Automation and Miniaturization of SNP Genotyping Assays - Practical Considerations and Solutions

Digilab Technical Note 103

INTRODUCTION:

A Single Nucleotide Polymorphism, or SNP is a single base genetic change, or variation, that can occur within a person's DNA sequence.

SNPs that appear in the promoter region, coding region, or at exon/intron boundaries may result in altered transcriptional regulation, altered amino acid sequence in the gene product, and altered mRNA editing (splicing), respectively. Investigating the frequency and phenotypic correlates with known SNPs harbours the potential to discover specific genes that are responsible for predisposition for disease risk, as well as offering value in forensics research and population genetics.

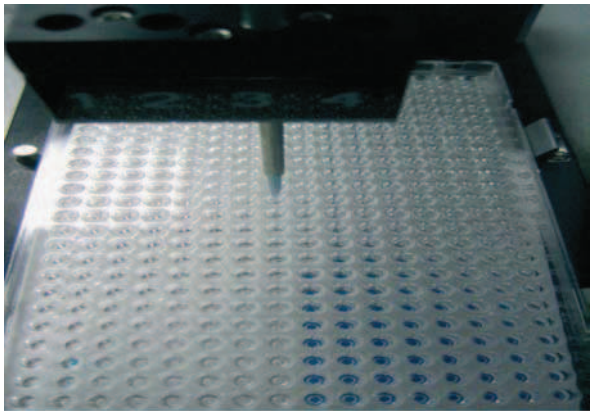
Many efforts to use SNP's for understanding genetic variability are now ongoing. High Throughput laboratories are undergoing research to map SNP's for disease diagnosis, pharmacogenomics, and agricultural genetics. The RIKEN center in Japan is now at a run rate of 100,000,000 wells per year. Given the increasing focus on SNP technology, there is an immediate need to increase throughput using robotic automation and to reduce reagent costs with nanoliter dispensing.

PRACTICAL CONSIDERATIONS:

Accuracy and Precision

When looking to both automate and miniaturize these assays the researcher must take into account the limitations of the liquid handling system. Due to the sensitivity of these assays, the first experiment to be run when miniaturizing is likely to be an optimisation assay with dispensing volumes of 250 nL - 2 µL per well. When this assay is set up across a 384 well plate, an aspiration volume of 96 µL - 800 µL will be required.

It is very important that accuracy and precision be maintained in moving from microliter to nanoliter dispening volumes. Precision will not be maintained at these dispense volumes when using a large syringe, a factor that will have great bearing on the success of these extremely sensitive assays.



Accuracy and Precision (con't)

In addition, careful consideration should be given to the components of the solution to be dispensed. The lower the volume the more challenging it will be to dispense certain reagents with accuracy and precision. The PCR Mastermix being dispensed in SNP genotyping assays contains both detergent and glycerol. The detergent effect will decrease the surface tension of the reagent making it more likely to form aerosols as it is dispensed while the glycerol effect will increase the chance of the reagent building up on the tip and not being dispensed precisely. Full control of the dispensing parameters of a liquid handling system will be needed to ensure optimal dispensing of these solutions.

Contamination

An important consideration when selecting a liquid handling system to set up SNP genotyping assays is determining how to ensure elimination of contamination. Carryover between wells must be eliminated and dispense speed must be controlled to minimize risk of aerosols in the system. These assays are likely to be set up with small amounts of DNA already lyophilised in the bottom of each well and again full control over dispense parameters will be necessary to ensure no sample from a single well contaminates those in surrounding wells. Within the liquid handling system itself, a process needs to be in place to ensure that the system fluid does not mix with the reagent being aspirated, as a dilution effect will occur with each assay leading to inaccuracies in results.

Reagent storage conditions

The reagents used in SNP genotyping assays also present problems to the researcher in terms of their storage conditions. The enzyme used is often light sensitive and is required to be stored frozen. Therefore it cannot simply be placed in a vial on the liquid handling system ready for aspiration, an alternative solution must be found. The PCR Mastermix is likely to be in a deepwell block and it is important that the liquid handling system has a tip capable of accessing these wells in addition to the shallower wells of a 384 well plate.

SOLUTIONS:

The systems from Digilab utilize proprietary synQUAD technology and are ideal for setting up SNP genotyping reactions.

synQUAD Technology

Delivering low volumes accurately and precisely with no contact between tip and destination requires a specially designed system. Unlike typical syringe pump technologies, the synQUAD principal of operation combines the opening and closing of a solenoid valve with the movement of a high resolution syringe pump (figure 1.) to release drops from a ceramic tip. A system fluid fills all the syringe pumps and lines providing a non-compressible environment. A stepper motor drives the syringe plunger and pushes the programmed volume of liquid into the closed solenoid valve. The actuation of the valve releases the pressure that has been created within the system and a drop is accelerated out and onto (or into) the target. The combination of the pressure generated by the syringe and the acceleration of the fluid by the valve allows very small drops to be released cleanly from the tip. Placing the valve as close as possible to the dispense tip produces the best results and the greatest control of the dispensing process.

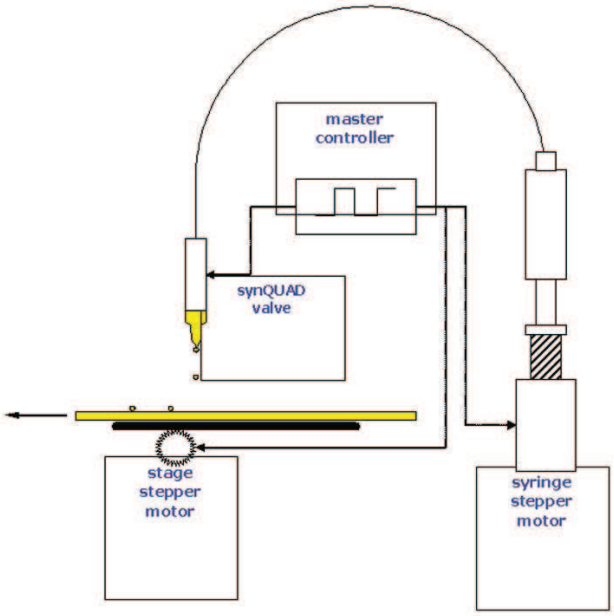


Figure 1. synQUAD - Synchronization of valve, syringe and stage

Accuracy and Precision

As discussed earlier, the liquid handling robot will need to dispense volumes as low as 250 nL with accuracy and precision while still being able to aspirate volumes as high as 800 uL. The Digilab synQUAD systems can utilize a 1 mL syringe enabling volumes from 250 nL - 2 µL to be dispensed as well as aspirations as high as 800 µL with the accuracy and precision required for sensitive SNP genotyping assays (Figure 2).

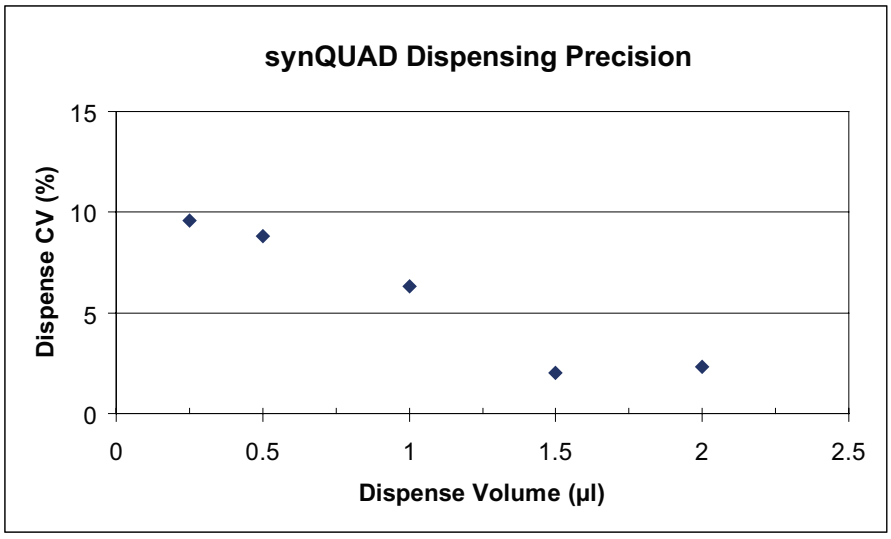


Figure 2. synQUAD dispensing 1% Tartrazine into 96 well plate for dispense tests, % CVs determined from Absorbance data. (1 mL syringe used)

The speed of the syringe and the opening and closing of the solenoid valve are the critical issues to be focused on in the control of nanoliter dispensing using syringe pumps. The Digilab synQUAD effectively addresses these problems allowing the scientist to control both parameters. This ensures optimal dispensing can be achieved when dispensing PCR mastermix containing 'difficult' components such as detergent and glycerol. The dispense speed should be slow enough to prevent aerosol production while fast enough that the glycerol effect does not challenge dispensing precision.

Contamination

Digilab synQUAD systems deliver non-contact dispensing thereby minimizing risk of carryover. Since the researcher has full control of both tip aperture and aspirate and dispense speed, it is simple to minimize the risk of aerosols being produced in the system and to eliminate risk of contamination between samples when dispensing solution into wells containing lyophilized DNA.

synQUAD dispensing utilizes the steady state pressure created by filling the syringe pumps and lines with fluid. To ensure no dilution of reagent from mixing with system fluid, the synQUAD channel can aspirate either an air or oil gap to separate the two solutions.

Reagent storage conditions

The Digilab liquid handling systems address the challenges of reagent storage in two ways. In order to avoid the light sensi tive frozen enzyme being exposed for longer than necessary it is possible to manually present this solution to the synQUAD tip. This ensures the solution is only out of its optimal storage conditions for the minimum time possible. The Digilab systems can easily access the PCR Mastermix in a deepwell plate as they can be configured with a deepwell tip ensuring the bottom of a deepwell plate can be accessed as well as the shallow wells of 384 well plates.

Miniaturization vs. Dilution

When choosing to miniaturize SNP genotyping assays the drive is often cost reduction due to the expense of some of the reagents required. The scientist may choose to either dilute the assay or miniaturize the whole assay to a lower volume.

If diluting the assay with a standard liquid handling robot it is important to consider that only one reagent is being diluted so the system will still need to be able to dispense higher, microliter volumes as well as the lower, nanoliter volume of the diluted reagent. These liquid handling robots are not dedicated to low volume dispensing and precision will become a critical issue. SNP genotyping assays are very sensitive and need to be optimised. They will behave differently at different volumes. The assay may not work if precision is compromised and a different volume is dispensed to that intended.

Digilab synQUAD systems are dedicated to low volume dispensing and have been used successfully in many laboratories to miniaturize the total assay volume with accuracy and precision.

Cost Savings of Miniaturization

In order to run statistically valid experiments at least 300 controls and 300 cases should be studied. It is likely that hundreds of SNPs will be investigated creating a study size of at least 60,000 assays ((300+300) x 100). Miniaturizing the total assay volume can therefore create significant cost savings for the user when viewed across the total number of assays being run.

To calculate how much you can save using Cartesian synQUAD technology for your SNP genotyping assays and for further information please contact info@digilabglobal.com.

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