GENOTYPING BY PCR PROTOCOL

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NAME OF PCR: **Sanger MirKO ES cell line MiR342 LRPCR** **MMRRC #** **036582-UCD**

Protocol: ***PCR protocol provided by Donating Investigator***

Reagent/ Constituent	Volume (µL)
Sterile H_20	13.44
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 (10µM)	0.5
Universal Primer (10µM)	0.5
Enzyme (black tube)	0.36
DNA extracted with ☐ NaOH ☒ Proteinase K ☒ Other: Qiagen DNEasy	1.0
TOTAL VOLUME OF REACTION:	**20.00µL**

Comments on protocol:

- SequelPrep Long PCR Kit, Invitrogen A10498
- 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 cycle in sequence	93	0:15	} **8x**
3. Annealing		68 to 60 (↓1°C/cycle)	0:30	
4. Elongation		68	9:00	
5. Denaturation		93	0:15	
6. Annealing	} steps 5-6-7 cycle in sequence	60	0:30	} **32x**
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)	AGCTCACTTACATTTTATTTATTTTCTCCT
3. 3' common fwd	TCTAGAAAGTATAGGAACTTCCATGGTC
4. 3' gene specific (LR4)	GTAGGCAAGAAGACATAATACAGAAAAG

Electrophoresis Protocol:

Agarose: 0.8% **V:** 90

Estimated Running Time (min): 90

Primer Combination	Band (bp)	Genotype
1 and 2 (5')	5,739 bp	Targeted
3 and 4 (3')	3,232 bp	Targeted

Note: the gene specific primers in this protocol have been provided by Wellcome Trust Sanger Institute.