

Flow-Through Pyrosequencing in a Microfluidic Device



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Pyrosequencing is a recent DNA sequencing technique based on DNA replication. Using special cascading chemistry, a light-emitting reaction is triggered each time a nucleotide, A, C, G or T, is incorporated into the complementary strand of DNA. The sequence of the DNA can be determined by iteratively introducing one type of nucleotide and detecting the emitted signal. This method is primarily used for sequencing short lengths of DNA (up to 100 bases) and detecting single nucleotide polymorphism.

Sequencing provides one option for the final identification step in an ongoing effort to start with a clinical sample and identify all known and unknown viruses within it. Although sequence data is considered the “gold standard” for microbial identification, the implementation of this technique is currently limited by time and cost. Since identification of a virus is improved with longer sequences, the 100-base cutoff is also a limitation. Therefore, other identification approaches, such as fragment length analysis in capillary

gel electrophoresis, are being pursued at LLNL. These processes, however, are less amenable to the rapid sample analysis needed to characterize the viral load within a single clinical sample.

By reducing to practice the microfluidic approach in which beads carrying a DNA template are washed with 200-nl volumes of the sequencing chemicals, we can reduce the sequencing cycle time per nucleotide, reduce the volume of expensive reagents used, and potentially increase the number of bases sequenced. This sequencing method is shown in Fig. 1.

Project Goals

The four major goals of this project were: 1) create a microfluidic chip for rapid, small-volume pyrosequencing; 2) replicate the biochemistry necessary to bind DNA to a magnetic polystyrene bead; 3) replicate the sequencing biochemistry; and 4) assemble an automated system to control sample loading, sequential nucleotide injection, and signal detection.

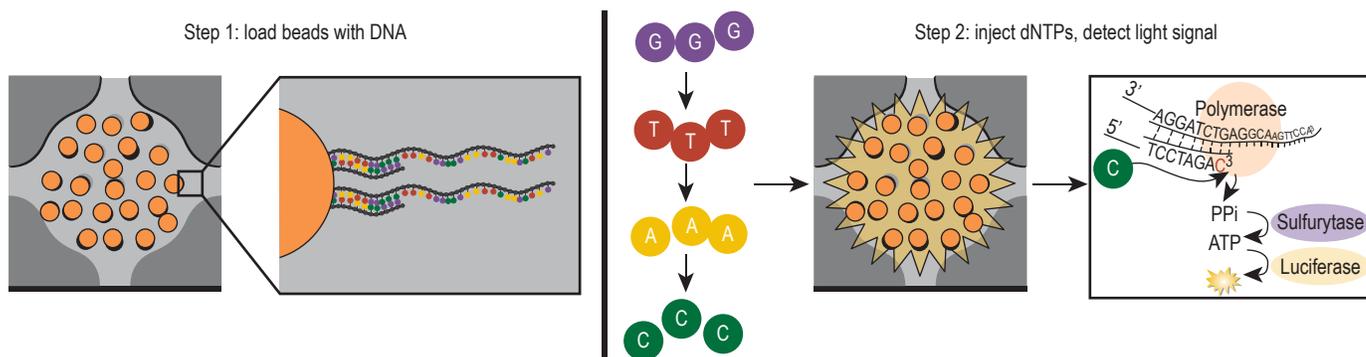


Figure 1. Description of microfluidic pyrosequencing technique. Step 1: The DNA template to be sequenced is bound to a magnetic polystyrene bead then loaded into the center sequencing chamber of the microfluidic chip. The particles are held in this region using an external permanent magnet. Step 2: Solutions containing dCTP, dATP, dTTP, and dGTP are sequentially injected into the chamber and washed over the bound DNA. Successful incorporation of a nucleotide initiates a cascade reaction resulting in the release of light.

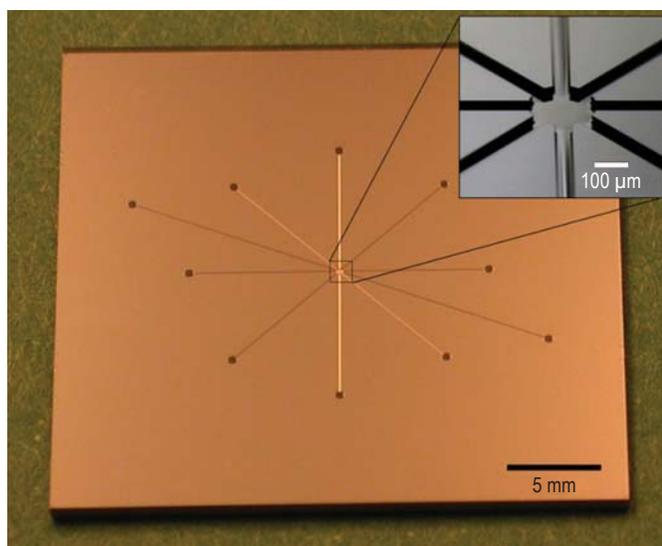


Figure 2. Example of the multiport microfluidic sequencing chip. The sequencing chamber has a 200- μm diameter and total volume of approximately 6 nl. The chips are microfabricated in a silicon substrate for high reflectivity and bonded to glass.

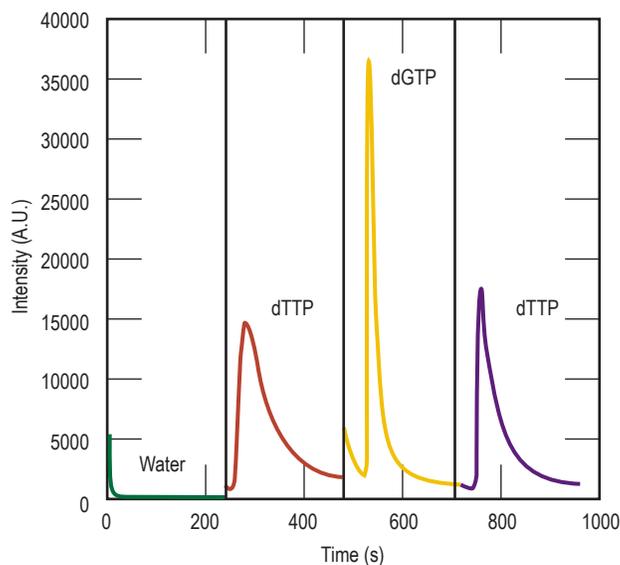


Figure 3. Example of the detected intensity signal from a DNA sequencing reaction. The height of each peak corresponds to the number of nucleotides incorporated.

Relevance to LLNL Mission

This project addresses needs identified by LLNL for the detection of viruses at low concentrations (10 to 1000 copies/ml). Specifically, this project addresses the need for rapid identification of individual viruses based on a low number of DNA sequences. This work has direct impact on LLNL's Viral Identification and Characterization Initiative (VICI).

FY2007 Accomplishments and Results

We accomplished the overall goals of this project by delivering an automated microfluidic system for sequencing bead-bound DNA. Specific results and accomplishments include the following.

1. Modeled sequencing chamber plans to optimize light capture from sequencing reactions.
2. Implemented two microfluidic sequencing chips. The first has dual input and output ports and a 1- μL volume. The second version has multiple input ports (up to 8) and two output ports with a 6-nl volume (Fig. 2). Figure 3 is an example of

the detected intensity signal from a DNA sequencing reaction.

3. Successfully bound DNA template to magnetic polystyrene beads using a biotin-streptavidin linker.
4. Demonstrated magnetic capture of the template-covered beads in the sequencing chamber.
5. Replicated the bioluminescence reporter system for identifying incorporation of dNTPs onto the existing DNA template.
6. Assembled an automated sequencing system with fine fluidic control, multiple sample injection, and a detection component for low signals.

Related References

1. Margulies, M., M. Egholm, *et al.*, "Genome Sequencing in Microfabricated High-Density Picolitre Reactors," *Nature*, **437**, 7057, pp. 376-380, 2005.
2. Ronaghi, M., "Pyrosequencing Sheds Light on DNA Sequencing," *Genome Research*, **11**, pp. 3-11, 2001.
3. Zhou, Z., A. C. Poe, *et al.*, "Pyrosequencing, a High-Throughput Method for

- Detecting Single Nucleotide Polymorphisms in the Dihydrofolate Reductase and Dihydropteroate Synthetase Genes of *Plasmodium Falciparum*," *Journal of Clinical Microbiology*, **44**, 11, pp. 3900-3910, 2006.
4. Russom, A., N. Tooke, *et al.*, "Pyrosequencing in a Microfluidic Flow-Through Device," *Analytical Chemistry*, **77**, pp. 7505-7511, 2005.