|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Mega-**  **scaffold** | **BACs/PCR products** | **scaffold-Gene-primer** | **Primer sequence** | **Product size (bp)** |
| 1 | 21A03 | 1-TFL2-F | TGAACAAGTGAAGCCAGACA | 501 |
| 1-TFL2-R | CCCCTGCCTAGATAATTGGT |
| 2 | Sc-02 | Lotus2-3F | GACTGCCAACCAACCGACACTCTGC | 4561 |
| Lotus2-3R | AACACCCACAACCCATAACCCTCCC |
| Lotus2-4F | CCATATTTTTGTCTAAAGCAACCCA | 5404 |
| Lotus2-4R | CTACAAGCCATCATTCCTAACCTATT |
| Lotus2-5F | CCATACCCGAGTTTAACCA | 3009 |
| Lotus2-5R | AGAACTTAGCAGACAGAGGACG |
| Lotus2-6F | TTGTGGGGAGGGATACTACTTA | 5030 |
| Lotus2-6R | ACTGCTCCAGAAAATGACACC |
| 3 | 10E06 | 3-VTC-F | CAAGTGGCTCAGTTTCTCGT | 474 |
| 3-VTC-R | ACTGCTTGGTGCTTAAGTGG |
| 4 | 06A17 | 4-PMI1-F | TGTTCAGACGGGTTTTCAAT | 557 |
| 4-PMI1-R | CAGAATACAACTGGCCCATC |
| 5 | 22O22 | 5-FY-F | CTAGGGGTGGGAAAAGACAT | 537 |
| 5-FY-R | TGGTAAGCACCCAACAAAAT |
| 6 | 02B06 | 6-WC-X-F | CCAAGGGTTAGATCCGAGTT | 550 |
| 6-WC-X-R | TAGGCAACTAGGCAACAAGG |
| 7 | Sc-07 | Lotus7-1F | TCATCCTTGAAACTTCCAAACGCTAC | 4126 |
| Lotus7-1R | TTTTGACAGGTCAGGGTCCAATACA |
| Lotus7-2F | AGACGAAGGCAGAACAACCAGACG | 6128 |
| Lotus7-2R | ACGAAATGAAGACAGAAACCGAAAG |
| Lotus7-4F | ATGACTAATGAGCAAGTAGCCCT | 4745 |
| Lotus7-4R | ACAGGAGCAGAGTTACCAGACC |
| Lotus7-5F | CCTAGAGGCTTTACATACGTTGC | 5161 |
| Lotus7-5R | CCCATTTCGGAGTCCTAACAA |
| 8 | Sc-08 | Lotus8-F | TGGAAGACACTGGCAAGATAGA | 4613 |
| Lotus8-R | GCCTCACCCATAGTTGTCAAG |
| Lotus8-1-F | GTGGAACGTGGAAGACGATAG | 5035 |
| Lotus8-1-R | ACAAGGTGACAAAAGTATCTAGGAC |
| Lotus8-2-F | CATGCAGCAATCTTCAACCCTA | 5536 |
| Lotus8-2-R | GTCCCACCTAACCCAAAATACG |
| 9 | Sc-09 | Lotus9-1F | GAATAGGTTCCAGTTTGTGC | 4392 |
| Lotus9-1R | CTCCCCATAGGTTGATAGAT |
| Lotus9-2F | GTCCGGCACAGTCATGTCTAC | 3486 |
| Lotus9-2R | TTGCAGGATGAGTGGGTTTAA |
| 10 | Sc-10 | Lotus10-1F | ATTTCGGTCGGTTCAGTTTGCT | 3317 |
| Lotus10-1R | TTTTAGGGAGGGACTCACCACC |
| Lotus10-2F | CACGCCTGACTGAAAACTTGA | 3789 |
| Lotus10-2R | GCCTTGTTCGTTTATTGGTATG |
| Lotus10-3F | TCATCCTACTTATTTCTGAGACGCCTAC | 3810 |
| Lotus10-3R | AAAACCACCCTTGCCCCACTAT |

**Suppl. Table 1. Primers used in the isolation of chromosome-specific markers.**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Chr.** | **Short arm (µm )** | **Long arm (µm )** | **Arm ratio** | **Location of 45S DNA** | **Location of**  **5S DNA** | **Total Length**  **(µm )** | **Relative length（%）** |
| 1 | 0.98±0.09 | 3.57±0.20 | 3.67±0.35 |  |  | 4.54±0.23 | 23.66±1.22 |
| 2 | 1.79±0.23 | 0.84±0.10 | 2.15±0.37 | Short arm |  | 2.62±0.25 | 13.67±1.32 |
| 3 | 0.69±0.07 | 1.92±0.11 | 2.81±0.30 | Long arm |  | 2.60±0.14 | 13.56±0.71 |
| 4 | 1.47±0.17 | 0.81±0.14 | 1.87±0.50 | Short arm |  | 2.28±0.20 | 11.87±1.06 |
| 5 | 0.95±0.07 | 1.35±0.09 | 1.43±0.13 |  | Long arm | 2.29±0.12 | 11.94±0.65 |
| 6 | 0.78±0.06 | 1.25±0.08 | 1.61±0.16 | Long arm |  | 2.03±0.11 | 10.63±0.60 |
| 7 | 0.65±0.06 | 0.86±0.06 | 1.34±0.16 |  |  | 1.51±0.08 | 7.87±0.43 |
| 8 | 0.56±0.07 | 0.76±0.12 | 1.37±0.21 |  |  | 1.32±0.16 | 6.85±0.85 |

**Suppl. Table 2. Karyotyping of the mitotic chromosomes in JianXuan 17.**

**Suppl. Table 3. Karyotyping of the mitotic chromosomes in DFH.**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Chr.** | **Short arm (µm )** | **Long arm (µm )** | **Arm ratio** | **Location of 45S DNA** | **Location of 5S DNA** | **Total Length**  **(µm )** | **Relative length（%）** |
| 1 | 0.86±0.11 | 3.70±0.21 | 4.33±0.44 |  |  | 4.56±0.29 | 23.75±1.50 |
| 2 | 0.88±0.12 | 1.76±0.19 | 2.05±0.47 | Short arm |  | 2.65±0.17 | 13.78±0.87 |
| 3 | 0.78±0.08 | 1.85±0.17 | 2.39±0.32 | Long arm |  | 2.63±0.21 | 13.67±1.09 |
| 4 | 0.83±0.11 | 1.47±0.30 | 1.79±0.34 | Short arm |  | 2.30±0.36 | 11.97±1.87 |
| 5 | 0.80±0.13 | 1.42±0.18 | 1.79±0.27 |  | Long arm | 2.22±0.26 | 11.55±1.37 |
| 6 | 0.77±0.15 | 1.18±0.13 | 1.59±0.31 | Long arm |  | 1.95±0.23 | 10.46±1.35 |
| 7 | 0.60±0.08 | 0.93±0.15 | 1.58±0.23 |  |  | 1.53±0.19 | 7.96±1.01 |
| 8 | 0.57±0.11 | 0.81±0.14 | 1.44±0.22 |  |  | 1.38±0.23 | 7.18±1.21 |

**Suppl. Table 4. Karyotyping of the mitotic chromosomes in Thailand.**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Chr.** | **Short arm (µm )** | **Long arm (µm )** | **Arm ratio** | **Location of 45S DNA** | **Location of 5S DNA** | **Total Length**  **(µm )** | **Relative length（%）** |
| 1 | 0.82±0.10 | 3.54±0.27 | 4.36±0.40 |  |  | 4.36±0.33 | 23.42±1.78 |
| 2 | 0.81±0.13 | 1.81±0.17 | 2.28±0.41 | Short arm |  | 2.63±0.23 | 14.12±1.21 |
| 3 | 1.76±0.20 | 0.82±0.16 | 2.25±0.57 | Long arm |  | 2.58±0.22 | 13.88±1.19 |
| 4 | 0.75±0.13 | 1.50±0.25 | 2.04±0.38 | Short arm |  | 2.24±0.33 | 12.06±1.76 |
| 5 | 0.75±0.10 | 1.45±0.16 | 1.96±0.30 |  | Long arm | 2.20±0.21 | 11.84±1.14 |
| 6 | 0.69±0.09 | 1.27±0.16 | 1.85±0.32 | Long arm |  | 1.96±0.21 | 10.51±1.11 |
| 7 | 0.58±0.08 | 0.92±0.17 | 1.60±0.35 |  |  | 1.50±0.20 | 8.08±1.06 |
| 8 | 0.44±0.10 | 0.69±0.10 | 1.63±0.42 |  |  | 1.13±0.17 | 6.09±0.92 |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **China Antique** | **JX17** | **DFH** | **Thailand** |
| **China Antique** | - | 3.9E-02 | 2.2E-4 | 7.6E-09 |
| **JX17** | - | - | 2.9E-02 | 5.5E-07 |
| **DFH** | - | - | - | 2.1E-3 |

**Suppl. Table 5. The significant assays (*p* values) of 5S signal gray values among China Antique, JX17, DFH, and Thailand.**