Recent advances in DNA marker/QTL (gene) discovery and marker detection technology have great impacts on crop improvement through marker-assisted selection. To discover and deploy the DNA markers in plant breeding programs, establishing low cost, high throughput, large scale and reliable and flexible marker genotyping platform is one of key factors, implementing high-throughput workflows for SNP genotyping platforms such as TaqMan and KASP assays requires accurate dispensing of assay master mixes and reagents. Speed and accuracy are critical factors to achieve the desired assay performance while utilizing a workflow that minimizes reagent utilization to allow for large-scale studies.

Here we demonstrate the use of the Mantis instrument within the marker/QTL discovery and molecular breeding processes in our soybean program.

Microdiaphragm Pump Technology

At the core of the Mantis is a patented microfluidic valve cluster that measures and dispenses discrete volumes of liquid. Pressure and vacuum open and close each valve on the silicon valve cluster. Each chip has two micro – diaphragms (100 nL / 500 nL or 1 μL / 5 μL) that can fill and dispense as rapidly as 10 times per second. The entire fluid path of a Mantis is disposable to eliminate cross – contamination risks.

The Mantis's software provides an easy to use, user friendly way to design and run your assay. Simply add reagents to the dispense list, define the volumes, and point and click on the virtual plate to define where you want your reagents to be dispensed. The Mantis software is capable of designing gradients and backfills in addition to being able to import .txt and .csv dispense lists.

Materials and Methods

Mantis Automated Dispensing Instrument

The Mantis is an easy to program, low volume, low dead-volume, non-contact reagent dispenser. The Mantis can be configured with up to (6) different reagents, has a dispense range of 100 nL – 2 mL, can dispense any volume into any well, and is compatible with any plate density up to 1536.

To minimize dead volume, reagents can plug directly into microfluidic chips. Dead volumes can be further reduced to 6 μL by using pipette tips as reagent reservoirs. This feature is ideal for dispensing expensive PCR reagents.

Experimental Design

Two BC1F2 populations that are segregating for FAD3A and FAD3C genes from the CX1512-44 source. Leaf samples were collected from greenhouse-grown seedlings or field-grown plants and arranged into 96-well plates. The leaf samples were dried at 55°C for 24hrs and were ground using GenoGrinder with a single BB (Daisy, Rogers AK) in the tube at 1600rpm for 2 min. DNA was then extracted from leaf powders.

TaqMan assay primers and probes were designed using Primer Express 3.0.1 software to detect mutant SNPs in FAD3A and FAD3C (CX1512-44). All assays consist of one primer pair to amplify amplicons range from 141-167 bp and two SNP-containing probes labeled with VIC (wild type) and FAM (mutant) dyes, respectively.

PCR reactions were conducted in a 4.0 μL reaction system with 2 μL sample DNA, 2 μL combination of 2x TaqMan Universal master mix (Life Technologies, Carlsbad CA) and 0.2 μL of 5x assay mix (final concentration of 0.255 μM for each primer and 0.05 μM for each probe). Master and assay mixes were dispensed into 384 well plates containing samples using the Mantis Regent Dispenser. Following PCR, the plates were read with Tecan M1000 Pro Infinite Reader (Tecan Group Ltd, Morrisville NC) and the output files were imported into Kluster Caller software for visualization of clusters and SNP calling.

Results

Soybean seed oil composition, especially palmitic, oleic and linolenic acids, is of tremendous interest to consumers because of its nutritional importance and industrial applications. Breeding high oleic and low linolenic soybean cultivars becomes one of important breeding goals. The SNP TaqMan marker assays we developed for the selection of favorable oil composition from certain sources were robust in separating wild types, mutants and heterozygotes. With the aid of Mantis Reagent Dispenser during the genotyping process, we have established this genotyping workflow which are accurate, efficient and cost effective. Therefore, the assays have been widely applied in soybean breeding programs to facilitate the marker-assisted selection of fatty acid traits with the improved efficiency and precision.

References

www.formulatrix.com/mantis

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