

A: KASPar genotyping Technology

1. Assay components:

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

B) KASP 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.

A) KASP Assay Mix

Allele-specific forward primers:

allele-1 5' → 3' C

allele-2 5' → 3' A

Reverse primer: 5' → 3'

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:

FAM-labelled oligo binds to new complementary tail sequence and is no longer quenched.

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

<https://info.biosearchtech.com/agrigenomics-pcr-based-kasp-genotyping>

B: AXIOM genotyping Technology

Target prep

Amplify

Fragment

Hybridization

Capture

+ Label

Labeled solution probe

Ligation

Differentiate

Signal amplification

Stain and image

Biomek FX[®] Target Prep Express System

Applied Biosystems™ Axiom™ 384-array layout

Applied Biosystems™ GeneTitan™ Multi-Channel (MC) Instrument

<https://www.thermofisher.com/fr/fr/home/life-science/microarray-analysis/agrigenomics-solutions-microarrays-gbs/axiom-genotyping-solution-agrigenomics.html>