

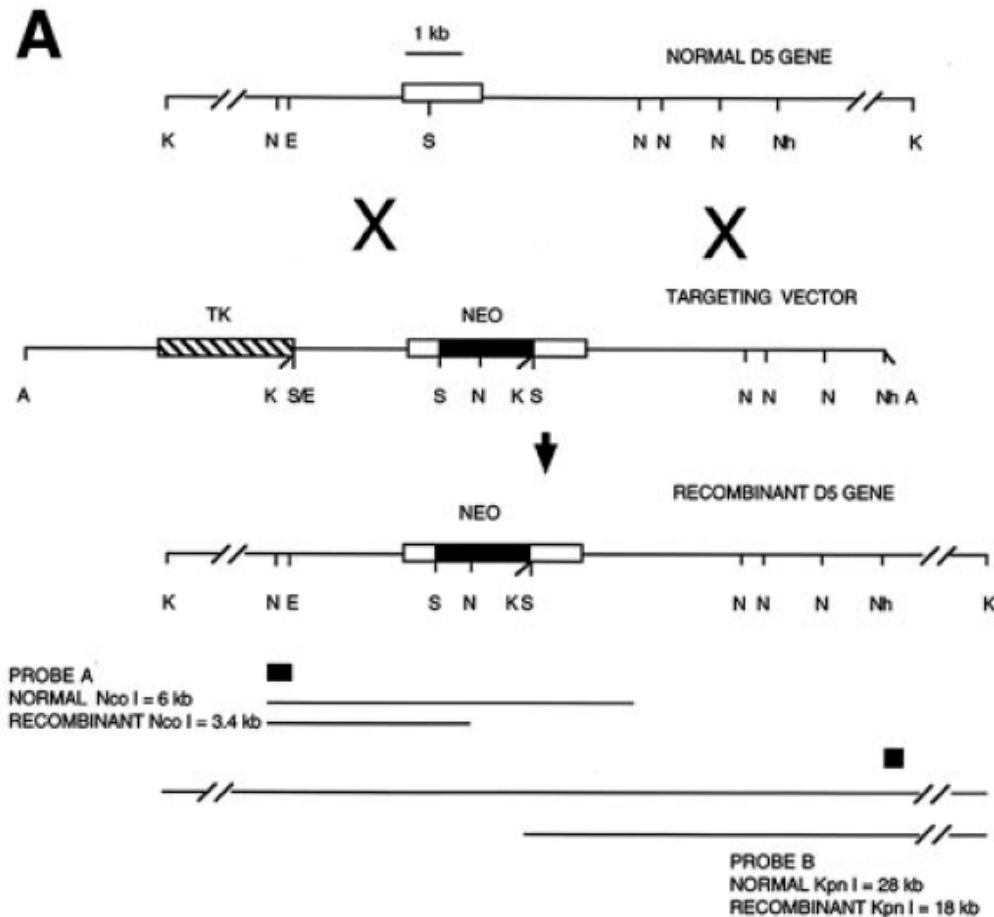
02.02.09 MS
08.02.10 HB updated
03.03.14 MLS

Genotyping Protocol: MMRRC 30628

Assay Type: PCR- can distinguish heterozygous animals from homozygous animals

DNA Extraction: DNA from tail snips was extracted using Qiagen's DNeasy Blood and Tissue kit (Cat# 69506). Kit directions for animal tissues were performed with a few minor modifications as follows: repeat AW1 and AW2 wash steps one time, elute in 200ul of AE buffer once.

Strain Characteristics: This strain has a neomycin resistance gene and a proximal linker containing a stop codon, which were ligated in reverse orientation into a unique *Sfi*I site within the dopamine receptor 5 gene (*Drd5*). This disrupts the reading frame within the coding region. Details can be found in Hollon et al (2002) J Neurosci 22:10801-10810.



Primer Information:

- | | |
|------------------------|--|
| 1) Name: M30628 D1B1 | Sequence: 5'-ACT CTC TTA ATC GTC TGG ACC TTG-3' |
| 2) Name: M30628 D1B2-1 | Sequence: 5'-GGA GGA GAT ACG GCG GAT CTG AAC-3' |
| 3) Name: M30628 D1B3 | Sequence: 5'-TGA TCA ACT AGT GCC CGG GCG GTA -3' |

Primer location: M30628 D1B1 and D1B2-1 are both located within the *Drd5* gene on Chromosome 5. M30628 D1B3 is located within the targeting vector.

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Assay name: Drd5 PCR

PCR Master Mix Components:

M30628 Mut:

component	manufacturer	concentration	μl/rxn
Buffer with MgCl ₂ (green cap)	Roche	10X	2
dNTP	Promega (Cat# U1515)	1.25mM	3.2
M30628 D1B1	Sigma	25μM	0.3
M30628 D1B3	Sigma	25μM	0.3
FastStart <i>Taq</i>	Roche (Cat# 12032953001)	5 U/μl	0.2
sterile water			13

M30628 WT:

component	manufacturer	concentration	μl/rxn
Buffer with MgCl ₂ (green cap)	Roche	10X	2
dNTP	Promega (Cat# U1515)	1.25mM	3.2
M30628 D1B1	Sigma	25μM	0.3
M30628 D1B2-1	Sigma	25μM	0.3
FastStart <i>Taq</i>	Roche (Cat# 12032953001)	5 U/μl	0.2
sterile water			13

PCR Setup:

Final Reaction: 19μl master mix & 1μl DNA template (10-20ng/μl)

All reactions were performed in 200μl thin walled PCR tubes and were run in Perkin Elmer 2400 thermocycler or Applied Biosystems 2700 thermocycler.

Cycle Parameters (both mixes):

- 1) 95°C 5 minutes
- 2) 94°C 1 minute
- 3) 66°C 1 minute
- 4) 72°C 1 minute
- 5) Repeat steps 2-4 34 times for a total of 35 cycles
- 6) 72°C 10 minutes
- 7) 4°C hold until refrigerate product

Product Analysis:

All products were analyzed on a 3% agarose gel with ethidium bromide staining.

Expected products:

WT PCR: 630bp

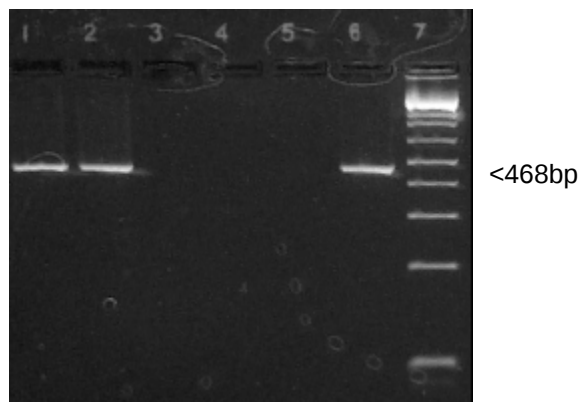
Mutant PCR: 468bp

Genotype	Mutant PCR	WT PCR
Heterozygous:	468bp	630bp
Homozygous Mutant:	468bp	no product
Wild Type:	no product	630bp

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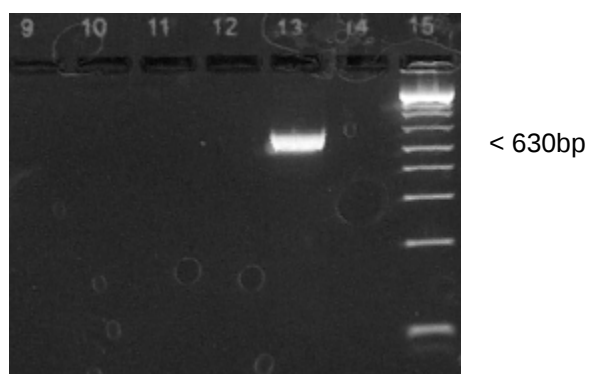
Example gels:

Mut :



Wells 1 and 2 are positive for the mutant allele. Wells 3 and 4 are blanks. Well 5 is a WT control and Well 6 is a homozygous control. Well 7 is 1Kb+ Ladder (Invitrogen Cat# 10787-018).

WT:



Wells 9 and 10 are negative for the WT allele. Wells 11 and 12 are blanks. Well 13 is a WT control and Well 14 is a homozygous control. Well 7 is 1Kb+ Ladder (Invitrogen Cat# 10787-018).