PCR-based human genotyping on high-throughput platforms using KlearKall Master mix

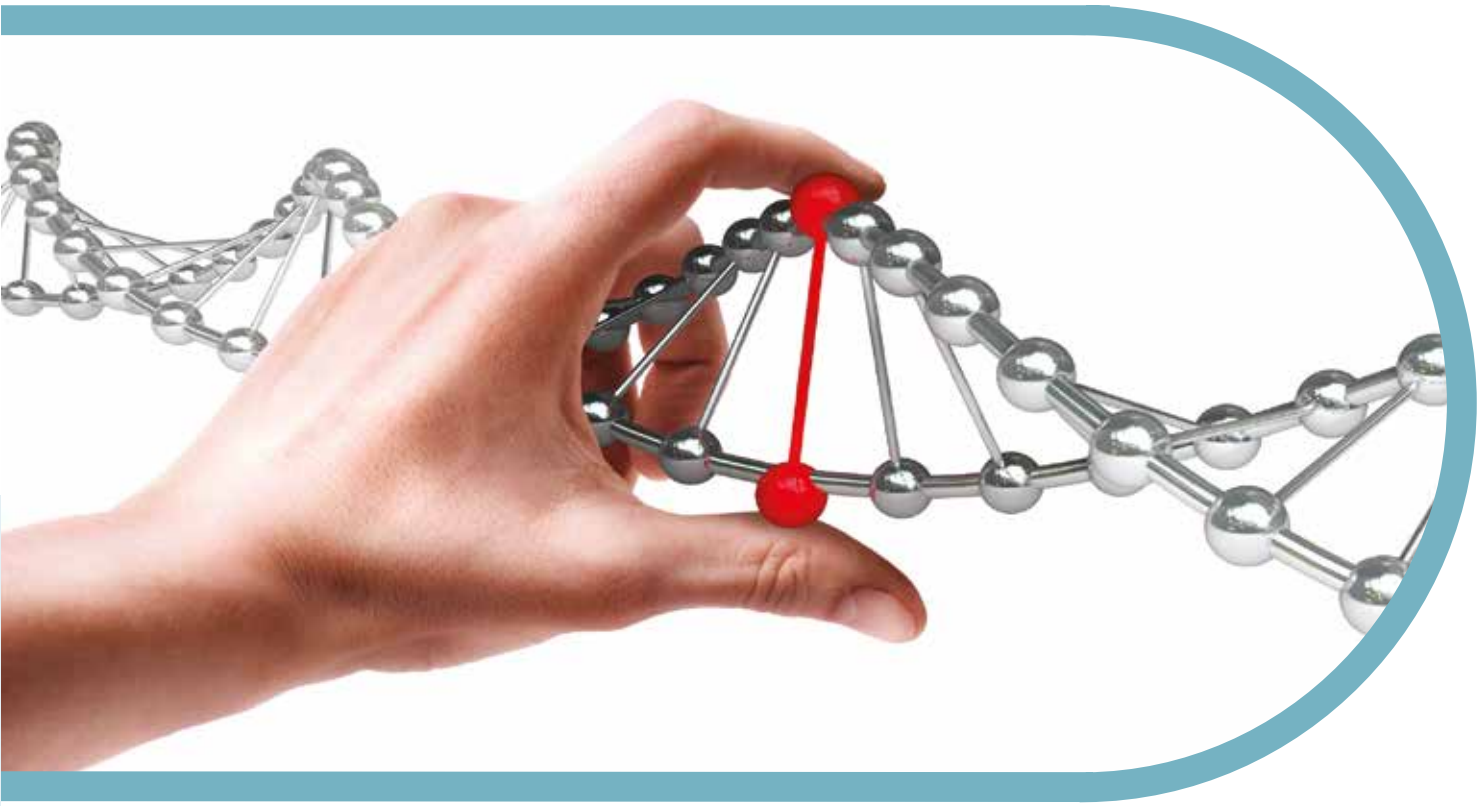
Designed by experts in PCR, KlearKall™ Master mix is a 2X PCR reaction mix that has been optimised for high-quality, end-point genotyping using hydrolysis probes. On both Nexar® Array Tape® and SNPline™ plate-based genotyping systems, KlearKall performance has been found to be consistently better than competitor mixes.

- Proven accuracy and reliability even on crude samples
- Low volume reactions (down to 1 µL) – reducing cost and increasing throughput
- Compatible with hydrolysis probe-based genotyping assays such as BHQ®, BHQplus®, and TaqMan®

Platform and competitor comparison

Summary

2X KlearKall Master mix (standard ROX) and six competitor PCR master mixes were used to run four SNP genotyping assays on purified human DNA samples. Performance was compared on the market leading high-throughput automated genotyping platforms LGC's SNPline and Douglas Scientific's Nexar. Experiments were carried out either on SNPline with 384- or 1536-well plates, or on Nexar with 384-well Array Tape.



Methods

SNP analysis was performed using TaqMan® MGB assays designed to four publically available human SNPs (rs11709077, rs9369425, rs10506440, rs4073). DNA was extracted from human whole blood samples using Kleargene™ (LGC Genomics).

Genotyping assays were run on a Douglas Scientific Nexar in-line liquid handling and assay processing system on 384-well Array Tape, in a 1.6 µL final reaction volume; and on an LGC SNPLine with 384- and 1536-well plates, in a 3.0 µL and 1.0 µL final reaction volume respectively.

Array Tape and plates were thermocycled using the times and temperatures specified in the individual manufacturer's instructions. The PCR protocol was the same across the two platforms.

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 2: Two-step thermal cycling program for KlearKall Master mix.

384- and 1536-well plates were thermocycled in a SNPline Hydrocycler™. For the Array Tape, thermal cycling was performed on a Hydrocycler adapted for Array Tape use. Fluorescence was measured after 38 PCR cycles. For data analysis, all data was normalised against the passive reference dye included in each master mix. Standard ROX / High ROX formulation of each master mix was used where available.

Conclusions

Across all platforms, KlearKall Master mix performed significantly better than, or as well as, competitor master mixes both in terms of cluster separation and amplification efficiency. KlearKall Master mix enables high-quality hydrolysis probe genotyping on high numbers of samples on both Array Tape and plate genotyping systems.

	KlearKall Master mix	Competitor A	Competitor B	Competitor C	Competitor D	Competitor E	Competitor F
rs11709077	+++++	++++	+++++	++++	++++	++++	++++
rs9369425	+++++	++++	+++++	+++++	+++++	+++++	++++
rs10506440	+++++	+++	++++	++++	++++	++++	+++
rs4073	++++	+++	++++	++++	+++	++++	+++

Table 1: Master mix performance with purified human DNA samples on SNPline using 384- and 1536-well plates, and Nexar with 384-well Array Tape. The table summarises data quality observed under the experimental conditions described in the Methods section.

Results

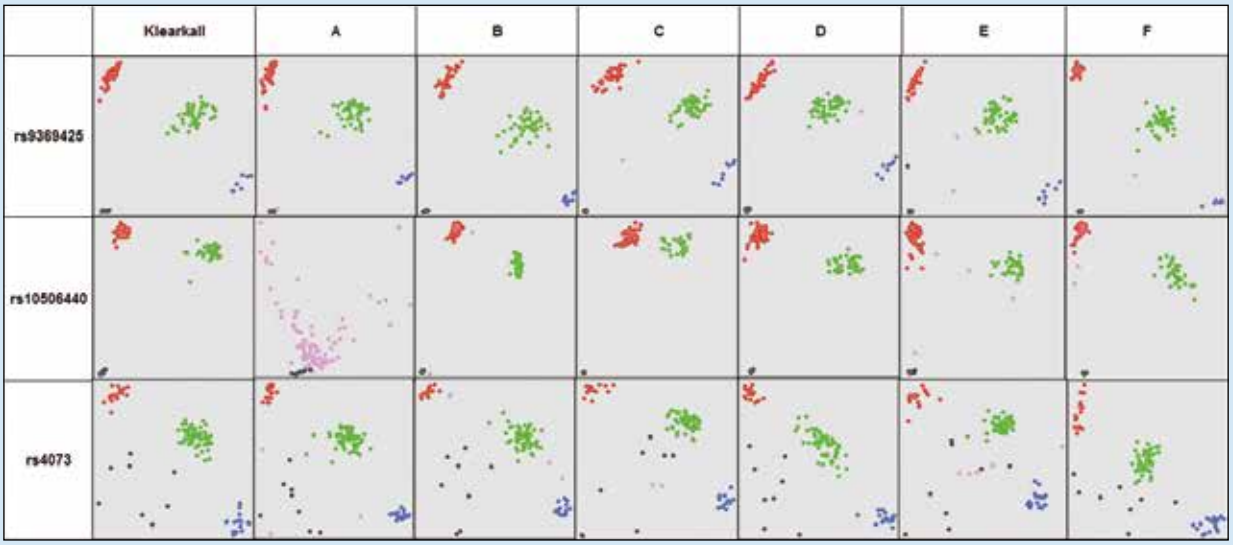


Figure 1: KlearKall vs. competitor mixes A-F on purified human DNA samples on Nexar Array Tape (1.6 µL reactions).

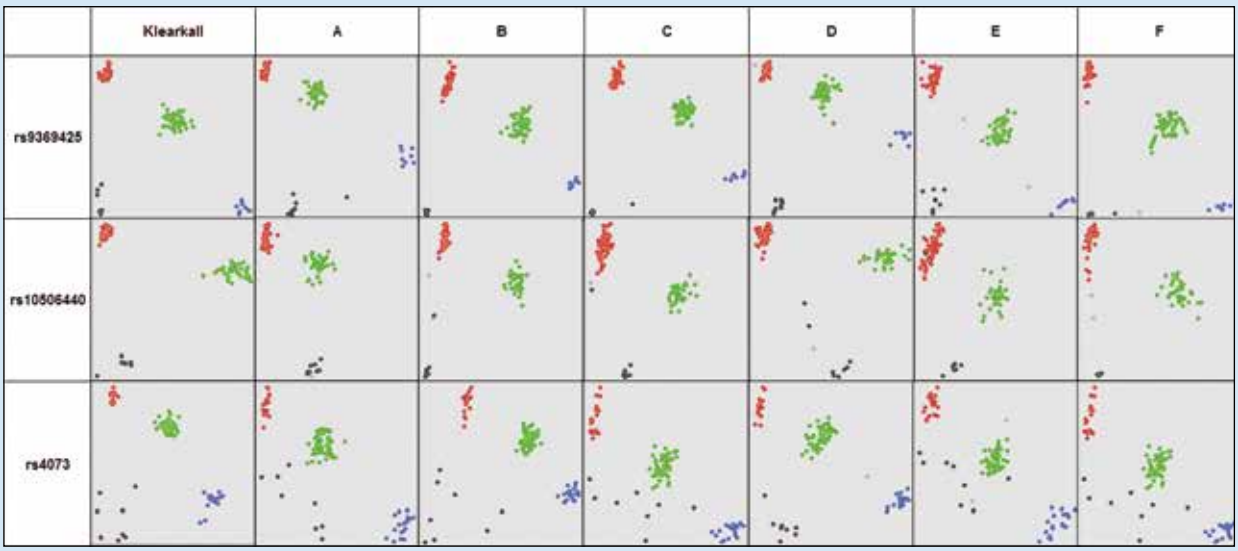


Figure 2: KlearKall vs. competitor mixes A-F on purified human DNA samples on 384-well plates (3 µL reactions).

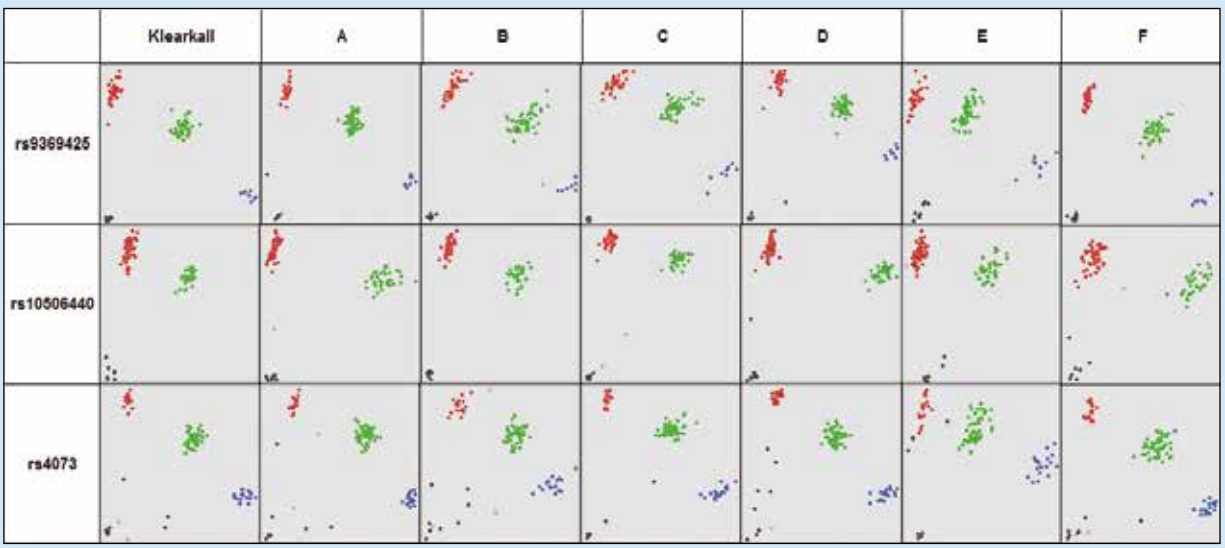


Figure 3: KlearKall vs. competitor mixes A-F on purified human DNA samples on 1536-well plates (1 µL reactions).

Analyse high numbers of samples quickly and accurately

Whether using Douglas Scientific's Array Tape technology, 384- or 1536-well plates, KlearKall is superior across all formats to deliver discrete clusters and high call rates for accurate and reproducible allelic discrimination, even with difficult assays.

- Contains KlearTaq™ Hot Start DNA polymerase plus optimised components, and passive reference dye ROX to enhance precision of data analysis
- Universal thermal cycling conditions for most assays and across platforms
- Bench top stability for ease and flexibility of use

Operates on most qPCR instruments and FRET-capable plate readers.

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 right through to actionable data.

Ordering information

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

*** KlearKall Master mix with standard ROX concentration was used throughout this study.**

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