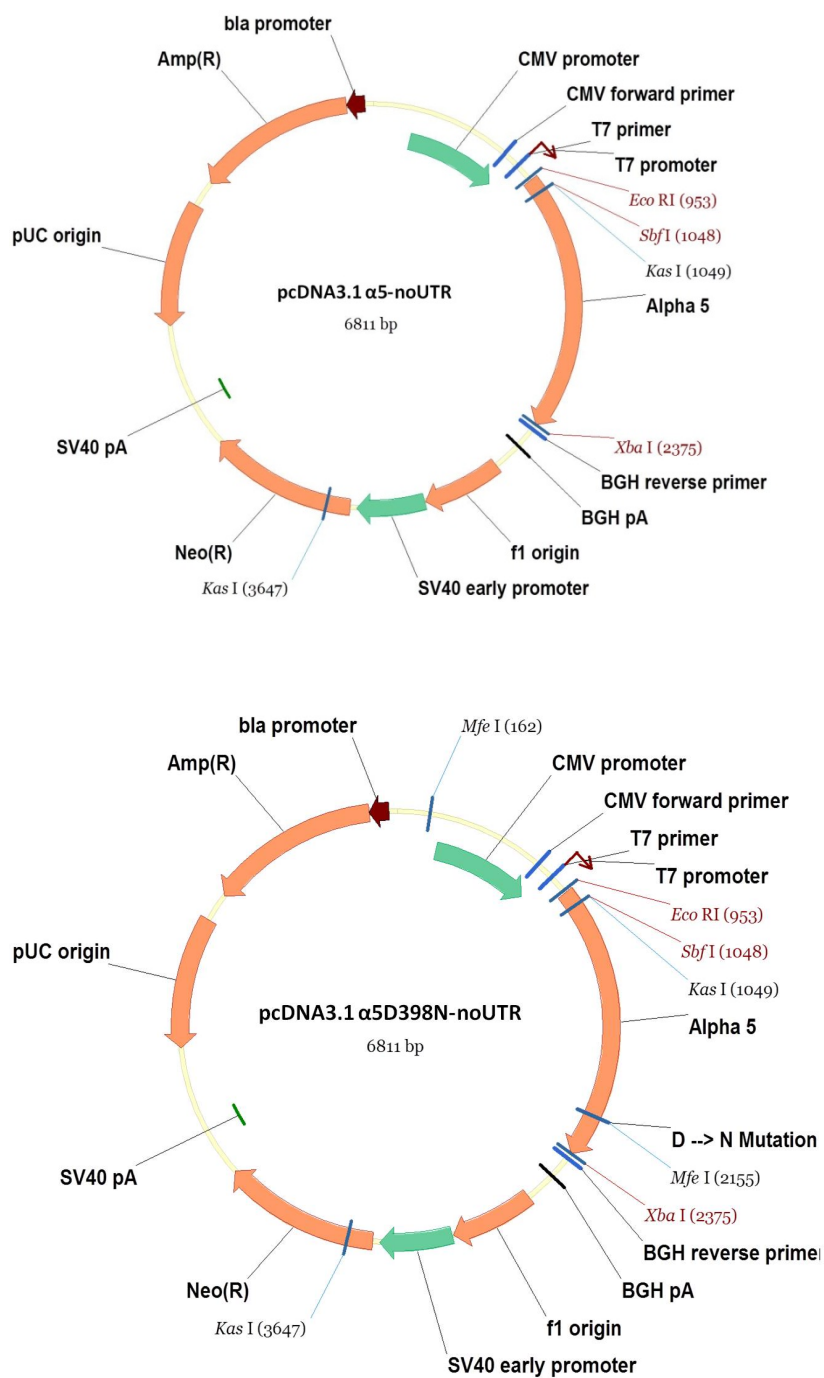


## Appendix i

### Plasmid Maps of *pcDNA3.1(+)* $\alpha 5$ -no UTR and *pcDNA3.1(+)* $\alpha 5D398N$ -no UTR



*PCR Protocol for extension of Chrna5*

PCR Mix:

	<b>reagent</b>	<b>μL</b>
alpha5	template	0.5
	quick solution	3
	10x buffer	5
	Forward primer 50μM	1
	Reverse primer 50μM	1
	dNTPs	1
	Pfu turbo hotstart polymerase	1.5
	dH2O	37
<hr/>		
	Total Volume	50

PCR Cycle Protocol

Quick change reaction Conditions:

<b>Temp</b>	<b>Time</b>
95°C	4min
95°C	30sec
60°C	2min
68°C	16min
68°C	10min
4°C	∞

Extension of *Chrna5* gene

<b>Temp</b>	<b>Time</b>
95°C	4min
95°C	30sec
52°C	2min
68°C	16min
68°C	10min
4°C	∞