

FESA genotyping
Stock

PCR Protocol
GE ko, GE knockout, GE -/-, GE cre
Granulocyte elastase 2 knockout, Ela2^{tm1(cre)Roes},

FESA code BGD EMMA No. 75

Protocol number 3

Comment

PCR reaction final [Mg] = 2mM

Reagent	Concentration	Volume per reaction (ul)
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Buffer	Qiagen Master mix	2 X	6
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Primer1	Mel135F	20uM	0.5
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Primer2	Mel684R	20uM	0.5
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Primer3	CreI _x	20uM	0.125
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MgCl ₂ (to achieve final concentration 2mM)	25mM	0.25
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H ₂ O	4.625
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Total Volume	12
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Add 1ul of DNA per reaction

PCR programme name GE-KO Book1/p51

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 31 cycles	
6	Final Extension	74	1 min
7	Hold at		4 forever
8	End		

PCR product size	Wild Type	'Mutant'	Gel
	615 bp	185 bp	2.5% Agarose in 1 x TBE

Primer sequences (5'- 3')

Mel135F CAT GAC ACC CCC ACT GTC GTG TCC

Mel684R TGG CAC CAC AGA AAT GAC CTC CAC

CreI_x TTT GGT GCA CGG TCA GCA GAT TGG