Abstract

Single nucleotide polymorphism (SNP) genotyping of maize samples is commonly performed in agricultural science to aide marker assisted selection, study heterosis and a variety of other biological behaviors. Its widespread adoption in agricultural science has been challenged with increasing reagent costs and labor intensive multi-step processes. Acoustic non-contact liquid handling using the Echo 525 liquid handler offers unique advantages to traditional processes by incorporating a tip-less solution to deliver reagents precisely and accurately. Assay miniaturization is enabled with high accuracy and precision at volumes as low as 25 nL. This study utilized the Echo 525 liquid handler to miniaturize a KASP genotyping assay for maize at a throughput meeting the demands of most high throughput production processes. The results demonstrate miniaturization with the Echo 525 liquid handler without compromising throughput targets.
Introduction

The Labcyte Echo 525 liquid handler distinguishes itself from traditional liquid handlers with the use of acoustic energy to move fluids instead of pipette tips or capillaries. The Echo 525 liquid handler is designed for rapid transfers of 25 nL droplets of biochemical and genomic reagents for assay assembly. These can be simple fluids (media for growing cells and buffers) or viscous solutions (lysis buffers, antibody solutions with glycerol, or transfection reagents). Larger volumes (microliter range or higher) are transferred rapidly by repeating 25 nL transfers hundreds of times per second. The Echo 525 platform enables contamination-free reagent transfer to precisely and accurately build assays. Assay volume miniaturization with the Echo 525 liquid handler retains high assay performance, allowing quantitative results at higher densities. The Echo liquid handler can be used to transfer any volume to any well.

KASP Assay for Maize Genotyping

To validate performance of the Echo 525 liquid handlers for the preparation of miniaturized SNP reactions, a KASP by Design kit for maize genotyping from LGC Genomics was tested. Samples and a master mix containing assay were assembled in a PCR plate using the Echo 525 liquid handler. The final reaction volume was 3.0 µL (1.5µL sample + 1.5 µL master mix with assay), a 70% reduction in volume from the standard 10 µL reaction volume.
Materials

- KASP by design primers and master mix
  - Kit contents include:
    - 1 x 96-well microtiter plate containing 44 validation samples pre-diluted to a concentration range appropriate for KASP genotyping reactions.
    - 1 x 500 µL tube of KASP V.4.0 2X Master mix 96/384, Std Rox, (Cat. no. KBS-1016-001) at a 2X concentration (sufficient for 100 reactions at 10µL).
    - 1 x tube of KASP by Design Primer mix (Cat. no. KBS-1013-001) (at 72X concentration)
- Roche LightCycler® 480 qPCR Thermal Cycler
  - Detection for KASP assay: Roche LightCycler 480 Dual Color Hydrolysis Probes Filter
    - 465 nm excitation and 510 nm detection, 533 nm excitation and 580 nm detection of dyes in KASP Assay
  - KASP genotyping thermal cycling conditions:
    - Initial denaturation hold at 94°C for 15 minutes
    - 10 cycles Touchdown PCR with denaturation at 94°C for 20 seconds and annealing/extension at 61°C for 60 seconds, decreasing to 55°C at 0.6°C/cycle
    - 36 cycles of PCR with denaturation at 94°C for 20 seconds, and annealing/extension at 55°C for 60 seconds
    - Final detection of 2 cycles, reading at 37°C after 5 second holds

Methods

The KASP genotyping assays were prepared and transferred by the Echo 525 Liquid Handler to a final reaction volume of 3 µL.

Prior to assay assembly, a master mixture was prepared manually by combining 300 µL 2X KASP reaction mix and 8.5 µL 72X assay equally into 5 wells of an Echo qualified 384 well source microplate for dispensing. Similarly, 44 samples from the KASP genotyping validation kit were transferred by pipette from the provided 96-well microplate into an Echo qualified 384 well source microplate.

The Echo 525 liquid handler was used to transfer 1.5 µL of the master mix into 96 wells of a 384-well qPCR plate from Roche and 1.5 µL of each of the 44 samples, in duplicate, to the same 96 wells of the 384-well qPCR plate.
Results

The KASP genotyping assay results for 44 samples were obtained and analyzed in a standard scatter plot, comparing the relative fluorescence for the SNP alleles in each sample to determine genotype. Experimental results show the expected pattern of sample grouping, with homozygous SNP groups for the FAM or Hex sorted alleles and a heterozygous SNP group with FAM and Hex alleles. All duplicate samples group together, and the results agree with the SNP genotypes provided by the vendor, LGC Genomics.

The Echo 525 Liquid Handler technology was used to assemble a KASP genotyping assay for maize, with a final volume of 3 µL – 70% lower than a standard reaction volume of 10 µL. The qPCR genotyping results show the expected sample grouping, with consistent calling of duplicate samples. These results demonstrate effective assay miniaturization using the Echo 525 liquid handler to conserve precious reagents and sample, while providing high quality SNP genotyping results. With the Echo 525 system, researchers can achieve optimal efficiency and performance from genotyping assays.

Summary

The Echo 525 Liquid Handler technology was used to assemble a KASP genotyping assay for maize, with a final volume of 3 µL – 70% lower than a standard reaction volume of 10 µL. The qPCR genotyping results show the expected sample grouping, with consistent calling of duplicate samples. These results demonstrate effective assay miniaturization using the Echo 525 liquid handler to conserve precious reagents and sample, while providing high quality SNP genotyping results. With the Echo 525 system, researchers can achieve optimal efficiency and performance from genotyping assays.