Appendix 1. Sequence information for all tagged PCR primers, T7 universal primers and anchor primers used in this study.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Primer ID | Primer sequence (5’-3’) | Length(bp) | %GC primera | Predictedamplicon size (bp)b |
| T7Prom | TAATACGACTCACTATAGGG | 20 | 40.0 | - |
| T7Term | GCTAGTTATTGCTCAGCGG | 19 | 52.6 | - |
| RB1gDNA\_T7Prom\_Ex1F | TAATACGACTCACTATAGGGacgccaggtttcccagttta | 40 | 45.0 | 709 |
| RB1gDNA\_T7Term\_Ex1R | GCTAGTTATTGCTCAGCGGgtcaagttgaagccgagacc | 39 | 53.8 |
| RB1gDNA\_T7Prom\_Ex2F | TAATACGACTCACTATAGGGtttggaatgaccatgaaaaaga | 42 | 35.7 | 503 |
| RB1gDNA\_T7Term\_Ex2R | GCTAGTTATTGCTCAGCGGaaatttcctctgggtaatggaa | 41 | 43.9 |
| RB1gDNA\_T7Prom\_Ex3F | TAATACGACTCACTATAGGGtgccatcagaaggatgtgtt | 40 | 42.5 | 468 |
| RB1gDNA\_T7Term\_Ex3R | GCTAGTTATTGCTCAGCGGaacggctccatgagagaatg | 39 | 51.3 |
| RB1gDNA\_T7Prom\_Ex4F | TAATACGACTCACTATAGGGttccttccaaaggatatagtagtga | 45 | 37.8 | 432 |
| RB1gDNA\_T7Term\_Ex4R | GCTAGTTATTGCTCAGCGGtgagctaacattaaaagggacaa | 42 | 42.9 |
| RB1gDNA\_T7Prom\_Ex5F | TAATACGACTCACTATAGGGtgagatgtcataaattgggaaaa | 43 | 34.9 | 483 |
| RB1gDNA\_T7Term\_Ex5R | GCTAGTTATTGCTCAGCGGgctgtcctgaatcaattccac | 40 | 50.0 |
| RB1gDNA\_T7Prom\_Ex6F | TAATACGACTCACTATAGGGaaattatgcaattaaaatggactgc | 45 | 33.3 | 390 |
| RB1gDNA\_T7Term\_Ex6R | GCTAGTTATTGCTCAGCGGaagccaagcagagaatgagg | 39 | 51.3 |
| RB1gDNA\_T7Prom\_Ex7F | TAATACGACTCACTATAGGGccatgctgatagtgattgttgaa | 43 | 39.5 | 396 |
| RB1gDNA\_T7Term\_Ex7R | GCTAGTTATTGCTCAGCGGgggcaaagtccatgtctgat | 39 | 51.3 |
| RB1gDNA\_T7Prom\_Ex8F | TAATACGACTCACTATAGGGtgatggatgtacaattgttcttat | 44 | 34.1 | 295 |
| RB1gDNA\_T7Term\_Ex8R | GCTAGTTATTGCTCAGCGGatattgttagggagaacttacatc | 43 | 41.9 |
| RB1gDNA\_T7Prom\_Ex9F | TAATACGACTCACTATAGGGatgcatgtgattgcacctgt | 40 | 42.5 | 481 |
| RB1gDNA\_T7Term\_Ex9R | GCTAGTTATTGCTCAGCGGtttcaccacaattctacttggcta | 43 | 44.2 |
| RB1gDNA\_T7Prom\_Ex10F | TAATACGACTCACTATAGGGattgcatgcgaactcagtgt | 40 | 42.5 | 511 |
| RB1gDNA\_T7Term\_Ex10R | GCTAGTTATTGCTCAGCGGtgcaaaaaggtaactgttatagga | 43 | 41.9 |
| RB1gDNA\_T7Prom\_Ex11F | TAATACGACTCACTATAGGGtgatgcataaagcacaaattgt | 42 | 35.7 | 257 |
| RB1gDNA\_T7Term\_Ex11R | GCTAGTTATTGCTCAGCGGaacgtgaacaaatctgaaacacta | 43 | 41.9 |
| RB1gDNA\_T7Prom\_Ex12F | TAATACGACTCACTATAGGGagacaagtgggaggcagtgt | 40 | 47.5 | 380 |
| RB1gDNA\_T7Term\_Ex12R | GCTAGTTATTGCTCAGCGGgcaagaaaagattatggataactaca | 45 | 40.0 |
| RB1gDNA\_T7Prom\_Ex13F | TAATACGACTCACTATAGGGttgctcattaacatccagtgaaa | 43 | 37.2 | 482 |
| RB1gDNA\_T7Term\_Ex13R | GCTAGTTATTGCTCAGCGGcacaggcagcagggatatag | 39 | 53.8 |
| RB1gDNA\_T7Prom\_Ex14F | TAATACGACTCACTATAGGGttgtgattttctaaaatagcagg | 43 | 34.9 | 250 |
| RB1gDNA\_T7Term\_Ex14R | GCTAGTTATTGCTCAGCGGcttgacctcctgatctgccc | 39 | 56.4 |
| RB1gDNA\_ T7Prom \_Ex15F | TAATACGACTCACTATAGGGggtttcaattaaacaacttc | 40 | 30.0 | 213 |
| RB1gDNA\_ T7Term \_Ex15R | GCTAGTTATTGCTCAGCGGattcagaagtttgctaaagg | 39 | 35.0 |
| RB1gDNA\_T7Prom\_Ex16F | TAATACGACTCACTATAGGGaaggtttcaattaaacaacttcttttt | 47 | 29.8 | 438 |
| RB1gDNA\_T7Term\_Ex16R | GCTAGTTATTGCTCAGCGGtggcttataatgaccaattacattc | 44 | 40.9 |
| RB1gDNA\_T7Prom\_Ex17F | TAATACGACTCACTATAGGGtcaaaattggaaggctatttcc | 42 | 38.1 | 593 |
| RB1gDNA\_T7Term\_Ex17R | GCTAGTTATTGCTCAGCGGggtgctcgattaaagctcca | 39 | 51.3 |
| RB1gDNA\_T7Prom\_Ex18F | TAATACGACTCACTATAGGGtttgtgtgtgggaagtacaaaaa | 43 | 37.2 | 522 |
| RB1gDNA\_T7Term\_Ex18R | GCTAGTTATTGCTCAGCGGtgactttatttgggtcatgtacctt | 44 | 43.2 |
| RB1gDNA\_T7Prom\_Ex19F | TAATACGACTCACTATAGGGaatctgtgattcttagccaacttgaaa | 47 | 36.2 | 303 |
| RB1gDNA\_T7Term\_Ex19R | GCTAGTTATTGCTCAGCGGatgatttgaacccagtcagcct | 41 | 48.8 |
| RB1gDNA\_T7Prom\_Ex20F | TAATACGACTCACTATAGGGttgtaattcaaaatgaacagtaaa | 44 | 29.5 | 281 |
| RB1gDNA\_T7Term\_Ex20R | GCTAGTTATTGCTCAGCGGcaagtaagtagggaggagagaagg | 43 | 51.2 |
| RB1gDNA\_T7Prom\_Ex21F | TAATACGACTCACTATAGGGgccttggtgatttgcatttt | 40 | 40.0 | 534 |
| RB1gDNA\_T7Term\_Ex21R | GCTAGTTATTGCTCAGCGGcccttatctttccaattctatttaag | 45 | 40.0 |
| RB1gDNA\_T7Prom\_Ex22F | TAATACGACTCACTATAGGGaatatgtgcttcttaccagtcaaa | 44 | 36.4 | 603 |
| RB1gDNA\_T7Term\_Ex22R | GCTAGTTATTGCTCAGCGGggatcaaaataatccccctctc | 41 | 48.8 |
| RB1gDNA\_T7Prom\_Ex23F | TAATACGACTCACTATAGGGaatatgtgcttcttaccagtcaaa | 44 | 36.4 | 738 |
| RB1gDNA\_T7Term\_Ex23R | GCTAGTTATTGCTCAGCGGtcttgcgttgcttaagtcgt | 39 | 48.7 |
| RB1gDNA\_T7Prom\_Ex24F | TAATACGACTCACTATAGGGtgattagacgggcactgttaga | 42 | 42.9 | 369 |
| RB1gDNA\_T7Term\_Ex24R | GCTAGTTATTGCTCAGCGGgcaatatgcctggatgaggt | 39 | 51.3 |
| RB1gDNA\_T7Prom\_Ex25F | TAATACGACTCACTATAGGGtttgcctgatttttgacacacc | 42 | 40.5 | 387 |
| RB1gDNA\_T7Term\_Ex25R | GCTAGTTATTGCTCAGCGGaacttggcatgaaagaaattgg | 41 | 43.9 |
| RB1gDNA\_T7Prom\_Ex26F | TAATACGACTCACTATAGGGtttgtgacatttatgttttagatgg | 45 | 33.3 | 418 |
| RB1gDNA\_T7Term\_Ex26R | GCTAGTTATTGCTCAGCGGccacaaatgtttgtagataaataacc | 45 | 40.0 |
| RB1gDNA\_T7Prom\_Ex27F | TAATACGACTCACTATAGGGaccactgcttttgcaaggtc | 40 | 45.0 | 620 |
| RB1gDNA\_T7Term\_Ex27R | GCTAGTTATTGCTCAGCGGaaaaacaagagcaaacatcacc | 41 | 43.9 |

T7Prom and T7Term represent both T7Promoter and T7Terminator universal sequencing primers that were tagged to all exon-specific PCR primer sequences. T7Prom was tagged to Forward primers while T7Term was tagged to Reverse primers. For each primer, sequences in uppercase belonged to T7Prom/Term and sequences in lowercase were exon-specific.

a %GC primer was calculated using the GC Calculator program available at <http://www.genomicsplace.com/gc_calc.html>.

b Predicted amplicon size (bp) was calculated using the UCSC In-Silico PCR program available at <https://genome.ucsc.edu/cgi-bin/hgPcr>. To account for T7 sequences that were present on both Forward and Reverse primers, additional 39bp was added to the amplicon size predicted by the aforementioned program.

|  |  |  |  |
| --- | --- | --- | --- |
| Anchor Primer ID | Primer sequence (5’-3’) | Length (bp) | %GC primera |
| RB1gDNA\_Ex2\_AnchorR | AATGAGAAAAAAAAATTTC | 19 | 15.8 |
| RB1gDNA\_Ex5\_AnchorR | AAAAATCTTTTTTTTTAAG | 19 | 10.5 |
| RB1gDNA\_Ex7\_AnchorF | AAATGTACATTTTTTTTTC | 19 | 15.8 |
| RB1gDNA\_Ex9\_AnchorR | TGGGCAAAAAAAAAATGTG | 19 | 31.6 |
| RB1gDNA\_Ex15\_AnchorF | CTTCTTTTTTTTTTTTTAA | 19 | 10.5 |
| RB1gDNA\_Ex15\_AnchorR | GGAAAAAAAAAAAGATTAT | 19 | 15.8 |
| RB1gDNA\_Ex16\_AnchorF | ATAATCTTTTTTTTTTTCC | 19 | 15.8 |
| RB1gDNA\_Ex17\_AnchorF | AGTTACTTTTTTTTTTCAT | 19 | 15.8 |
| RB1gDNA\_Ex22\_AnchorF | TTTTTTTTTTTTACTGTTC | 19 | 15.8 |

a %GC primer was calculated using the GC Calculator program available at <http://www.genomicsplace.com/gc_calc.html>