

Figure S2 Confirmation of the specificity of primers F2/R2. (A) Comparison of the *Rf4* sequence in A619 and F2/R2 amplifying sequence. A619-Rf4 indicated the genome sequence of *Rf4* in the inbred line A619 and the number in it represent for bases position. (B) The search result of F2/R2 amplifying sequence in maize genome. The red bar represented for the position of F2/R2 amplifying sequence in maize B73 genome.

**S2A**

|                          |        |   |        |
|--------------------------|--------|---|--------|
| A619-Rf4                 | (1152) | ACCTGCTGCACTCGCTCGAGCGCGGGCGGGTCAATCCCGTCA CGGCGCGCGCGAGTCCACCATCGACTACTTCTCTGGG GGGCGGATCCCCACCA   | 100    |
| F2/R2 amplifying product |        | ACCTGCTGCACTCGCTCGAGCGCGGGCGGGTCAATCCCGTCA CGGCGCGCGCGAGTCCACCATCGACTACTTCTCTGGG GGGCGGATCCCCACCA   | 100    |
| Consensus                |        | acctgctgcaactcgctcgagcgcggcgggggtcaatcccgtcacggcgcgcgcgagtcacacatcgactacttctctcggcgcgcgatccccacca   |        |
| A619-Rf4                 |        | GCAGGCCATGCAGTACGAGCGGTGCGGCCCGCGGGGGCCACCAACAGTACACCATGGACATGTTCCGGCGACTACTGCGAGGGCAACTACCCACCC    | 200    |
| F2/R2 amplifying product |        | GCAGGCCATGCAGTACGAGCGGTGCGGCCCGCGGGGGCCACCAACAGTACACCATGGACATGTTCCGGCGACTACTGCGAGGGCAACTACCCACCC    | 200    |
| Consensus                |        | gcaggccatgcagtacgagcgcgtgcccgcggcgggggccaccacagtcacacatggacatgttccggcgactactgagcgggcaactaccceacc    |        |
| A619-Rf4                 |        | GCCGAGCCGTACATCCGCGGGACAATGACTGGAGCCCTCGTGTTCGGGGCCACCGACGACGACTCGGCCGTGCCTACATGCCCGGGGGCACTTTG     | 300    |
| F2/R2 amplifying product |        | GCCGAGCCGTACATCCGCGGGACAATGACTGGAGCCCTCGTGTTCGGGGCCACCGACGACGACTCGGCCGTGCCTACATGCCCGGGGGCACTTTG     | 300    |
| Consensus                |        | gccgagccgtacatccgcgggacaatgactggagccctcgtgttcggggccaccgacgacgactcggccgctgacctacatgccccgggggcaactttg |        |
| A619-Rf4                 |        | AGACCTCCCCCGCGCCACCGCCACCGCGCGCGAGGAAGCGGGCAGGGCGCTGGGGCGGGCTTCCATGCTGTGCTGGCCAACGGCGTCGAGAA        | 400    |
| F2/R2 amplifying product |        | AGACCTCCCCCGCGCCACCGCCACCGCGCGCGAGGAAGCGGGCAGGGCGCTGGGGCGGGCTTCCATGCTGTGCTGGCCAACGGCGTCGAGAA        | 400    |
| Consensus                |        | agacctccccgcggcgccacgcgccaccgcccgcggcaggaagcggggcaaggcgctggggcggggttccatgctgtgctggccaacggcgctcgagaa |        |
| A619-Rf4                 |        | GAAGGAGAAGCAGCGCCGGCTGCGGCTCACCGAGAAGTTTACGGCCCTCATGCACCTCATACCCAACGTTACGAAGTCTGTACGGCTACTTGCGC     | 496    |
| F2/R2 amplifying product |        | GAAGGAGAAGCAGCGCCGGCTGCGGCTCACCGAGAAGTTTACGGCCCTCATGCACCTCATACCCAACGTTACGAAGTCTGTACGGCTACTTGCGC     | 496    |
| Consensus                |        | gaaggagaagcagcgcggctgcggtcaccgagaagtttacggccctcatgcacctcataccaacgttacgaaggtcgtacgggtacttgccg        | (1647) |

