

FESA genotyping
Stock

PCR Protocol
**CG knockout, CG -/-, Cathepsin G mutant,
Ctsg-/-**

FESA code BEX EMMA no. 52

Protocol number 2

Comment

PCR reaction final [Mg] = 1.5mM	Reagent	Concentration	Volume per reaction (ul)
Buffer	Qiagen Master mix	2 X	6
Primer1	CGwt rev	20uM	0.5
Primer2	CG6s	20uM	0.5
Primer3	CG7as	20uM	0.5
MgCl2 (to achieve final concentration 1.5 mM)		25mM	0
H2O			4.5
Total Volume			12

Add 1ul of DNA per reaction

PCR programme name CATH bk 1/p38

Cycling parameters

STEP number	Objective	Temp oC	Time
1	Initial Denaturation	94	1 min
2	Denaturation	94	30 sec
3	Annealing	55	30 sec
4	Extension	72	30 sec
5 = 2 to 4	Amplification	x 30 cycles	
6	Final Extension	72	1 min
7	Hold at	4	forever
8	End		

PCR product size Wild Type 'Mutant' Gel
330 bp 442 bp 2.5% Agarose in 1 x TBE

Primer sequences (5'- 3')

CGwt rev TTA GGA CGA AGT CTT CTC GC

CG6s GTG ACA AAG GTC TGG GAA GGA GAC G

CG7as CAG GAT TGA AGG GAT GGA GAC CTG