

PROTOCOLS & PRIMER SEQUENCES FOR AMPLIFICATION OF PRESENILIN 1 & PRESENILIN2

1. PCR Conditions For Amplification Of Exons Of Presenilin 1 and Presenilin 2 From Genomic DNA (Except for Exon 5 of Presenilin 2)

Reagents Per Reaction:

H2O	9.90 μ l
10x Gold Buffer	2.0 μ l
MgCl ₂ (25mM)	2.0 μ l
dNTPs (2.5mM)	1.0 μ l
Forward Primer (5uM)	0.5 μ l
Reverse Primer (5uM)	0.5 μ l
AmpliTaq Gold (5U/ μ l)	0.1 μ l
DNA (10ng/ μ l)	4.0 μ l
Total Volume:	20 μ l

PCR were performed on PE 9700 thermocycler with following cycling Parameters: Initial denature at 94°C for 12min, 10 cycles of touch-down PCR (denature: 94°C for 15s; annealing: 65-55°C for 15s; extension: 72°C for 45s), followed by 30 cycles of regular PCR with annealing at 55°C with a final extension at 72°C for 5min.

2. PCR Amplification of Presenilin 2 Exon 5 using FailSafe PCR System (EPICENTRE Biotechnologies, Madison, WI)

Reagents Per Reaction for Exon 5 of Presenilin 2:

H2O	3.0 μ l
2xPre Mix H	10.0 μ l
dNTPs (2.5mM)	1.0 μ l
Forward Primer (5uM)	0.5 μ l
Reverse Primer (5uM)	0.5 μ l
Failsafe Enzyme mix	1.0 μ l
DNA (10ng/ μ l)	4.0 μ l
Total Volume:	20 μ l

PCR were performed on PE 9700 thermocycler with following cycling Parameters: Initial denature at 94°C for 4min; 10 cycles of touch-down PCR (denature: 94°C for 15s; annealing: 65-55°C for 15s; extension: 72°C for 60s), followed by 30 cycles of regular PCR with annealing at 55°C with a final extension at 72°C for 5min.

Primers Used To Amplify Exons Of Presenelin 1

PSEN1 EXONS	FORWARD	REVERSE
3	CATTCCTGGTTTACAAATTGGTCT	CAGAGGTGAGGGGAGATGATAA
4	GGTAAAAGAGAGGACCTGAATGC	GATGGGCTCTCCAGCTAAGTC
5	CTAGATTGGTGAGTTGGGGAAA	CCACAGTGAGGAGGAAGAAAAC
6	GCGACAAAGTGAGACCCTGT	TGTGGAACAATTAACACTTCAGAGT
7	GCACAGTTGATATAGGTTATGGTAAAA	TGGGATGTACACGTTACCATT
8	GTTACCTGCCATTTATTTCA	AAAGAGATCTGCAGGAGTTCCA
9	CCTGCTAAAACCAAAGAGAACC	TGGGCATTATCATAGTTCTCAAG
9b	CAGGCCAGAAAATGCAC	TCCGATTTCCAGAGCAAAGTTGT
10	GAAATAAAGGAGAAAATAGCTGTTG	AGTTACATGTGATATAGAATTAGACTG
11	TGAAGCCTAATTTTGTATATCATTT	AGGTGAGGTCCAATTATCAAA
12	TCTGTTCTAAAATTTAACCCCAAAG	CAAATCGTTTTCTATAGGCACCTC

Primers Used To Amplify Exons Of Presenilin 2

PSEN2 EXONS	FORWARD	REVERSE
2	TGCACCACTGTACTCCAACG	AGGACCAGGTCTCCTGAGGT
3	GACAGGCATCTCTTGGAAGC	CGCCAATCCTCATCATTAC
4	GCTTCATCCAGCTCCAAATC	AGTGACCAACACAGGCTGCT
5	AGGACAGGAACTGCTCATGG	GGGTGGGGAGGAGTACAAAA
6	TCCAGCGTAGGCATGAAGTA	CACTTCCACTCCCCTGTGTC
7	AGTGAAGGTCGGGGAAGG	ACAAAGGCGACTCTCTCAGG
8	GGGCCTGCTCTAAGTTGTGA	CTCCCTGGCTTCTGAAAGTG
9	CACAACGGCCTCCTAACAAT	AATCTTCAAGCCGCATGTGT
10	GTGTCAGGTGCTGGTGCTC	CGCTGCAGGCATCTTCTACT
11	TCCCAGTCCACATCTTAGC	CGCTCCCTGCTCTTGTCC
12	ACTGGTCCTCGAACAAGCTC	ACTCCCGAGCACACTCTTTG