KlearKall™ Master mix for ultra high-throughput genotyping on crude plant samples to enable precision breeding

SNP genotyping is a powerful tool to accelerate breeding programmes for agricultural crop improvement. The crude DNA extracts often used in such high-throughput genotyping projects are rapid and cost-effective to prepare, but can pose significant challenges to downstream analysis due to carry-over of endogenous PCR inhibitors from the original plant material.

Designed by experts in PCR, KlearKall™ Master mix is a 2X PCR reaction mix that has been optimised to perform high-quality end-point genotyping with hydrolysis probes on large numbers of samples in low (1 µL) reaction volumes. KlearKall™ performance on both the Nexar® System using Array Tape® and the SNPline™ with microplates has been found to be significantly better than, or equal to, competitor mixes in terms of both cluster separation and amplification efficiency.

Platform and competitor comparison

Summary

2X KlearKall Master mix and six competitor PCR master mixes were used to assess SNP genotyping performance on a range of different crude plant DNA extract samples at the low reactions volumes (1 – 3 µL). The plant types selected are those typically used for ultra high-throughput agricultural applications. Assays were run on the market-leading high-throughput automated genotyping platforms: LGC's SNPline and Douglas Scientific's Nexar, with 384- and 1536-well plates, or 384-well Array Tape.

Methods

SNP analysis was performed on seeds from five different crops using 3 different TaqMan® MGB assays per plant sample. Plant crude extracts were prepared from leaves using the sodium hydroxide-based "Hotshot" method.

Genotyping assays were run on a Douglas Scientific Nexar in-line liquid handling and assay processing system using 384-well Array Tape, in a 1.6 µL final reaction volume; and LGC SNPLine for 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	35 - 45 cycles
	60º C	60 sec	

Table 1: Two-step thermal cycling programs 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 and cluster plots were assessed after cluster formation was complete. For data analysis, 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 where available.

For each plant species, the cluster plots are taken from same number of PCR cycles across tape and plates formats. Numbers of PCR cycles used for each species differ out of necessity due to differences in the composition of the crude lysates generated from each sample type.

	KlearKall	Competitor A	Competitor B	Competitor C	Competitor D	Competitor E	Competitor F
Wheat	+++++	+++	++++	++++	++++	++++	+++
Rapeseed	+++++	+++	++++	+++	++++	+++	+++
Maize	+++++	+++	++++	++++	+++++	++++	++
Soybean	+++++	+++	+++++	++++	+++++	++++	++++
Sunflower	+++++	+++	+++++	+++	+++++	++++	+++

Table 2: KlearKall vs. competitor mix A-F performance on Hot shot, crude plant extracts across plates and tape combined.

Results

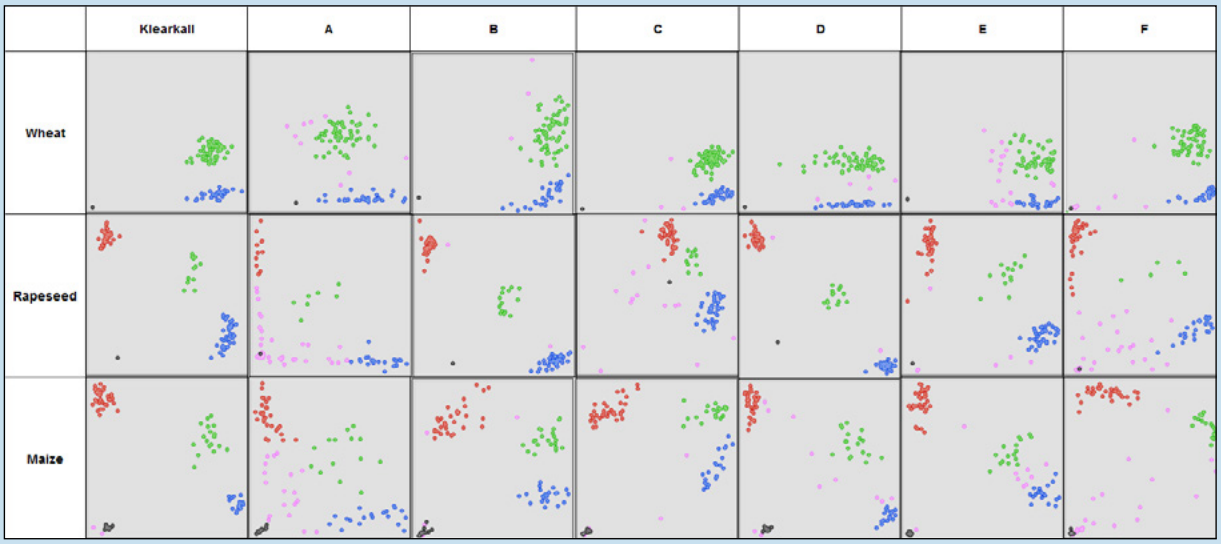

Figure 1: Klearkall vs. competitor mixes A-F on crude DNA lysate samples on Nexar Array Tape (1.6 µL reactions).

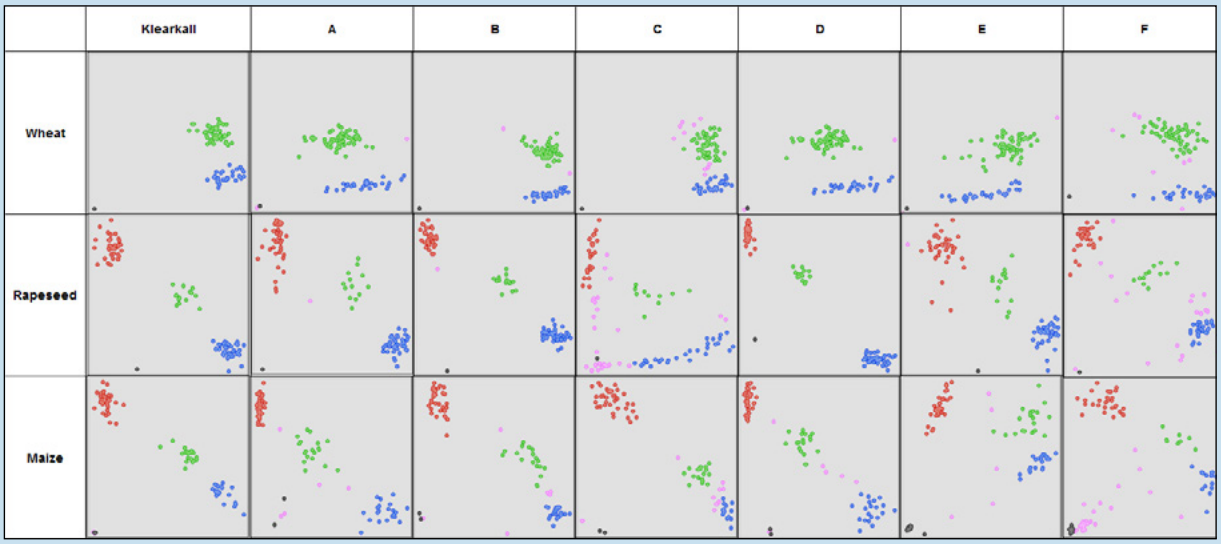

Figure 2: Klearkall vs. competitor mixes A-F on crude DNA lysate samples on 384-well plates (3 µL reactions).

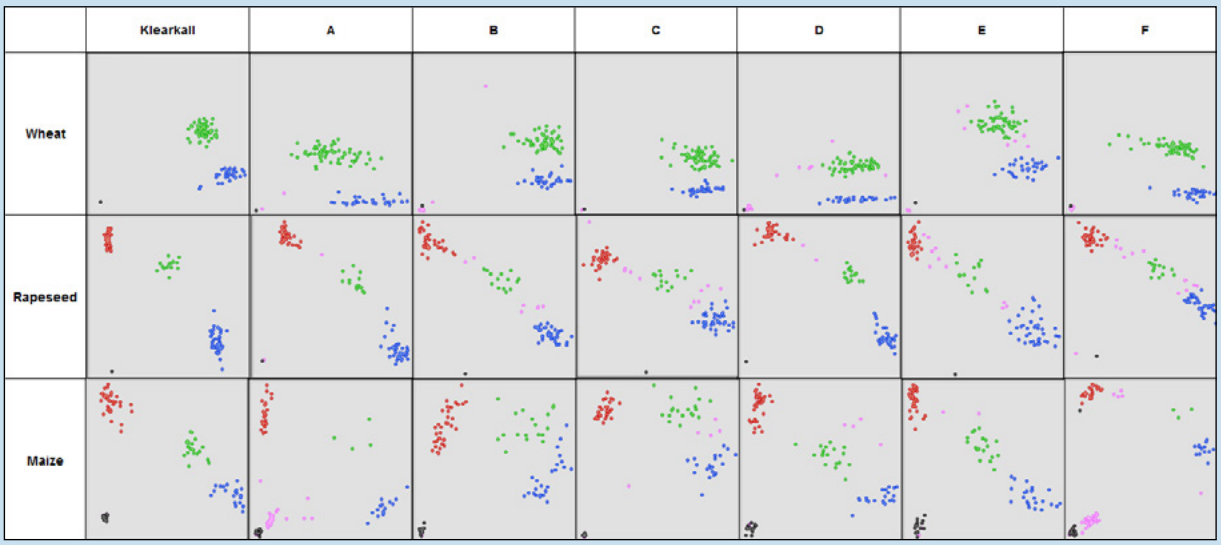

Figure 3: Klearkall vs. competitor mixes A-F on crude DNA lysate samples on 1536-well plates (1 µL reactions).

Conclusions

Klearkall showed excellent performance with crude DNA samples on 384- and 1536-well plates as well as the Array Tape. Data quality with Klearkall was either better than, or equivalent to, competitor master mixes on crude samples from: wheat, rapeseed, maize, soybean and sunflower across Array Tape, 1536-well or 384-well plates on crude DNA extracts run in low volumes.

KlearKall Master mix - 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
- Proven accuracy and reliability even on crude "Hotshot" extracts
- Supports low volume reactions (down to 1 µL) – reducing cost and increasing throughput
- Compatible with hydrolysis probe-based genotyping assays such BHQ®, BHQplus®, and TaqMan®
- 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 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.**

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