Table S5. Fragments used to assess the accuracy of Illumina sequence data. For each fragment the start and approximate length of the usable sequence in the *S. melililoti* reference genome is given (*S. medicae* in parentheses). If strains differed in the length of high-quality sequence, the range of fragment lengths is given. *S. meliloti* strains: KH12g, KH16b, KH46c. *S. medicae* strains: KH36d, KH53a, KH53b. PCR conditions were: Cycle 1: 34 cycles 95 ºC for 0:30, 55 ºC 1:00, 72 ºC 2:00. Cycles 2 – 4 are the same but with 52, 60, or 50 ºC annealing temperature.

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| --- | --- | --- | --- | --- | --- | --- |
| Fragment | Replicon | Primers | PCR cycle | Start | Length | Strains |
| asd | Chrom | F: CGGCCGGGAGATGCTGAACA  R: ATGCGCTTGGTGAACTTCTTG | 1 | 3617425 (3347795) | 401 | KH12g KH16b KH36d KH46c KH53a KH53b |
| edD | Chrom | F: GGCATCATCACCTCCTACAA  R: CGGCGTGCCGGGATT | 1 | 767751 (318657) | 441 | KH12g KH16b KH36d KH46c KH53a KH53b |
| gap | Chrom | F: CGGTCCGGTCGAGACCAA  R: CGGTAGAGATCCTTGTGCAT | 1 | 2975612 (2755361) | 376 | KH12g KH16b KH36d KH46c KH53a KH53b |
| glnD | Chrom | F: GTGCGCTGCCACATGCAYTT  R: CCGGRTCRCGCTTGAA | 1 | 32205 (32205) | 216 | KH12g KH16b KH36d KH46c KH53a KH53b |
| gnd | Chrom | F: GGGCCGGCTCAACTCCTA  R: CGGCATCGGCAGGTT | 1 | 2091418 | 288 | KH12g, KH16b, KH46c |
| nodD1 | pSymA / pSMED02 | F: TTCAGGGTTCTCTAATAGG  R: AAGTTAAKGCTCTTGGC | 4 | (1105981) | 903 | KH36d, KH53a, KH53b |
| nodD3 | pSymA / pSMED02 | F: TTCGCCTTTACTGATTGGTCGG  R: ATCCGTCAATGTCTAACGC | 1 | 462810 (1132988) | 882 | KH16b, KH46c, KH53a, KH53b |
| nodH | pSymA / pSMED02 | F: TAGAAGCACGAAACTAGCAAA CG  R: AAGCTCAAAGAACCTCGCG | 1 | 468267 (1119739) | 676 | KH12g, KH16b, KH36d, KH46c, KH53b |
| nodP2 | pSymB / pSMED01 | F: ATTGCGATATGTCTCTTCCC  R: ATCGACTTGTTGTCGTGC | 2 | 786682 (1046202) | 399 – 899 | KH12g KH16b KH36d KH46c KH53a KH53b |
| nolR | Chrom | F: TCTTCGCGAACGATTCCTTCGC  R: TCATGGCGCGATCATGTAGCACG | 3 | 2594603 | 829 | KH12g, KH16b, KH46c |
| nuoE1 | Chrom | F: GCGCGCKCAGGAGCAGGA  R: CGCAGGCGCCCTGACATT | 1 | 1381488 (963622) | 204 | KH12g KH16b KH36d KH46c KH53a KH53b |
| ordL2 | Chrom | F: GCGGCGCGGTCGTCAT  R: CGCCATGGCCGGAATA | 1 | 771164 (322085) | 337 | KH12g KH16b KH36d KH46c KH53a KH53b |
| recA | Chrom | F: CCGGTTCGCTCGGCCTCGATA  R: CGCCCATCTCGCCCTCGATTT | 1 | 1948820 (1607544) | 229 | KH12g KH16b KH36d KH46c KH53a KH53b |
| sucA | Chrom | F: GCTCGGCCTCGAATA  R: CCGTCAGCGACAGGT | 1 | 3315057 (3089072) | 432 | KH12g KH16b KH36d KH46c KH53a KH53b |
| syrM | pSymA / pSMED02 | F: ATATTGTGACGACCTGG  R: ATTCATTATGCTCACCCATCC | 1 | 466411 (1126442) | 977 – 986 | KH16b, KH36d, KH53a |
| val\_a4 | pSymA | F: AAAGCAGCCGATATCCAGAA  AAGCCGCGAATATCGAAGTA | 2 | 989078 | 578 – 591 | KH12g, KH16b, KH46c |
| val\_b1 | pSymB | F: AGCTGTCTTTCGGCGTAATG  R: GCCATGACCTCCTTCGAT | 1 | 937793 | 481 – 570 | KH12g, KH16b, KH46c |
| val\_b2 | pSymB | F: CATCAATCCTTCGCTGTCCT  R: TGGAAA ACTTGTGCAGAATGAC | 2 | 342840 | 557 – 586 | KH12g, KH16b |
| val\_b3 | pSymB | F: AAGGAACATCTGGGCCTGC  R: TTTCCTCGGCGCTTTCG | 1 | 1635399 | 404 - 531 | KH12g, KH16b, KH46c |
| val\_b4 | pSymB | F: ATA AGGCTGCCGAGGTTCTT  R: ATGATGACCCCAGGATTGC | 1 | 633180 | 429 – 455 | KH16b, KH46c |
| val\_b5 | pSymB | F: ATTGCCGGAGAAGTTCATGT  R: AGGAGCCGATGGTTTTCAAG | 2 | 1677556 | 392 | KH16b |
| val\_p01\_1 | pSMED01 | F: GAGAGGTCGCACTCGTCAAC  R: TGCTGACTGTCTCTCGTATGC | 1 | (1104074) | 425 – 565 | KH36d, KH53b |
| val\_p02\_1 | pSMED02 | F: GGTGACAGATGGCCGAGAT  R: CTCGACGATCGGGCTCTTAC | 1 | (761420) | 403 – 467 | KH36d, KH53b |
| zwf | Chr | F: GGGGGCACCGGCGATCTTG  R: AGCGCAGTGCCATCAGATTCT | 1 | 770205 | 404 | KH12g, KH16b, KH46c |