PCR: Theoden (amplifluor)

Amplifluor: Yes

Primers

Nucleotide sequence (5` --> 3`)

Primer	Sequence
Theoden-1	GAAGGTCGGAGTCAACGGATTGATTCTCCTGGCTGTGAACACTA
Theoden-2	GAAGGTGACCAAGTTCATGCTATTCTCCTGGCTGTGAACACTG
Theoden-R	TCAGAAGGCAGGATGTTCCAT

Reagents

Reagents/Constituents	Commercial name	Stock Concentration	20x Primer mix concentration (µM)	Volume (µL)
DNA Sample				0.7
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: **Theoden-1**			0.5	
Primer: **Theoden-2**			0.5	
Primer: **Theoden-R**			7.5	
Amplifluor primer volume				1.5
Water				4.95
Taq Polymerase	Platinum Taq (5U/µl)			0.05

Comments on protocol: DNA Sample (ear punch).

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

Final MgCl2 conc. is 2.5mM

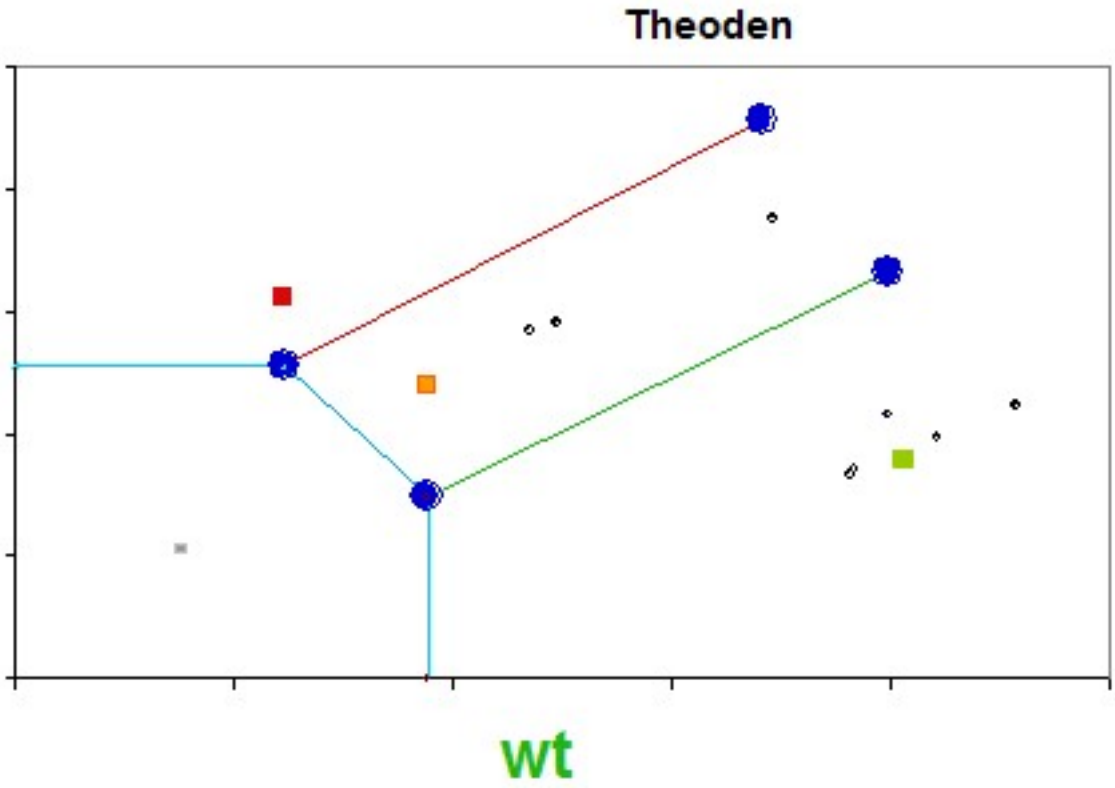
Strategy

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): **35**		
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


Theoden
wt