PCR: KENOBI (amplifluor)

Amplifluor: Yes

Primers

Nucleotide sequence (5` --> 3`)

Primer	Sequence
Kenobi-1	GAAGGTGACCAAGTTCATGCTTGCAGGAACCCTAATATCACATGGTGA
Kenobi-2	GAAGGTCGGAGTCAACGGATTGCAGGAACCCTAATATCACATGGTGG
Kenobi-R	TGATGTTAGACTGAAGGCTGAA

Reagents

Reagents/Constituents	Commercial name	Stock Concentration	20x Primer mix concentration (µM)	Volume (µL)
DNA Sample				2.0
10x Buffer	10x Reaction Mix S Plus			1.0
dNTPs		2.5 mM		0.8
20x amplifluor SNP FAM Primer				
20x amplifluor SNP JOE Primer				
Primer: **Kenobi-1**			1.5	
Primer: **Kenobi-2**			0.5	
Primer: **Kenobi-R**			22.5	
Amplifluor primer volume				0.5
Water				3.9
Taq Polymerase	Platinum Taq (5U/µl)			0.1

Comments on protocol: DNA Sample (cleaned ear punch in dH20-see cleanup protocol).

Reactions to be done at room temperature with reagents added in order.

Final MgCl2 conc. is 2.5mM.

Reagent order: dH2O, DNTPs, 20x amplifluor SNP FAM Primer, 20x amplifluor SNP JOE Primer, 20x Kenobi specific primer mix, 10x Reaction Mix S Plus, 10mM MgCl, Platinum Taq (5u/ul), DNA sample.

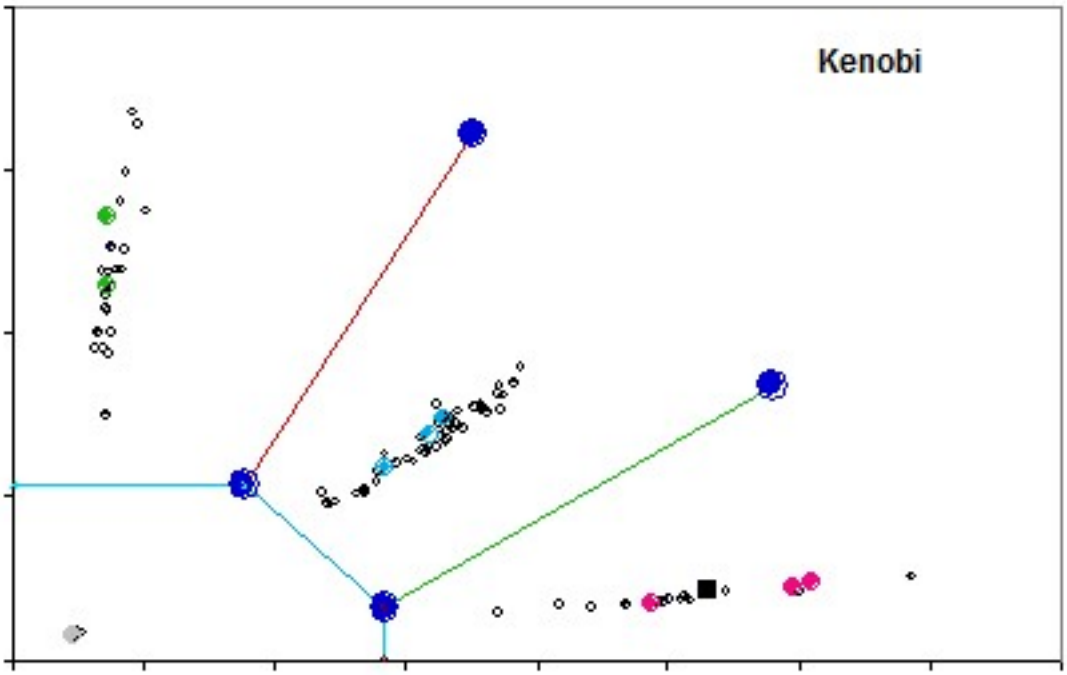
Strategy

Steps	Temp (°C)	Time (seconds)
Hot start: **Yes**		
Initiation/Melting	94.0	240
Denaturation	94.0	10
Annealing	60.0	20
Elongation	72.0	40
Number of cycles (repeat denature, anneal & elgonate): **30**		
Strand completion (i.e. 72°C, 10 min)	72.0	180
Finish (i.e. 4°C, indefinite)	10.0	Hold

Electrophoresis

Running buffer:	
% Agarose:	
Volt:	
Estimated running time (min):	

Image



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