**Appendix 2**. **Primers used to screen candidate genes within the LD interval on CFA25**. **A**. Primer information used for *SAG* screening. **B**. Primer information used for *KCNJ13* screening. **C**. Primer information used for the screening of the non-stop mutation in SAG. In bold and lower case is the base before the last that was altered from the database in C1 and C2 forward primers. In red is the mutated base.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **#** | **Forward primer name**  | **Forward primer sequence** | **Reverse primer name**  | **Reverse primer sequence** | **Size** **bp** | **Targeted sequence** | **Amplicon location on CFA 25** |
| **A. SAG gene screening** |
| 1 | SAG F1 | CCCAAAGAGGGTTAGGATAAACAGC  | SAG R1 | ACCACACAGAAAAGGCAAAACTGG  | 487 | Exon 1 (non-coding 5’UTR) | 47,814,374- 47,814,860 |
| 2 | SAG F2 | ATGGCCTTATTTCGTCAAACATCG  | SAG R2 | GGCACCCCATGTCTATTCTTGAAA  | 351 | Exon 2 (5’UTR and coding) | 47,815,466- 47,815,816 |
| 3 | SAG F3 | CTCCCCTTGACCCTATTGCCTTT  | SAG R3 | TCATCTCCTATTTCTACCCCCTGGT  | 648 | Intron 2 | 47,818,665- 47,819,312 |
| 4 | SAG F4 | GGCCACTTAGCATCTACATCTGTTC  | SAG R4 | GAAATTGTTTTCAATCAGCCAGTG  | 262 | Exon 3 | 47,820,607- 47,820,868 |
| 5 | SAG F5 | GCTGAACGTGGAACCTCCTTAAAA  | SAG R5 | AGCACTAAGACCAGACCAGACCAG  | 212 | Exon 4 | 47,823,499- 47,823,710 |
| 6 | SAG F6 | AGTGTCAGCGGTTACCCCATGTT  | SAG R6 | TCTCCTTCACTCCTGGTCACACTG  | 359 | Exon 5 | 47,825,286- 47,825,644 |
| 7 | SAG F7 | GTTTTGCTTGCTCGCTCTCTTCTC  | SAG R7 | TTTGGTTTTTGCCTGATTTCTGTG  | 388 | Exon 6 | 47,827,092- 47,827,479 |
| 8 | SAG F8 | ATGATAAACCTGGCTCCTTCCTGT  | SAG R8 | GTCCACGACCGAACACACTGG  | 540 | Intron 6 | 47,828,272- 47,828,811 |
| 9 | SAG F9 | GAGGGACAACTAAATGGAGGCAGA  | SAG R9 | CGAAGAAGAAGCAAACTGGAGAGG  | 617 | Exon 7 | 47,828,898- 47,829,514 |
| 10 | SAG F10 | ATGGGGTCCTTCCTCTTCTGG  | SAG R10 | CACGGTCGGTATGTTGAGACTTTG  | 753 | Exon 8 | 47,830,044- 47,830,796 |
| 11 | SAG F11 | CCCACTCATTCCCCCTCACTG  | SAG R11 | TGTGCTGCCTCTGTCAACAAAAAT  | 797 | Exon 9 | 47,830,880- 47,831,676 |
| 12 | SAG F12 | ACAGCCATTAGACAGGGAAGACCA  | SAG R12 | GAAGTGAGAAAAGGGGAAGGAAGC  | 294 | Exon 10 | 47,833,870- 47,834,163 |
| 13 | SAG F13 | GAGCAGGGACTTGAGGGAATGTC  | SAG R13 | GTGAGACGAGAAGGGGAGCAGAC  | 422 | Exon 11 | 47,835,843- 47,836,264 |
| 14 | SAG F14 | CAGAGGAAGGAGCCAAGGAAGATG  | SAG R14 | ATCATTCAGGAAAGGAAGGGGAGA  | 494 | Exon 12 | 47,838,861- 47,839,354 |
| 15 | SAG F15 | TGGGAAAAGTGAGTGTGTGTTTGC  | SAG R15 | AGAAGGCAGTGGGCATCGTTT  | 828 | Exon 13 | 47,839,505- 47,840,332 |
| 16 | SAG F16 | TCTGGTTTGTTTTGTTTTGAATCTCG  | SAG R16 | CAGGGCAGCATTTGAGGATGAC  | 336 | Exon 14 | 47,841,528- 47,841,863 |
| 17 | SAG F17 | AAAAGCAAAGGGCAGGGGTGAC  | SAG R17 | CAAGGGAGAGAAAATGAAGGAACC  | 666 | Exon 15 | 47,844,729- 47,845,394 |
| 18 | SAG F18 | GCTTGGTCCGTGCTCCATCAT  | SAG R18 | CTTCCCTCCCCCACCTGAGAC  | 481 | Exon 16 and 3UTR | 47,845,438- 47,845,918 |
| **B. KCNJ13 gene screening** |
| 1 | KCNJ135UTRF | AACAAACCAGCATTCTTTTCCACA | KCNJ135UTRR | TGCTTTCAGTAGGGCTATTACTCCA | 450 | Exon 1 (non-coding 5’UTR) | 47,387,670- 47,388,119 |
| 2 | KCNJ13\_intron1F | GAGCTGTTAGACTGGGAAAGCCTA | KCNJ13\_intron2R | TATCCCTTCTCCAAACCACTTGTA | 664 | Exon 2 (5’UTR and coding) | 47,382,095- 47,382,758 |
| 3 | KCNJ13\_intron2F | TCTTCAGGCAAATGAGACTATCCAA | KCNJ13\_3UTRR | AGTAGCTGCATAACTGGCTGGGTA | 848 | Exon 3 and 3’UTR | 47,379,501- 47,380,348 |
| 4 | KCNJ13\_F4 | ATCCGAAGGCAAGTGATGATTAGG | KCNJ13\_R4 | GACCTCTTTGAGCACCATCCATTT | 343 | Exon 2 | 47,382,500- 47,382,842 |
| 5 | KCNJ13\_F5 | TCAGAGATACCGGAGGATGGTCAC | KCNJ13\_R5 | GGAGCATTTGTATGGCAAGTAGGG | 389 | Exon 2 | 47,382,182- 47,382,570 |
| 6 | KCNJ13\_F6 | GCTGAGATGAATGGTGATCTGGAA | KCNJ13\_R6 | TACCGGCTGATAATGCAAATGAA | 341 | Exon 2 | 47,382,037- 47,382,377 |
| 7 | KCNJ13\_F7 | TCTTCAGGCAAATGAGACTATCCAA | KCNJ13\_R7 | GCTGGAGCAGAGTAGCAAGAGGAC | 477 | Exon 3 | 47,379,872-47,380,348 |
| 8 | KCNJ13\_F8 | TCAGGAAAGGGAAAATGGTGAACT | KCNJ13\_R8 | CTTGGAGACCAGAGGAGTTGGAAG | 370 | Exon 3 | 47,379,651-47,380,020 |
| 9 | KCNJ13\_F9 | GGGGTTCCAAAGGTGAATATCAAA | KCNJ13\_R9 | TTGCAGATGAAAATGAGAGATTGTGA | 553 | Exon 3 and 3’UTR | 47,379,181-47,379,733 |
| **C. Allele-specific extension test for the non-stop mutation in SAG** |
|  | **Forward primer name**  | **Forward primer sequence** | **Reverse primer name**  | **Reverse primer sequence** | **Expected size in normal allele** | **Expected size in affected allele** | **Amplicon location on chromosome 25** |
| 1 | Bas\_AS\_wt\_F | ACCGGGAGGCCATGGATGA**t**T | SAG R18 | CTTCCCTCCCCCACCTGAGAC | 259 | - | 47,845,660-47,845,918 |
| 2 | Bas\_AS\_mut\_F | ACCGGGAGGCCATGGATGA**t**C | Bas\_R1 | CAAATAGGGACTTGGACCGAACG | - | 531 | 47,845,660-47,846,190 |

**Appendix 3.** **Genotype calls for the significant locus on CFA25**. Allele are represented as A or B. Colored in green are homozygous genotypes for the A allele; Colored in orange are homozygous genotypes for the B allele; Colored in yellow are the heterozygous genotypes AB. NA= call is not available. SNPs within the *SAG* gene are marked in red. SNPs with the highest –log10(P) values are highlighted in purple. The homozygous block is boxed. The beginning and end of the homozygous block identified by homozygousity analysis are bold.

[See separately submitted Excel spreadsheet, for tabulated data]