Application Note

Tools for High Throughput Genomics

Robotics Worklists for Creating 1536-Well Real Time PCR Assays.

Introduction

As the field of genomics increasingly become higher throughput, the demand for lower volume assays and more effective automated control of these experiments increases. Several manufacturers have recently expanded their offerings of PCR plates to include 1536-well versions, however, the handling of large numbers of samples and primers creates a difficulty in programming automated liquid handlers to perform all the proper combinations of reactions in a logical order and with a logical plate layout, as well as with the desired number of experimental replicates. In order to provide maximum flexibility for the user, it is desirable to utilize a worklist that the user can generate and modify to create custom layouts for higher density plates. Coupling this worklist to a software platform that is capable of translating the required plate map into actions by the liquid handler, as well as aiding in the visualization of this process for the user, results in an automation platform that is able to fully utilize the benefits of the lowered reagent costs and increased speed that higher density PCR platforms enable.

Problem

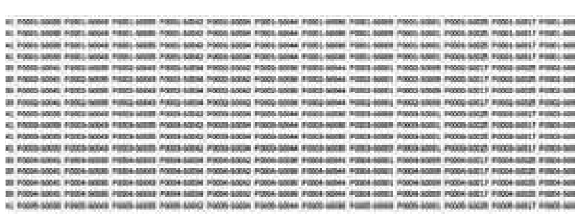
Many reactions involved in genomics applications are combinatorial in nature, requiring the reaction of M samples against N primer sets. Commonly, several replicates of each combination are also required for statistical validation. While a logical set-up for these reactions can be fairly easily comprehended for 384 reactions, scaling up to 1536 reactions can mean the loss of full comprehension of the data sets involved. For a few examples, one may need to set-up an experiment testing 4 primers against 128 samples in triplicate, or 32 primers against 24 samples in duplicate, even 1536 primers against one sample. Adding further complexity, one may wish to pool experiments running 8 primers against 24 samples in triplicate plus 10 primers against 48 samples in duplicate. Confusion may result in all but the most mathematically and computer savvy scientist, causing inefficiencies and errors in the assay development process. Newly available capabilities in the Nanobuilder software platform (used in conjunction with the Innovadyne Nanodrop or Nanodrop Express liquid handlers) can process vendor- or user-(LIMS) supplied plate map data sets in a csv file format presented in an easily visualized well-geometry format, with each of the 1536 destination wells containing all information about chemicals within the wells and their corresponding source plate and well. The Nanobuilder software, then splits this data into sample and primer information and converts this into aspirate and dispense sequences that can be optimized by time (minimizing path length and maximizing the number of concurrent dispenses) or by required plate lay-out. So, rather than the traditional 'Worklist' approach whereby the worklist describes each aspiration step and each dispense step in a series of commands, the 'csv file plate map' data set approach describes the desired end-result. The actual commands and micro-management of the aspiration and dispense steps are delegated to the Nanodrop instrument. In order to assist in the logical set-up of these data sets, IDEX Health and Sciences provides a large number of previously developed probe/primer combinations and the ability to modify or customize these for the user's needs.

Experimental

The software that utilizes this worklisting feature, Nanobuilder, was developed by IDEX Health and Sciences, LLC – Innovadyne and is the control software for the Innovadyne family of low-volume liquid handlers. An Innovadyne Nanodrop II or Nanodrop Express instrument can be used to run the resulting protocols. The Innovadyne instruments are also non-contact, aspirate and dispense instruments, with a unique architecture that eliminates the need for reagents to contact any moving parts and have individually addressable solenoids (for dispensing) and syringe valves (for aspiration) to make such combinatorial protocols feasible. Dispensing platforms include the utilization of a plate nest that allows dispensing to occur in multiple plate orientations depending on the particular data set being used.

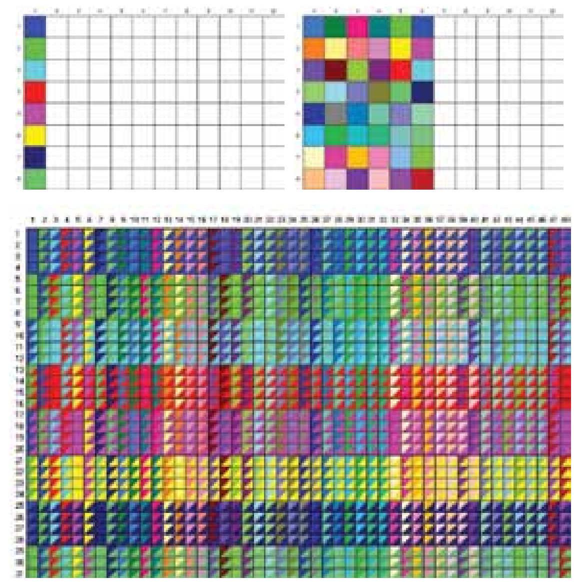
Workflow/Case Study

In a 1536-well plate, there are seemingly an infinite number of different sample/primer combinations that can be tested. As a representative sample of a non-trivial data set to program and visualize, we will use the case of 48 DNA samples tested against 8 different probe/primer sets (or vice versa) with 4 replicates of each reaction condition. The source plates are assumed for this example to be in 96-well plate format. The first step for a user is to identify the most logical and/or quickest pattern with which to dispense each of the primers and samples, for instance reducing the number of times the robot needs to aspirate. Innovadyne employs a tool that assists in setting up the csv file for this dispense pattern and the corresponding source plate layout, however, many LIMS systems would be capable of setting up the file as well (see Picture 2).

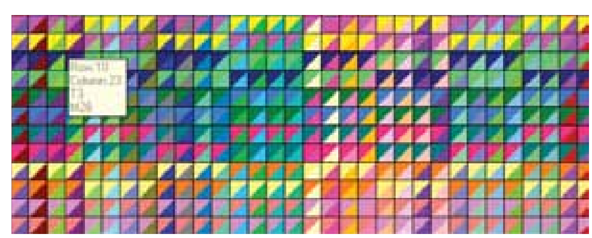


Picture 2: Partial csv file of showing the source well assignations for each well in a 1536-well layout

Following the generation of the csv file, a utility program supplied by IDEX, PatternViewer, allows for the importation of that file to make chemical assignments to each well of the source plates (optional) and to generate color-coded pictures in a .jpg format. Source and dispense plates may be viewed with 'per well' information containing either the source well, chemical, or color representation of the individual reagent within that well (up to 16 source plates can be addressed). For an example of the color-coded plate maps generated for this particular example, see Picture 3. Passing the cursor over one of the destination well, pops up an informational bubble containing the source well address of both the primer and the sample contained within, as well as a chemical identification for each (if assigned) (see picture 4).



Picture 3: Sample and Primer source plate maps (top) and resulting dispense plate map (bottom). Each well of the 1536-well dispense map shows the color corresponding to the sample source well in the top left and the color corresponding to the primer source well in the bottom right.



Picture 4: Dispense Plate map with source well identification information for an individual well.

For this particular protocol, the dispensing of primers works best dispensing by columns, whereas the sample dispensing is most efficient via rows. The switch in dispensing directions is accomplished with the use of a rotatable plate nest that allows the destination plate to be oriented in either direction. Now the user has a coded source and destination plate map that can be used to visually understand the dispensing protocol, as well as a csv file that can be used by the dispensing robot to run the protocol.

Nanobuilder Dispensing Protocols

A dispensing protocol may then be used by the Innovadyne liquid handler, specifically designed for use with PCR-type reactions. This protocol provides reasonable dispense condition defaults, but still allows the user to optimize these conditions, including cleaning routines and dispense volumes. In addition to the optimization of those basic parameters, the protocol utilizes the csv file generated in the previous steps to control the source wells aspirated from and the destination wells dispensed into. The program will also let the user know, for each set of source material, whether the destination plate is required to be in a landscape or a portrait dispense orientation. A barcode reader can be integrated into the work flow as well. The program calls aspirate and cleaning routines specific to the material being dispensed (e.g. a more thorough wash for DNA samples). Finally, the protocol calculates the aspirate volume needed and tells the robot where to aspirate and dispense, as well as when to wash.

Once the liquid handler has performed its appropriate tasks, the plate is thermocycled and analyzed. To aid in the analysis of the results, the csv file generated from the above steps can be imported to other software such as the Roche Lightcycler™ 1536 analysis program and used to correlate the raw data results to the dispense pattern used by the liquid handler.

Conclusions/Future Work

It can now be seen that a mechanism exists which allows the user to create target plate and source map set-up for complex PCR reactions in a more visual and intuitive manner. The Innovadyne software platform can process a vendor- or user- (LIMS) supplied plate map data set in a csv file format presented in an easy to understand 1536-well geometry. The software splits the data into sample and primer aspirate and dispense sequences that can be optimized by time (minimizing path length and maximizing the number of concurrent dispenses) or by required plate layout. These sequences are used by the Innovadyne liquid handlers to accomplish a dispensing protocol for high density plates fully optimized for such factors as time and reduced reagent loss. Further optimizing the speed of dispense is the utilization of a rotatable plate nest that allows dispensing to occur in two different plate orientations depending on the particular data set used. Future work includes the automation of this plate nest and for the release of a PatternMaker utility that would allow for the manual creation of the csv files by the user. This capability would allow the user to simply define the number of reagents and replicates, as well as the instrument configuration, and it would be able to suggest logical plate lay-outs that may be then be tested for maximum efficiency.

IN16/IDX1095-1/05.10