

**GENOTYPING BY PCR PROTOCOL  
MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS**

[mmrrc@ucdavis.edu](mailto:mmrrc@ucdavis.edu)

530-754-MMRRC

**NAME OF PCR:** Keck MirKO ES cell line Mir34a (miR-34a) **MMRRC #** 036876-UCD

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

Reagent/ Constituent	Volume (µL)
Sterile H <sub>2</sub> 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/ <input type="checkbox"/> NaOH <input checked="" type="checkbox"/> Proteinase K <input checked="" type="checkbox"/> Other: Qiagen DNEasy	2.0
<b>TOTAL VOLUME OF REACTION:</b>	<b>25.00µL</b>

**Comments on protocol:**

**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	} <b>8x</b>
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	} <b>32x</b>
7. Elongation } steps 5-6-7 cycle in sequence	68	9:00 (↑20sec/cycle)	
8. Finish	4	∞	

**Primers:**

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

**Electrophoresis Protocol:**

**Agarose:** 1% **V:** 90

**Estimated Running Time (min):** 90

Genotype	Expected Band
Wild-type	2093 bp
Mutant	1793 bp

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530-754-MMRRC

**NAME OF PCR:** Keck MirKO ES cell line Mir34a (miR-34a) **MMRRC #** 036876-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
<b>TOTAL VOLUME OF REACTION:</b>	<b>25.00 µL</b>

**Comments on protocol:**

**Strategy:**

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting <span style="float:right">HOT START? <input type="checkbox"/></span>	94	5:00	1
2. Denaturation	94	0:15	
3. Annealing <span style="float:right">steps 2-3-4 cycle in sequence</span>	65 to 55 (↓1°C/cycle)	0:30	<b>40x</b>
4. Elongation	72	0:40	
5. Amplification	72	5:00	1
6. Finish	15	∞	n/a

**Primers:**

Name	Nucleotide Sequence (5' - 3')
1. F	GGGTACTAATTACAGCATGGAAGG
2. R	CGTGTGTCTGTCTGTCTGTAAGAA

**Electrophoresis Protocol:**

**Agarose:** 1% **V:** 90

**Estimated Running Time:** 90 min.

Genotype	Expected Band
Wild-type	384 bp
Mutant	476 bp