Thermal Gradient Announces Breakthrough in Fast PCR

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Thermal Gradient is developing technology for performing PCR thermal cycling "As Fast as Nature Will Allow" in a micro-device that is simple and inexpensive enough to regarded as a disposable.

These efforts have now culminated in demonstrating the fastest PCR over achieved in such a device: 4.4 minutes for 30 cycles for an 80bp target.

Thermal Gradient's micro-fluidic flow-through thermal cycling device is unique. It is imminently mass-producible by well-established fabrication technologies, with novel features that overcome fundamental drawbacks of previous approaches. It is also entirely passive: There are no *in situ* heaters, pumps, valves, wells and other features that add complexity and cost to the device. To carry out PCR simply place the device between a pair of heated platens and pump sample mix through it.

The company set out to prove very fast PCR was possible in the first generation design. It measured 5 mm x 12 mm x 2 mm and was composed of alternating layers of high and low thermal conductivity material with channels etched in the former and vias through the latter. See Figure 1. The layers are arranged so that the denaturing portion of the PCR process occurs in the top conducting layer, annealing occurs in the bottom conducting layer and extension in the middle layer. This unique three dimensional geometry sets the Thermal Gradient design apart from other approaches. Its simplicity renders it mass-producible and it requires only two static, external heat sources to establish the necessary temperatures for PCR. In addition, the thermal efficiency of the design ensures that the temperature transitions occur virtually instantaneously, backing the company's claim of "PCR as fast as nature will allow".

The low-cost fabrication techniques for making the device come from other, well established industries. Without further invention and with very little modification, the first generation design could be commercialized. Many of the processes and equipment used at the Infotonics Center to produce the first generation devices were also used in commercial production for other industries, notably the manufacture of printer ink jet cartridges.

The first generation devices were tested in the summer of 2005 (the test fixture is shown in Figure 2) and the first amplifications occurred in late July, establishing the feasibility of the design. The company communicated that finding in press releases and private communications to many parties. They then set out to determine the factors that contributed to performance and maximize it.

Thermal Gradient's researchers chose to amplify an 80 bp fragment of PUC18/19, a 2686 bp cloning vector plasmid. The PCR recipe was: 0.3 μM primers, 0.2mM dNTP's, 0.1 U/μl Promega Flexi Taq, 2.5mM $MgCl_2$, 1% Tween 20 and 1X Promega Taq buffer. Template concentration was 3.4M/μl, 340K/μl and 34K/μl. Cycle times ranged from 9.2 min down to 4.4 min. Sample size was 20 μl. Temperature protocol for the device was nominally 95°C / 55°C / 75°C. Positive and negative controls were run in a Hybaid MBS

using a protocol of 2 min initial denaturation followed by 30 cycles of 20 seconds at 95°C, 20 seconds at 55°C and 30 seconds at 75°C.

The device channel design was as follows: 120 nl of initial denaturation followed by 30 cycles of 30 nl denaturation, 30 nl annealing and 60 nl of extension. At a cycle time of 9.2 minutes, therefore, the sample had an 18 second initial denaturation followed by 30 cycles consisting of 4.5 seconds each, denaturation and annealing, and 9 seconds extension.

Agarose gel results are shown in Figure 3. The trend of increasing product with template concentration and cycle time is apparent. At 3.4M template molecules per μl, amplification was still apparent even at the minimum cycle time tested, 4.4 minutes.

It was expected that the length of the fragment would affect minimum cycle time so an additional primer set was designed to create a long fragment on the same PUC18/19 template molecule. At 259 bp the new, long fragment was abut three times the length of the standard 80 bp fragment. In principle the same amplification efficiency should occur with this fragment at three times the cycle time of the shorter fragment. That turned out to be right, as seen in Figure 4. With an initial template concentration of 340K the standard fragment had visible amplification at 5.9 minutes whereas with the 259 bp fragment it was first visible in the 18.5 min run.

While the company is extremely pleased with the performance of the first generation design it is certain the technology can go faster. "The first generation design is by no means optimized; the second will be much better", say the Founders. "But we think we have shown that it has a great deal of promise with advantages for many applications". The company believes that their technology, now demonstrated as feasible and practical, can enable new applications previously considered impractical do to speed, complexity and cost factors. Examples are:

Automated, Multi-test, PCR Analyzers. For the first time a molecular diagnostic or molecular biology instrument can be built that is as convenient and productive as present high-end high-throughput clinical chemistry or immunodiagnostic instruments. They could, for example, have multiple, replaceable on-board assays, menu-selected through sample bar coding, automatic sample prep and handling and test throughput rates measured in minutes.

Point-of-Collection PCR. Small console or hand-held devices to run molecular diagnostic tests in doctors' offices and small clinics while patients wait minutes for results. *In situ* testing in surgical theatres, crime scenes, food processing centers, potential bio-terrorist targets, battle fields. Laboratory automation PCR devices attached to robotic end-effectors: aspirate the sample mix and dispense the PCR output.

The company was assisted by its partners in this endeavor: the Infotonics Technology Center of Canandaigua, NY, a NYS Center of Excellence; the Functional Genomics Center of the University of Rochester; and its local investment backers, the Trillium Group of Pittsford, NY. It has also been supported by Brooks Biomedical Consulting, who provided assay design and guidance in all biological areas.

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Thermal Gradient is also in partnership with the Department of Homeland Security. Shortly after incorporating, the company and its partners joined with Lighthouse Biosciences and Diffinity Genomics to propose and eventually to win approval for a project to develop a second generation design of the device for the HSARPA Office of Science and Technology. The team was honored to be chosen as the only component technology selected for the IBADS program.

Thermal Gradient believes that all of this and much more is possible with the right strategic partners. The company is now ready to tell interested parties much more about the technology and to discuss the mutual opportunities that the Thermal Gradient device makes possible. Contact them at the address below.

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Figure 1. First generation device, shown in exploded view and to scale with a common 1.25" paper clip.

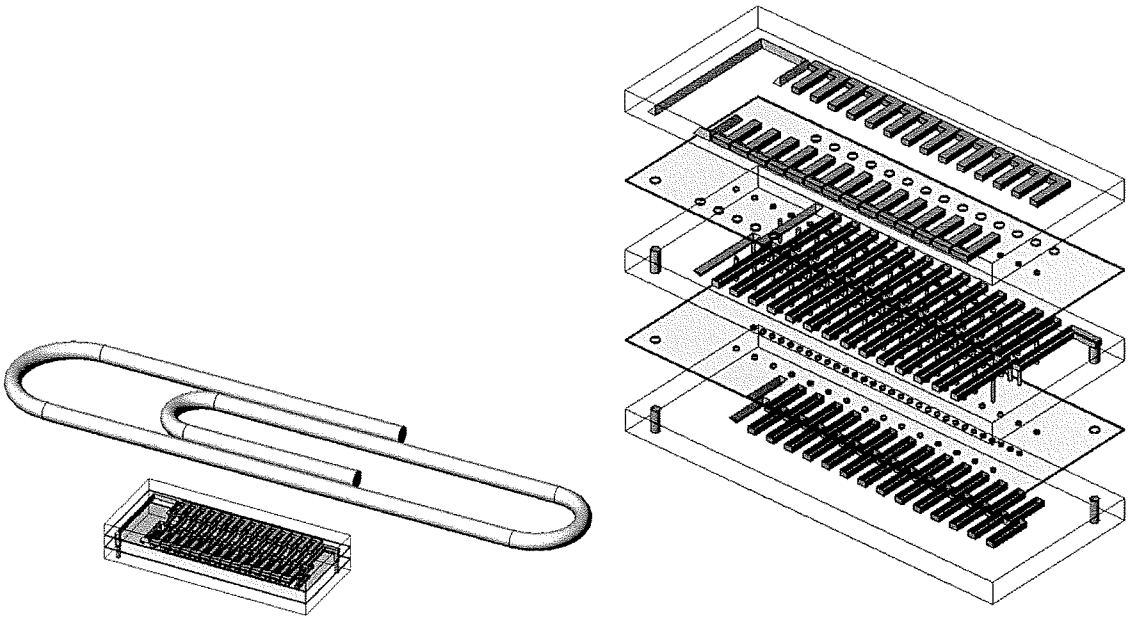

Figure 2. First generation test fixture. Note: 1. Position of device, in green, between upper and lower platens; 2. Locations of heater rods in holes through platens. (Lower runs obliquely to axis); 3. Location of inlet channel through lower platen in far right cross section.

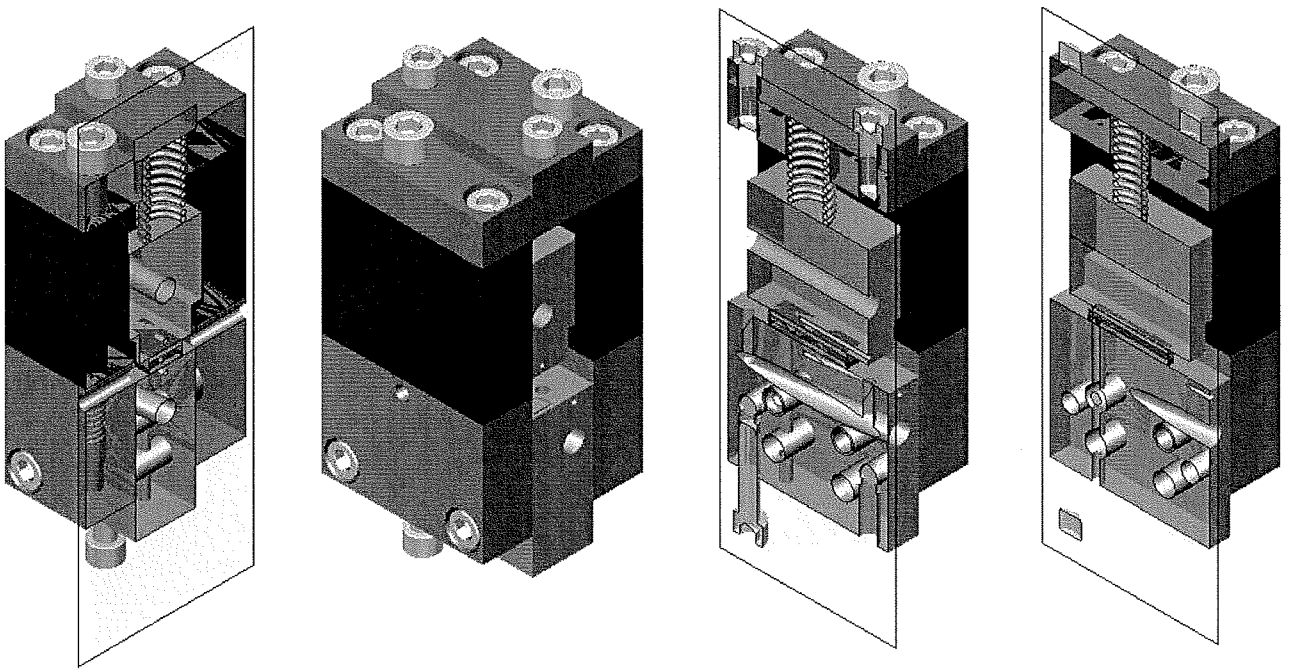

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Figure 3. Agarose gel (4%) results of reactions with standard fragment at various cycle times, in minutes, and three initial template concentrations. Amplicon was an 80 bp segment from a 2686 bp plasmid template (cloning vector PUC18/19). Conditions: 95°C / 55°C / 75°C; time ratios 1:1:2.

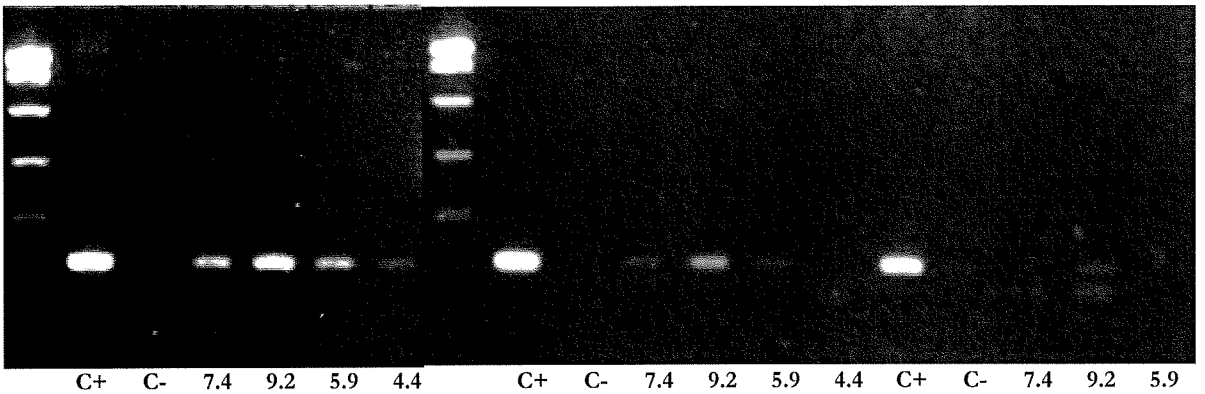

Figure 4. Agarose gel results of reactions with long fragment at various cycle times, in minutes and one initial template concentration. Amplicon was a 259 bp segment from the PUC18/19 plasmid. Conditions otherwise identical to standard fragment runs.