GENOTYPING BY PCR PROTOCOL

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NAME OF PCR: **Sanger MirKO ES cell line Mir15b/Mir16-2 cluster 3N1** **MMRRC #** **036335-UCD**

Protocol: SequelPrep long PCR kit, Invitrogen A10498

Reagent/ Constituent	Volume (µL)
Sterile H_20	13.24
10X Buffer (green tube)	2.0
Enhancer A (Red tube)	1.0
Enhancer B (Yellow tube)	1.0
DMSO (brown tube)	0.2
Gene specific primer (10uM)	0.5
Universal primer (10uM)	0.5
Enzyme (black tube)	0.36
DNA extracted with ☐ NaOH ☒ Proteinase K ☒ Other: Qiagen DNEasy	1.0
TOTAL VOLUME OF REACTION:	**20µL**

Comments on protocol:

- May decrease Elongation time for shorter PCR fragments per manufacturer's suggestion i.e. 1 minute/kb of sequence.

Strategy:

Steps		Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting	HOT START?	93	3:00	**1**
2. Denaturation	steps 2-3-4 will cycle in sequence	93	0:15	**8x**
3. Annealing		68 to 60 (↓1°C/cycle)	0:30	
4. Elongation		68	9:00	
5. Denaturation	steps 5-6-7 will cycle in sequence	93	0:15	**32x**
6. Annealing		60	0:30	
7. Elongation		68	9:00 (↑20sec/cycle)	
8. Finish		4	∞	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')
1: 5' common rev	ATAGCATACATTATACGAAGTTATCACTGG
2: 5' gene specific (LR1)	AGTGTCACTTACACAAAATAGTTCAAAAAT
3: 3' common fwd	TCTAGAAAGTATAGGAACTTCCATGGTC
4: 3' gene specific (LR4)	TGACAACCTAGAACTTTTCTTGTAGTTTTA

Electrophoresis Protocol:

% Agarose: 0.8 **V:** 90

Estimated Running Time (min): 90

Primer Combination	Band (bp)	Genotype
1 and 2 (5')		Targeted
3 and 4 (3')		Targeted

Note: the gene specific primers in this protocol have been provided by Wellcome Trust Sanger Institute. Expected band size not provided.