

FESA genotyping
Stock

PCR Protocol

Date 25/4/06

Talin ko, Tln1<hyg/+>/C57

Tln1<Tm1ULB>

FESA code BBX EMMA No. 185

Protocol number 1

Comment homozygotes embryonic lethal 7.5dpc

PCR reaction final[Mg] = 1.5mM

Reagent	Concentration	Volume per reaction (ul)
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Buffer	Qiagen Master mix	2 X	6
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Primer1	5'TD2	20uM	1
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Primer2	pgk3	20uM	0.5
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Primer3	Tex2b	20uM	0.5
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H2O			4
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Total Volume			12
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Add 1ul of DNA per reaction

PCR programme name TALIN book 1/p2

Cycling parameters

STEP number	Objective	Temp oC	Time
1	Initial Denaturation	94	2 min
2	Denaturation	94	15 sec
3	Annealing	60	30 sec
4	Extension	72	1 min
5 = 2 to 4	Amplification	x 35 cycles	
6	Hold at	4	forever
7	End		

PCR product size	Wild Type	'Mutant'	Gel
	361 bp	446 bp	2.5% Agarose in 1 x TBE

Primer sequences (5'- 3')

5'TD2 5' GCA TTG GGA ATG TGG TGA AGA CG

pgk3 3' WT GAA AGA GCC CAA AGT CGT TGG

pgk3 3'Hyg CCA GAA AGC GAA GGA GCA AAG C