

FESA genotyping
Stock

TbRIIfI

PCR Protocol

date 25/4/06

FESA code	BFC	EMMA No.	174
Protocol number	4		
Comment	weak bands		

PCR reaction final [Mg] = 2 mM	Reagent	Concentration	Volume per reaction (ul)
Buffer	Qiagen Master mix	2 X	6
Primer1	e2f	20 uM	0.5
Primer2	e3r	20 uM	0.5
MgCl2 (to achieve final concentration 2mM)		25mM	0.25
H2O			4.5
Total Volume			12

Add 1ul of DNA per reaction

PCR programme name	TBRIIFL	ref	Book1/p51
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Cycling parameters
STEP number

Objective	Temp 0C	Time
Initial Denaturation	94	2 min
Denaturation	94	30 sec
Annealing	63	30 sec
Extension	72	2 min
Amplification	x cycles	28
Final Extension	72	2 min
Hold at	4	forever
End		

PCR product size	Wild Type	'Mutant'	Gel
	1.5 kb	1.75 kb	0.9% Agarose in 1 x TBE

Primer sequences (5'-3')

e2f	CAT GAA GTC TGC GTG GCC GTG TG
e3r	TGT AAT CGT TGC ACT CTT CCA TGT