs

Q: Do I have to re-optimize my PCR reactions when adding LCGreen Plus dye to my PCR reactions or when I use LightScanner Master Mix?

A: Yes. The addition of LCGreen dye to the PCR reaction stabilizes the dsDNA molecules such that the T_m is elevated 1–3°C. We recommend running a gradient PCR experiment to determine the optimal PCR conditions when using LCGreen Plus dye or any LightScanner Master Mix.

Q: Can I analyze samples that were preserved and extracted by different methods?

A: They can be melted on the same plate, but they should be analyzed in different subsets. Significant differences have been observed between different sample types.

Q: What is the small peak that looks like a bump at the end of the melt profile. How do I eliminate it?

A: The bump is called a “toe,” and it corresponds to co-amplification of undesired PCR products that contain a higher T_m than the amplicon. To increase the quality of your PCR reactions try one or a combination of the following:

1. Decrease primer concentration by 20%.
2. Increase annealing temperature by 1–2°C.
3. Minimize input of DNA to no more than 20 ng total.
4. Decrease cycle number if desired PCR product is robust.

Q: What is the best setting for the normalization cursors during data analysis?

A: When using linear correction, the distance between cursors should be a minimum of 0.5°C.

Q: What is the lowest volume that can be used in 96-well plates for detection on the LightScanner?

A: We recommend no less than 10 μL with a 20 μL oil overlay.

Q: My melt curves are wavy. Is there something wrong with my LightScanner?

A: A common cause is improper sealing film or the presence of air bubbles in sample wells. To avoid bubbles in your reactions, centrifuge each plate for approximately 2 min. at 2000 rpm before placing it in the LightScanner. Use one of the approved sealing films found in Appendix B of the LightScanner Operator Manual.

Q: Can I add mineral oil after PCR instead of before?

A: No. Using mineral oil before PCR is best for preventing evaporation during PCR and is required for high resolution melting. In a 10- μL reactions, evaporation of just 1 μL may cause a 10% change in reaction concentrations and ratios, which may significantly impact PCR efficiency.

Q: What is the recommended amplicon size range when using the High Sensitivity Master Mix?

A: The recommended range for this master mix is 40–120 base pairs.

Q: Can the High Sensitivity Master Mix with the internal calibrators be used with amplicons larger than 120 bp?

A: Yes, however larger PCR products inherently produce greater fluorescent signals, which may hinder detection of the low amplitude temperature calibrators. Larger amplicons also melt at higher temperatures, and this may affect the ability to use the high T_m calibrator peak at approximately 92°C.

Q: What should the T_m of my LunaProbe be relative to the T_m of my primers?

A: The highest T_m of the probe should be at least 2°C less than the lowest primer T_m . If the highest probe T_m is greater than that of the primers, the probe can inhibit PCR.

Q: How long should my LunaProbe be and are there any restrictions relative to amplicon length?

A: The probe should be between 20–35 bases, and it should be at least 10% the length of the amplicon to ensure detection of the probe/target duplex.

Q: What type of plates should I use with the LightScanner?

A: Use only white-welled, black-shell plates with the LightScanner. Clear plates allow fluorescence bleed-over from well to well, black plates quench fluorescence and increase noise, and completely white plates reduce the quality of the data.

More troubleshooting questions and answers can be found at <http://www.idahotech.com/LightScanner/SupportForm-LS.html>.

Questions regarding LightScanner use should be directed to Luke Stewart (luke_stewart@idahotech.com or 801-556-5346) or to Mike Wall (mike_wall@idahotech.com, +1-801-736-6354 x. 424, 800-735-6544 x. 424).



Idaho Technology Receives AOAC Approval for Listeria LT Detection Kit



Idaho Technology is proud to announce that its *Listeria* test used with the R.A.P.I.D. LT Food Security System (FSS) has been granted Performance Tested Methods Status by the AOAC Research

Institute (Certificate No. 010901). The assay uses real-time PCR technology to identify the presence of the *Listeria* bacterium in food and environmental samples. Each year approximately 2,500 people become seriously infected with *Listeria* and 500 die from the disease.

The validation of this rapid and accurate screening tool for *Listeria* is an important development for all food manufacturers since *Listeria* can grow in refrigerated temperatures and affect ready-to-eat foods. It will enable food manufacturers to release products without fear of potential outbreaks or possible food recalls. The assay is intended for use by trained laboratory personnel.

“Our aim is to provide food companies with cost efficient, rapid, and accurate pathogen identification to enhance their productivity and success. The validation of the *Listeria* test joins our growing portfolio of assays that accomplish this goal,” states ITI Vice President of Product Development, David Nielsen.

The system marks a milestone in real-time PCR testing of food and environmental surface pathogens as this platform enables detection of *Listeria* in as little as 35 minutes after only 24 hours of enrichment. The complete system provides the easiest end-to-end protocol for PCR-based detection methods, as well as the only AOAC-approved post-enrichment optional pooling protocol. The 5:1 sample pooling approach enables the cost per test to approach that of traditional methods.

The *Listeria* test is recommended for use with the R.A.P.I.D. LT FSS, which combines rapid air thermocycling and a real-time fluorimeter to identify test food and environmental samples. In addition to the instrument, robust freeze-dried reagents have been designed and optimized to run on this instrument and provide precise results. The R.A.P.I.D. LT FSS represents a significant improvement over traditional microbiology tests that currently require 5–7 days.

For more information about the Listeria LT Detection Kit and the R.A.P.I.D. LT FSS, please contact Tiffany Colton (tiffany_colton@idahotech.com, or on +1-801-736-6354 x. 478, 800-735-6544 x. 478).

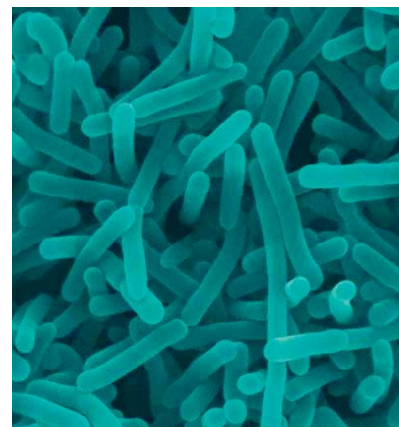


Photo of the Quarter

Frosty Window, Park City
(photographer: Lyle Nay)

Dates to Remember

March

25–29 **Annual Clinical Genetics Meeting (ACMG)**
Booth # 213
Tampa, Florida
<http://www.acmgmeeting.net>

April

13–17 **SPIE Defense, Security, and Sensing Symposium, Booth #1522**
Orlando, Florida
<http://spie.org/x24718.xml>

17–22 **25th Clinical Virology Workshop and Symposium**
Daytona Beach, Florida
<http://hsc.usf.edu/nocms/medicine/medmicro/virology>

18–22 **American Association for Cancer Research Denver, Colorado**
<http://www.aacr.org/home/scientists/meetings--workshops/aacr-100th-annual-meeting-2009.aspx>

22–24 **United Fresh, Booth #832**
Las Vegas, Nevada
<http://www.unitedfreshshows.com>

27–29 **Food Safety Summit, Booth #825**
Washington, D.C.
http://www.foodsafetysummit.com/HTML/BNP_GUID_9-5-2006_A_10000000000000258013

May

5–8 **2009 Association of Public Health Laboratories Annual Meeting, Booth #306**
Anchorage, Alaska
<http://www.aphl.org/profdev/conferences/2009annualmeeting/Pages/default.aspx>

17–21 **American Society for Microbiology Booth #1212**
Philadelphia, PA
<http://gm.asm.org>

Department of State Note: The R.A.P.I.D. System, RAZOR Instrument, and Light-Scanner 32 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.



The FilmArray™: Multiplex PCR Made Simple

The Idaho Technology FilmArray is the latest in user-friendly automated multiplex PCR. Fourth quarter of 2008 saw significant progress toward completion of the FilmArray—our highly user-friendly, automated, multiplex PCR system. In the 3 months since the last update we have:

- Locked down the final list of targets for the respiratory panel.
- Redesigned the software user-interface streamlining the workflow process.
- Completed the redesign of the FilmArray disposable pouch making it more robust, easier to use, and easier to manufacture.
- Improved the FilmArray optics system vastly increasing the signal-to-noise ratio.

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

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

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