GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

mmrrc@ucdavis.edu
530-754-MMRRC

NAME OF PCR: B6.FVB(Cg)-Tg(Drd1a-cre)266Gsat/Mmcd **MMRRC #** 034259-UCD

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

Protocol:

Reagent/ Constituent	Volume (µL)
Water	11.275
10x Buffer	2.5
$MgCl_2$ (25mM)	1.7
Betaine (5M)	6.5
dNTPs (10mM)	0.5
DMSO	0.325
Primer 1 (20µM)	0.5
Primer 2 (20µM)	0.5
Taq Polymerase (5 Units/µL)	0.2
DNA Sample	1.0
TOTAL VOLUME OF REACTION:	**25µL**

Comments on protocol:

- Use Touch-Down cycling protocol-first 10 cycles anneal at 65° C decreasing in temperature by 1.0 ° C; next 30 cycles anneal at 55° C.
- Betaine/DMSO is standardized due to high GC content in promoter regions and protocol may be tested without. Also, may adjust $MgCl_2$ to increase reaction or decrease non specific amplifications.

Strategy:

Steps		Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? CHECK HERE ☐		94	5:00	**1**
2. Denaturation		94	0:15	
3. Annealing	steps 2-3-4 will 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		4	Hold	n/a

Primers:

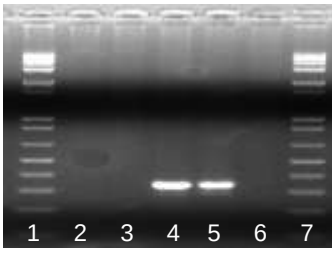
Name	Nucleotide Sequence (5' - 3')
1: Drd1a F1	GCT ATG GAG ATG CTC CTG ATG GAA
2: CreGS R1	CGGCAAACGGACAGAAGCATT

Electrophoresis Protocol:

% Agarose: 1.5 **V:** 90

Estimated Running Time (min): 90

Primer Combination	Band	Genotype
1 and 2	340 bp	transgenic



Lanes
1 & 7: 1 kb+ ladder (Invitrogen, Cat. #10787-026)
2: H_20
3: Wild Type Control
4 & 5: Drd1a tg/+
6: Other GENSAT line