

An introduction to KASP

KASP™ genotyping technology utilises a unique form of competitive allele-specific PCR (polymerase chain reaction) that enables highly accurate bi-allelic scoring of SNPs (single nucleotide polymorphisms) and InDels (Insertions and Deletions) at specific loci across a wide range of genomic DNA samples, including those of complex genomes. KASP technology delivers extremely high levels of assay robustness and accuracy with significant cost savings.

Superb accuracy and performance

- Accuracy >99.8% based on independent assessment
- Industry leading SNP & InDel assay conversion rate (>90%).

Tremendous flexibility

- Flexible primer design which increases the rate of successful assay development
- Supports low-, medium- and high-throughput studies and individual repeat assays
- Compatibility with a wide range of liquid handling systems and thermal cyclers; signal can be read on most FRET (fluorescent resonant energy transfer) capable plate readers and qPCR machines.

Breakthrough cost savings

- KASP uses a universal reporting system where the labelled components are present in the KASP Master mix
- Eliminates the need for expensive labelled assay-specific primers or probes
- Requires only 10 ng DNA per sample per SNP (based on human genome size)
- Low reaction volumes keep reagent costs to a minimum
- Cost benefits enable you to perform more assays overall, improving the quality of your data.

KASP genotyping chemistry can be accessed in two ways:

1. In reagent kit form for use in your own laboratory (requires a FRET-capable plate reader or qPCR machine)
2. Through our genotyping service laboratories in Europe and North America.

Assembled as a homogeneous assay in 96-, 384- or **1536-well PCR plates**, bi-allelic discrimination is achieved through the competitive binding of two allele-specific forward primers, each with a unique tail sequence that corresponds with one of two universal FRET cassettes; one labelled with FAM™ dye and the other with HEX™ dye.



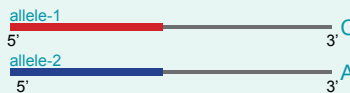
How KASP works

1) Assay components:

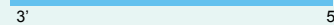
KASP uses three components: test DNA with the SNP of interest; KASP Assay Mix containing two different, allele-specific, competing forward primers with unique tail sequences and one reverse primer; the KASP Master mix containing FRET cassette plus Taq polymerase in an optimised buffer solution.

A) KASP Assay mix

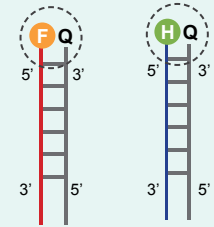
Allele-specific forward primers:



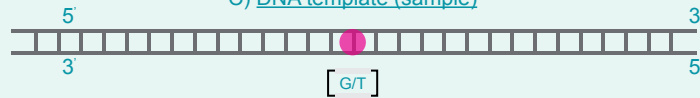
Reverse primer:



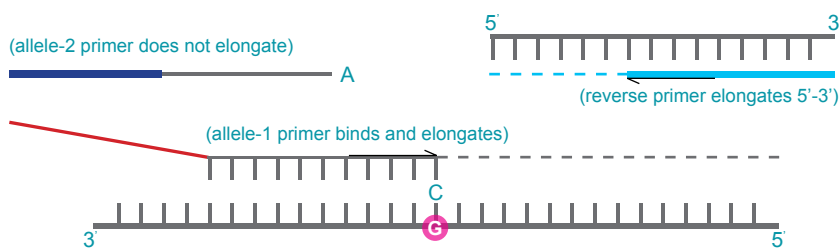
B) KASP Master mix



C) DNA template (sample)

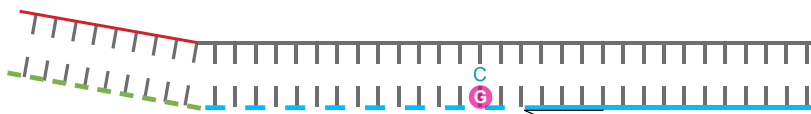


2) Denatured template and annealing components – PCR round 1:



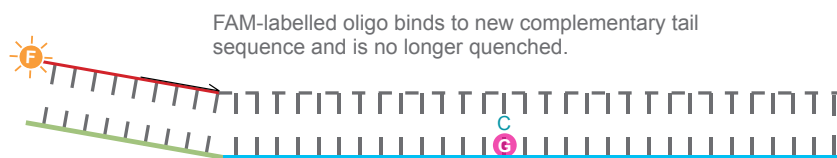
In the first round of PCR, one of the allele-specific primers matches the target SNP and, with the common reverse primer, amplifies the target region.

3) Complement of allele-specific tail sequence generated – PCR round 2:



(Reverse primer binds, elongates and makes a complementary copy of the allele-1 tail.)

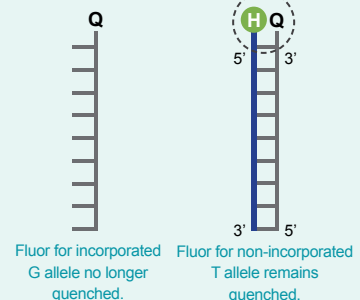
4) Signal generation – PCR round 3:



In further rounds of PCR, levels of allele-specific tail increase. The fluor labelled part of the FRET cassette is complementary to new tail sequences and binds, releasing the fluor from the quencher to generate a fluorescent signal.

Legend

- Allele-1 tail FAM-labelled oligo sequence
- Allele-2 tail HEX-labelled oligo sequence
- Common reverse primer
- F FAM dye
- H HEX dye
- Target SNP
- Q Quencher



KASP genotyping assay components

Running a KASP genotyping assay is easy. Simply add the specific KASP Assay mix and the universal KASP Master mix to your DNA sample in either a 96-, 384-, or 1536-well PCR plate, seal the plate and run the PCR thermal cycle. At the end of the thermal cycle, conduct an end-point read using either a qPCR machine or separate FRET-capable plate reader. No separation steps are required. The graphic on the next page illustrates the KASP genotyping workflow.

1. KASP Assay mix

The KASP Assay mix contains three assay-specific non-labelled primers: two allele-specific forward primers plus one common reverse primer. The sequences for these primers are generated via our proprietary Kraken™ software.

KASP Assay mix can be purchased in two formats:

1. KASP by Design (KBD)
– designed *in-silico*
2. KASP on Demand (KOD)
– validated and optimised in our laboratories. KOD assays are, guaranteed to generate good genotyping data when used in conjunction with DNA of suitable quality and KASP Master mix.

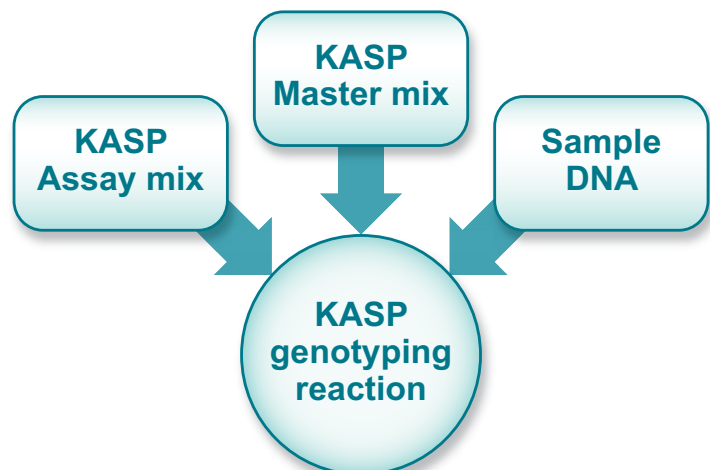
KASP Assay mix is supplied in a single 2D barcoded tube containing sufficient Assay mix to run a minimum of 2500 x 10 µL reactions - the recommended volume for 96-well PCR plates. If run in 384-well PCR plates, the total reaction volume can be reduced to 5 µL, doubling the number of reactions and cutting the cost per data point in half.



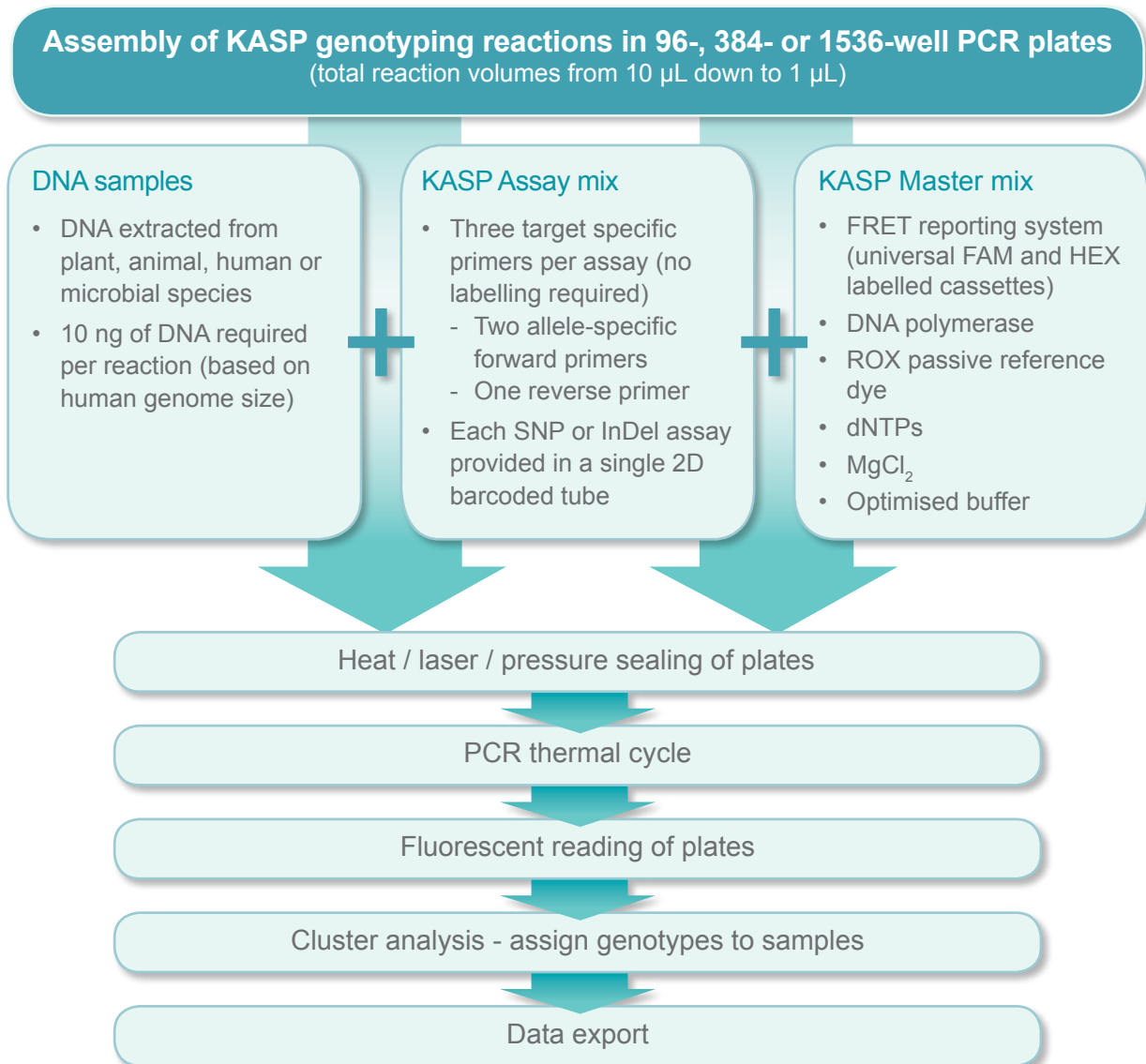
For breakthrough savings, customers can utilise our SNPLine workflow for high-throughput PCR (see SNPLine brochure), that we developed for use in our own service laboratories and that enables 1 µL reactions in 1536-well PCR plates. SNPLine workflow customers can achieve a 10-fold reduction in the cost per data point, while increasing throughput up to 500,000 data points per day.

2. KASP Master mix

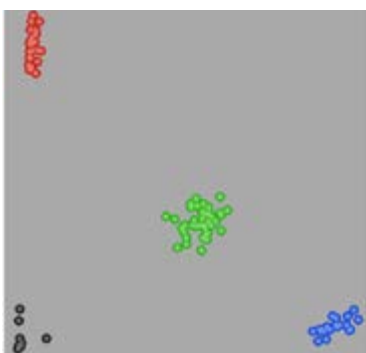
The KASP Master mix contains the universal FRET cassettes, ROX™ passive reference dye, KASPTaq™ DNA polymerase, free nucleotides and MgCl₂ in an optimised buffer solution. KASP Master mix is universal to every KASP genotyping assay and is offered in a range of standard packaging sizes to address varying throughput needs. Custom packaging solutions are also available if needed.



KASP genotyping workflow



Following completion of the KASP PCR, reaction plates are read and the data analysed using any cluster analysis viewing software. Detected signals are plotted as a graph, with samples of the same genotype clustering together. In our genotyping service laboratories, cluster analysis is performed using our proprietary Kraken software. An example of the data generated is shown below.

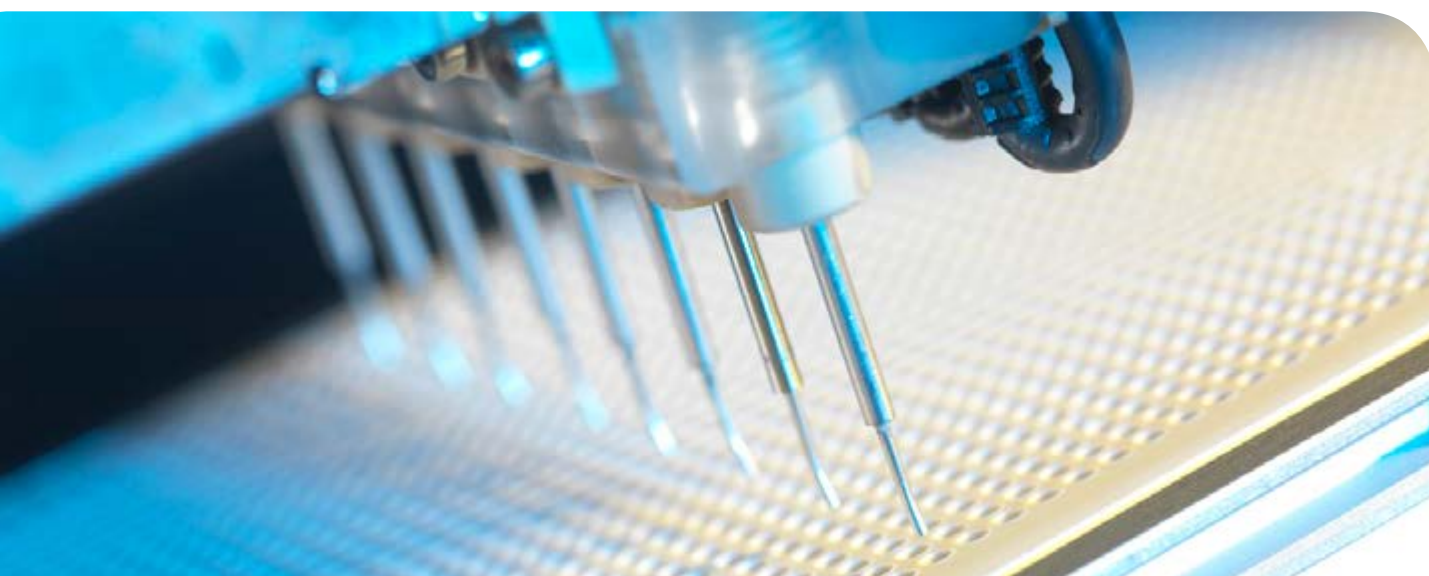


Red	Sample is homozygous for the HEX allele
Green	Sample is heterozygous: one FAM allele and one HEX allele
Blue	Sample is homozygous for the FAM allele

KASP genotyping - product and service options

	Product options		Service
	KBD - KASP by Design	KOD - KASP on Demand	Genotyping service using KASP
Relative cost and value provided	+	++	+++
Lead times	1 - 3 weeks	Standard: 4 - 6 weeks Fast: 1 - 3 weeks	Quoted based on scope (# of SNPs / # of samples) Typically 4 - 6 weeks
Customer submits reference sequences	Yes	Yes	Yes
Target-specific KASP primer sequences are designed for each assay using proprietary Kraken software	Yes	Yes	Yes
Target specific primer sequences and common reverse primers prepared	Yes	Yes	Yes
Primers pooled into a single 2D barcoded tube to create the KASP Assay mix	Yes	Yes	Yes
Assay validation is performed in our service lab with customer supplied DNA. Should any assay fail, assay conditions and / or primer sequences will be optimised until the assay works	No ¹ (>90% of assays work based on standard conditions)	Yes ² (Validation testing accounts for KBD/ KOD price variance)	Yes ² (Validation testing captured as set-up charge – this charge is not applicable on repeat orders)
LGC provides a money-back guarantee that each assay will work	No	Yes	Yes
LGC service lab runs customer samples and provides genotyping data	No	No	Yes

- 1) Our proprietary Kraken software has an industry leading SNP- to- assay conversion rate that exceeds 90%. This means that, on average, 9 out of 10 KBD assays will work based on standard conditions, without any optimisation.
- 2) Once validation has been completed for a particular SNP, it does not need to be repeated. If a customer orders a set of assays as KOD products, the next time they order this same set, they would order the assays as KBD products since the validation work has already been completed. The same holds true for service orders; the set-up charge applies only to the first order.





Ordering information

KASP Assay mix examples

Catalogue number	Description and quantity
KBS-1013-001	KASP by Design Assay mix, 2,500 x 10 µL reactions (2-3 week delivery)
KBS-1013-101	KASP by Design Assay mix, Mega InDels, 2,500 x 10 µL reactions (2-3 week delivery)
KBS-1005-001	KASP on Demand Assay mix, 2,500 x 10 µL reactions, standard service (4-6 week delivery)
KBS-1006-001	KASP on Demand Assay mix, 5,000 x 10 µL reactions, standard service (4-6 week delivery)
KBS-1008-001	KASP on Demand Assay mix, 10,000 x 10 µL reactions, standard service (4-6 week delivery)
KBS-1009-001	KASP on Demand Assay mix, 2,500 x 10 µL reactions, rapid service (2-3 week delivery)
KBS-1010-001	KASP on Demand Assay mix, 5,000 x 10 µL reactions, rapid service (2-3 week delivery)
KBS-1011-001	KASP on Demand Assay mix, 10,000 x 10 µL reactions, rapid service (2-3 week delivery)

KASP Master mix examples

Catalogue number	Description and quantity
KBS-1016-001	KASP 2x Master mix 96 / 384, standard ROX, 500 x 10 µL reactions (2.5 mL)
KBS-1016-002	KASP 2x Master mix 96 / 384, standard ROX, 5,000 x 10 µL reactions (25 mL)
KBS-1016-003	KASP 2x Master mix 96 / 384, standard ROX, 50,000 x 10 µL reactions (250 mL)
KBS-1016-004	KASP 2x Master mix 96 / 384, standard ROX, 500,000 x 10 µL reactions (2.5 L)
KBS-1016-005	KASP 2x Master mix 96 / 384, standard ROX, 100,000 x 10 µL reactions (500 mL)
KBS-1016-006	KASP 2x Master mix 96 / 384, standard ROX, 200,000 x 10 µL reactions (1 L)
KBS-1016-007	KASP 1x Master mix 96 / 384, standard ROX, 100,000 x 10 µL reactions (1 L)

Note: ROX is added to KASP Master mix as a passive reference dye. Different qPCR instruments have differing requirements for ROX; please contact tech.support@igcggenomics.com to determine the correct version for your instrument. In addition, for SNPLine customers only, we offer a separate formulation for use with 1536-well PCR plates. All formulations are readily available. For ordering information, please enquire.

Software and barcode reading

Catalogue number	Description and quantity
KBS-0101-002	KlusterCaller, genotyping calling software
KBS-0101-001	Kraken full LIMS system for genotyping (site license)
KBS-0025-001	Cyclops 2D single tube reader
KBS-0025-002	ZTS-01 96-rack 2D reader

Products and services overview

Services

- KASP SNP and InDel genotyping
- DNA and RNA extraction services
- Sanger sequencing
- Next-generation sequencing services (Roche 454, Illumina HiSeq)
- Whole Genome Amplification (WGA)

Products

- KASP SNP and InDel genotyping chemistry
- DNA extraction products (sbeadex™, Kleargene™ and mag™ kits)
- Enzymes and PCR reagents (KlearKall™, KlearTaq™, KlearTaq™ HiFi)
- Whole Genome Amplification (WGA) kits and services
- DNA shearing instruments (Covaris)
- Microtitre plates and seals for heat and laser sealing (96, 384 & 1536)
- SNPLINE PCR workflow instrumentation:
 - Plate heat sealers (Kube™)
 - Plate laser sealer (Fusion3™)
 - Thermal cycling instruments (Hydrocycler™)
 - Assay dispensing systems (Meridian™)
 - Software (SNPviewer™, KlusterCaller™, Kraken™)
 - DNA extraction instruments (oKtopure™ and Genespin™)
 - DNA plate replicating robot (repliKator™)



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