

α 4- nAChR subunit null mutation from Dr John Drago

On C57Bl6 background (10 generations)

Reference: Ross SA, Wong JYF, Clifford JJ, Kinsella A, Massalas JS, Horne MK, Scheffer TE, Kola I, Waddington JL, Berkovic SF, Drago, J. (2000) Phenotypic characterization of α 4 neuronal nicotinic acetylcholine receptor subunit knockout mice. *J Neurosci* **20**:6431–6441.

α 4 PCR Protocol

Mix	Reagent	Master Mix
10.25 μ L	Molecular Grade Water	
2.5 μ L	10X PCR Buffer (no MgCl ₂)	Applied Biosystems
2.5 μ L	MgCl ₂ (25mM)	Applied Biosystems
2.5 μ L	DMSO (100X)	
1.0 μ L	dNTP Mix	BioRad
1.0 μ L	α 4 K01 (10 uM)	Oligos etc.
1.0 μ L	α 4 K02 (10 uM)	“
1.0 μ L	GMK01 (10 uM)	“
1.0 μ L	GMK02 (10uM)	“
0.25 μ L	Amplitaq Gold Polymerase	
2.0 μ L	Genomic DNA	
25 μL Total Volume		

Thermal Cycler Program

Updated: 4/08/05

95° 7 minutes
94° 1 minute
55° 2 minutes
72° 3 minutes
72° 7 minutes
4° Hold

} 25 cycles

α 4 Bands

+/+ 385bp
+/- 385bp and 280bp
-/- 280bp

Primer Sequences:

A4KO1 (forward) -5'- CAA TGT ACA CAA CCG CTC AC -3'
A4KO2 (reverse) -5'- ACT GCT ATT GGG TGG GTG AC -3'
GMK01(neo forward) -5'-CTT GGG TGG AGA GGC TAT TC
GMK02 (neo rev.) -5'- AGG TGA GAT GAC AGG AGA TC -3'