

FESA genotyping  
Stock

p47 phox

PCR Protocol

FESA code BHZ EMMA No. 255  
 protocol number 6  
 Comment Two separate PCRs need to be run simultaneously.  
 One for Wild Type and one for 'Mutant'

**MUTANT' = B**

PCR reaction	Reagent	Concentration	Volume per reaction (ul)
Buffer	Qiagen Master mix		6
Primer1	p47	20 uM	0.5
Primer2	neo3633R	20uM	0.5
MgCl2 (to achieve final conc 2mM)		25 mM	0.25
H2O			4.75
Total Volume			<u>12</u>

Add 1ul of DNA per reaction

PCR programme name PHOXKOMU

Cycling parameters

STEP number	Objective	Temp 0C	Time
1	Initial Denaturation	94	2 min
2	Denaturation	94	30 sec
3	Annealing	65	30 sec
4	Extension	74	90 sec
2 to 4	Amplification	x cycles	38
5	Hold at	4	forever

PCR product size Mutant' Gel  
 1200 bp 2 % Agarose in 1 x TBE

Primer sequences (5'-3')

p47 ATTCACCGAGATCTACGAGTTCCATGT  
 neo3633R TGCGTGCAATCCATCTTGTTCAATGG

**WILD TYPE = A**

PCR reaction	Reagent	Concentration	Volume per reaction (ul)
Buffer	Qiagen Master mix		6
Primer1	p47TAS2	20 uM	0.5
Primer2	p47TS2	20uM	0.5
MgCl2 (to achieve final conc 2.0mM)		25 mM	0.25
H2O			4.75
Total Volume			<u>12</u>

Add 1ul of DNA per reaction

PCR programme name PHOXKOWT

Cycling parameters

STEP number	Objective	Temp 0C	Time
1	Initial Denaturation	94	2 min
2	Denaturation	94	30 sec
3	Annealing	65	30 sec
4	Extension	74	30 sec
2 to 4	Amplification	x cycles	38
5	Hold at	4	forever

PCR product size Wild type Gel  
 400 bp 2 % Agarose in 1 x TBE

Primer sequences (5'-3')

p47TAS2 GGCAGTTTCAGGTCATCAGGCCGC  
 p47TS2 GGCCGGCGAGATCCACACAGAGAAC