FESA genotyping PCR Protocol

Stock G6pdx<a-m1Neu>

FESA code NE

Protocol number 12

Comment PCR followed by Restriction enzyme digest of product. ReddyMixPCR master mix (2.5mM MgCl2) is a 1.1 x mix, obtained from Abgene. This includes the *Taq* DNA polymerase plus loading buffer and dye for running on the 3% gel.

ref. Nichol C et al (2000) An embryo protective role for glucose-6-phosphate dehydrogenase in developmental oxidative stress and chemical teratogenesis. The FESAB Journal 14:111-127.

PCR reaction	Reagent	Concentration		Volume per reaction (ul)
Buffer	**ReddyMix PCR Master mix. See comments above**	1.1X		24
Primer1	G6PD sense	20 uM		0.45
Primer2	G6PD antisense	20 uM		0.45
MgCl2 (to achieve final concentration 2.5 mM)				0
H2O				0
Total Volume				26.9

Add 2ul of DNA (30-40ng/ul) per reaction

PCR programme name G6PD

Cycling parameters			
STEP number	**Objective**	**Temp oC**	**Time**

1	Initial Denaturation	94	2 min
2	Denaturation	94	20 sec
3	Annealing	58	20 sec
4	Extension	72	30 sec
5 = 2 to 4	Amplification	x cycles	35 total
6	Final Extension	72	5min
7	Hold at	4	forever
8	End		

PCR product size

Wild Type	'Mutant'	Gel
269bp same as 'Mutant'	269bp	3% Agarose in 1 x TBE buffer

After amplification of PCR products run 7 ul of the reaction mix on a 3% gel to confirm size, then digest with Dde1 see below.

Primer sequences (5'- 3')

G6PD sense	GGAAACTGGCTGTGCGCTAC	20 mer
G6PD antisense	TCAGCTCCGGCTCTCTTCTG	20 mer

Digest of PCR product with Dde1 restriction enzyme

Constituents	**Reagent**	**Concentration**		**Volume per reaction (ul)**
PCR product mix	PCR reaction mix after amplification			5
Enzyme	Dde1	10U/ul		1
Buffer	NE buffer 3(supplied with enzyme)	10X		1
H2O				4
Total volume				11

Incubate at 37oC for 1 hour

Enzyme purchased form New England BioLabs. 500 Units (10,000 U/ml) supplied with some 10X NE buffer 3.

To prove the presence of homozygous females (or hemizygous males?) the digestion of controls need to be complete so that no undigested 296bp band remains.

Ideally run a heterozygous female sample if available. Suitable controls in this instance were 101/H, C3H/HeH and the hybrid of these two 3H1. Presumably a heterozygous female will have all three bands, but there must be complete digestion in the controls (2 bands).

PCR product size after digestion	Wild Type	'Mutant'	Gel	
Run 10 ul of digest on gel	both 214bp and 55bp	269bp, = undigested for homozygotes	3% Agarose in 1 x TBE buffer	