

KBioscience Competitive Allele-Specific Polymerase chain reaction (KASPar) assay

SNPs (Single nucleotide polymorphisms) are one of the most common types of genetic variation in eukaryotic genomes. They can be used for construction of high-resolution genetic maps, investigation of population evolutionary history, discovery of marker-trait associations in association mapping experiments. SNPs are found to be involved in the etiology of many human diseases and are becoming of particular interest in pharmacogenetics. Because SNPs are conserved during evolution, they have been proposed as markers for use in quantitative trait loci (QTL) analysis and in association studies in place of microsatellites. The use of SNPs is being extended in the HapMap project, which aims to provide the minimal set of SNPs needed to genotype the human genome. SNPs can also provide a genetic fingerprint for use in identity testing. The increase in interest in SNPs has been reflected by the furious development of a diverse range of SNP genotyping methods.

SNP genotyping is the measurement of genetic variations of SNPs between members of a species. It is a form of genotyping, which is the measurement of more general genetic variation. The development of SNP-based genotyping platforms has led to an increase in the number of protocols available for analyzing the genetic variation in numerous species.

(http://en.wikipedia.org/wiki/SNP_genotyping)

KASPar assay is the patented SNP genotyping system from KBioscience based on FRET. FRET (Fluorescent Resonance Energy Transfer) allows for the detection of SNP's without the need for a separation step. Coupled with the power of competitive allele specific PCR, the KASP system offers a truly superior system for determination of SNP or insertion / deletion genotypes in your laboratory. The system was originally developed at KBioscience for use in our Laboratory Services division, and has been used for a number of years undergoing continued improvement over time. It offers the simplest and most cost effective way to determine SNP genotypes in the laboratory.

(<http://www.kbioscience.co.uk/reagents/KASP/KASP.html>)

Sequence used for KASP assay design:

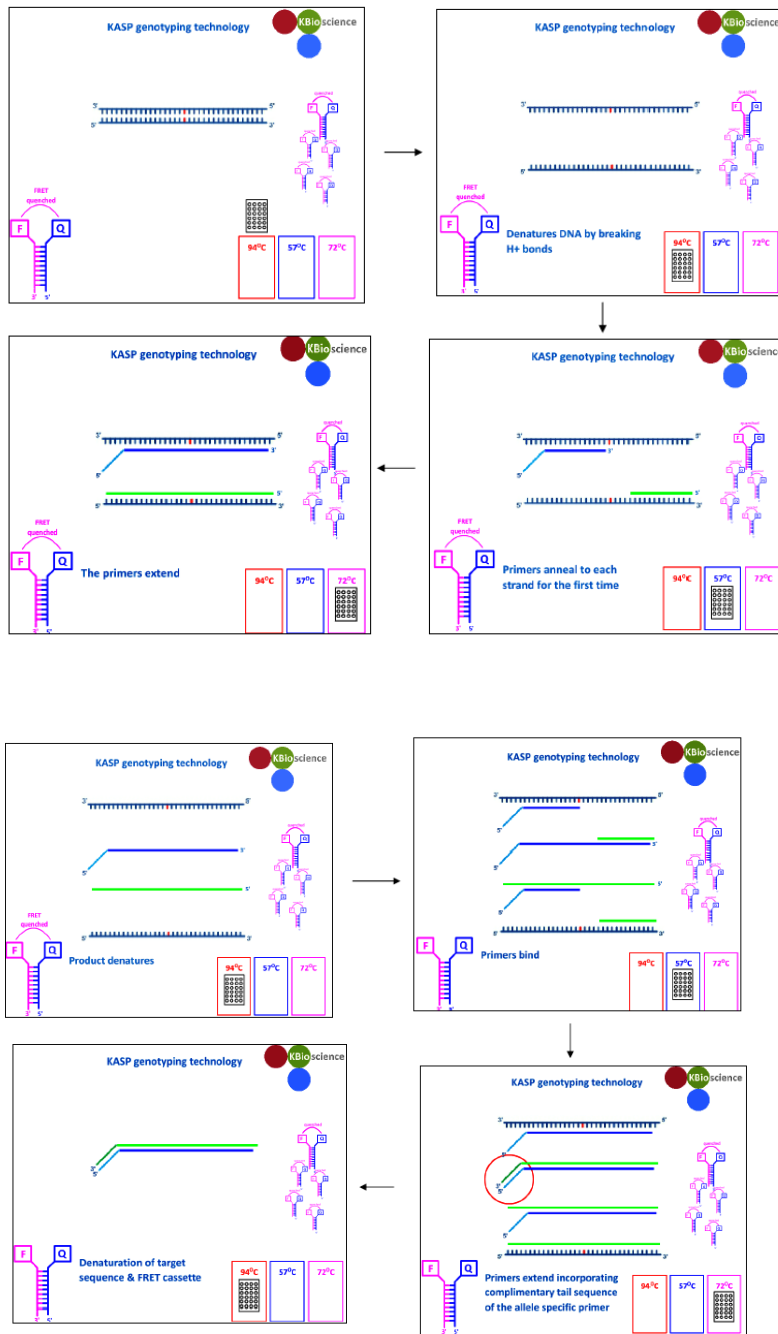
CAGACGCTTAAGCGGTAGGGCGCCGTGGCAGCGCCTTAGAAATATSCCCGCTTTAGTCTCTTATA
GGCGTCGCTTAAGCGTCAGGGCGGGTGGTGCTCGCTTTAGGTTGCTTTACTGCCTTATAAACCAT
GCATGAAATGTTTGCTTAGTATCAGTTGAATATTTCTYCATATCAATGTTATAGGATTTACCTGG
TTTTGTAGCTTGTACGACTTACCATTACCAATTTGACCCAATAGTCTGTGCTACAATTAGCTG
AATATTTTCATGGAATATTTCTCCCGTGTTACATTGTTGCTTATCCGCTTAACCGAGACTCTGGAY
ATTAAGCTGCRTTTTAAATTCAGTATTTAATTTTGAATAGTAGCATTATCTCATCTTCTCTGT
GTNNNANCNCNCATATCTGGCAGCAATTTGCGAGGACGATTACAAAGATCTGAGCGAAGTCA
GAAGGATACTGCAAGAACACCTGAAGGTAAGCTTT[W]CCCGGCTGCACCTTGTGTATAAATCCC
ATGCAGCTGCTCTAACAGCAATCCTGTATTTTCCAAGGCATGC

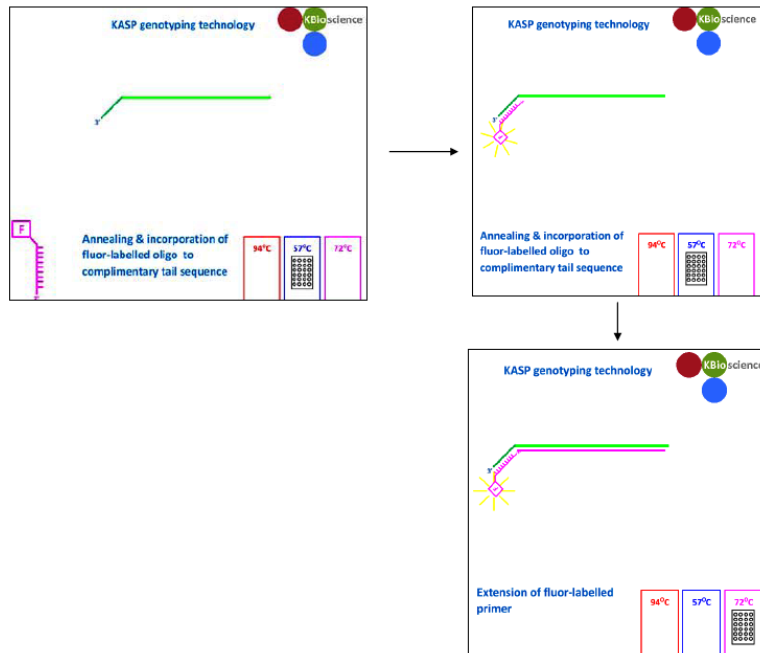
W: A/T

Allele FAM: A

Allele Vic: T

KASP: How it works





KASP assay reaction set up:

DNA (5ng/ul) – 4ul
 2x Reaction mix – 4ul
 Assay mix - 0.11ul
 Total – 8.11ul

1. Transfer 4ul of provided DNA samples into low-profile 8-Tube PCR strips
2. Prepare a master mix: 40ul of 2x reaction mix (brown tube) and 1.1ul of assay mix (clear tube). Pipette gently, try to avoid bubbles
3. Add 4.2ul of master mix to DNA samples
4. Cover the strip with provided optical 8-cap strip
5. Spin if needed in a minifuge
6. Place in the CFX 96

KASP assay instrument (CFX 96) protocol programming:

1. 94°C for 15:00
 2. 94°C for 0:10
 3. 60°C for 1:00
 4. GOTO 2, more 35 times
 5. 35°C for 0:30
- + Plate Read
 END

When reaction is complete, evaluate the results in the Allelic Discrimination mode. Select RFU instead of Cq values.