Peptides: The Wave of the Future
Michal Lebl and Richard A. Houghten (Editors)
American Peptide Society, 2001

Fully Automated Parallel Oligonucleotide Synthesizer

Michal Lebl[1,2], Christine Burger[2], Brett Ellman[2], David Heiner[2], Georges Ibrahim[2], Aaron Jones[2], Mark Nibbe[2], Jaylynn Pires[2], Petr Mudra[3], Vít Pokorný[3], Pavel Poncar[3] and Karel Ženíšek[3]

[1]Spyder Instruments Inc., San Diego, CA 92121, USA
[2]Illumina, Inc., San Diego, CA 92121, USA
[3]Institute of Organic Chemistry and Biochemistry, 166 10 Prague 6, Czech Republic

Introduction

The oligonucleotide and peptide synthesis technology is of major strategic importance in the field of genomics and proteomics. Commercially available single channel synthesizers cannot satisfy the demand of emerging technologies. Currently, there are several instruments for parallel synthesis of oligonucleotides such as: (i) a 96-channel instrument based on a microtiter plate format developed by scientists at Stanford University [1]; (ii) PolyPlex machine produced by the company GeneMachines [2]; or synthesizer using two microtiterplates for simultaneous synthesis of 192 oligonucleotides, developed at The University of Texas Southwestern Medical Center at Dallas, and sold under name MerMade by company BioAutomation [3]. While these technologies meet the modest requirements of most experiments today, they are inadequate for the manufacturing needs looming in the very near future. Current synthesis technologies do not meet the need for manufacturing large numbers of oligonucleotides (tens of thousands to millions of sequences) cost-effectively. Our goal was to fill this gap and build the parallel (and economical) synthesizer capable of preparation of needed numbers of oligonucleotides.

Results and Discussion

We have devised a new technological concept for the automation of the solid-phase synthesis of large compound array [4]. The key feature of this technology is a new method for separation of the solid support from reagent solutions, termed “tilted plate centrifugation”, which uses centrifugation as a means of liquid removal in conjunction with the use of tilted microtiter plates as reaction vessels. The plates are mounted on a centrifugal plate and slightly tilted down towards the center of centrifugation, thus generating a pocket in each well, in which the solid support is collected during centrifugation, while the supernatant solutions are expelled from the wells. An essential feature of this approach is that well-to-well cross-contamination with reagent solution or resin is avoided by the fact that the plates are tilted, while the direction of centrifugation is horizontal. Consequently, any liquid or resin expelled from the wells is either captured in the inter-well space of the plate, or collected on the wall of the centrifugal drum. The fact that the cross contamination is not an issue we have proven by analyzing all products prepared on the microtiterplate by HPLC/MS.

We have built high-throughput synthesizer “Oligator™ 768” (Figure 1). Wash solutions and reagents common to all synthesized species are delivered automatically through a multichannel distributor connected to a serially linked four-port selector valves. Building blocks and other specific reagents are delivered individually to the respective wells by distribution through the banks of solenoids. An array of nozzles is placed on the actuator (single axis robotic arm). Each nozzle in the array is connected through the solenoid valve to a pressurized bottle (argon, 0.2 atm) and can be positioned (as a part of the array) above the particular well of a given microtiterplate. We

are using four arrays of eight nozzles for the delivery of four phosphoramidites and one array of eight nozzles for the delivery of the activator solution. Each array serves in parallel the wells of one column of microtiterplate. The arrays are placed in the grid, which covers five columns of the microtiterplate. The oligonucleotide synthesizer requires extremely fast building block and reagent delivery since prolonged times may destroy the prepared intermediate. Due to the fact that all five arrays of nozzles can be operated at the same time, the delivery of the reagents is fast – one plate can be serviced in 8 s. The synthesis is performed under inert atmosphere.

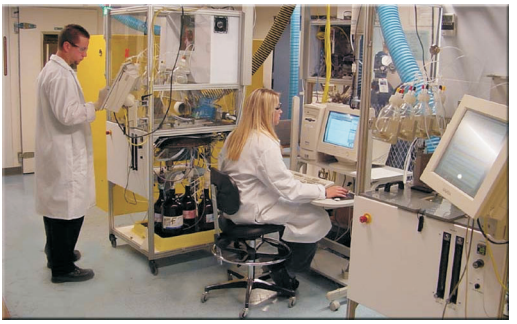

Fig. 1. Prototypes of second generation Oligator™ 768 being tested at Illumina, Inc.

Oligator™ 768 was tested in the synthesis of about 100,000 individual oligonucleotides of the lengths spanning from 20 bases up to 85 bases. Oligonucleotides were analyzed by ion exchange and reversed phase HPLC, gel electrophoresis and mass spectroscopy. As the ion exchange HPLC was shown to separate full-length oligonucleotide from the $n - 1$ product, we were able to calculate step coupling yield of most of the products. Analysis of average step coupling yield showed 98.9%, which is above the industry-wide accepted value of 98%. Multifacility survey of 71 DNA core facilities have shown that only 85% of tested laboratories provided products with average coupling efficiency higher than 98% [5].

In conclusion, we have designed and built automated synthesizers using the tilted plate centrifugation technology. Eight microtiterplates are processed simultaneously, providing thus a synthesizer with a capacity of 768 parallel syntheses. The instruments are capable of unattended continuous operation, providing thus a capacity of close to a million of 20-mer oligonucleotides in a year per instrument.

References

1. Lashkari, D.A., Hunickesmith, S.P., Norgren, R.M., Davis, R.W., Brennan, T. *Proc. Natl. Acad. Sci. USA* **92**, 7912–7915 (1995).
2. http://www.genemachines.com/PolyPlex.html.
3. Rayner, S., Brignac, S., Bumeister, R., Belosudtsev, Y., Ward, T., Grant, O., O'Brie, K., Evans, G.A., Garner, H.R. *Genome Res*. **8**, 741–747 (1998).
4. Lebl, M. *Bioorg. Med. Chem. Lett*. **9**, 1305–1310 (1999).
5. Pon, R.T., Buck, G.A., Hager, K.M., Naewe, C.W., Niece, R.L., Robertson, M, Smith, A.J. *BioTechniques* **21**, 680–685 (1996).