

GENOTYPING BY PCR PROTOCOL
MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS
mmrrc@ucdavis.edu
 530-754-MMRRC

NAME OF PCR: 129S-Hspa1a/Hspa1b^{tm1Dix}/Mmucd MMRRC # 000372-UCD

DNA Extraction Method: ☐ NaOH ☒ Proteinase K ☐ Other: _____

Protocol: Mutant

Reagent/ Constituent	Volume (μL)
Water	16.0
10x Buffer w/o MgCl ₂	2.5
25mM MgCl ₂	1.8
dNTPs (stock concentration is 10mM)	0.7
Primer 1 (stock concentration is 10μM) F12489	0.8
Primer 2 (stock concentration is 10μM) R13269	0.8
Taq Polymerase	0.4
DNA Sample	2.0
TOTAL VOLUME OF REACTION:	25μL

Comments on protocol:

Run two separate reactions for wild-type and knockout. At original anneal temp of 61°C, you will see extra bands. We recommend experimentation to find optimal MgCl₂ concentration and annealing temperature for each individual lab.

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? CHECK HERE ☐	94	2:00	1
2. Denaturation	94	1:00	} 35x
3. Annealing } steps 2-3-4 will cycle in sequence	57	0:30	
4. Elongation	72	0:30	
5. Amplification	72	7:00	1
6. Finish	25	n/a	n/a

Primers:

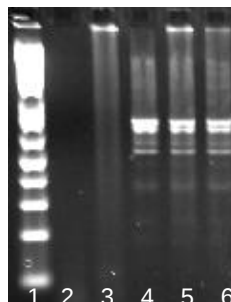
Primer Name	Nucleotide Sequence (5' - 3')
1: F12489	GAA CGG AGG ATA AAG TTA GG
2: R13269	AGT ACA CAG TGC CAA GAC G

Electrophoresis Protocol:

% Agarose: 2 mV: 80

Estimated Running Time (min): 90

Number	Band	Genotype
1	780 bp (doublet)	KO or Het
2	No band	Wild-type



Lanes:
 1. 1 Kb+ Ladder
 2. H₂O
 3. B6 wild-type +/+
 4-6. HSP KO -/-

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NAME OF PCR: 129S-Hspa1a/Hspa1b^{tm1Dix}/Mmcd MMRRC # 000372-UCDDNA Extraction Method: ☐ NaOH ☒ Proteinase K ☐ Other: _____**Protocol: Wild-type**

Reagent/ Constituent	Volume (μL)
Water	19.0
10x Buffer w/ 15mM MgCl ₂	2.5
dNTPs (stock concentration is 10mM)	0.5
Primer 1 (stock concentration is 10μM) F191	0.8
Primer 2 (stock concentration is 10μM) R644	0.8
Taq Polymerase	0.4
DNA Sample	1.0
TOTAL VOLUME OF REACTION:	25μL

Comments on protocol:**Strategy:**

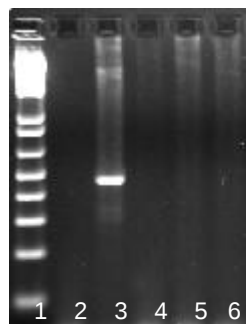
Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? CHECK HERE ☐	94	2:00	1
2. Denaturation	94	1:00	} 35x
3. Annealing	56	1:00	
4. Elongation	72	0:30	
5. Amplification	72	7:00	1
6. Finish	25	n/a	n/a

Primers:

Primer Name	Nucleotide Sequence (5' - 3')
1: F191	GTA CAC TTT AAA CTC CCT CC
2: R644	CTG CTT CTC TTG TCT TCG

Electrophoresis Protocol:% Agarose: 2 mV: 80Estimated Running Time (min): 90

Number	Band	Genotype
1	454 bp	Wild-type or HET
2	No band	KO Mutant



Lanes:
1. 1 Kb+ Ladder
2. H₂O
3. B6 wild-type +/+
4-6. HSP KO -/-