GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

mmrrc@ucdavis.edu

530-754-MMRRC

NAME OF PCR: **Keck MirKO ES cell line Mirc26** (miR-30b/ miR-30d) **MMRRC #** **036834-UCD**

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

Reagent/ Constituent	Volume (µL)
Sterile H_20	14.6
10X Buffer	2.5
dNTPs (stock concentration is 10mM)	0.5
DMSO	1.2
Primer 1 (stock concentration is 10µM) F-pr	1.3
Primer 2 (stock concentration is 10µM) R-pr	1.3
Primer 3 (stock concentration is 10µM) Universal primer	1.3
Taq Polymerase	0.3
DNA extracted w/ ☐ NaOH ☒ Proteinase K ☒ Other: Qiagen DNEasy	2.0
TOTAL VOLUME OF REACTION:	**25.00µL**

Comments on protocol:

.

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. F	GTGCCTTACAGCCCTTTCTTTA
2. R	ACAAAGGAACAGAGCAAATGGT
3. Reverse Universal	GTGGTATCGTTATGCGCCTT

Electrophoresis Protocol:

Agarose: 1% **V:** 90

Estimated Running Time (min): 90

Genotype	Expected Band
Wild-type	2405 bp
Mutant	1973 bp

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NAME OF PCR: **Keck MirKO ES cell line Mirc26** (miR-30b/ miR-30d) **MMRRC #** **036834-UCD**

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

Reagent/Constituent	Volume (µL)
Water	16.0
10x Buffer	2.5
dNTPs (stock concentration is 10mM)	0.5
DMSO	1.2
Primer 1 (stock concentration is 10µM) F-pr	1.3
Primer 2 (stock concentration is 10µM) R-pr	1.3
Taq Polymerase	0.2
DNA (50-200ng/ µL) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	2.0
TOTAL VOLUME OF REACTION:	**25.00 µL**

Comments on protocol:

Strategy:

Steps	Temp (^{o}C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	94	5:00	**1**
2. Denaturation	94	0:15	
3. Annealing steps 2-3-4 cycle in sequence	65 to 55 (↓1^{o}C/cycle)	0:30	**40x**
4. Elongation	72	0:40	
5. Amplification	72	5:00	**1**
6. Finish	15	∞	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')
1. F	TCCTGGTCTCCACTTCTTTGTC
2. R	GATCAACCTGTCTGCCTGGTA

Electrophoresis Protocol:

Agarose: 1% **V:** 90

Estimated Running Time: 90 **min.**

Genotype	Expected Band
Wild-type	212 bp
Mutant	304 bp