

GENOTYPING BY PCR PROTOCOL
MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS
 2795 2nd Street, Suite 400, Davis, CA 95618
mmrrc@ucdavis.edu
 530-754-MMRRC

NAME OF PCR: B6;129-*Htr3a*^{tm1Ckn}/Mmcd, **MMRRC #** 009966-UCD

Protocol: PCR Only

Reagent/ Constituent	Volume (μL)
Water	16.25
10x Buffer (without 15mM MgCl ₂)	2.5
MgCl ₂ (stock concentration is 25mM)	2.5
dNTPs (stock concentration is 10mM)	0.5
Primer 1 (stock concentration is 10μM) HTin55a	0.5
Primer 2 (stock concentration is 10μM) HTin53a	0.5
Taq Polymerase 5 Units/μL	1.25
DNA Sample	1.0
TOTAL VOLUME OF REACTION:	25μL

Comments on protocol:

This protocol can be used to differentiate between wild-type and mutants, but PCR-RFLP protocol (attached) is preferential.

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐	95	2:00	1
2. Denaturation	94	0:30	} 30x
3. Annealing	60	1:00	
4. Elongation	72	0:50	
5. Amplification	72	5:00	1
6. Finish	25	∞	n/a

Primers:

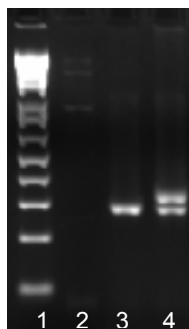
Primer Name	Nucleotide Sequence (5' - 3')
1: HTin55a	AAC TCT AAC AAA GAA ACA TAG AAG GTT GTT TGG AAG G
2: HTin53a	CAA TCA TAG AAC CTT CGA GCA TAG AAG GTG G

Electrophoresis Protocol:

% Agarose: 2 mV: 110

Estimated Running Time (min): 60

Expected Bands	Genotype
300 bp	WT +/+
300 / 340 bp	Mutant



Lanes:
 1. 1Kb+ ladder
 2. H₂O
 3. B6
 4. +/-

GENOTYPING BY PCR PROTOCOL
MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS
 2795 2nd Street, Suite 400, Davis, CA 95618
mmrrc@ucdavis.edu
 530-754-MMRRC

NAME OF PCR: B6;129-Htr3a^{tm1Ckn}/Mmcd, MMRRC # 009966-UCD

Protocol: PCR-RFLP

Reagent/ Constituent	Volume (μL)
Water	16.25
10x Buffer (without 15mM MgCl ₂)	2.5
MgCl ₂ (stock concentration is 25mM)	2.5
dNTPs (stock concentration is 10mM)	0.5
Primer 1 (stock concentration is 10μM) 800F	0.5
Primer 2 (stock concentration is 10μM) 1050R	0.5
Taq Polymerase 5 Units/μL	1.25
DNA Sample	1.0
TOTAL VOLUME OF REACTION:	25μL

Comments on protocol:

Digest 5ul of PCR product with 1ul (10 units) of DdeI restriction enzyme for a total volume of 25 ul. Run restriction product on Hi-Resolution agarose.

Strategy:

Steps	Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? CHECK HERE ☐	95	2:00	1
2. Denaturation	94	0:30	} 30-35x
3. Annealing	63	1:00	
4. Elongation	72	0:30	
5. Amplification	72	5:00	1
6. Finish	25	∞	n/a

Primers:

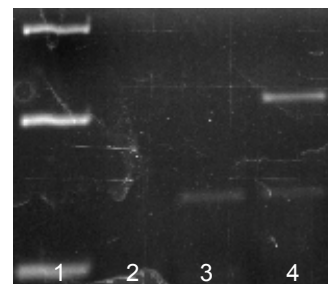
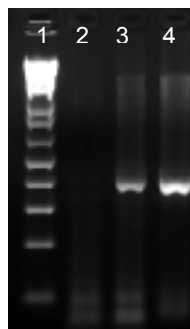
Primer Name	Nucleotide Sequence (5' - 3')
1: 800F	CCG GAG GCC TTT ATT CTA CGC AGT CAG CCT C
2: 1050R	CTG GCC GCT GTA GGT CCT GCT TAT GCA CC

Electrophoresis Protocol:

% Agarose: 2 mV: 110

Estimated Running Time (min): 60

Expected Bands	Genotype
150 bp	WT +/+
150 / 210 bp	Heterozygous +/-
360 bp	PCR Product



Lanes:
 1. 1Kb+ ladder, 2. H₂O, 3. B6, 4. +/-