

Appendix

Genomic DNA extraction

Label sterile tubes; one tube for each sample.

Add 380 μ l extraction buffer to each sterile tube.

Extraction buffer consisted of the following reagents: 30 ml 10X TNE, 30 ml 1M Tris pH8, 270 ml dH₂O, 20 ml 10% SDS (OR 8 ml 25% SDS). Refrigeration not necessary.

Add a sample to each tube.

Add 20 μ l Proteinase K to each tube.

Vortex each tube.

Place tubes in a waterbath at 55 °C for 3 hours – overnight.

Vortex occasionally during this period.

Picofuge each sample.

Transfer the liquid to labelled sterile tubes.

Add 200 μ l 5M NaCl to each tube.

Vortex each tube.

Shake vigorously for approximately 20 minutes.

Add 600 μ l IAC (Isoamyl alcohol:Chloroform = 1:24) in a fume cupboard to each tube.

Vortex each tube.

Shake for a further 10 minutes.

Vortex each tube.

Centrifuge at 4000 rpm for 10 minutes.

Transfer the supernatant to labelled sterile tubes (a volume of approximately 400 μ l) in a fume cupboard.

Add 650 μ l Isopropanol (100 % propan-2-ol) to each tube.

Invert slowly twice, faster 3 times and again slowly twice. DNA may be visible at this stage.

Centrifuge at 12000 rpm for 10 minutes.

Pour off supernatant from each tube.

Add 1 ml cold 70% ethanol to each tube.

Flick each tube to dislodge the DNA pellet.

Invert each tube.

Centrifuge at 12000 rpm for a further 5 minutes.

Pour off the ethanol.

Picofuge each tube.

Remove the remaining ethanol with a micropipette.

Dry each tube (lids off) on a hot plate at 55 °C for 10 – 15 minutes or as soon as dry.

Add 150 µl dH₂O to each tube.

Flick each tube to resuspend the extracted DNA.

Picofuge each tube.

Freeze each tube.

1% Agarose Gel

0.8 g Agarose I

80 ml 0.5X TBE

Once the gel had been poured and had set, the DNA was heated at 55 °C for 3 minutes.

Five microlitres of 1X dye were added to each well of a microtitre plate lid, along with 2 µl of sample DNA. The gel was run at 125 V for approximately 30 minutes. Once rinsed in water, the gel was placed in ethidium bromide staining medium and quietly shaken for 30 minutes. After another rinse, the gel was placed above UV light and a photograph was taken of the florescent part of the gel.

Table 5: Information related to the samples that were used in the present study.

Colony	Ind ID	Sex	Status*	EF12	DMR3	DMR4	DMR5	DMR7	NCAM
L03	1	F	O	CC	HQ	JJ	BF	BC	AA
	2	F	O	CC	HK	JJ	BF	BC	AB
	3	M	O	AC	HH	JJ	BE	BB	AA
	4	F	R	CC	HQ	CJ	EF	BB	AB
	5	F	O	CC	ND	JJ	EF	BC	AB
	6	F	O	CC	HQ	CJ	BE	BC	AA
	7	F	O	AC	HH	CJ	BE	BC	AA
	8	F	O	AC	HH	JJ	FF	BB	AA
	9	F	O	CC	HH	JJ	BF	BC	AB
	10	M	O	CC	GQ	JJ	BE	BC	AB
	11	F	O	CC	GQ	CJ	BE	BC	AB
	12	M	O	CC	HH	JJ	FF	BB	AA
L01	1	F	O	CE	CC	FH	BE	EE	CD
	2	M	O	CE	CC	CK	BE	BE	DD
	3	F	R	EE	CC	HK	BB	BE	DD
	4	F	O	DE	CC	FK	BB	EE	DD
	5	F	O	CE	CC	CK	BG	BE	DD
	6	F	ND	CC	CC	CH	BG	BE	DD
	7	M	ND	CD	CC	FH	BE	EE	DD
	8	M	O	DE	CC	FK	BG	EE	DD
GRP	1	F	O	BC	ND	DJ	BE	DF	DD
	2	F	R	BC	ND	DH	AB	DF	DD
	3	F	O	BB	HM	HJ	AB	BD	AD
	4	M	O	BC	BH	DJ	AA	DF	AD
	5	M	O	BC	BH	DH	AE	BD	DD
	6	M	O	BC	BH	BD	AE	BF	CD
	7	M	O	BB	HH	BH	AA	BD	CD
	8	ND	ND	BB	BM	BJ	AB	BD	DD
	9	ND	O	BC	ND	DJ	AB	BD	DD
	10	M	O	BD	JP	DF	BD	GG	AC
GAS	1	M	O	BB	JJ	DF	BB	AH	AC
	2	M	O	BD	JP	FK	BB	CG	AC
	3	F	O	BD	FP	DF	BB	AG	AC
	4	F	O	BB	JJ	DF	BD	CG	AC
	5	F	O	BB	FJ	DF	BB	CG	AC
	6	F	O	BB	JJ	DF	BD	AG	AC
	7	F	R	BD	FJ	DK	BD	AG	AA
	8	ND	ND	EF	JL	CG	BB	BC	CC
	9	ND	ND	BD	JJ	GL	BD	AG	AC
	10	ND	O	BB	FP	ND	BB	AC	AC
G42S	1	M	O	CD	EN	EF	DH	BC	CC
	2	M	O	CE	AE	EE	CD	BC	AC
	3	F	ND	CE	AD	CE	BC	BB	AA
	4	F	ND	DE	AE	EF	BH	BB	AC
	5	F	R	CC	CE	CE	CH	BC	AC
	6	ND	O	CC	CO	CE	CH	BB	AC

* O = offspring of reproductive female, R = reproductive individual, ND = not determined.

Table 6: Key (given by locus) to the codes used for allele sizes in the preceding text.

EF12		DMR3		DMR4		DMR5		DMR7		NCAM	
Code	Allele Size (bp)	Code	Allele Size (bp)	Code	Allele Size (bp)	Code	Allele Size (bp)	Code	Allele Size (bp)	Code	Allele Size (bp)
A	121	A	902	A	195	A	248	A	132	A	238
B	123	B	912	B	203	B	250	B	134	B	244
C	125	C	914	C	205	C	252	C	136	C	248
D	127	D	916	D	209	D	254	D	138	D	250
E	135	E	918	E	213	E	256	E	140	E	
F	139	F	920	F	215	F	258	F	142		
		G	922	G	217	G	260	G	148		
		H	924	H	219	H	268	H	152		
		J	926	J	221						
		K	928	K	227						
		L	930	L	229						
		M	934								
		N	936								
		O	938								
		P	946								
		Q	950								