

α 5- nAChR subunit null mutation from Dr Art Beaudet

On C57Bl6 background (10 generations)

Reference: Salas R, Orr-Urtreger A, Broide RS, Beaudet A, Paylor R, and DeBiasi M (2003) The nicotinic acetylcholine receptor subunit α 5 mediates short-term effects of nicotine *in vivo*. *Mol Pharmacol* **63**:1059–1066.

α 5 PCR Protocol

Volume of Reagent	Reagent
14.25 μ L	Molecular Grade Water
2.5 μ L	10X PCR Buffer (noMgCl ₂)
2.5 μ L	MgCl ₂ (25mM)
1.5 μ L	DMSO (100X)
0.5 μ L	dNTP Mix (10mM each dNTP)
0.25 μ L	α 5 wt Forward Primer (10 μ M)
0.25 μ L	α 5 wt Reverse Primer (10 μ M)
0.5 μ L	α 5 Neo-1 Primer (10 μ M)
0.5 μ L	α 5 Neo-2 Primer (10 μ M)
0.25 μ L	Amplitaq Gold Polymerase
2.0 μ L	Genomic DNA
25 μL Total Volume	(Buffer, MgCl ₂ and Polymerase from Applied Biosystems, dNTPs from Bio-Rad)

Thermal Cycler Program Updated: 4/11/05

94° 10 minutes	
94° 30 seconds	} 65-57 degrees/ 2 degree increments/ 2 cycles each
65° 30 seconds	
72° 1 minute	
94° 30 seconds	} 20 cycles
55° 30 seconds	
72° 1 minute	
72° 7 minutes	
4° Hold	

α 5 Bands

+/+ 190 bp
+/- 290 and 190bp
-/- 290bp

Primer Sequences:

α 5 wt Forward Primer: 5'-CACTGTCACCTTGGACGCAGCC -3'
 α 5 wt Reverse Primer: 5'-GTTCCCCTTGCTCCCCATTGC-3'
Neo-1 Primer: 5'-CTTTTTGTCAAGACCGACCTGTCCG-3'
Neo-2 Primer: 5'-CTCGATGCGATGTTTCGCTTGGTG-3'