

Echo Application Highlight Miniaturized Genotyping Reactions

The Echo[®] liquid handler enables low volume genotyping reactions in a 384-well format with as little as 500 nL total reaction volume. Precise and accurate drop placement eliminates cross-contamination, while offering the flexibility to transfer from any well of a source plate to any well of a destination plate. Cost benefits from reduction of tip waste and decrease in reagent consumption can be realized without sacrificed data quality.

- Miniaturize PCR to save reagent cost and waste
- Increase throughput with lower volume 384-well PCR
- Contamination-free reagent and sample transfer
- Eliminate pipette tips and washing to save consumables cost and waste

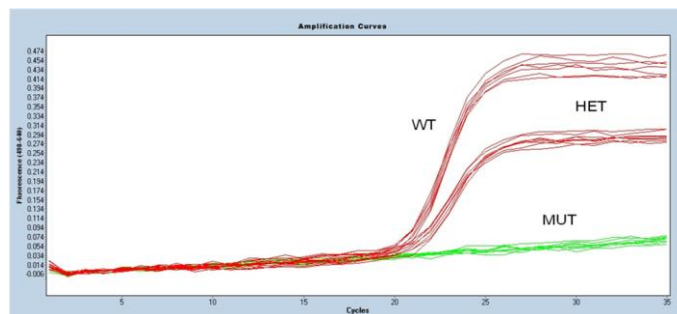


Miniaturized genotyping reactions

Component	Vol. (nL)/rxn	Vol. (nL)/rxn	Vol. (nL)/rxn	Vol. (nL)/rxn	Final Conc.	Final React. Conc.
Water	712.5	536	356.25	78.125	1.5X	1.125X
5X DNA Probes Master	450	337.5	225	112.5	1.5X	1.125X
Primer Mix (20X)	112.5	84.5	56.25	28.125	1.5X	1.125X
Genotyping Probe, 10X	225	167	112.5	56.25	1.5X	1.125X
Total MM Volume Dispensed	1500	1125	750	375	1.5X	1.125X
DNA Plasmid Dispensed						
WT plasmid	500	375	250	125		
Heterozygote	500	375	250	125		
Mutation	500	375	250	125		
Total Reaction Volume	2000	1500	1000	500		

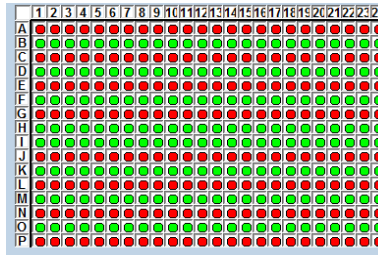
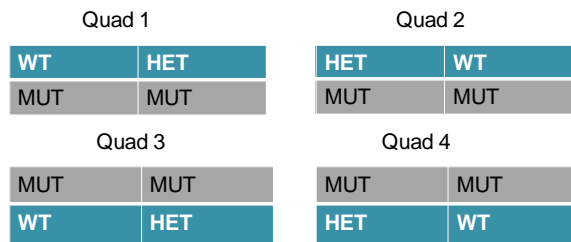
- Miniaturize PCR as low as 500 nL
- Simple assay setup allows for varying conditions within one microplate.

Figure 1: Quantification of wild-type, heterozygote and mutant in 1.0 uL reaction volume



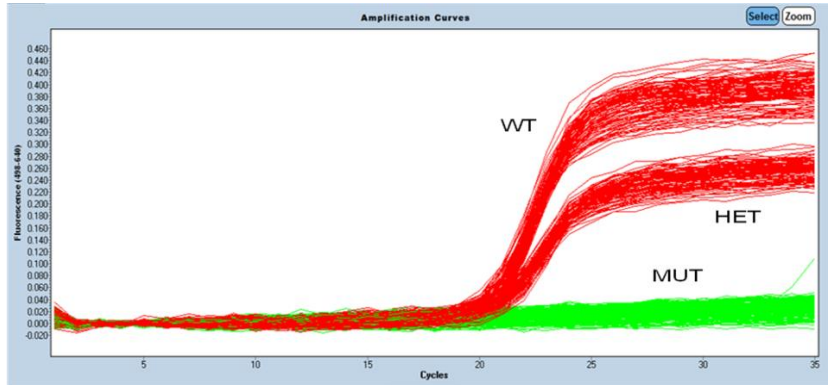
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Zero Cross-Contamination Results



Zero cross-contamination schematic for alternating WT, HET, and MUT plasmid plate.

Positive/Negative Call by the LightCycler 480. Red circles indicate positives. Green circles indicate negatives. There were no positive calls for mutant genes indicating a 100% call rate and zero cross contamination.



Zero cross-contamination with multiple transfer steps

Reactions were dispensed in 2000 nL final reaction volumes in a two step process utilizing the Echo liquid handler. DNA Probes Master mix including the primer mix and genotyping probe was dispensed, followed by alternating plasmid DNA (wild-type (WT), heterozygote (HET), and mutation (MUT)) in an alternating pattern, shown below.

- Starting with only a few source wells, the Echo liquid handler can transfer from any well to any well with zero cross contamination.
- Tipless transfer of reagents can occur without adherence to a format imposed by traditional liquid handlers.
- Zero cross-contamination with multiple transfer steps of primer, probe, mastermix and samples.

The Labcyte Echo 500 series revolutionizes liquid transfer by using acoustic energy to eject fluids. The Echo liquid handler is completely touchless—no tips or nozzles, and no material contacts the same as it moves from source to destination. Low-volume, accurate and precise transfer at volumes of 2.5 nL and up allow for assay miniaturization to previously unattainable volumes. Acoustic transfer is compatible with a wide range of fluid types, enabling high-throughput total PCR assay assembly for applications including genotyping, gene expression and DNA sequencing. Transfer of samples from any well of a source to any destination further minimizes sample consumption and simplifies assay setup and execution.