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Rapid Detection of RNA at POC by PyroScript® Amplification

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Abstract

RNA viruses are infectious agents that pose a growing threat to human health and well being. Nucleic acid tests (NATs) to target the RNA component of the virion are now the diagnostic gold standard to detect these important pathogens. NATs allow higher specificity and sensitivity compared to alternatives based on antibodies. As new infectious agents like the 2009 H1N1 swine flu emerge, new NATs are far easier to develop. Treatment and management of diseases caused by these pathogens like influenza, the common cold, Dengue Fever, and HIV could be significantly improved by rapid detection and typing of the viruses that cause them at point of care (POC). This need is not well served by the currently available NAT methods like RT-PCR which require instrumentation, technical training and may take a 24 hour turn around time; or by rapid antibody-based tests which can miss 50 to 60% of cases of influenza. A significant market exists for a rapid, simple, sensitive NAT diagnostic amenable to POC and near-POC settings such as clinics. We believe there is also direct consumer demand for similar tests for these and other infectious agents.

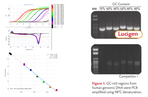
Lucigen has made significant progress developing rapid tests for RNA pathogens that do not require any instrumentation. The fully integrated form of these tests will be highly reliable and as simple to operate and interpret as a home pregnancy test. Our current assays are based on single enzyme, isothermal RNA amplification using the Lucigen proprietary PyroScript® polymerase and detection by lateral flow. We have developed a test that detects influenza A and B RNA after 30-40 minutes with results read out on a lateral flow test cassette. The PyroScript test has been verified externally using nucleic acids extracted from residual clinical nasal swab samples.

Lucigen has also incorporated the enzyme into PyroScript RT-PCR 2X Master Mix, a complete one-step RT-PCR pre-mix . PyroScript RT-PCR 2X Master Mix is optimized for sensitive, specific and accurate RNA detection with the added benefits of reduced hands-on time and set-up error combined with improved reagent stability. Application of the Pyrophage RT polymerase to molecular diagnostics improves the speed and accuracy of testing for human health and disease.

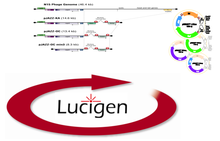
About Lucigen

Lucigen Corporation was founded in 1998 as a molecular biology and genomics technology development firm with fundamental skills in nucleic acid isolation and cloning, sequencing, amplification and detection. Lucigen Corporation delivers advanced molecular technology, tools, and services to life scientists by inventing solutions to the most difficult problems in DNA cloning, amplification, detection and protein expression.

Amplification and Detection
PyroScript™ RT-PCR and EconoTaq® Master Mixes



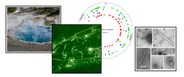
Cloning the "Unclonable"
pJAZZ® Linear Vector, CloneSmart®, GC Cloning Systems

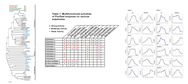


Competent Cells
Phage Display, Expression, Custom

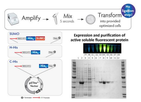
Highest Efficiency
Electrocompetent Cells available
ER2738, DS20 (NEB5α F'), and TG1

Metagenomics of Thermophilic Organisms
Random Shear BAC and NexGen Library Construction





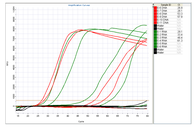
Expression Systems
Expresso™ Cloning and Expression Systems

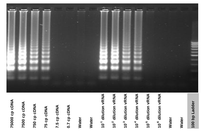


Enzyme Discovery

PyroScript Isothermal Amplification

Isothermal Detection of Influenza



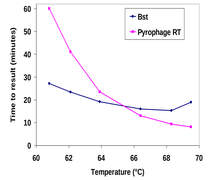


RT-Isothermal amplification with PyroScript RT of a 10-fold dilution series of Influenza B cDNA and Influenza B vRNA.

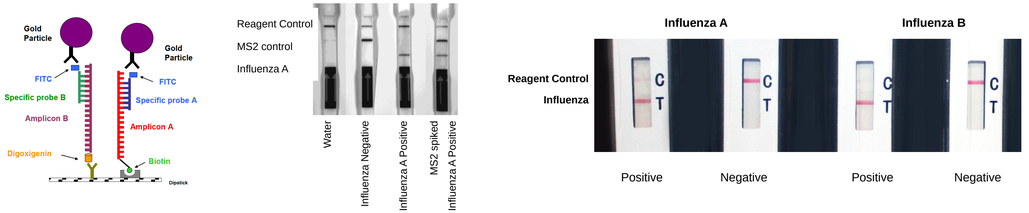
Amplification was detected in real-time by EvaGreen (left) and by agarose gel (right).

2X Faster Isothermal Amplification

- Isothermal amplification at various temperatures.
- Amplification detected in real-time by EvaGreen.
- PyroScript at 70°C reduces test time by 50 percent.



Lateral Flow Detection of Influenza



A. Nucleic Acid Lateral Flow (NALF) Device.

Amplification products labeled with biotin (green) or digoxigenin (yellow) enter NALF device by capillary action and bind to lines of streptavidin (gray) or anti-digoxygenin Ab (gold). Amplified products are hybridized to fluorescein (FITC)-labeled specific probes. FITC (blue) is detected by anti-FITC Abs labeled with colloidal gold particles (purple) resulting in accumulation of dark color at the test lines if amplicon is present.

B. Duplex Detection of Influenza A and MS2 control RNA

C. NALF Detection of Influenza A and Influenza B

RT Isothermal Amplification performed using PyroScript. Detection by NALF. Total time for amplification detection is ~30 minutes including NALF.

Acknowledgements

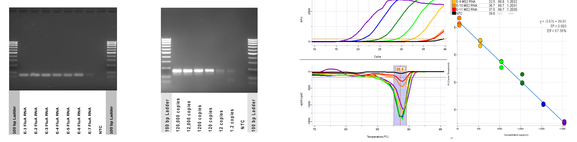
This work was supported by DOE, NSF and NIH grants. Sequencing was performed by JGI.

PyroScript RT-PCR Master Mix Kit

- **2X RT-PCR Master Mix**
  - Buffer
  - dNTPs
  - PyroScript Polymerase
- **RNA control**
  - Direct RT-PCR control
  - Extractable Control
- **Control Primer Set**

- Fast - Amplify viral and cellular RNA in 30 minutes!
- High Temperature - Reverse Transcription at > 70°C.
- Convenient - Just add primers and RNA template.

Sensitive RT-PCR/ RT-qPCR



Left: Single-enzyme, one-step RT-PCR amplification of a 10^1 to 10^7-fold dilution series of Influenza A vRNA and a water no target control (NTC).

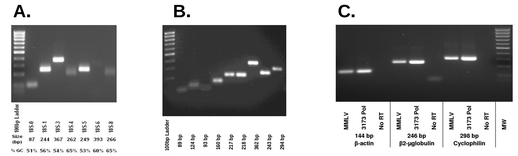
Middle: RT-PCR amplification of a 10-fold dilution series of MS2 vRNA from 120,000 copies to 1.2 copies and a water no target control (NTC).

Right: Real-time PCR Analysis using EvaGreen of reactions in Middle Panel

- One-step single-enzyme RT-PCR cycling conditions:

15 sec @ 94˚C, (10s @ 94˚C, 30s @ 72˚C)*40

Human and Viral RNA



A. 18S rRNA sequences amplified from 100 pg total A549 RNA. Primers targeting amplicons from 51 to 65% GC and from 87 to 393 bp in length were tested.

B. MS2 Phage RNA was amplified by 40 cycles of RT-PCR without background. Products of 89 to 362 bp were amplified.

C. Total human Liver RNA (1 µg) was reverse transcribed by Moloney Murine Leukemia Virus or by PyroScript, then PCR amplified using Lucigen EconoTaq® PLUS Master Mix. Shown are targets of 144, 246 and 298 bp.

Conclusions

- **PyroScript performs sensitive, specific single-enzyme RT Isothermal Amplification**
- **PyroScript is faster for Isothermal Amplification**
- **Influenza A and B detection in about 30 minutes at POC by PyroScript and NALF**
- **PyroScript RT-PCR 2X Master Mix is a robust and convenient RT-PCR solution**