

FESA genotyping  
**Stock**

**Grancalcin**  
Gca <tm1Roes>

PCR Protocol

9/11/06

FESA code BQA EMMA No. 1194

Protocol number 10

Comment

PCR reaction Reagent Concentration Volume per reaction (ul)

Buffer Qiagen Master mix 2 X 6

Primer1 m285' 20uM 0.5

Primer2 neo 3633r 20uM 0.25

Primer3 R3a 20uM 0.75

MgCl2 (to achieve final concentration 2.0 mM) 25mM 0.25

H2O 4.25

Total Volume 

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12

Add 1ul of DNA per reaction

PCR programme name GCA bk1/p154

Cycling parameters

STEP number	Objective	Temp oC	Time
1	Initial Denaturation	94	2 min
2	Denaturation	94	30 sec
3	Annealing	65	30 sec
4	Extension	74	1 min
5 = 2 to 4	Amplification	x cycles	38
6	Final Extension	74	1 min
7	Hold at	4	forever
8	End		

PCR product size Wild Type 'Mutant' Gel  
600 bp 1000 bp 2% Agarose in 1 x TBE

Primer sequences (5'- 3')

m285 GCA CAT TTC ACC TAC CCC GAG TC

neo 3633r TGC GTG CAA TCC ATC TTG TTC AAT GG

R3a CCA ACA TGG CAA TCA TAA TTC TGC