

(E)-Table 1 - Ohtake et al. PCR primer and probe sequences, expected product sizes, and annealing conditions for synuclein exon amplifications and TaqMan analysis

Primers	Forward	Reverse	PCR (bp)	Tm (°C)	Ref.	Gene Bank #
a3f/a3r	gtctcacactttggagggttc	cacccatcacacataccctgactc	395	60	23	U46897
a4f/a4r	gctaattcagcaatttaaggctag	gatatgttcttagatgctcag	216	60	23	U46898
a5f/a5r	cgtatggctagtggaaagtgg	ccccacagtaagtatctgc	325	60	23 / *	U46899
a6f/a6r	cggaggcattgtggagtttag	ccacgtaatgagcatgttagagac	373	60	23	U46990
a7-2f/a7-1r	atgatcattaaatggtcatccg	tcaactgctgtatggaagacttcg	431	60	*	U46901 / *
b2f/b2r	agtgcacccgtgcccgtgtac	agccctgcagccccagaaac	198	65	24	AF053134
b3f/b3r	cagcgcagagtccctaaatg	cggccagatcatccgcctaa	139	55	24	AF053135
b4f/b4r	tttccccctggctcccaaac	ctgcatgtgcgggtcagaag	197	60	24	AF053135
b5f/b5r	tcctcacgagtccgtaccct	agctaggacggcagcaatca	186	60	24	AF053136
b6f/b6r	aaactccctcccttttgc	cgtcgctcgatctcggt	403	55	24	AF053136

* We developed primers A5R and A7-1R based upon sequence information present in Genbank. The A7-2F primer sequence did not appear in Genbank. We therefore generated the A7-2F primer after sequencing this region of the α -synuclein gene using previously described primer information (21).

PCR = polymerase chain reaction product size

Tm = melting temperature; used as the annealing temperature for the PCR

Ref. = number of reference

Primers for V70M allele detection:

SNCB-Taq-4F 5'-ggatcgcttaccttcagatcagtag-3'
 SNCB-Taq-4R 5'-accaaggaaacaggcctcacat-3'

Taqman Probes for V70M detection:

Wild type: SNCB-MGB-W (FAM)-AGAGAACACAGCTCC
 Mutant allele: SNCB-MGB-V (VIC)-AGAACATAGCTCCTCCCA

PCR conditions for allelic discrimination analysis:

Stage 1: UNG activation --- 50°C, 2 min

Stage 2: AmpliTaq Gold activation --- 95°C, 10 min

Stage 3: PCR (35 cycles) --- denature 92°C, 15 sec; anneal/extend 60°C, 1 min

{for questions regarding this data, email laspada@u.washington.edu}