
AUTOMATIC OLIGONUCLEOTIDE SYNTHESIZER UTILIZING THE CONCEPT OF PARALLEL PROCESSING

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Described is a system synthesizing DNA in an array of 36 384-well microtiterplates with filters. The plates are placed in holders on a perimeter of a rotating table and positioned under arrays of nozzles delivering individual reagents. Delivery of reagents is verified by cameras as well as the progress of the synthesis is monitored by camera inspection of the plates after delivery of deblocking reagent. As every plate on the table is in a different stage of the synthesis cycle (one cycle of the synthesis equals one turn of the table), the synthesizer is performing all synthetic operations simultaneously and all stations are utilized to the maximum capacity. Since every plate can be taken out independently of the other plates, the synthesizer can be called a "continuous synthesizer".

INTRODUCTION

Genomic technologies are enabled by fast and economical preparation of synthetic oligonucleotides. Solid phase synthesis of these molecules is one of the most efficient and well developed chemistries¹. Therefore, the challenge in automation of the synthesis of oligonucleotides is in the level of parallelization of the synthetic processes and in the lowering their costs. Illumina needed millions of DNA probes and the cost of available machinery and/or custom synthesized oligos was a major stumbling block in the development of the genotyping technology based on the random distribution and subsequent decoding of micron-sized silica beads² immobilized in the genotyping "chips".

RESULTS

We have applied in our first generation of DNA synthesizers the technology of the so-called tilted centrifugation and achieved the throughput of 768 oligos per synthesizer run³. The technology of tilted centrifugation⁴, in which the solid support is kept in the synthetic vessel (well of the microtiterplate) and the reagent solutions are removed by decantation over the edge of the well, was later improved by melting the CPG (controlled-pore glass) to the bottom of the plate wells. This improvement allowed us to use V-shape wells of 384 well plates and remove the liquids completely by centrifugation without the need to apply any tilt in the centrifugation step.

In this way we improved throughput of our synthesizer to 3024 oligos per run. However, this still did not fulfill the need required by the manufacturing of our chips and increase of the productivity was achieved only by building multiple synthesizers.

We have found the solution in the application of the concept of parallelization of all synthetic processes. In the next generation of our synthesizers we abandoned the concept of centrifugation for liquid removal and we worked with the 384-well filter plates. Thirty-six of these plates were placed on the perimeter of two-meter diameter rotor in special holders allowing their placement under delivery heads and application of vacuum for emptying the wells by filtration. Synthetic protocol was distributed into 36 stations placed above the area through which the plates were carried around by the rotor. The stations were either active – stations in which reagents were added or removed – or passive – stations in which only incubation occurred. In addition, in several stations the plates were inspected by the digital camera to establish whether the reagent was added correctly or whether the reaction (deblocking reaction which creates orange coloring) proceeded to completion. In this arrangement all steps of the synthesis are performed at the same time in the different location of the rotor and no time is wasted by waiting for the completion of the previous step. In two special locations of the rotor the plate holders can be taken out and replaced by the new holder with fresh synthetic substrate (CPG) and the synthesis in the new plate can be started. Therefore, the synthesizer can work on the different oligonucleotides of various length (obviously the oligos of the similar length should be placed in one microtiterplate) and does not have to stop between different batches of oligos. The batch is defined as an individual microtiterplate. Delivery of solutions is achieved by passing the plate wells under an array of 24 nozzles actuated “on the fly” to deliver single digit microliter amount of the reagents in 100 milliseconds per well. Reagents are stored in mini columns located in close proximity to solenoid valves connected to the nozzles. These columns are continually refilled from the large volume storage of reagents and kept under constant pressure. All nozzles are periodically calibrated so that the volume defined by the back pressure and solenoid actuated time is kept constant. Delivery of liquid is verified by the camera and plate is ready for the visit in the next station. One operation, or “tick” of the instrument is about 9 seconds, which gives the instrument the capacity of 3,686,400 couplings per day.

Figure 1 is a schematic diagram of the rotary table and dispensing stations. The plate modules and their associated plate holders can be loaded onto the synthesizer either manually or using an automated robotic arm. A

unique bar code is assigned to each microtiter plate so that each plate is automatically tracked from the time it is placed onto the synthesizer until it is removed. A computer control system monitors the progress of each microtiter plate as it moves onto and off the rotary plate. A bar code scanner is associated with the automated handling device such that each plate which is loaded or unloaded from the synthesizer is scanned and tracked within a LIMS. Each well of the microtiter plate is assigned a different oligonucleotide to be synthesized. Based on this assignment, a LIMS instructs each dispenser to output the proper reaction solutions into the proper well.

Once a plate holder is loaded onto a plate module on the rotary table, the plate module enters below the wash station dispenser and the valves within the dispenser output a wash solution into the microtiter wells. After a wash solution is dispensed into each well, and it has been allowed to incubate for a desired time, the solution is removed by vacuum. The vacuum is in continuous communication with the plate modules as the rotary table rotates. A valve is placed between the vacuum source and the plate for attenuated communication between the vacuum source and the plate. The rotary union allows a vacuum line to communicate with each of the microtiter plates and to travel with each of the microtiter plates while the rotor is moving and while the vacuum source is maintained at a static location. The vacuum supply to each multi-well plate is controlled by separate valves. An electrical slip ring of the rotary union contains a series of electrical circuits allowing operating solenoids to travel with each of the multi-well plates. By connecting a solenoid valve to each of the moving plates, the vacuum to each plate is turned on or off by the system at any time during the synthesis cycle.

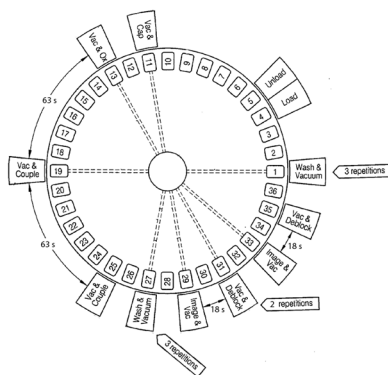


FIG. 1

Schematic diagram of the array of stations within the continuous oligonucleotide synthesizer

Prior to evacuation of the ACN wash, an image of the multi-well plate is taken by one of the digital cameras, and sent for processing to the image processing system. The image processing system analyzes the image to determine if each well of the microtiter plate was properly treated with a wash. If a well is not found to have a wash solution, then that well can be marked as bad, or can be marked to be treated with an additional wash at a later cycle. The image processing system is used in a similar fashion to determine if solution has been sufficiently removed from a multi-well plate based on an image acquired after an evacuation step.

Following the wash step, the plate modules move to a position under the deblock dispenser. This dispenser dispenses a deblocking solution containing, trichloroacetic acid that removes a dimethoxytrityl (DMT) group from the last nucleotide. The release of DMT is readily identifiable by a bright orange color that is imaged by another camera. The optical density of the orange color of each well in the microtiterplate is determined from the image and used to evaluate coupling efficiency. The plate module is then evacuated to remove the deblocking solution. The delivery of deblocking solution and imaging of the plate is repeated twice more before the plate is washed by acetonitrile three times.

After being washed, the plate module travels under the coupling dispenser where the appropriate nucleotide is added to each of the wells. The dispenser is composed of four banks of 24 nozzles delivering A, C, G, and T nucleotides into each of the wells of the microtiter plate. The next dispenser is composed of three banks which can be used to deliver modified nucleotides, and one bank delivering an activator (dicyanoimidazole).

Following delivery from the two coupling dispensers and incubation, the plate module moves to an oxidation dispenser. This solution is imaged and evacuated, and the plate module moves to a capping mixture dispenser for the final stage in the synthesis cycle.

Illumina is using two of these world's highest throughput DNA synthesizers and tested their performance by the synthesis of more than 40 million oligonucleotides up to 110 bases long.

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