Superior high-throughput genotyping with KlearKall Master mix and BHQplus probes

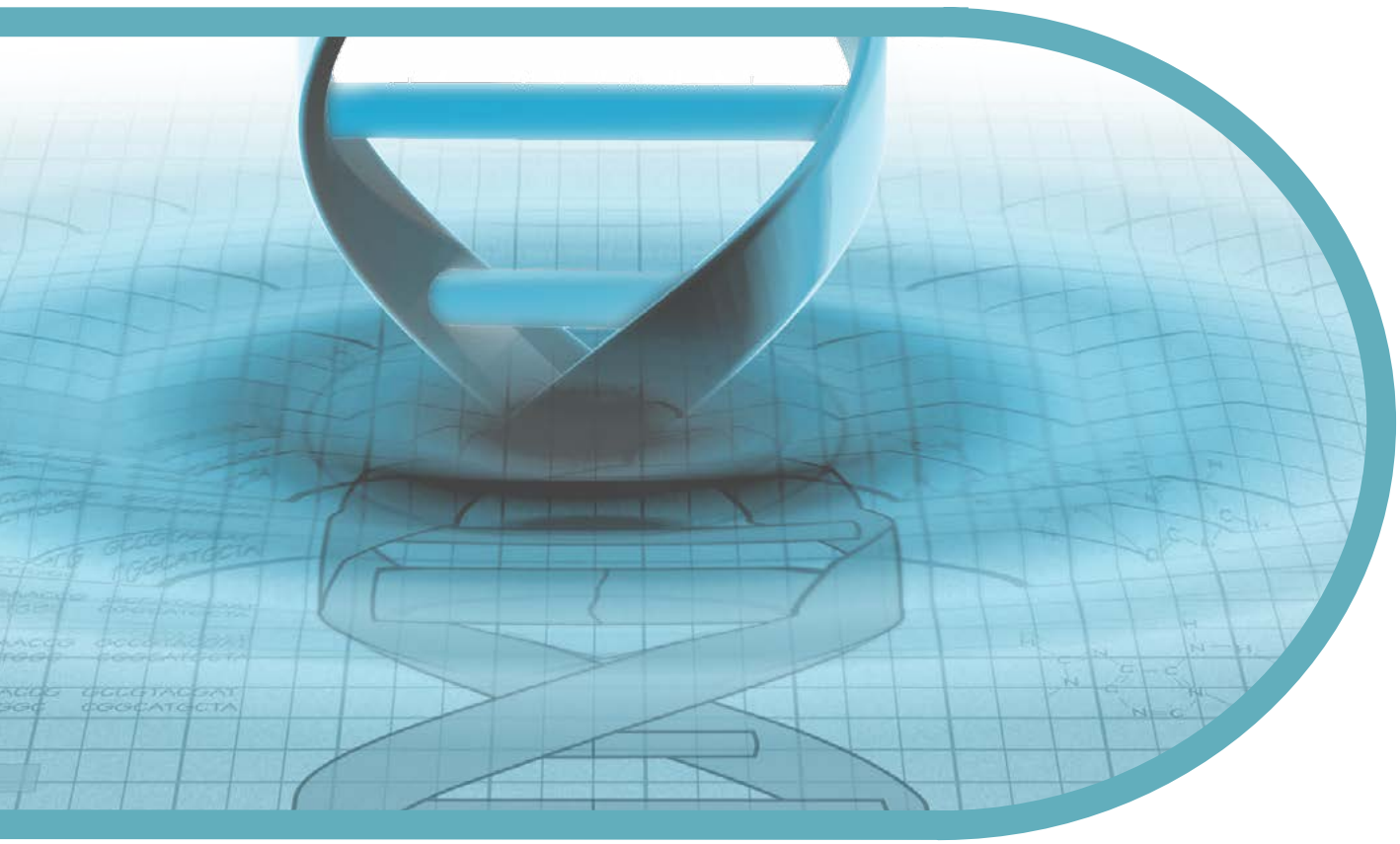
- KlearKall Master™ mix was benchmarked for high-throughput SNP genotyping with BHQplus® probes on purified human DNA samples and crude plant extracts.
- KlearKall delivered discrete clusters and high call rates, even with difficult samples and low reaction volumes.
- KlearKall outperformed competitor PCR mixes overall.

High-throughput genotyping of SNPs (single nucleotide polymorphisms) is used to analyse large numbers of human samples in genome-wide association studies and for validating any disease-linked genetic variants found. Analysis of large numbers of samples accurately and efficiently is fundamental to this process.

In agriculture, using SNPs as desirable trait markers is a powerful tool to accelerate breeding programs for crop improvement. Crude DNA extracts are commonly used for this as they are rapid and cost effective to prepare. However, crude extracts produce poor quality DNA and carry debris and endogenous PCR inhibitors. A high-quality PCR master mix, optimised for these unfavourable reaction conditions, is a key component in determining assay robustness and genotyping data quality.

Designed by experts in PCR, KlearKall Master mix is a 2X PCR reaction mix that has been optimised to perform high-quality, high-throughput endpoint genotyping with hydrolysis probes in low (1 µL) reaction volumes.

BHQplus probes are an advanced probe technology for PCR that offers the benefits of traditional MGB probes without their expense. As with MGB probes, BHQplus probes form highly stable duplexes with DNA targets, allowing shorter probes to be used for hybridisation-based assays. Due to their shortened lengths, BHQplus probes achieve an enhanced target specificity making them ideal for SNP discrimination.



KlearKall performance with BHQplus genotyping assays was assessed and compared alongside various commercially available PCR master mixes, in low volume, high-throughput SNP genotyping reactions (down to 1 µL). KlearKall performance on both the Nexar® System using Array Tape® and the SNPline™ with 1536-well microplates has been found to be significantly better than, or equal to, competitor mixes in terms of both cluster separation and amplification efficiency.

BHQplus probes with KlearKall Master mix – high numbers of samples analysed quickly and accurately

KlearKall

- Tight clusters and high call rates for accurate, reproducible genotyping
- Excellent data quality even on crude plant extracts
- Ideal for BHQplus, BHQ® and TaqMan® MGB genotyping assays
- KlearKall and BHQplus duplex for 4-colour detection
- Universal thermal cycling conditions
- Validated on LGC SNPline and Douglas Scientific Nexar high-throughput platforms as well as other leading platforms.

BHQplus probes

- Small but powerful - duplex stabilisers permit the design of shorter dual-labelled probes for enhanced allelic discrimination.
- High fidelity - shorter sequences offer increased protection against hybridising to a mismatch
- Black Hole Quencher® dye extinguishes fluorescence until target hybridisation
- Design your BHQplus probe through our free, web-based RealTimeDesign™ software
- An economical alternative for probe-based SNP genotyping

Platform and competitor comparison

Summary

The SNP genotyping performance of BHQplus assays was compared using 2X KlearKall Master mix and six competitor master mixes at low reaction volumes. A variety of assays were performed on human DNA samples purified from whole blood, and crude plant DNA extracts from agricultural crop species. All assays were run on the market-leading, high-throughput automated genotyping platforms: LGC's SNPline and Douglas Scientific's Nexar, in 1536-well plates or 384-well Array Tape respectively.

Human whole blood samples

DNA was extracted from human whole blood samples using Kleargene™ (LGC Genomics). SNP analysis was performed using BHQplus assays designed to two publically-available human SNPs (rs10506440, rs4073)

Plant crude extracts

Plant crude extracts were prepared from the seeds and leaves of five different crops (wheat, rapeseed, maize, soybean and sunflower) using the sodium hydroxide-based "HotShot" method.

HotShot protocol overview: Grind seed / leaf to a powder; add NaOH and mix thoroughly; incubate; mix thoroughly then pellet the debris by centrifugation. Finally, remove aliquot of liquid and dilute in TE buffer. Genotyping of three SNPs for each crop was performed using BHQplus assays.

Genotyping

All genotyping assays were run on a) Douglas Scientific Nexar + 384-well Array Tape (1.6 µL reaction volume) and b) LGC SNPLine + 1536-well plates (1.0 µL reaction volume). Thermocycling was performed using times and temperatures specified in each master mix manufacturer's instructions and replicated on both platforms. Thermocycling was carried out in a SNPline Hydrocycler™, adapted for Array Tape when used.

For the human assays, fluorescence was measured and cluster plots assessed after 38 PCR cycles. For the plant assays, fluorescence was measured and cluster plots assessed after cluster formation was complete. For each plant species, the cluster plots in Figures 3 and 4 are taken from same number of PCR cycles across tape and plate formats. The number of PCR cycles used for each species differs out of necessity due to differences in the composition of the crude lysates generated from each sample type. All data was normalised against the passive reference dye included in each master mix. Standard ROX or high ROX formulation of each master mix was used.

Step	Temperature	Time	Number of cycles
1	95º C	15 min	1 cycle
2	95º C	15 sec	38 cycles
	60º C	60 sec	

Table 1: Two-step thermal cycling programmes for KlearKall Master mix.

Genotyping from purified human DNA samples in high-throughput, low-volume reactions

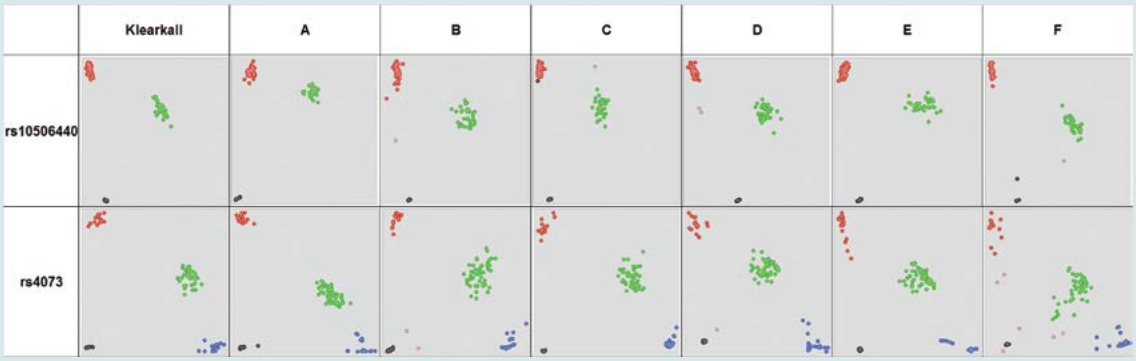


Figure 1: KlearKall vs. competitor mixes A-F on purified human DNA samples on Nexar Array Tape (1.0 µL reactions).

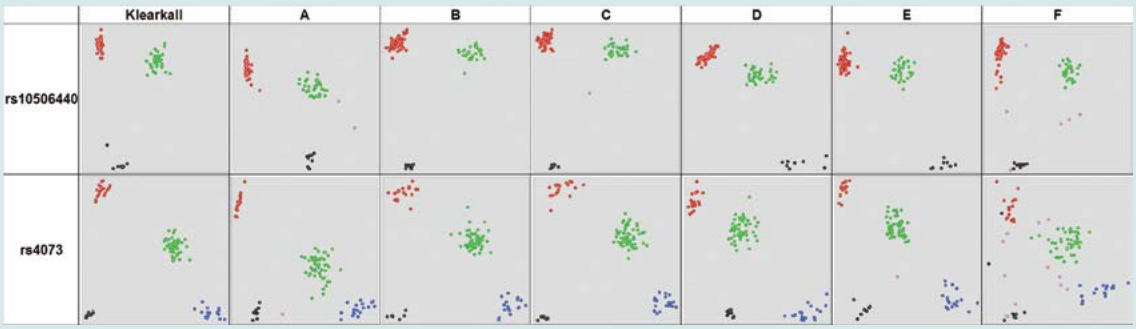


Figure 2: KlearKall vs. competitor mixes A-F on purified human DNA samples on 1536-well plates (1.0 µL reactions).

Genotyping crude plant extracts in high-throughput – poor quality DNA in low volume reactions

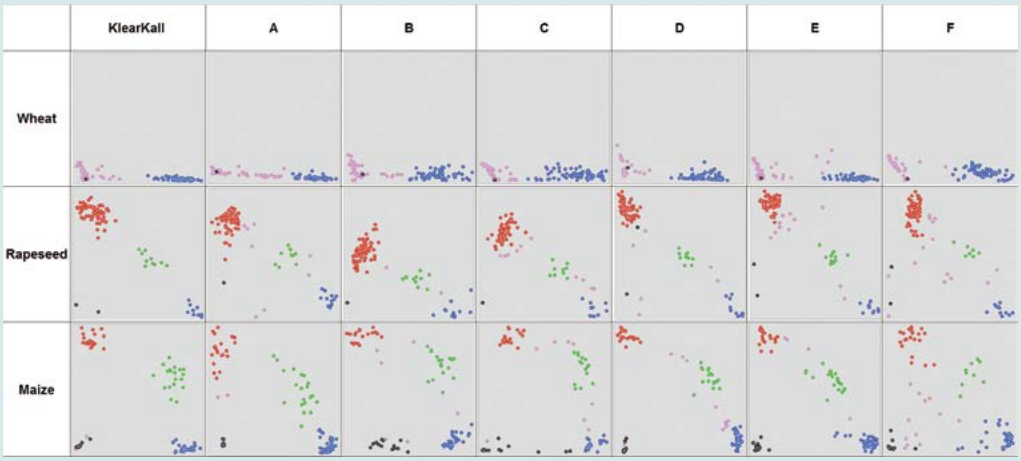


Figure 3: KlearKall vs. competitor mixes A-F on crude DNA lysate samples on Nexar Array Tape (1.6 µL reactions).

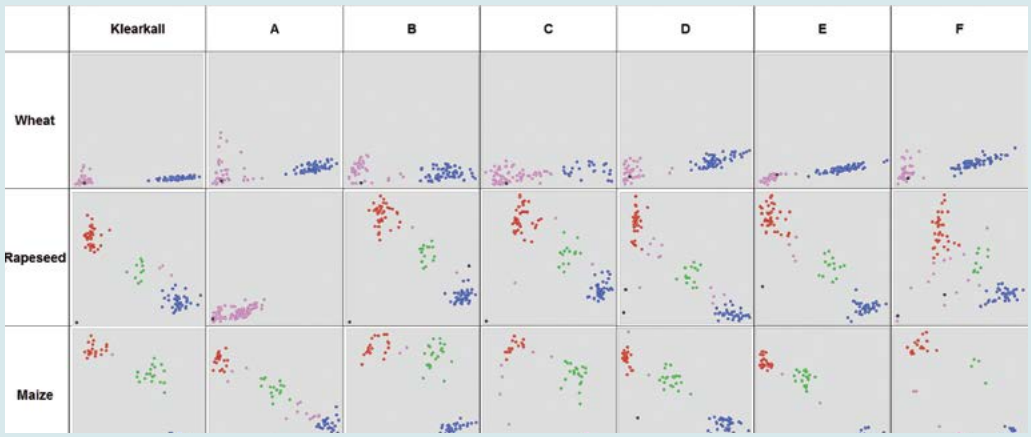


Figure 4: KlearKall vs. competitor mixes A-F on crude DNA lysate samples on 1536-well plates (1 µL reactions).

KlearKall for BHQplus Duplex

KlearKall Master mix performance was also tested on rapeseed crude extract samples with duplex BHQplus reactions (two assays in the same reaction) on SNPline 1536-well plates, 1 µL final reaction volume.

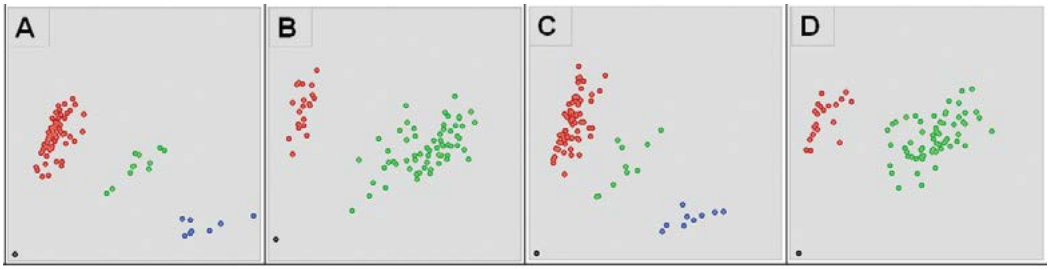


Figure 5 : KlearKall Master mix with duplex BHQplus assays on rapeseed crude extract, SNPline 1536-well plates with 1 µL final reaction volume. A - Assay 1 (Singleplex with FAM/CAL Fluor® Orange 560); B - Assay 2 (Singleplex with CAL Fluor Red 610 Quasar® 670); C - Assay 1 (Duplex with FAM/CAL Fluor Orange 560); D - Assay 2 (Duplex with CAL Fluor Red 610/Quasar 670).

Conclusions

- KlearKall Master mix was successfully benchmarked for high-throughput SNP genotyping with BHQplus probes on purified human DNA samples and crude extracts of wheat, rapeseed, maize, soybean and sunflower.
- KlearKall delivered discrete clusters and high call rates for accurate, reproducible allelic discrimination, even with difficult samples and low reaction volumes.
- KlearKall was superior across genotyping platforms, and outperformed competitor PCR mixes overall.

LGC – a complete solution for your high-throughput genotyping workflow

With the acquisition of Biosearch Technologies, LGC has become a single, unified source for sample preparation, PCR reagents, probes, consumables and instrumentation. With the combined PCR expertise of LGC and Biosearch, we can supply everything you need to run your assays at your own facility. Alternatively, take advantage of our experienced service laboratories for a convenient and efficient service from sample through to actionable data.

Ordering information

Product code	Product name*	Volume
KBS-1002-001	2X KlearKall 100 Std ROX**	2.5 mL
KBS-1002-003	2X KlearKall 1000 Std ROX**	25 mL
KBS-1002-007	2X KlearKall 8000 Std ROX**	200 mL
KBS-1002-100	2X KlearKall 100 Low ROX	2.5 mL
KBS-1002-102	2X KlearKall 1000 Low ROX	25 mL
KBS-1001-001	2X KlearKall 100 No ROX	2.5 mL
KBS-1001-003	2X KlearKall 1000 No ROX	25 mL

*** The number of reactions referred to in the product name is calculated based on 50 µL total reaction volumes.**
**** KlearKall Master mix with standard ROX concentration was used throughout this study.**