

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 Mir325 (miR-325) MMRRC # 036858-UCD

Protocol: **FRT**

PCR protocol provided by Donating Investigator

Reagent/ Constituent	Volume (μL)
Sterile H ₂ O	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	} 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	} 32x
6. Annealing } steps 5-6-7 cycle in sequence	60	0:30	
7. Elongation	68	9:00 (↑20sec/cycle)	
8. Finish	4	∞	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')
1. F	TTCATTGAAATAAAGCAGATGACA
2. R	AAACCTTCTCTCTTTGTGAATGC
3. Reverse Universal	GTGGTATCGTTATGCGCCTT

Electrophoresis Protocol:

Agarose: 1% V: 90

Estimated Running Time (min): 90

Genotype	Expected Band
Wild-type	2071 bp
Mutant	1720 bp

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530-754-MMRRC

NAME OF PCR: Keck MirKO ES cell line Mir325 (miR-325) **MMRRC #** 036858-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 (°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°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	GAGTAAGCAAAGGGCAGTGATT
2. R	AACTACCACACCTATGCCATCC

Electrophoresis Protocol:

Agarose: 1% **V:** 90

Estimated Running Time: 90 min.

Genotype	Expected Band
Wild-type	205 bp
Mutant	297 bp