**TableS2: PCR conditions and primer sequences for mutations identified in the *RB1* gene**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Patient No. | Exon/ Intron | Type of mutation | c.DNA | Amino acid change | Primer Name | Primer Sequence / strand identity | Ta (0C) |
| RB1 | Ex 1 | Indel | c.46\_68delGCCGCCGCGGAACCCCCGGCACC | - | SPRB1-E1-F | 5’-TTTTCTCAGGGGACGTTG -3’(+) | 55 |
|  |  |  |  |  | SPRB1-E1-R | 5’-ATCCTGTCACCATTCTGC -3’(-) |  |
| RB2 | Ex 2 | Indel | c.178\_182delTTATG | p.Leu60SerfsTer48 | SPRB1-E2-F | 5’-GTCACTGGCGTTGAATTG -3’(+) | 55 |
|  |  |  |  |  | SPRB1-E2-R | 5’-CCAATACTCCATCCACAG -3’(-) |  |
| RB3 | Ex 4 | Indel | c.400\_407delTTACTAAA | p.Leu134ArgfsTer3 | SPRB1-E4-F | 5’-GAGGTGTAAGTTGAAGGC -3’ | 56 |
|  |  |  |  |  | SPRB1-E4-R | 5’-TGAGCTAACATTAAAAGGGAC -3’ |  |
| RB4 | Int 5 | SS | c.539+1G>A | - | SPRB1-E5-F | 5’- GATCAGCTGGGTGTTTTC -3’(+) | 56 |
|  |  |  |  |  | SPRB1-E5-R | 5’- CAGAGTTGGTGATATGCTG -3’(-) |  |
| RB5 | Ex 6 | Indel | c.540-6\_545delTAATAGGATATC | - | SPRB1-E6-F | 5’-TTGCAGCTTCTCATGGTC -3’(+) | 56 |
|  |  |  |  |  | SPRB1-E6-R | 5’-AGAATGAGGGAGGAGTAC -3’(-) |  |
| RB6 | Ex 8-11 | LD | c.719-2574\_1127+678delinsC | p.Lys240ArgfsTer3 | SPRB1-E7-F | 5’-GGATATACTCTACCCTGCGA-3’ (+) | 61 |
|  |  |  |  |  | SPRB1-E11-R | 5’-GAGGGAATATCAAGTACGAGG-3’ (-) |  |
| RB7 | Ex 8 | NS | c.751C>T | p.Arg251Ter | SPRB1-E8-F | 5’- TTGGGAGCAGAGTAGAAG-3’ (+) | 55-56 |
|  |  |  |  |  | SPRB1-E8-R | 5’- CTGCAAAAGAGTTAGCAC-3’ (-) |  |
| RB8 | Int 9 | SS | c.940-1G>A | - | SPRB1-E10-F | 5’- TTGCATGCGAACTCAGTG -3’(+) | 56 |
|  |  |  |  |  | SPRB1-E10-R | 5’- CCATGTGCAATACCTGTC -3’(-) |  |
| RB9 | Ex 11 | NS | c.1072C>T | p.Arg358Ter | SPRB1-E11-F | 5’-AGGGAGAAAGCATCTGAC -3’(+) | 56 |
|  |  |  |  |  | SPRB1-E11-R | 5’-GAGGGAATATCAAGTACGAGG -3’(-) |  |
| RB10 | Int 12 | SS | c.1215+1G>A | - | SPRB1-E12-F | 5’-GGAGGCAGTGTATTTGAAG -3’(+) | 54 |
|  |  |  |  |  | SPRB1-E12-R | 5’-GAAGAATGGTGAGCAAGG -3’(-) |  |
| RB11 | Ex 13 | Indel | c.1281delA | p.Glu428ArgfsTer29 | SPRB1-E13-F | 5’- GAGGCTCTTTCTGATGGATGG-3’(+) | 59 |
|  |  |  |  |  | SPRB1-E13-R | 3’- ATAGCAGCATACACAGGCAG-5’(-) |  |
| RB12 | Ex 14 | NS | c.1333C>T | p.Arg445Ter | SPRB1-E14-F | 5’-CTGGGCACATAACCTATC -3’(+) | 56 |
|  |  |  |  |  | SPRB1-E15-R | 5’-GCCATTACAACCTCAAGAG -3’(-) |  |
| RB13 | Ex 14 | NS | c.1363C>T | p.Arg455Ter | SPRB1-E14-F | 5’-CTGGGCACATAACCTATC -3’(+) | 56 |
|  |  |  |  |  | SPRB1-E15-R | 5’-GCCATTACAACCTCAAGAG -3’(-) |  |
| RB14 | Ex 14 | NS | c.1363C>T | p.Arg455Ter | SPRB1-E14-F | 5’-CTGGGCACATAACCTATC -3’(+) | 56 |
|  |  |  |  |  | SPRB1-E15-R | 5’-GCCATTACAACCTCAAGAG -3’(-) |  |
| RB15 | Ex 14 | NS | c.1363C>T | p.Arg455Ter | SPRB1-E14-F | 5’-CTGGGCACATAACCTATC -3’(+) | 56 |
|  |  |  |  |  | SPRB1-E15-R | 5’-GCCATTACAACCTCAAGAG -3’(-) |  |
| RB16 | Int 15 | Indel | c.1421+5\_1421+17delTTTTTTACTTTT | - | SPRB1-E14-F | 5’-CTGGGCACATAACCTATC -3’(+) | 56 |
|  |  |  |  |  | SPRB1-E15-R | 5’-GCCATTACAACCTCAAGAG -3’(-) |  |
| RB17 | Ex 17 | NS | c.1654C>T | p.Arg552Ter | SPRB1-E17-F | 5’-TCCTATGAGTCCGTAGAC -3’(+) | 53 |
|  |  |  |  |  | SPRB1-E17-R | 5’-CCCTATATGTTCTTGAGG -3’(-) |  |
| RB18 | Ex 17 | MS | c.1689G>C | p.Trp563Cys | SPRB1-E17-F | 5’-TCCTATGAGTCCGTAGAC -3’(+) | 53 |
|  |  |  |  |  | SPRB1-E17-R | 5’-CCCTATATGTTCTTGAGG -3’(-) |  |
| RB19 | Int 17 | SS | c.1696-3T>G | p.Asp566ArgfsTer45 | SPRB1-E18-F | 5’-GCCACTGTCAATTGTGCC-3’(+) | 55 |
|  |  |  |  |  | SPRB1-E18-R | 3’-CAGTAGCACTCTGTACTC-5’(-) |  |
| RB20 | Ex 18 | NS | c.1723C>T | p.Gln575Ter | SPRB1-E18-2F | 5’-TTTGTGTGTGGGAAGTAC -3’(+) | 56 |
|  |  |  |  |  | SPRB1-E18-2R | 5’-CAGTAGCACTCTGTACTC -3’(-) |  |
| RB21 | Ex 18 | NS | c.1735C>T | p.Arg579Ter | SPRB1-E18-2F | 5’-TTTGTGTGTGGGAAGTAC -3’(+) | 56 |
|  |  |  |  |  | SPRB1-E18-2R | 5’-CAGTAGCACTCTGTACTC -3’(-) |  |
| RB22 | Ex 18 | NS | c.1735C>T | p.Arg579Ter | SPRB1-E18-2F | 5’-TTTGTGTGTGGGAAGTAC -3’(+) | 56 |
|  |  |  |  |  | SPRB1-E18-2R | 5’-CAGTAGCACTCTGTACTC -3’(-) |  |
| RB23 | Ex 19 | Indel | c.1953T > TA | p.K653fs | SPRB1-E19-F | 5’- GCTGGAATCACCTTATGG -3’(+) | 54 |
|  |  |  |  |  | SPRB1-E19-R | 5’- GTGACTTGCCCAGTAATG -3’(-) |  |
| RB24 | Ex 20 | Indel | c.1959\_1960dupA | p.Val654SerFsTer14 | SPRB1-E19-F | 5’- GCTGGAATCACCTTATGG -3’(+) | 54 |
|  |  |  |  |  | SPRB1-E19-R | 5’- GTGACTTGCCCAGTAATG -3’(-) |  |
| RB25 | Ex 20 | MS | c.2104C>A | p.Gln702Lys | SPRB1-E20-F | 5’- TATTCCCACAGTGTATCG -3’(+) | 53 |
|  |  |  |  |  | SPRB1-E20-R | 5’- CAAGTAAGTAGGGAGGAG -3’(-) |  |
| RB26 | Ex 21 | MS | c.2134T>C | p.Cys712Arg | SPRB1-E21-F | 5’- TGAGCCTTGGTGATTTGC -3’(+) | 60 |
|  |  |  |  |  | SPRB1-E21-R | 5’- GAAGGTAAACCGGAAACAC -3’(-) |  |
| RB27 | Int 22 | SS | c.2325+1G>A | **-** | SPRB1-E22-F | 5’- CCTCAGACATTCAAACGTG -3’(+) | 53 |
|  |  |  |  |  | SPRB1-E22-R | 5’- CACCTTGATCTTGGAGTC -3’(-) |  |
| RB28 | Ex 23 | NS | c.2359C>T | p.Arg787Ter | SPRB1-E22-F | 5’- CCTCAGACATTCAAACGTG -3’(+) | 53 |
|  |  |  |  |  | SPRB1-E22-R | 5’- CACCTTGATCTTGGAGTC -3’(-) |  |
| RB31 | \*Ex 25-27 | LD | c.2643\_(\*1915+3849)del |  | SPRB1-E25-F | 5’-CCTCACTGTTGAATCTATCC -3’(+) | 55 |
|  |  |  |  |  | SPRB1-E25-R | 5’- CTGTTCTGGCCATTAGAC-3’(-) |  |

Abbreviations: Indel: small insertion or deletion, LD: Large deletion, MS: Missense, NS: Nonsense, SS: Splice site, Ta(0C): Annealing temperature in degree Celsius, \*Partial deletion of exon 25 with complete deletion of exons 26 and 27.