

GENOTYPING BY PCR PROTOCOL
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 530-754-MMRRC

NAME OF PCR: STOCK *Maib*^{m1Gsb}/Imucd, (*Kr*^{ENU}) MMRRC # 000022-UCD

Protocol:

Reagent/ Constituent	Volume (μL)
Water	34.3
10x Buffer (without 15mM MgCl ₂)	2.5
MgCl ₂ (stock concentration is 25mM)	1.7
dNTPs (stock concentration is 10mM)	0.5
Primer 1 (stock concentration is 10μM)	3.0
Primer 2 (stock concentration is 10μM)	3.0
Taq Polymerase	1.0
DNA Sample	4.0
TOTAL VOLUME OF REACTION:	50μL

Comments on protocol:

- Proceed to restriction enzyme digestion if a strong band is present after PCR reaction. Do not add loading dye to sample as be used later! Note-an approximately 400bp artifact may be present.
- **Digest with Earl Enzyme:** use 27μl of PCR product with 7μl of water, 4μl of appropriate 10x buffer, and 2 μl of Earl enzyme. Incubate at 37 °C for 2 hours and run on high-resolution agarose gel.

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	94	2:00	1
2. Denaturation	94	0:45	} 30x
3. Annealing	56	0:45	
4. Elongation	72	1:00	
5. Amplification	72	10:00	1
6. Finish	4	∞	n/a

Primers:

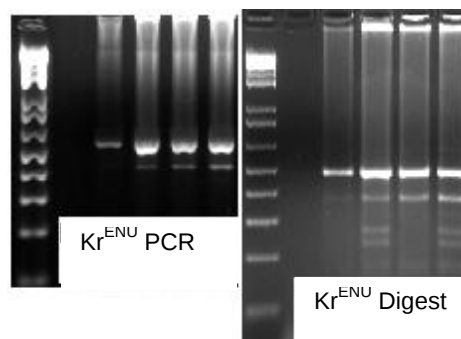
Primer Name	Nucleotide Sequence (5' - 3')
1: <i>Kr</i> ^{ENU} Up	CCG CCG TCC AGC GCT GTC GCC AGT C
2: <i>Kr</i> ^{ENU} Low	GCC GGA CCC GCC AGG ACT

Electrophoresis Protocol:

% Agarose: 2 mV: 80

Estimated Running Time (min): 90

Expected Bands	Genotype
513 bp	<i>Kr</i> ^{ENU} PCR
513 bp	Wild-type, PCR and digest
253 / 260 bp	Digested <i>Kr</i> ^{ENU}



Kr^{ENU} PCR : 1kb+ ladder, H₂O, B6, het control, 2 sample lanes
Kr^{ENU} Digest (of this PCR): 1kb+ ladder, H₂O, B6, het control, wild-type sample, het sample