KASP genotyping technology

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BIOSEARCH™ TECHNOLOGIES
GENOMIC ANALYSIS BY LGC

An introduction to KASP

KASP™ genotyping technology from LGC, Biosearch Technologies™ 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/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 and 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
- Compatible 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 simplicity and flexibility

- KASP uses a universal reporting system, which simplifies assay design and eliminates time-intensive probe design steps
- 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 is available as a service in Europe and North America or as *in silico* wet lab validated and non-validated kits.

KASP by Design (KBD)	KASP on Demand (KOD)
Designed *in silico* No validation	Designed *in silico* Optimised and validated

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 probes; one labelled with FAM™ dye and the other with HEX™ dye.

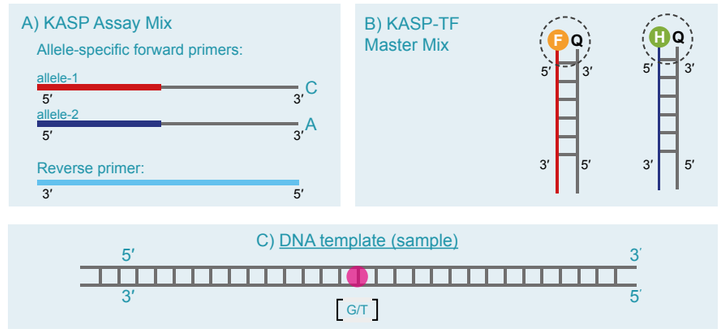
How KASP works

1. Assay components:

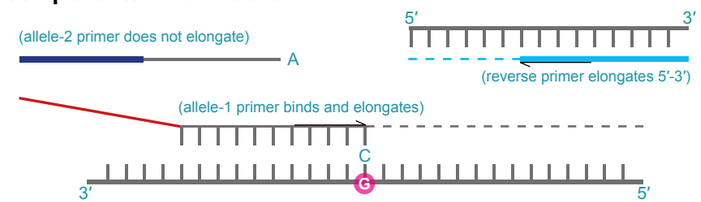
A) KASP Assay Mix: consists of 2 allele specific primers and 1 reverse primer.

B) KASP-TF Master Mix: contains universal fluorescent probes, Taq polymerase and dNTP's in an optimised buffer solution.

C) Sample DNA: DNA contains the SNP of interest.

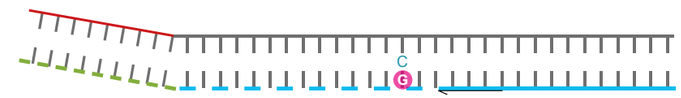


2. Denatured template and annealing components – PCR round 1:



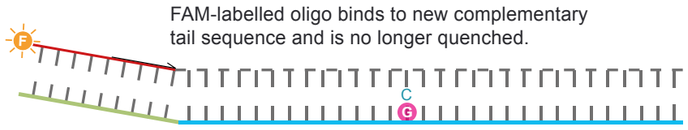
In the first round of PCR, one of the allele-specific primers matches the target SNP and with the common reverse primer, amplifies the target region.

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

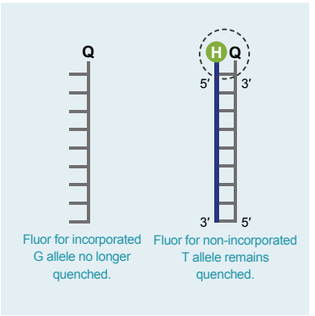


(Reverse primer binds, elongates and makes a complementary copy of the allele-1 tail.)

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

Designing a KASP genotyping assay is fast and easy. KASP uses a universal reporting system that eliminates the need to design assay-specific fluorescent probes. Simply create your Assay mix consisting of your assay-specific primers, add your DNA sample and KASP-TF Master Mix. You can run KASP in 96-, 384- or 1536-well PCR plates. To run, seal the plate and run your PCR cycling protocol. At the end of the thermal cycle, conduct an end-point PCR read using either a qPCR instrument or FRET-capable plate reader. No separation by agarose gel is required.

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:

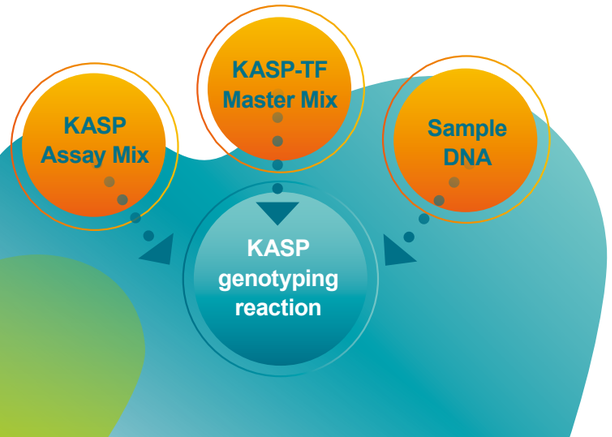
1. **KASP by Design (KBD)**
   – designed *in silico*

2. **KASP on Demand (KOD)**
   – validated and optimised in our laboratories. KOD assays are guaranteed to generate accurate genotyping data when used in conjuction with DNA of suitable quality and KASP-TF 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, 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-TF Master Mix

KASP-TF Master Mix is formulated without Triton™ X-100 (Triton-Free), and contains the universal FRET cassettes, ROX™ passive reference dye, KASPTaq™ DNA polymerase, free nucleotides and $MgCl_2$ in an optimised buffer solution. KASP-TF 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

Assembly of KASP genotyping reactions in 96-, 384- or 1536-well PCR plates
(total reaction volumes from 10 µL down to 1 µL)

DNA samples
- DNA extracted from plant, animal, human or microbial species
- 10 ng of DNA required per reaction (based on human genome size)

KASP Assay Mix
- Three target specific primers per assay (no labelling required)
  - Two allele-specific forward primers
  - One reverse primer
- Each SNP or InDel assay provided in a single 2D barcoded tube

KASP-TF Master Mix
- FRET reporting system (universal FAM and HEX labelled cassettes)
- DNA polymerase
- ROX passive reference dye
- dNTPs
- $MgCl_2$
- Optimised buffer

Heat/laser/pressure sealing of plates

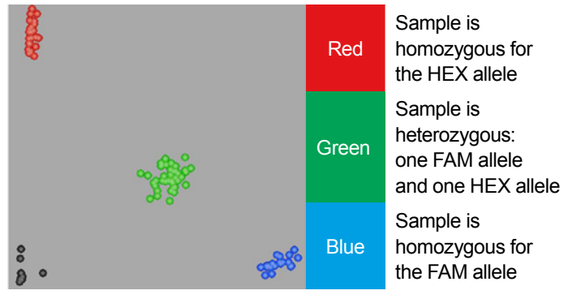
PCR thermal cycle

Fluorescent reading of plates

Cluster analysis - assign genotypes to samples

Data export

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 on the right.



Sample is homozygous for the HEX allele

Sample is heterozygous: one FAM allele and one HEX allele

Sample is homozygous for the FAM allele

KASP products and services

	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	No1 (>90% of assays work based on standard conditions)	Yes2 (Validation testing accounts for KBD/KOD price variance)	Yes2 (Validation testing captured as set-up charge – this charge is not applicable on repeat orders)
Biosearch Technologies provides a money-back guarantee that each assay will work	No	Yes	No
Biosearch Technologies 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

Request a free KASP trial kit at:
biosearchtech.com/kasp-free-trial-kit

KASP Assay Mix	
Catalogue number	Description and quantity
KBS-2300-001	KASP by Design Primer Mix, 2500 × 10 µL Reactions, Standard Service
KBS-2300-002	KASP by Design Primer Mix, 2500 × 10 µL Reactions, Standard Service, Manual Design
KBS-2400-001	KASP on Demand Primer Mix, 2500 × 10 µL Reactions, Standard Service
KBS-2400-002	KASP on Demand Primer Mix, 2500 × 10 µL Reactions, Rapid Service
KBS-2400-003	KASP on Demand Primer Mix, 2500 × 10 µL Reactions, Standard Service Manual Design (3 Primers)

KASP-TF Master Mix examples	
Catalogue number	Description and quantity
KBS-1050-101	KASP-TF V4.0 2X Master Mix 96/384, Standard ROX (2.5 mL)
KBS-1050-102	KASP-TF V4.0 2X Master Mix 96/384, Standard ROX (25 mL)
KBS-1050-103	KASP-TF V4.0 2X Master Mix 96/384, Standard ROX (250 mL) (10 × 25 mL)
KBS-1050-111	KASP-TF V4.0 2X Master Mix 1536, Standard ROX (2.5 mL)
KBS-1050-112	KASP-TF V4.0 2X Master Mix 1536, Standard ROX (25 mL)
KBS-1050-113	KASP-TF V4.0 2X Master Mix 1536, Standard ROX (250 mL) (10 × 25 mL)
KBS-1050-164	KASP-TF V4.0 Trial Kit (Standard ROX)

ROX is added to KASP-TF Master Mix as a passive reference dye. Different qPCR instruments have differing requirements for ROX; please contact techsupport@lgcgroup.com to determine the correct version for your instrument. Formulations for use with 1536-well PCR plates are compatible with SNPline™ instrumentation. Please enquire for ordering information.

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

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Accelerated science.

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