The New Era of KASP Method for Genotyping

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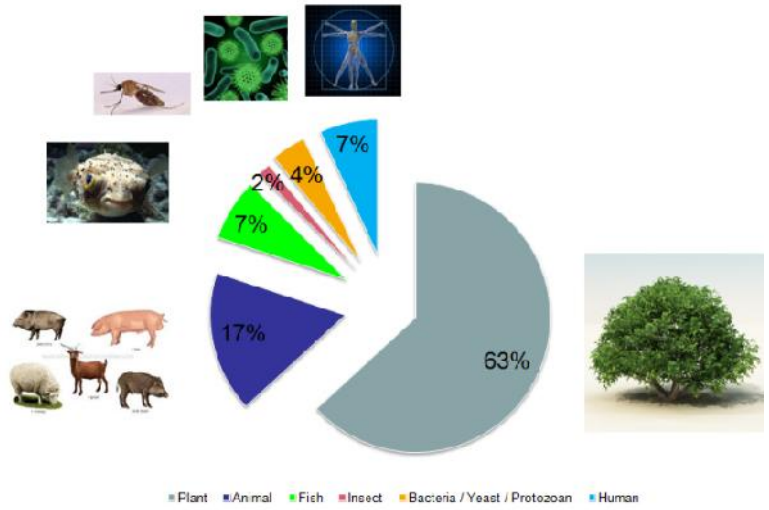
20/12/60

Outline

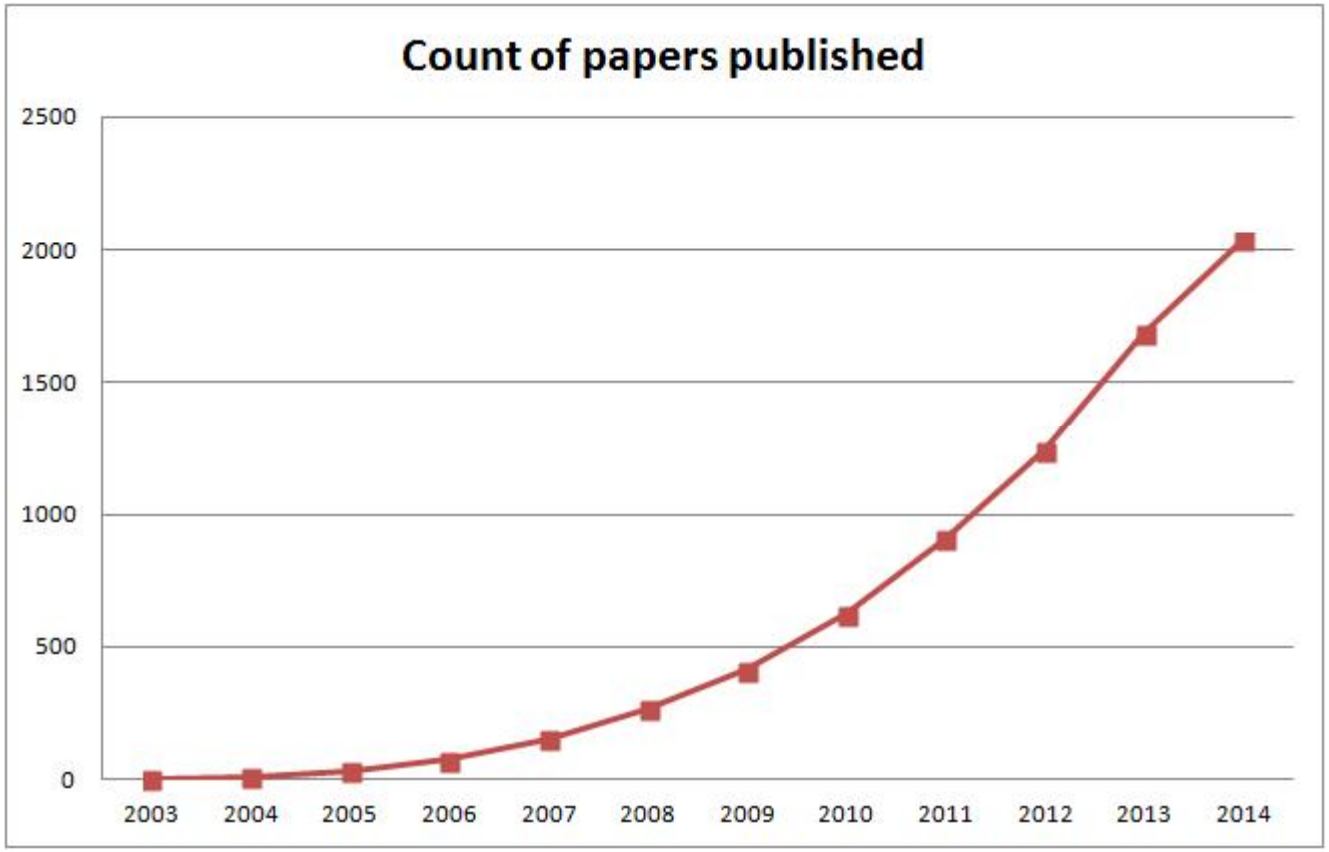
- Introduction
- Principal of KASP Method
- Summary

KASP Assays in Various Fields

- > 360,000 unique KASP assays developed in our laboratories
- > 25,000 Validated Human assays.
- Multiple assays to key genetic diseases such as Cystic Fibrosis and Breast & Ovarian Cancer
- Extensive Panels for key field crops (~13,600 assays)



KASP Scientific Publications



KASP Publication Reference

Crop Science

Title : QTL Mapping and Molecular Breeding for Developing Stress Resilient Maize for Sub-Saharan Africa (Oct 2014)

- KASP good at fingerprinting due to simplicity of data and low drop out rate

- CIMMYT routinely uses KASP, generating in excess of a million data points annually for different purposes

-The KASP average genotyping error rate in positive control DNA samples varied between 0.7 to 1.6%

CIMMYT : The International Maize and Wheat Improvement Center

Electronic Journal of Biotechnology

One-step, codominant detection of imidazolinone resistance mutations in weedy rice (*Oryza sativa* L.)

To assess the suitability and validity of KASP method for detection of RWR compare to AS-PCR

The AS-PCR had specificity of 0.97, sensitivity of 0.95 whereas KASP, both parameters were 1.00

Thus, the method (KASP) validated here for timely and accurate detection of RWR is a valuable and cost-effective tool for decision making in Clearfield rice management and regional surveillance of RWR in the framework of a sustainable use of this production system.

Recherche uO

Thèses uOttawa

Molecular Marker Applications in Oat (Avena Sativa L.) Breeding and Germplasm Diagnostics

GBS will likely provide a good source for future KASP™ marker design

Therefore, we recommend KASP™ for rapid cultivar identification and GBS for more conclusive identification and/or for purity assessment”

The present work can also be considered as a resource for breeders. Firstly, KASP™ and GBS have been developed for rapid cultivar identification and purity assessments

Mol Breeding
DOI 10.1007/s11032-013-9917-x

REVIEW

Single nucleotide polymorphism genotyping using Kompetitive Allele Specific PCR (KASP): overview of the technology and its application in crop improvement

Kassa Semagn · Raman Babu · Sarah Hearne · Michael Olsen

Received: 5 April 2013 / Accepted: 1 July 2013

Abstract Single nucleotide polymorphism (SNP) data can be obtained using one of the numerous uniplex or multiplex SNP genotyping platforms that combine a variety of chemistries, detection methods, and reaction formats. Kompetitive Allele Specific PCR (KASP) is one of the uniplex SNP genotyping platforms, and has evolved to be a global benchmark technology. However, there are no publications relating either to the technology itself or to its application in crop improvement programs. In this review, we provide an overview of the different aspects of the KASP genotyping platform, discuss its application in crop improvement, and compare it with the chip-based Illumina GoldenGate platform. The International Maize and Wheat Improvement Center routinely uses KASP, generating in excess of a million data points annually for crop improvement purposes. We found that (1) 81 % of the SNPs used in a custom-designed GoldenGate assay were transferable to KASP; (2) using KASP, negative controls (no template) consistently clustered together and rarely produced signals exceeding the threshold values for allele calling, in contrast to the situation observed using GoldenGate assays; (3) KASP's average genotyping error in positive control DNA samples was 0.7–1.6 %, which is lower than that observed using GoldenGate (2.0–2.4 %); (4) KASP genotyping costs for marker-assisted recurrent selection were 7.9–46.1 % cheaper than those of the BeadXpress and GoldenGate platforms; and (5) KASP offers cost-effective and scalable flexibility in applications that require small to moderate numbers of markers, such as quality control analysis, quantitative trait loci (QTL) mapping in bi-parental populations, marker-assisted recurrent selection, marker-assisted backcrossing, and QTL fine mapping.

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DOI: 10.1038/ncomms5871 OPEN

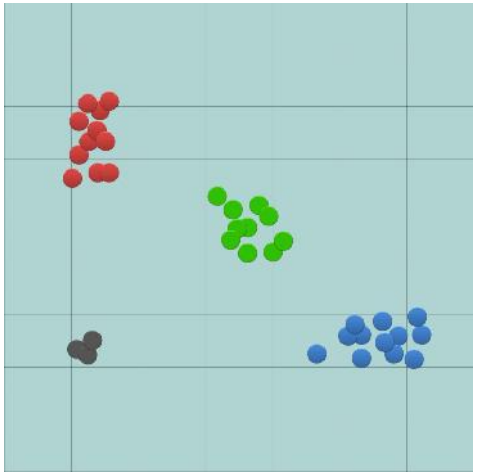
A rare variant in *APOC3* is associated with plasma triglyceride and VLDL levels in Europeans

For ALSPAC, the entire cohort (10,145 participants, including 38 carriers of the rare A allele) was genotyped using KASP with a genotyping accuracy of 100%

ALSPC : The Avon Longitudinal Study of Parents and Children

KASP Overview

- Competitive Allele-Specific PCR
- Endpoint PCR-based genotyping chemistry
- Genotyping Suitable for :
  - Single nucleotide polymorphisms (SNPs) and
  - INDELs (include large INDELs)

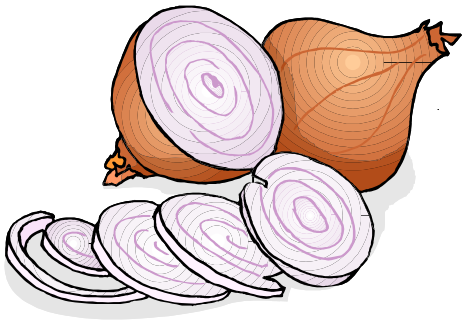


DNA requirements

- Most KASP assays will function with 5 - 50 ng DNA per reaction
- For human DNA (genome = 3000 Mbp), we would recommend using 5 ng / µL concentration (wet DNA) as a starting point

5-50 ng / µL

DNA Requirements Vary with Genome Size



Onion

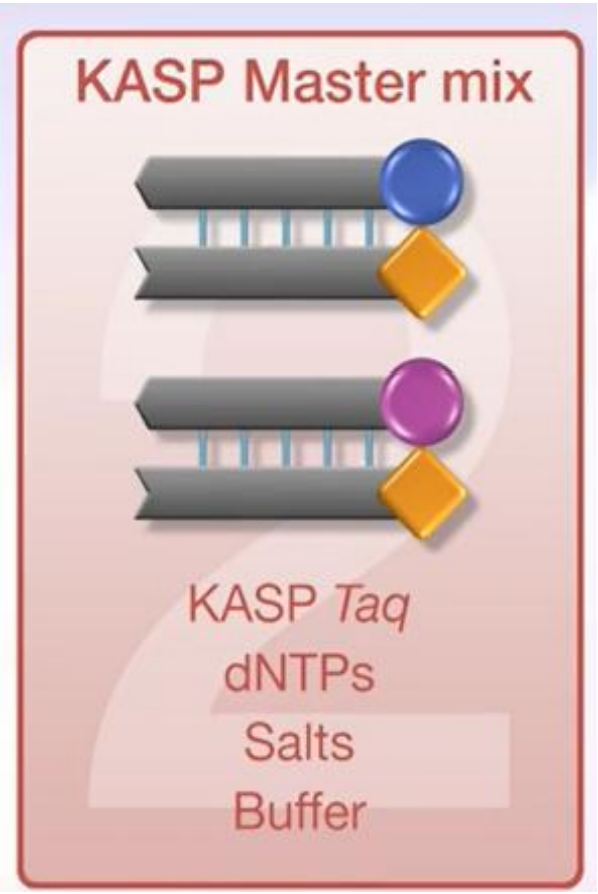
Genome size = 16400 Mbp

16400 / 3000 Mbp = 5.5

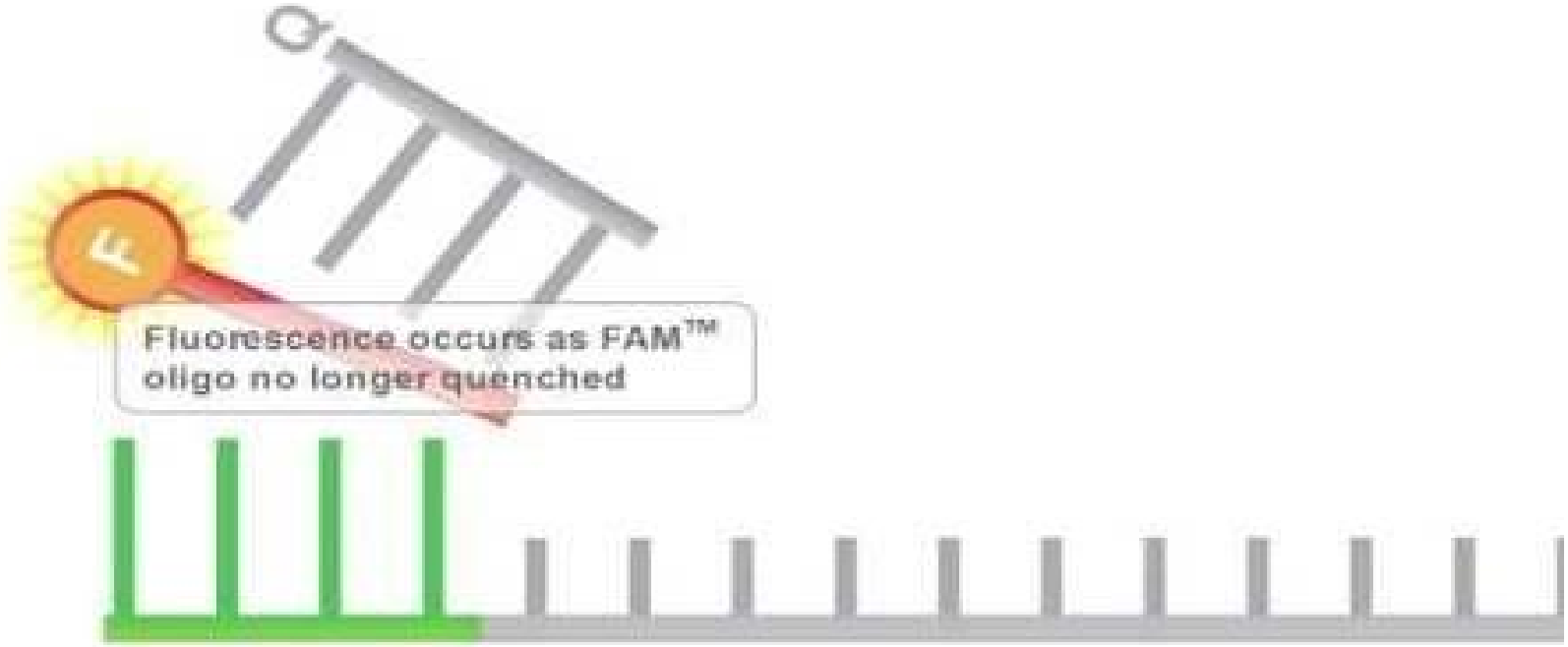
5 ng / µL x 5.5 = 27.5 ng / µL

- The concentration of DNA required will vary based upon the genome size of your organism
- For a species with a larger genome size, you will require a greater mass of DNA per reaction
- Divide the genome size of your organism by the size of the human genome (3000 Mbp), and use the resulting number to multiply the amount of DNA that is required.

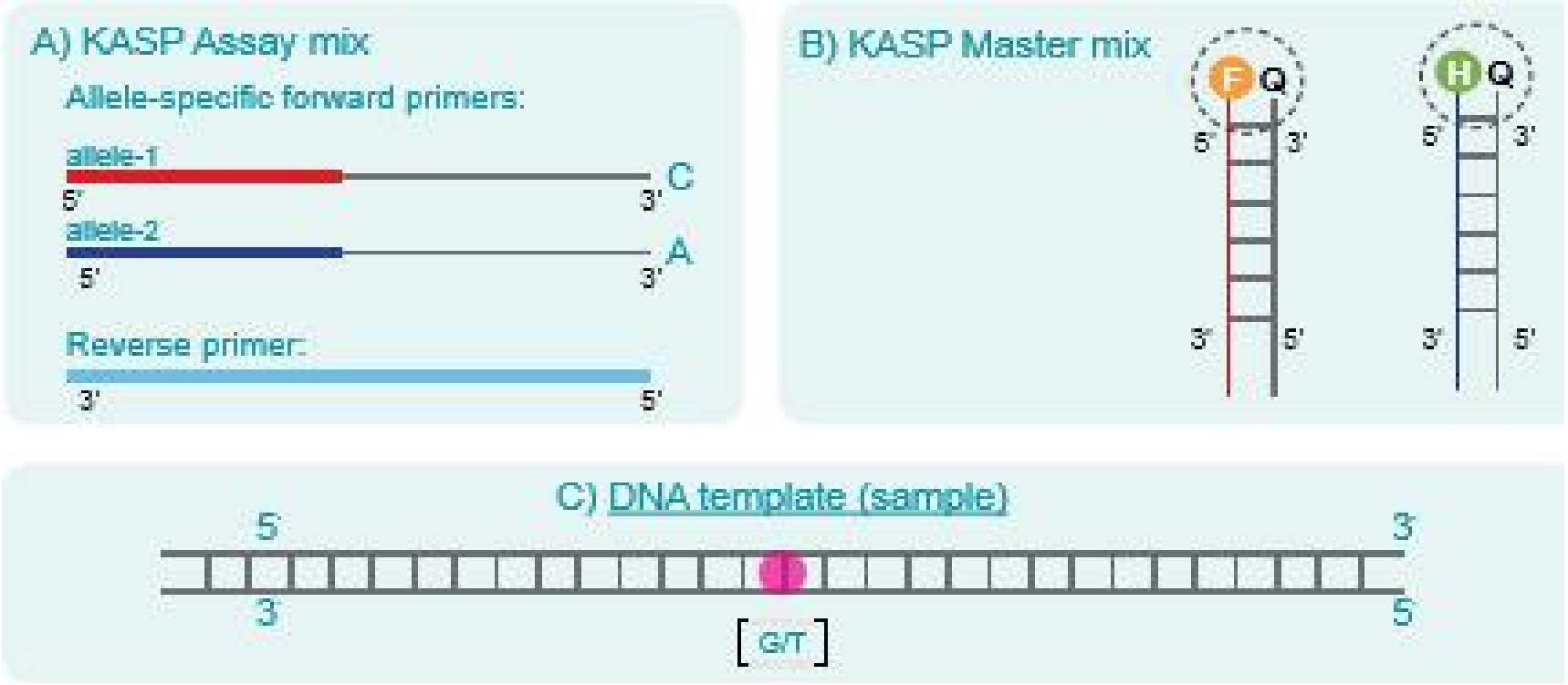
The KASP reaction components



How KASP work!

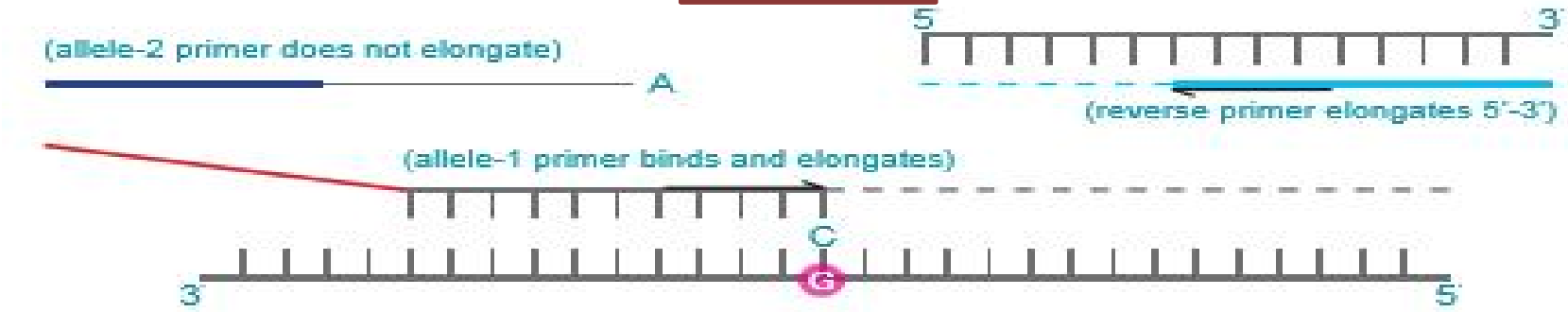


Principal of KASP genotyping assay



Principal of KASP genotyping assay

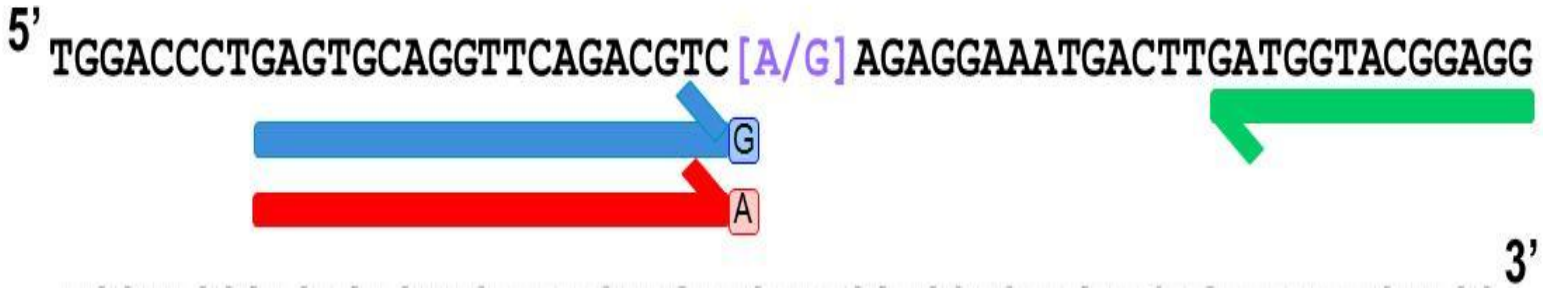
PCR round 1

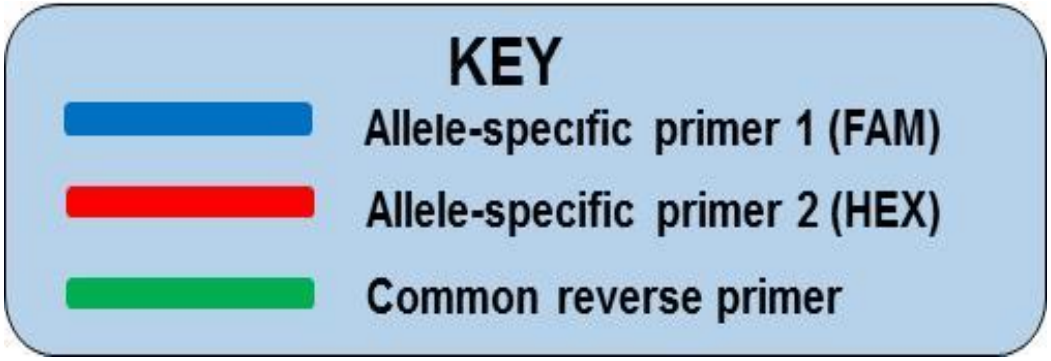


PCR round 2

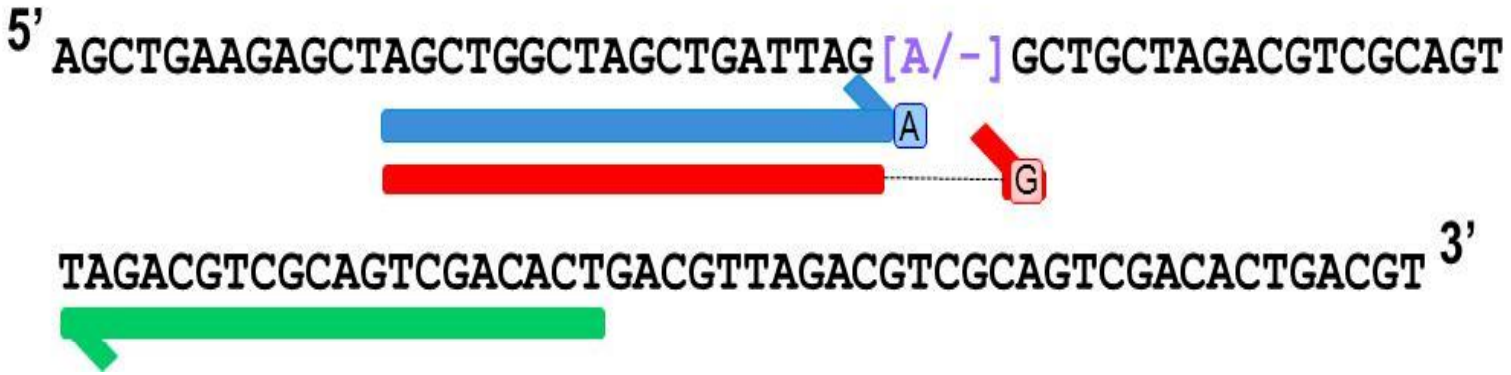
PCR round 3

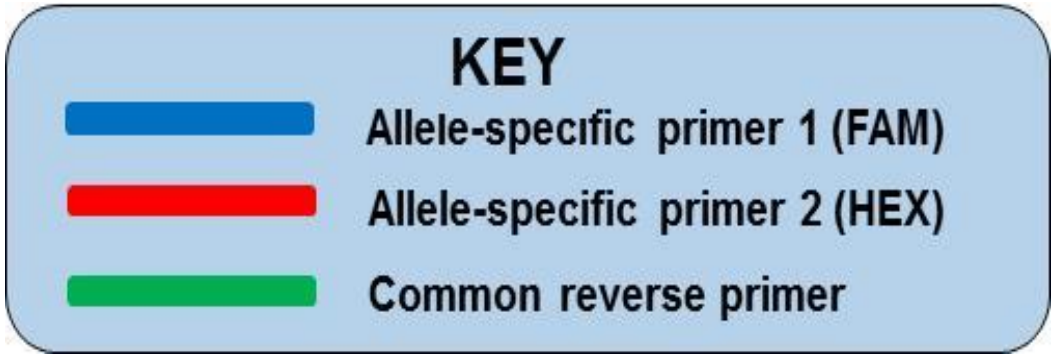
SNP Genotyping



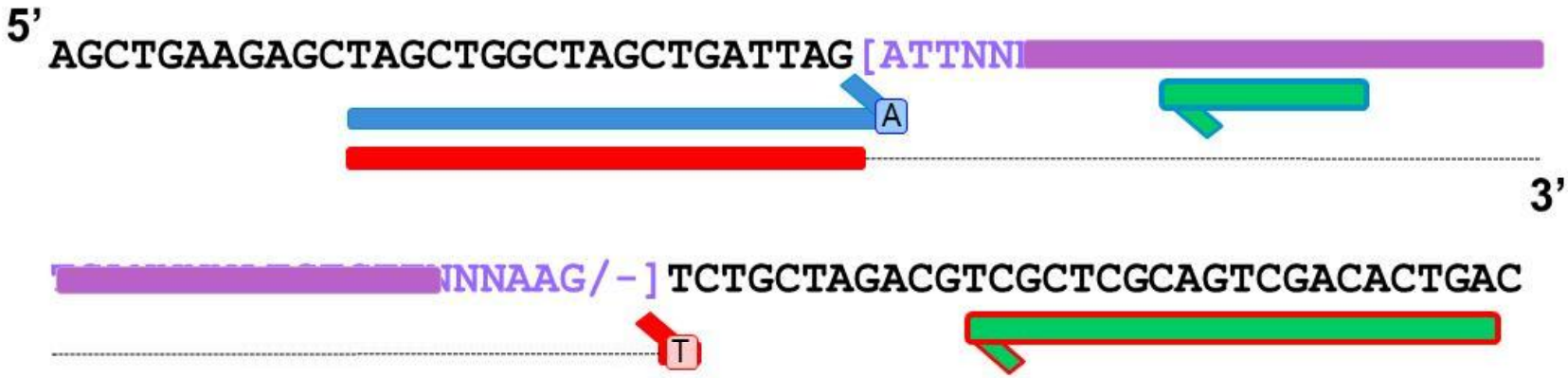


Single Base Insertion / Deletion





Large Insertion or Deletion



KEY

- Allele-specific primer 1 (FAM)
- Allele-specific primer 2 (HEX)
- Reverse primer 1
- Reverse primer 2
- Represents large insertion

KASP assay using which instrument ?

- Thermal Cycler + FRET-capable Plate Reader
- qPCR Instrument

Running KASP on qPCR instruments

ABI: 7000-series, Viia7, Quant Studio-series

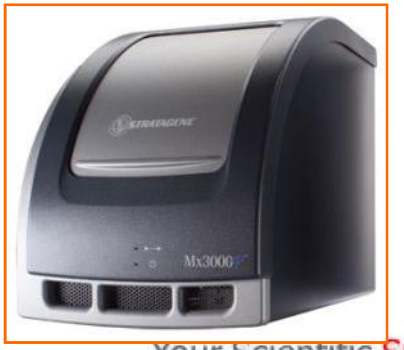
BIO-RAD: CFX, iCycler iQ, MyiQ & MyiQ 2

Illumina:
Eco RT-PCR

Roche: LC480 vI and vII, LC 2.0 & LC96

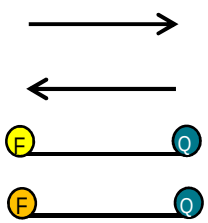
Thermo Scientific: PikoReal

Agilent: Mx3000 / 3500P & Mx4000P



KASP vs Taqman®

Taqman® components:



1 x Forward primer

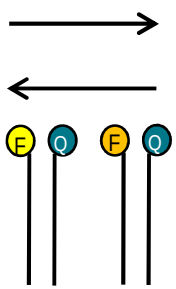
1x Reverse primer

Dual labelled probe 1

Dual labelled probe 2

Reaction mix

KASP Components



2 x Allele-specific forward primer

1x Reverse primer

Reaction mix (containing fluorescent quencher cassettes)

Both chemistries are used to detect single nucleotide polymorphisms (SNPs)

KASP advantages:

- Increased design flexibility- only primers unique in every assay; not detection system
  - able to design to complex regions (more common in plants)
  - able to design to large insertion/deletion sites (phenotypic mutations)
- Reduced Fluor-Quencher Probe costs
  - Taqman has 2 expensive dual labelled probes that need to be synthesised for every assay
  - KASP detection system uses a generic FRET cassette in reaction mix, rather than being specific for every assay

Product and Service Options

	Product Options		Service
	KBD (KASP by Design)	KOD (KASP on Demand)	Service using KASP
Relative cost and value provided	+	++	+++
Lead times	1-3 wk	Standard : 4-6 wk Fast :1-3 wk	Typically 4-6 wk
Assay validation is performed in our service lab with customer supplied DNA.	No	Yes	Yes
Money-back guarantee	No	Yes	Yes
LGC service lab runs customer samples and provides genotyping data	No	No	Yes

KASP Assay Design and Validation

- A standard KASP genotyping assay takes a few minutes to design. For specialist designs such as speciation/pathogen detection assays this can be up to1-2 days. This is required due to:
  - A requirement for high sensitivity
  - High levels of homology with similar species.
- Once assays have been ordered and assembled, validation can take from a few hours to several weeks depending on:
  - Sensitivity requirements (may require redesign to improve sensitivity);
  - Specificity studies – it may take several weeks to ensure no cross-reactivity with large panels of other species.
- Chances of success are improved by initially ordering multiple assay designs, particularly for complex targets. Since KASP assays are relatively inexpensive, this is usually the best option.

Advantage

- 99.8% Accuracy
- > 90% Call rate
- Cost-effective
- Suitable for SNP, INDEL genotyping
- Compatibility with a wide range of qPCR machine and Thermal cycler with FRET-capable plate reader

Summary

- Suitable for detection of a wide variety of targets including animal and human targets
- Highly specific and sensitive detection
- Use for genotyping and pathogen/species detection


KASP Master mix
X
FAM
Q
Y
HEX
Q
KASP
Taq