

PCR Genotyping of GluR6-ΔECS mice

Date: 9/20/2007

only mutant alleles will be amplified in hets; do separate PCR to confirm presence of ECS

Reaction mixture	Volume (μl)	9x Mix (ul)	Samples	Controls
gDNA	1		4/11	5145
dH2O	12.1	108.9	5/1	5146
10 x PCR buffer	2.5	22.5	6/11	TE
5x Q Solution	5	45	6/12	
dNTPs (10 mM)	0.2	1.8	6/15	
Primer R6/8490B+	2.5	22.5		
Primer R6/9395B-	1.5	13.5		
Taq (Qiagen)	0.2	1.8		
Total	25	216		

Mix factor: 9

Cycling Conditions

Program Name: GLUR6-DE

Temperature	Time	
95 °C	4:00	} 34 cycles
95 °C	0:25	
59 °C	1:00	
72 °C	1:00	
72 °C	10:00	
7 °C	hold	

Run PCR products on a 1.5% agarose gel

WT : 800 bp

GluR6 editing mutant: 400 bp

Primer Sequences

R6/9395B-

TGG CCC ATC TTA CAC TTC AGT TCA TCT TAC

R6/8490B+

TGG AGT TCT CTC AGG TCT GAA GGG ATA CAC

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this PCR detects the ECS region, and therefore WT alleles only

<u>Reaction mixture</u>	<u>Volume (μl)</u>	<u>9x Mix (ul)</u>	<u>Samples</u>	<u>Controls</u>
gDNA	1		4/11	5145
dH ₂ O	13.2	118.8	5/1	5146
10x PCR buffer	2.5	22.5	6/11	TE
5x Q Solution	5	45	6/12	
dNTPs (10 mM)	1	9	6/15	
Sense Primer	1	9		
Antisense Primer	1	9		
Taq (Qiagen)	0.3	2.7		
Total	25	216		

Mix factor: 9

Cycling Conditions

Program Name: ECS

<u>Temperature</u>	<u>Time</u>	
94 °C	2:00	} 35 cycles
94 °C	1:00	
53 °C	1:00	
72 °C	1:00	
72 °C	7:00	
7 °C	hold	

Run PCR products on a 1.5% agarose gel