|  |  |  |
| --- | --- | --- |
| **Amplicon 1** | **FOR** | **GTTTTTGTATATGTTTTTTTTTTG** |
| **REV** | **AATAAAAATCTATCAAACAAACC** |
| **Amplicon 2** | **FOR** | **AGTTATTGTTTTTTTGAAAATTT** |
| **REV** | **CTAACCTTTTAAAATCACTAATTA** |
|  |  |  |  |  |  |
|  |  | **TAG sequence** | **spacer** | **barcode** | **unique sequence** |
| **Amplicon 3** | **FOR** | **CGTATCGCCTCCCTCGCGCCA** | **TCAG** | **NNNNNNNNNN** | **TTTTTTTTTAATTAGTGATTTTAA** |
| **REV** | **CTATGCGCCTTGCCAGCCCGC** | **TCAG** | **NNNNNNNNNN** | **CATAAAAAATTAAACTAAAAAACC** |
| **Amplicon 4** | **FOR** | **CGTATCGCCTCCCTCGCGCCA** | **TCAG** | **NNNNNNNNNN** | **GGTTTTTTAGTTTAATTTTTTATG** |
| **REV** | **CTATGCGCCTTGCCAGCCCGC** | **TCAG** | **NNNNNNNNNN** | **TAAATCCTCCTCAAAAAAAAAC** |

Table S1. Bisulphite treated DNA primer sequences. Amplicons 1 and 2 analysed by Sanger sequencing only. Amplicons 3 and 4 analysed by NGS.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Reads (n)** |  |  | **Reads (n)** |
| **Sample** | **Amplicon 2** | **Amplicon 3** |  | **Sample** | **Amplicon 2** | **Amplicon 3** |
| **664** | 1201 | 1187 |   | **2504** | 1547 | 2300 |
| **935** | 3118 | 1573 |   | **2198** | 640 | 688 |
| **1342** | 1757 | 1364 |   | **2933** | 464 | 1192 |
| **1706** | 1680 | 1160 |   | **2958** | 397 | 527 |
| **2149** | 2982 | 1442 |   | **3093** | 465 | 1337 |
| **2898** | 1923 | 1144 |   | **3138** | 615 | 1628 |
| **3531** | 3121 | 1020 |   | **3297** | 794 | 1226 |
| **3532** | 1734 | 1283 |   | **3587** | 427 | 810 |
| **4170** | 2374 | 1615 |   | **3718** | 685 | 585 |
| **4220** | 2962 | 1258 |   | **3727** | 340 | 825 |
| **4861** | 1605 | 1897 |   | **3818** | 495 | 745 |
| **5387** | 954 | 1417 |   | **4029** | 983 | 835 |
| **5492** | 1787 | 1490 |   | **4128** | 951 | 924 |
| **17921** | 1172 | 1712 |   | **1323** | 169 | 586 |
| **1867** | 872 | 1324 |   | **4207** | 557 | 723 |
| **1986** | 1305 | 1830 |   | **4271** | 542 | 557 |
| **M5616** | 1502 | 1560 |   | **4631** | 825 | 615 |
| **3208** | 1310 | 1513 |   | **4927** | 546 | 1098 |
|   |   |   |   | **5811** | 438 | 850 |
|   |   |   | **Total Reads** | **45239** | **43840** |
|  |  |   | **Average Reads/sample** | **1223** | **1185** |

Table S2 Amplicons spanning the RB1 promoter region analysed by *NGS* after amplification of bisulphite treated DNA, number of reads per sample

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Sample ID** | **RB1 gene** **status** | **RB1** **promoter**  | **CpGs methylated****(c.-1183-c.-259 )** | **CTCF CpGs** **methylated** **(c.-240-c.-208 )** | **Core promoter CpGs** **methylated** **(c.-203-c.-170 )** | **CpGs methylated****(c.-167-c.+77 )** | **DNA methylation** **Boundary CpGs\*** |
| **4170** | RB+/+ | PrE-/E- | 38/38 | 8/8 | 7/7 | 39/40 | ND |
| **1867** | RB+/+ | PrE-/E- | 38/38 | 8/8 | 7/7 | 40/40 | ND |
| **2933** | RB+/+ | PrE-/E- | 38/38 | 8/8 | 7/7 | 37/40 | ND |
| **4271** | RB+/+ | PrE-/E- | 37/38 | 6/8 | 5/7 | 31/40 | ND |
| **4927** | RB+/+ | PrE-/E- | 38/38 | 8/8 | 7/7 | 19/40 | ND |
| **3093** | RB+/+ | PrE-/E- | 37/38 | 8/8 | 7/7 | 38/40 | ND |
| **3138** | RB+/+ | PrE-/E- | 38/38 | 8/8 | 6/7 | 39/40 | ND |
| **5492** | RB+/+ | PrE-/E- | 38/38 | 8/8 | 7/7 | 40/40 | ND |
| **1986** | RB+/+ | PrE-/E- | 38/38 | 8/8 | 7/7 | 40/40 | ND |
| **2504** | RB+/+ | PrE-/E- | 38/38 | 8/8 | 7/7 | 5/40 | c.-140 |
| **3718** | RB+/+ | PrE-/E- | 38/38 | 8/8 | 7/7 | 40/40 | ND |
| **3818** | RB+/+ | PrE-/E- | 38/38 | 8/8 | 7/7 | 40/40 | ND |
| **3297** | RB+/+ | PrE-/E- | 38/38 | 8/8 | 7/7 | 39/40 | ND |
| **3727** | RB+/+ | PrE-/E- | 38/38 | 8/8 | 7/7 | 40/40 | ND |
| **4207** | RB+/+ | PrE-/E- | 38/38 | 8/8 | 7/7 | 40/40 | ND |
| **3587** | RB+/+ | PrE-/E- | 38/38 | 8/8 | 7/7 | 39/40 | ND |
| **4029** | RB+/+ | PrE-/E- | 38/38 | 8/8 | 7/7 | 9/40 | c.-136 |
| **4128** | RB+/+ | PrE-/E- | 38/38 | 8/8 | 7/7 | 13/40 | c.-119 |
| **5811** | RB+/+ | PrE-/E- | 38/38 | 8/8 | 7/7 | 40/40 | ND |
| **6361** | RB+/+ | PrE-/E- | 38/38 | 8/8 | 7/7 | 40/40 | ND |
| **9825** | RB+/+ | PrE-/E- | 38/38 | 8/8 | 7/7 | 40/40 | ND |
| **6930** | RB+/+ | PrE-/E- | 38/38 | 8/8 | 7/7 | 40/40 | ND |
| **1049** | RB+/c.1363C>T | PrE-/E+ | 38/38 | 8/8 | 7/7 | 40/40 | ND |
| **5387** | RB+/c.610delG | PrE-/E+ | 38/38 | 8/8 | 7/7 | 40/40 | ND |
| **4861** | RB+/c.109\_112delinsCCAGA | PrE-/E+ | 35/42 | 8/8 | 5/7 | 7/40 | c.-119 |
| **2198** | RB+/c.2490-?\_2520+?del | PrE-/E+ | 38/38 | 8/8 | 6/7 | 2/40 | c.-136 |
| **2898** | RB+/c.763C>T | PrE-/E+ | 38/38 | 8/8 | 7/7 | 40/40 | ND |
| **4631** | RB+/? | PrE-/E+ | 38/38 | 8/8 | 7/7 | 28/40 | c.+5 |
| **2958** | RB+/? | PrE-/E+ | 38/38 | 8/8 | 6/7 | 0/40 | c.-182 |
| **1792-1** | RB+/c.2536insC | PrE-/E+ | 38/38 | 8/8 | 7/7 | 40/40 | ND |

Table.S3 Summary characteristics of retinoblastomas with at least one epigenetically silenced *RB1* promoter (*PrE-/E-*, and*PrE-/E+*). CpGs counted as methylated includes any CpG showing at least partial methylation (>10% per allele). ND=Not determined if distal to c.+77. \*DNA methylation boundary location where CpGs transition from significant methylation to weak or no methylation downstream. All DNA specimens analysed were extracted from sporadic retinoblastomas.