

## **$\alpha$ 4-S6'F nAChR subunit gain-of-function mutation from Dr John Drago**

Mixed background

Reference: Teper Y, Whyte D, Cahir E, Lester HA, Grady SR, Marks MJ, Cohen BN, Fonck C, McClure-Begley T, McIntosh JM, Labarca C, Lawrence A, Chen F, Gantois I, Davies PJ, Petrou S, Murphy M, Waddington J, Horne MK, Berkovic SF, Drago J. (2007) Nicotine-induced dystonic arousal complex in a mouse line harboring a human autosomal-dominant nocturnal frontal lobe epilepsy mutation. *J Neurosci.* **27**:10128-42.

### **$\alpha$ 4S6'F PCR Protocol**

<b>Volume of Reagent</b>	<b>Reagent</b>
13.75 $\mu$ L	Molecular Grade Water
2.5 $\mu$ L	10X PCR Buffer
2.5 $\mu$ L	MgCl <sub>2</sub> (25mM)
2.5 $\mu$ L	DMSO (100X)
1.0 $\mu$ L	dNTP Mix (10mM each dNTP)
0.75 $\mu$ L	S6'F UP (10 $\mu$ M)
0.75 $\mu$ L	S6'F Down (10 $\mu$ M)
0.25 $\mu$ L	Amplitaq Gold Polymerase
2.0 $\mu$ L	Genomic DNA
<b>25.0 <math>\mu</math>L Total Volume</b>	(Buffer, MgCl <sub>2</sub> and Polymerase from PE Biosystems)

### **Thermal Cycler Program**

Updated: 11/2/07

94° 10 minutes  
 94° 30 seconds }  
 65° 30 seconds } -2°/2 cycles/ to 57°  
 72° 1 minute }  
 94° 30 seconds }  
 55° 30 seconds } 21 cycles  
 72° 1 minute }  
 72° 7 minutes  
 4° Hold

### **$\alpha$ 4S6'F Bands**

+/+ 200bp  
 +/- 200bp and 240bp  
 -/- 240bp

### **Primer Sequences**

S6'F UP Primer: 5'-GAGGGTACATAGTGTAGTCT-3'  
 S6'F Down Primer: 3'-AAACAGCCTGAACACTTCAA-3'