

GENOTYPING BY PCR PROTOCOL
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NAME OF PCR: Sanger MirKO ES cell line Mir490, LRPCR **MMRRC #** 036376-UCD

Protocol: SequelPrep Long PCR Kit, Invitrogen A10498

Reagent/ Constituent	Volume (μL)
Sterile H ₂ O	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:

- 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	93	0:15	} 8x
3. Annealing } steps 2-3-4 cycle in sequence	68 to 60 (↓1°C/cycle)	0:30	
4. Elongation	68	9:00	
5. Denaturation	93	0:15	
6. Annealing	60	0:30	} 32x
7. Elongation } steps 5-6-7 cycle in sequence	68	9:00 (↑20sec/cycle)	
8. Finish	4	∞	

Primers:

Name	Nucleotide Sequence (5' - 3')
1. 5' common rev	ATAGCATACATTATACGAAGTTATCACTGG
2. 5' gene specific (LR1)	AAATTTCACTTACTAGGGTTTAAGTATTCTG
3. 3' common fwd	TCTAGAAAGTATAGGAAGTCCATGGTC
4. 3' gene specific (LR4)	TAAGTATTAAAACCACTTACCAGAAGTTGA

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.