

# SNP Analysis by Real-Time PCR Set-Up in High-Throughput Service Facility



Savings by adoption of robotic liquid handling using the 4LAB™ and 384-well consumables

## INTRODUCTION:

Single nucleotide polymorphisms (SNP) are the most common type of genetic variation among people. Each SNP represents a difference in a single DNA base. These genetic variations between individuals (particularly in non-coding parts of the genome) are exploited in DNA fingerprinting, which is used in forensic science. Also, these genetic variations underlie differences in our susceptibility to disease.

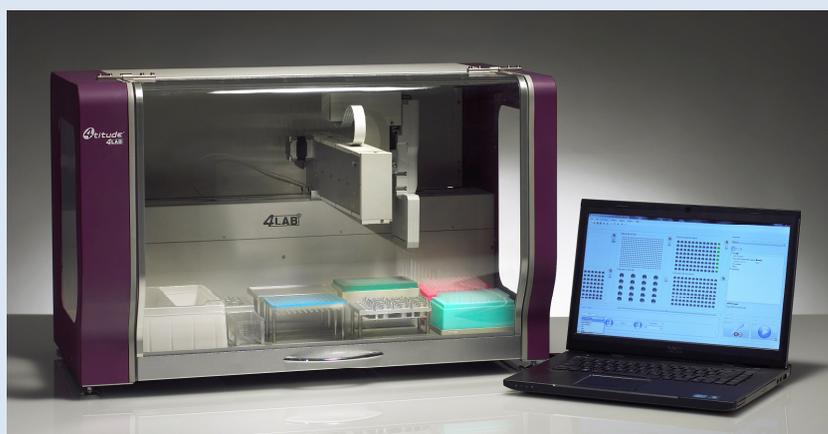


Figure 1: 4LAB™ Automated Pipetting System

The severity of illness and the way our body responds to treatments are also manifestations of genetic variations. For example, a single base mutation in the APOE (apolipoprotein E) gene is associated with a higher risk for Alzheimer disease

Seeing Bioscience Co ([www.seeingbioscience.com](http://www.seeingbioscience.com)) is a sequencing and contract research provider based in Taiwan. Since April 2011 they established a SNP assay project. By adopting the 4LAB™ and 384-well PCR plate, the reagent and labour costs were significantly reduced. Human errors during sample processing could be eliminated and accurate and precise results obtained.

## MATERIALS AND METHODS:

### Reagents and Consumables:

- Purified DNA samples
- TaqMan probes (Life Technologies)
- TaqMan Universal PCR Master Mix (Life Technologies)
- 96-well PCR plates (4ti-0910/C) and 384-well PCR plates (4ti-0384)
- PCR plate seal (4ti-0560)

### Equipment:

- 4LAB™ Automated Pipetting System
- Life Technologies 7900HT Fast Real-Time PCR system with 384well compatibility
- General laboratory equipment

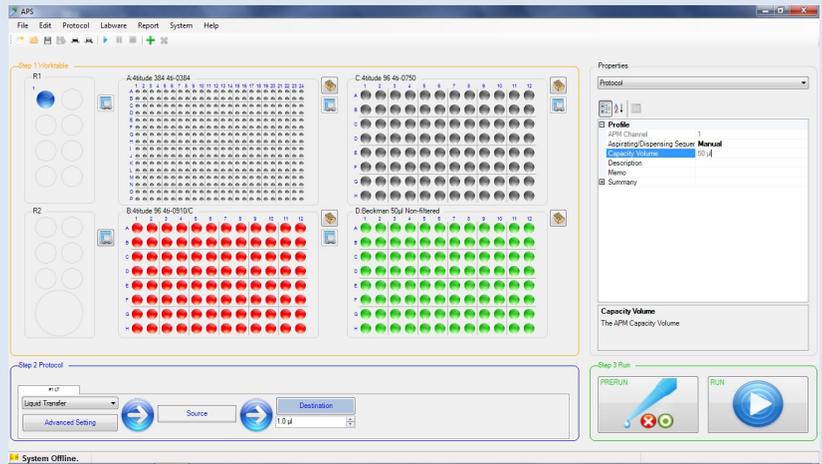
### Method:

1. Use 1-channel 200µl pipette to mix TaqMan probe and combine TaqMan Universal PCR Master Mix well in a 2ml screw cap tube. Transfer the mixture to 96-well PCR plate.
2. Place 96-well PCR plate with mixture on Area B of 4LAB™.
3. Use 8-channel 50µl automated pipetting module (APM) to transfer 5µl TaqMan mixture from 96-well PCR plate to all wells of a 384-well PCR plate on Area A.



Figure 2: FrameStar® 384-well PCR Plate, 4ti-0384

- Place 384-well PCR plate with TaqMan mixture on Area B and 2x 96-well PCR microplates with 96 DNA samples on Area A and C of 4LAB™. Use 8-channel 50µl automated pipetting module (APM) to transfer 2µl DNA samples in the 2 x 96well PCR microplates to a 384 well PCR plate on Area B.
- Repeat step 3) with two additional 96-well PCR plates with DNA samples
- Perform real-time PCR assay on the 384-well PCR plate which is loaded with 384 DNA samples and TaqMan mixture.



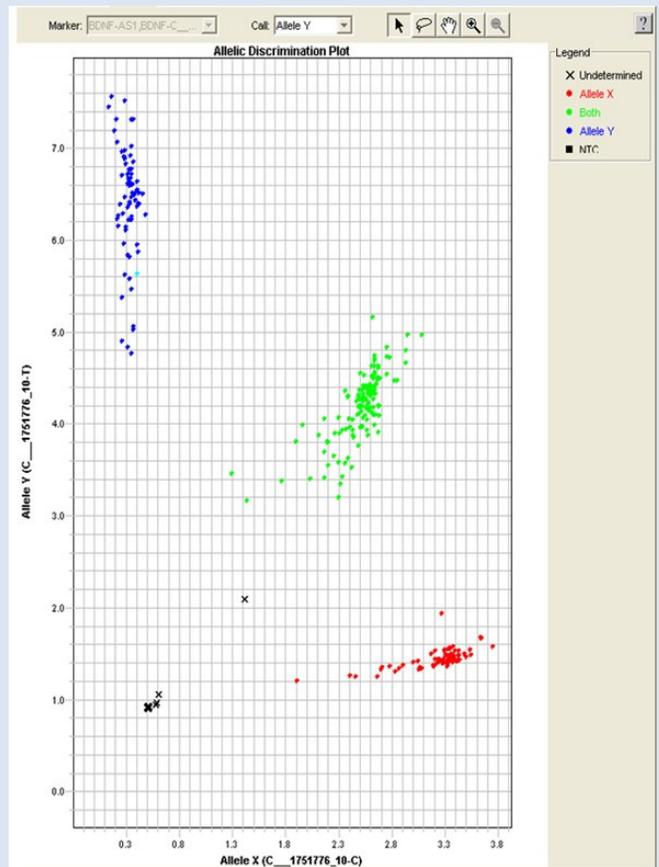
**Figure 3: Automate loading 384 DNA samples from 4 x 96-well PCR plates to 384-well PCR plates with the 4LAB™**

## RESULTS AND DISCUSSION:

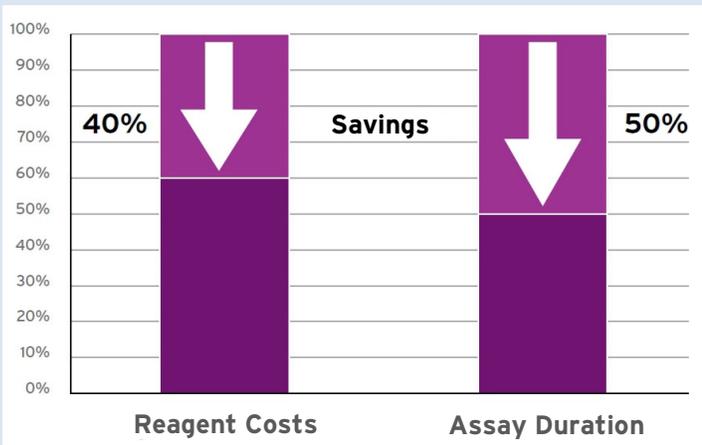
The assay delivered accurate and reproducible results and continues to be used to obtain data for contract research clients. Allele results could be clearly distinguished see Figure 4.

The adoption of the 4LAB™ and the use of 384-well plates allowed for a 40% reduction in reagent costs because mastermix volume was reduced from 10µl to 5µl. Additional consumable savings were made since use of 96-well PCR plates could be limited. Because a single run of the 384-well plate could process the same number of samples than 4 separate runs of 96-well plates the use of the real-time PCR instrument could be optimized and overall time savings of 50% for the assay processing implemented.

The SNP project will be extended for another year.



**Figure 4: The SNP results: red refers to allele X; green refers both alleles; blue refers to allele Y; black refers to No Template Control (NTC)**



**Figure 5: Seeing Bioscience cut reagent costs by 40% and assay duration by 50% by adopting the 4LAB™ Automated Pipetting System in their SNP assay project**