Kompetitive Allele Specific PCR (KASP) with BioRad Software

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Abstract:

A short guide to primer design with HEX/FAM tags and a basic KASP protocol using LGC Genomics KASP master mix and BioRad analyzation software.

Keywords: KASP, Primer design, Hex/Fam, PCR

Primer Design:

1. Design regular primers over the mutation you wish to test. The primer sequence must include this mutation to be specific.
2. Copy and paste the FAM and HEX tags to your wild type and mutant alleles. Pay attention to which tag is which.

FAM tag: GAAGGTGACCAAGTTCATGCT

HEX tag: GAAGGTCGGAGTCAACGGATT

**Example: IAA16 GG to RR mutation Primers** in *Bassia scoparia*: (Order these sequences through your primer manager)

|  |  |
| --- | --- |
| Bs\_IAA16\_S\_FP(Hex) | GAAGGTCGGAGTCAACGGATT TGTTCTTCAGGACACAAGTTGTAGG |
| Bs\_IAA16\_R\_FP(Fam) | GAAGGTGACCAAGTTCATGCT TGTTCTTCAGGACACAAGTTGTAAA |
| Bs\_IAA16\_RP | AGTTTGATCATCGGACGTCTTCTT |

1. Remove 2x KASP master mix from freezer, place on ice (LGC Genomics, Beverly, MA, USA).
   * This volume of 432 ul contains KASP enzyme, buffer, cofactors, dNTPs
2. Thaw primers; one reverse primer, two forward primers, each specific to a SNP and corresponding fluorophore
3. Make primer mix:

|  |  |
| --- | --- |
| EACH forward primer | 18 μl |
| Reverse primer | 45 μl |
| H2O | 69 μl |
| Total primer mix | 150 |

1. Add 12 ul of primer mix to the 2x KASP tube, this now the KASP master mix.
2. Add 4 ul of KASP master mix to each sample well of a 96 well plate
3. Add 4 ul of template DNA @ 5-20 ng/ul.
4. Include 3 NTCs, as well as appropriate controls

Bio-Rad machine and software

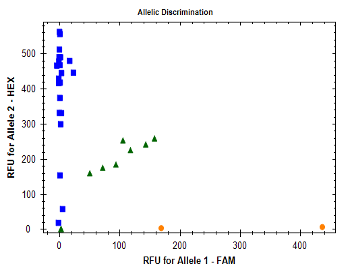
1. Set up thermocycling conditions according to the protocol:

|  |  |  |
| --- | --- | --- |
| Step | Temperature | Length |
| 1 | 94 C | 15 minutes |
| 2 | 94 C | 20 seconds |
| 3 | 61 C | 1 min |
| 4 | Go to step 2 | 10x |
| 5 | 94 C | 20 seconds |
| 6 | 55 C | 1 min |
| 7 | 30 C | 10 seconds |
| 8 | Go to 5 | 35x (take read each cycle) |

1. Make sure that each well of the plate has both FAM and HEX fluorophores selected
2. Click Start Run

Analysis

1. On the “Allelic Discrimination” tab the relative florescence units (RFU) for FAM and HEX are displayed, these are used to make a call on the SNP(s) present in each sample.
2. This can be compared to the controls for identification of genotype or species.

Example output of allelic discrimination:

Y axis: wild type, X axis: mutant