

# GENOTYPING BY PCR PROTOCOL

## MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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530-754-MMRRC

**NAME OF PCR:** Sanger MirKO ES cell line Mir721, LRPCR **MMRRC #** 036388-UCD

**Protocol:** SequelPrep Long PCR Kit, Invitrogen A10498

Reagent/ Constituent	Volume (µL)
Sterile H <sub>2</sub> 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 <input type="checkbox"/> NaOH <input checked="" type="checkbox"/> Proteinase K <input checked="" type="checkbox"/> Other: Qiagen DNEasy	1.0
<b>TOTAL VOLUME OF REACTION:</b>	<b>20.00µL</b>

### 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 <span style="float: right;">HOT START? <input checked="" type="checkbox"/></span>	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)	TAAATGTTTATATTAAGTGCTGCTAGAGG
3. 3' common fwd	TCTAGAAAGTATAGGAACTTCCATGGTC
4. 3' gene specific (LR4)	GAGCTATTATATATCCTTAGCCCTCTTTCT

### 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.