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Kompetitive Allele Specific PCR (KASP) with BioRad Software

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Protocol status: Working
We use this protocol and it's working

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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.

Attachments

DOCX

590-1238.docx

38KB

Materials

Materials

- KASP master mix
- primer mix

Primer Design

1 Design regular primers over the mutation you wish to test.

Note

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*

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Note

Order these sequences through your primer manager.

	A	B
	Bs_IAA16_S_FP(Hex)	GAAGGTCGGAGTCAACGGATT TGTTCTTCAGGACACAAGTTGTAGG
	Bs_IAA16_R_FP(Fam)	GAAGGTGACCAAGTTCATGCT TGTTCTTCAGGACACAAGTTGTAAA
	Bs_IAA16_RP	AGTTTGATCATCGGACGTCTTCTT

Remove 2x KASP master mix from freezer, place On ice (LGC Genomics, Beverly, MA, USA).

- This volume of 432 µL contains KASP enzyme, buffer, cofactors, dNTPs.

4 Thaw primers; one reverse primer, two forward primers, each specific to a SNP and corresponding fluorophore.

5 Make primer mix:

	A	B
	EACH forward primer	18 µl
	Reverse primer	45 µl
	H2O	69 µl
	Total primer mix	150

6 Add 12 µL of primer mix to the 2x KASP tube, this now the KASP master mix.

7 Add 4 µL of KASP master mix to each sample well of a 96 well plate.

8 Add 4 µL of template DNA @ 5 ng/µl - 20 ng/µl .

9 Include 3 NTCs, as well as appropriate controls.

Bio-Rad machine and software

10 Set up thermocycling conditions according to the protocol:

	A	B	C
	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)

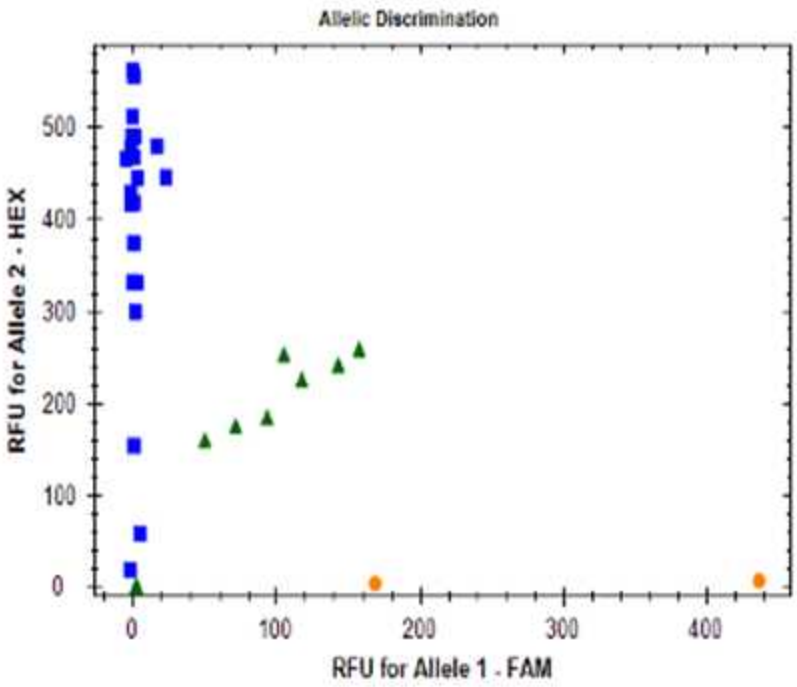
11 Make sure that each well of the plate has both FAM and HEX fluorophores selected.

12 Click Start Run.

Analysis

13 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.

14 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