

# Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Shattuck TM, Välimäki S, Obara T, et al. Somatic and Germ-Line Mutations of the *HRPT2* Gene in Sporadic Parathyroid Carcinoma. *N Engl J Med* 2003;349:1722-9.

**Supplementary Appendix 1. Primers and Conditions for Polymerase-Chain-Reaction (PCR) Amplification and Sequencing of the *HRPT2* Gene in Parathyroid-Carcinoma DNA.\***

Exon	Forward Primer	Reverse Primer	PCR Product Size <i>bp</i>	Annealing Temperature $^{\circ}\text{C}$
1	GAGGACGGCTGTTAGTGCT (PCR)† CGAAGGAGGAGGAGGAAGAG (SEQ)†	CCCCTTCTTTCCTTACCCTA	451	55
2	TAAATGAATCCAGCCTGAAG	AGGCCAGACCCTGTCTCT	251	58
3	AAAGTGCTGGGATTCTAGG	TGGACAAAAATGAAGGTAGG	599	58
4-5	AACATGTTTTTG CAGAGCTG (PCR)† ACCTAGAGAAAATCACCATA (SEQ)†	CTCCTCAGGTTACTGCAATC	451	55
6	GGCCTAAAGACTGATACC	CGAACTTAAGAGCAAAGAGG	351	55
7	GAATGCCTGCTGTGAAAA	TGTGAAGGAGCTTGCAATTT	502	55
8	CTCTGGATCAATATCTTAGTAGTGG	GTCTTCAACGTTACTACTGTC	348	55
9	ATGGTCATGCTACTGCACTC	CCAACCCTTACCCTTAAACA	251	55
10	CAGAGATAGTCTTAACCAGCTTC	CTTCAACATGTGCTACTCACAT	384	55
11	AACATGTTCACTGGAGTAACC	TGCACTGTTACGATCTTTTG	251	55
12-13	TGGTAACTGAAACTGCAGA	GTATCTCAATATCCTACGTACAGG	713	58
14	ATCTTCCCATTTTCATCACG	CCCCATCTCTTAAAAAGCAA	321	55
15	TGCCTAAGGGATTATAGTAGC	ACATCATATGCGCAGAACT	281	55
16	GGCGTGATAAACCTGAAT	GAAAGAAGGGAATTAGGGAA	401	55
17	GAGGAGTGTTATTCTAGCTTATTC	GATCAATCTGTGACCTTCTTCA	301	55

\* Primer sequences are listed from the 5' to the 3' ends. Primers were constructed on the basis of the alignment of the complementary DNA sequence (GenBank accession number XM-029845) with the genomic sequence of AL390863 and AL139133. Sequences of primers 1 forward and 17 reverse are located within 5' and 3' untranslated regions, whereas all other primers are derived from intronic sequences.<sup>20</sup> The PCR conditions were as follows for all reactions except those involving exons 12 to 13: a 20- $\mu\text{l}$  reaction mixture with 25 ng of DNA, 20 pmol of each primer, 200 mM of each deoxyribonucleoside triphosphate, 1.25 U of Amplitaq Gold DNA Polymerase (Applied Biosystems), 1 $\times$  PCR buffer (Applied Biosystems), and 1.0 to 1.5 mM magnesium chloride was subjected to initial denaturation at 95 $^{\circ}\text{C}$  for 10 minutes, 35 cycles of denaturation at 95 $^{\circ}\text{C}$  for 30 seconds, annealing as noted above for 30 seconds, and elongation at 72 $^{\circ}\text{C}$  for 30 seconds, with a final period of extension at 72 $^{\circ}\text{C}$  for 10 minutes. The PCR conditions for exons 12 to 13 were as follows: a 50- $\mu\text{l}$  reaction mixture with 25 ng of DNA, 50 pmol of each primer, 200 mM of each deoxyribonucleoside triphosphate, 2 U of Eppendorf HotMaster DNA Polymerase (Brinkman), and 1 $\times$  HotMaster *Taq* buffer with 2.5 mM magnesium (Brinkman) was subjected to initial denaturation at 96 $^{\circ}\text{C}$  for 6 minutes, 35 cycles of denaturation at 95 $^{\circ}\text{C}$  for 30 seconds, annealing at 58 $^{\circ}\text{C}$  for 30 seconds, and elongation at 72 $^{\circ}\text{C}$  for 45 seconds, with a final period of extension at 72 $^{\circ}\text{C}$  for 10 minutes.

† Different primers were used for PCR and sequencing (SEQ) reactions. For all other exons, the same primers were used for both sequencing and PCR.