GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS
2795 2nd Street, Suite 400, Davis, CA 95618
mmrrc@ucdavis.edu
530-754-MMRRC

NAME OF PCR: **Sanger MirKO ES cell line Mir453, LRPCR** **MMRRC #** **036374-UCD**

Protocol: SequelPrep Long PCR Kit, Invitrogen A10498

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:

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

Primers:

Name	Nucleotide Sequence (5' - 3')
1. 5' common rev	ATAGCATACATTATACGAAGTTATCACTGG
2. 5' gene specific (LR1)	ATAGACTTCCATTTTGTTTCTAAATTGACC
3. 3' common fwd	TCTAGAAAGTATAGGAACTTCCATGGTC
4. 3' gene specific (LR4)	GGCCATGTAATACTCATCATAAATAAAAT

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.

**Targeting confirmation at the 3' end may not have been successful with these primers; primers will be redesigned if genetic analysis is requested with the associated clones. Please contact mmrrc@ucdavis.edu if you have questions or concerns.