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CHCHD2 T61I Mouse Genotyping

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Protocol status: Working
We use this protocol and it's working

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Disclaimer

The **protocols.io** team notes that research involving animals and humans must be conducted according to internationally-accepted standards and should always have prior approval from an Institutional Ethics Committee or Board.

Abstract

To genotype CHCHD2 T61I point mutant mice by qPCR.

Mouse Tail Lysis

1 **Materials**
- Isoflurane
- Sanitized scissors
- DirectPCR lysis reagent (mouse tail, Viagen Biotech, 102-T)
- Proteinase K – Recombinant, PCR grade (Roche, 03115844001)
- Microcentrifuge tubes
- Incubator
- Water bath

2 Anesthetize mice with isoflurane.

3 Cut no longer than 2 mm of distal tail from mice.

4 Place each tail in a sterile, labeled tube.

5 Add 200 µl of DirectPCR lysis reagent.

6 Add 10 µl of proteinase K and mix well.

7 Incubate the tails at 55°C overnight.

8 To deactivate proteinase K, incubate tails for 45 min in an 85°C water bath.

9 Store extracted DNA at -20°C until use.

qPCR Genotyping

10 **Materials**
- Kapa Probe Fast qPCR Master Mix (2X) kit (Kapa Biosystems, KK4715)
- EndPoint-F Primer: TGAAGATGGCCCAGATCTG

- EndPoint-R Primer: CTGAAGCCCCCAGTGAT
- WT-Probe: (5′ JOE) TTAGATGGCTACCACCGCG (3′ Iowa Black)
- MT-Probe: (5′ 6-FAM) TTAGATGGCGATCACCGCG (3′ Iowa Black)
- Nuclease-free water

11 Make Reaction Buffer:

	A	B	C
	Reagent	**Volume (µl)**	**Final Concentration**
	qPCR Master Mix (2X)	10	1X
	High ROX (50X)	0.4	1X
	EndPoint-F (10 µM)	0.8	0.4 µM
	EndPoint-R (10 µM)	0.8	0.4 µM
	WT-Probe (10 µM)	0.4	0.2 µM
	MT-Probe (10 µM)	0.4	0.2 µM
	DNA		100 ng
	Nuclease-free water	Make up to 20 µl	
	Total	20 µl	

12 Open SDS 2.4 software, create a new experiment and choose “standard curve”.

13 Load the plate.

14 Set thermal cycling as follows:

Step	Temperature (°C)	Duration
1	95	3 min
2	95	10 s
3	60	30 s
4	Repeat steps 2-3 for 40 cycles	
5	4	∞

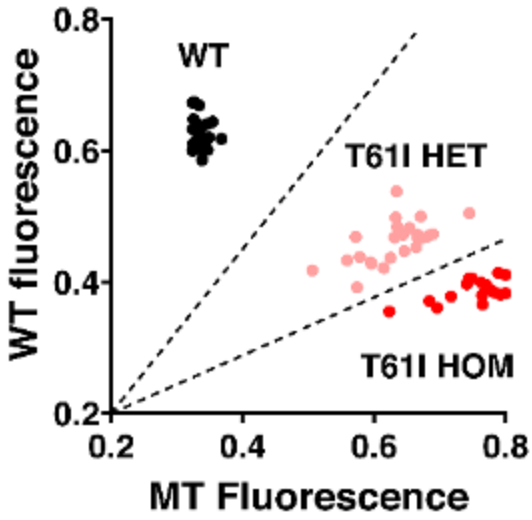
15 In the Setup tab, add detectors for FAM and JOE signal and start the program.

16 After amplification is done, create a new experiment and choose “allelic discrimination”.

17 Assign the markers which detect FAM and JOE signals.

18 Start “Post Read”.

19 **Expected Results:**
When plotted WT against MT fluorescence signal, each genotype would show a distinct ratio/slope as below:


0.8
0.6
0.4
0.2
WT fluorescence
WT
T61I HET
T61I HOM
0.2
0.4
0.6
0.8
MT Fluorescence