

Mutations in the CDS and promoter of *BjuA07.CLVI* cause a multilocular trait in *Brassica juncea*

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Supplementary Table S1 Primer sequences of molecular markers developed in this study

Marker	Sequence (5'-3')
ln10 F	GCGCTAGACTTCCTTGTGTG
ln10 R	CCTTAGCCTCTGTGTCTTCG
ln11 F	GCAAGTCTGATCGTAGCGTC
ln11 R	TTCACCTCCAAGAGAGAACC
ln12 F	GCTTCTGTACTGCTTCCACC
ln12 R	ATAGCCACCGAGTCGAATTG
ln13 F	ATCGTAAGCCATCCATCCTC
ln13 R	ATACCACCAGGCAACATGAG
ln14 F	CATGATCCATGTTGTGCTCC
ln14 R	TCTCTTCGCTGCTCTTGTTG
ln15 F	ACGCTTCCATTGTCCAGAAC
ln15 R	GCGACGTGGTACGGATTATG
ln16 F	GCATACGATCAGCGATAGTG
ln16 R	TAGACCTTCTTCTGCTGTGC
FL-F	CTCCAGATCATCATCATCAT
FL-R	CACAGCAGAAGAAGGTCTAC
qP-F	CGGCGAATTGACAAACCTAGAGGT
qP-R	AGGACTTGGAGGTTTCGGCATGT
β -actin F	GAGCTCCGTGTTGCCCTGAAGA
β -actin R	TGGATAGCAACATACATGGCAGGGACA
pro-F	CCCAAGCTTCAGCAGAAGAAGGTCTACAA
pro-R	TCCCCCGGGAAAAGCTGTCTTTTGAGAGA
M13 (-47)	CGCCAGGGTTTTCCAGTCACGAC
TF	CCGGAATTCAGTTAGACTCCTCGGATACG
PB-F	TACtctagaATGTACGTCGGCTACTTCAACAGCTAC
PB-R	TACtctagaGGTTAGGTTGTTAGCACTCGTGTTGAT

Supplementary Fig.S1 Frequency distribution with respect to bilocular silique percentage in untransformed control (the left column) and 25 T0 plants (the second to the last column) carrying the candidate gene.

Supplementary Fig.S2 The examination of insertion fragment and genetic background of some T1 individuals derived from one of the T0 plants. (a) The examination of insertion fragment by the specific primer TF and M13 (-47). Lanes 1-4 represent the non-transgenic multilocular plants (negative control), lanes 5-6 represent the non-transgenic bilocular plants (positive control), lanes 7-38 represent the bilocular plants in transgenic T1 progenies, lanes 39-48 represent the multilocular plants in T1 progenies, M represent the DNA ladder, ladders from top to bottom are 2000bp, 1000bp, 750bp, 500bp, 250bp and 100bp. (b) The examination of genetic background using molecular marker IP18. Lanes 1-4 represent the non-transgenic bilocular plants (positive control), lanes 5-36 represent the bilocular plant in transgenic T1 progenies, lanes 37-46 represent the multilocular plants in T1 progenies, M 100-bp DNA ladder.

Supplementary Fig.S3 Comparison of the BjuA07.CLV1 and BjuA07.clv1 coding sequences (c) and genomic sequences (g). The coding sequences of BjuA07.CLV1 and BjuA07.clv1 were isolated by cDNA sequencing by using RNA from buds of NIL (BL) and NIL (ML) in BC3F5. Sequences were aligned using GeneDoc. Black boxes and gray ones indicate identical residues and similar residues, respectively.

Supplementary Fig.S4 Amino acid sequence comparison of BjuA07.CLV1 from Duoshi and BjuA07.clv1 from Tayou2. GeneDoc was used for sequence alignment. Identical and similar residues are shown as black and grey boxes, respectively.

Supplementary Fig. S5 Comparison of a putative upstream promoter region between the BjuA07.CLV1 of Tayou2 and BjuA07.clv1 of Duoshi. GeneDoc was

used for sequence alignment. Black boxes and gray ones indicate identical residues and similar residues, respectively.

Figure S1

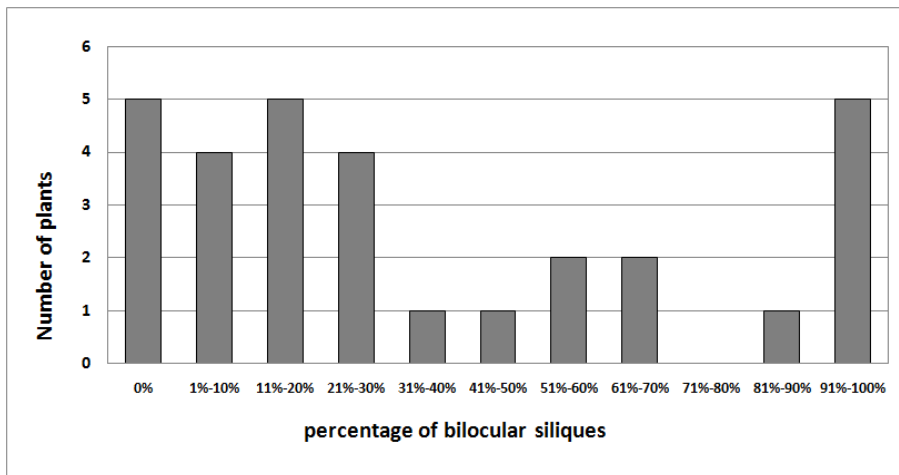


Figure S2

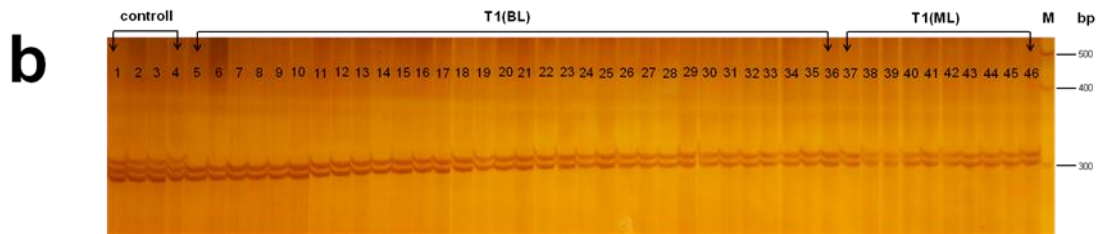
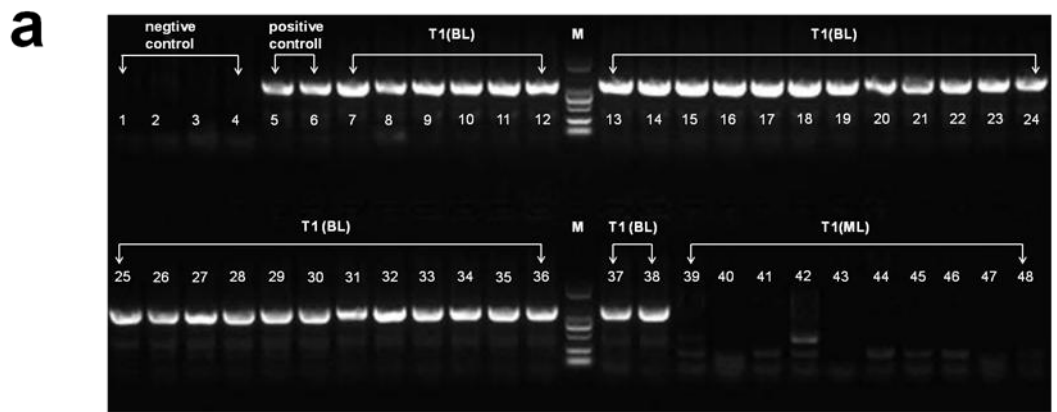


Figure S3

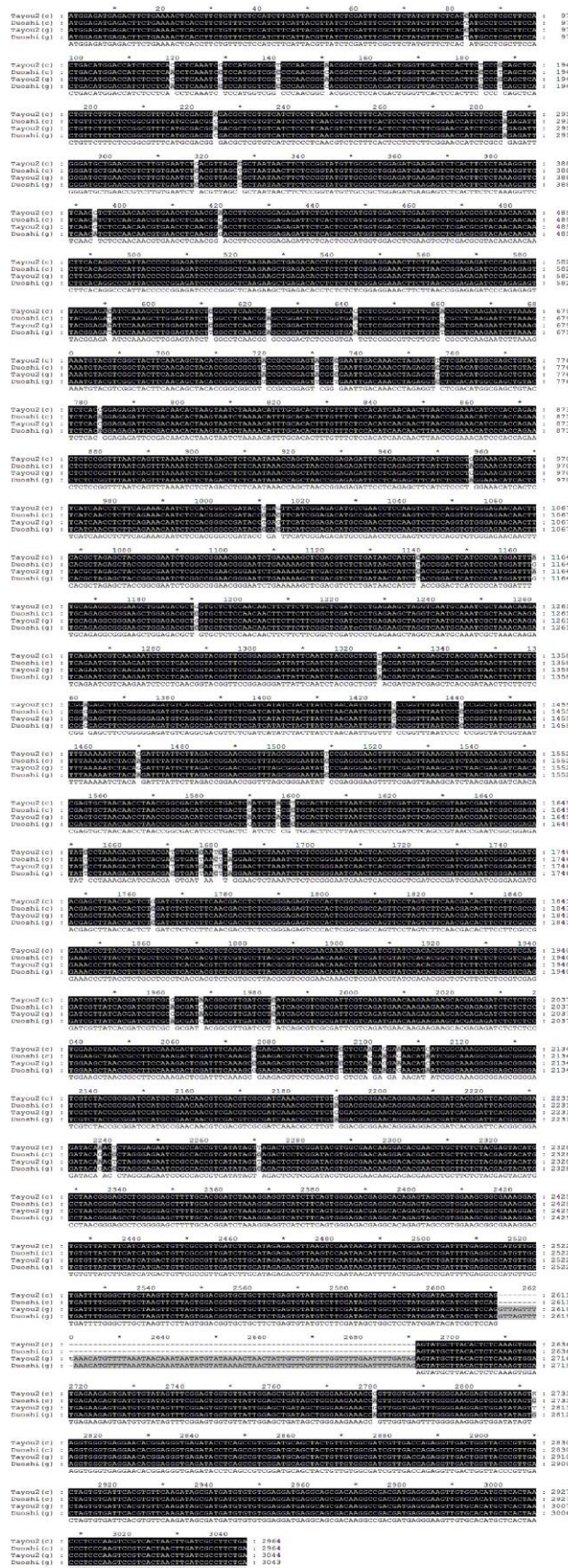


Figure S4

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*          *          *          *          *          *          *          *
20         40         60         80         100
Tayou2 : MEMRLKTHLLFLHLHYVISISLLCFSSCLASTDMDHLLLRKSSMVGPNGLHLDVHSTSPAHCSFSGVSCDGDARVISLNVSFPLFGTISPEIGMLN : 101
Duoshi  : MEMRLKTHLLFLHLHYVISISLLCFSSCLASTDMDHLLLRKSSMVGPNGLHLDVHSTSPAHCSFSGVSCDGDARVISLNVSFPLFGTISPEIGMLN : 101
MEMRLKTHLLFLHLHYVISISLLCFSSCLASTDMDHLLLRKSSMVGPNGLHLDVHSTSPAHCSFSGVSCDGDARVISLNVSFPLFGTISPEIGMLN

*          *          *          *          *          *          *          *
120        140        160        180        200
Tayou2 : RLVLNLAANNFSGMLPEMKSLTSLKVLNVSNNVNLCTFPFGRIITPMVDLEVLDAYNNNFTGPLPEIPGLKLRHLGLGNFLTGEIPESYGDIQSLE : 202
Duoshi  : RLVLNLAANNFSGMLPEMKSLTSLKVLNVSNNVNLCTFPFGRIITPMVDLEVLDAYNNNFTGPLPEIPGLKLRHLGLGNFLTGEIPESYGDIQSLE : 202
RLVLNLAANNFSGMLPEMKSLTSLKVLNVSNNVNLCTFPFGRIITPMVDLEVLDAYNNNFTGPLPEIPGLKLRHLGLGNFLTGEIPESYGDIQSLE

*          *          *          *          *          *          *          *
220        240        260        280        300
Tayou2 : YLGLNGAGLSGESPAFLSRLKLNKEMVGYFNSTYGGVPPPEYGEINLEVLDMASCTLTGEIPTTLSNLRHLHTLFLHINNLGNIPPELSGLISLKSLDL : 303
Duoshi  : YLGLNGAGLSGESPAFLSRLKLNKEMVGYFNSTYGGVPPPEYGEINLEVLDMASCTLTGEIPTTLSNLRHLHTLFLHINNLGNIPPELSGLISLKSLDL : 303
YLGLNGAGLSGESPAFLSRLKLNKEMVGYFNSTYGGVPPPEYGEINLEVLDMASCTLTGEIPTTLSNLRHLHTLFLHINNLGNIPPELSGLISLKSLDL

*          *          *          *          *          *          *          *
320        340        360        380        400
Tayou2 : SINQLTGEIQSFTSLGNITLNLFRNHLGPIFFIGDMPNLQVLQWENNFTLELPANLGRNGNKKLDVSDNHLTGLIPMDLCRGGKLETIVLSNFF : 404
Duoshi  : SINQLTGEIQSFTSLGNITLNLFRNHLGPIFFIGDMPNLQVLQWENNFTLELPANLGRNGNKKLDVSDNHLTGLIPMDLCRGGKLETIVLSNFF : 404
SINQLTGEIQSFTSLGNITLNLFRNHLGPIFFIGDMPNLQVLQWENNFTLELPANLGRNGNKKLDVSDNHLTGLIPMDLCRGGKLETIVLSNFF

*          *          *          *          *          *          *          *
420        440        460        480        500
Tayou2 : FGSIPKLGQCCKSLNKIRIVKNLNGTVPEGLFNPLVVTIIELDNFFSGELPGEMSGDVLVDHIYLSNNWF3GLIPPAIGNFKNLQDLFLDRNRFSGNIPR : 505
Duoshi  : FGSIPKLGQCCKSLNKIRIVKNLNGTVPEGLFNPLVVTIIELDNFFSGELPGEMSGDVLVDHIYLSNNWF3GLIPPAIGNFKNLQDLFLDRNRFSGNIPR : 505
FGSIPKLGQCCKSLNKIRIVKNLNGTVPEGLFNPLVVTIIELDNFFSGELPGEMSGDVLVDHIYLSNNWF3GLIPPAIGNFKNLQDLFLDRNRFSGNIPR

*          *          *          *          *          *          *          *
520        540        560        580        600
Tayou2 : EVFELKHLTKINTSANNLTGDIPIPSISRCTSLISVDLSRNRIGGDIKDIHDV6NLGTLNLSGNQLTGSIPIGIKWTSITLTLDSFNLSGRVPLGGQFL : 606
Duoshi  : EVFELKHLTKINTSANNLTGDIPIPSISRCTSLISVDLSRNRIGGDIKDIHDV6NLGTLNLSGNQLTGSIPIGIKWTSITLTLDSFNLSGRVPLGGQFL : 606
EVFELKHLTKINTSANNLTGDIPIPSISRCTSLISVDLSRNRIGGDIKDIHDV6NLGTLNLSGNQLTGSIPIGIKWTSITLTLDSFNLSGRVPLGGQFL

*          *          *          *          *          *          *          *
620        640        660        680        700
Tayou2 : VENTDSFAGNPLYCLPHHVSCILTRPEQTSRDIHTALFSPSRIVITIVAATLILISVAIRQMKNKKHERLSWKLTAFQRLDFKAEDVLECLQEENIGK : 707
Duoshi  : VENTDSFAGNPLYCLPHHVSCILTRPEQTSRDIHTALFSPSRIVITIVAATLILISVAIRQMKNKKHERLSWKLTAFQRLDFKAEDVLECLQEENIGK : 707
VENTDSFAGNPLYCLPHHVSCILTRPEQTSRDIHTALFSPSRIVITIVAATLILISVAIRQMKNKKHERLSWKLTAFQRLDFKAEDVLECLQEENIGK

*          *          *          *          *          *          *          *
720        740        760        780        800
Tayou2 : GGAGIVYRGSMPNNVDVAIKRVLGRGTGRSDHGFTAIEIQLGRIRHRHIVRLLGYVANKDTNLLLYEYMPNGSLGELLHGSRGGHLQWETRHRVAVEAARG : 808
Duoshi  : GGAGIVYRGSMPNNVDVAIKRVLGRGTGRSDHGFTAIEIQLGRIRHRHIVRLLGYVANKDTNLLLYEYMPNGSLGELLHGSRGGHLQWETRHRVAVEAARG : 808
GGAGIVYRGSMPNNVDVAIKRVLGRGTGRSDHGFTAIEIQLGRIRHRHIVRLLGYVANKDTNLLLYEYMPNGSLGELLHGSRGGHLQWETRHRVAVEAARG

*          *          *          *          *          *          *          *
820        840        860        880        900
Tayou2 : LCVLHHDCSPLILHRDVKSNNILLDSDFEAHVADFLAKFLVDGAASECMSSIAGSYGYIAPEYAYTLKVDKSDVYSFGVWLELILAGKKPVGEGVDF : 909
Duoshi  : LCVLHHDCSPLILHRDVKSNNILLDSDFEAHVADFLAKFLVDGAASECMSSIAGSYGYIAPEYAYTLKVDKSDVYSFGVWLELILAGKKPVGEGVDF : 909
LCVLHHDCSPLILHRDVKSNNILLDSDFEAHVADFLAKFLVDGAASECMSSIAGSYGYIAPEYAYTLKVDKSDVYSFGVWLELILAGKKPVGEGVDF

*          *          *          *          *          *          *          *
920        940        960        980
Tayou2 : IVRWVRNTEGEIQPSDAATVVAIVDQRLTGYPLTSVIHVFKIAMMVEDEAATRPTMREVVHMLTNPVKSVTNLIAF* : 987
Duoshi  : IVRWVRNTEGEIQPSDAATVVAIVDQRLTGYPLTSVIHVFKIAMMVEDEAATRPTMREVVHMLTNPVKSVTNLIAF* : 987
IVRWVRNTEGEIQPSDAATVVAIVDQRLTGYPLTSVIHVFKIAMMVEDEAATRPTMREVVHMLTNPVKSVTNLIAF
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Figure S5

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Tayou2 (p) : CACAGCAGAAGAAGGCTACAAAGAAAGTGGAGTGCACAAAGACAAAGAAAGGAGCGTTGACTATTAGATTGAAGCCAAAATAGAAGGGGACAGATT : 97
Duooshi (p) : CACAGCAGAAGAAGGCTACAAAGAAAGTGGAGTGCACAAAGACAAAGAAAGGAGCGTTGACTATTAGATTGAAGCCAAAATAGAAGGGGACAGATT : 97
          *      20      *      40      *      60      *      80      *
Tayou2 (p) : TGTCTCTTTTGGAAAGGACACAGACAATCTTTTATACGGGCCATTTAATAACTAGGCCCTACTTAATAAGCCCAATTAACCCCTCTTTGTCCTTTT : 194
Duooshi (p) : TGTCTCTTTTGGAAAGGACACAGACAATCTTTTATACGGGCCATTTAATAACTAGGCCCTACTTAATAAGCCCAATTAACCCCTCTTTGTCCTTTT : 194
          *      100     *      120     *      140     *      160     *      180     *
Tayou2 (p) : AAGTTTTTAAAATAATTTCCCAATTTCCGACACGCTGACGATAGGGGTGGGCACCTTAACCCGATATCCGAAGTGGCCACCCGAAACCCGATCCGAAA : 291
Duooshi (p) : AAGTTTTTAAAATAATTTCCCAATTTCCGACACGCTGACGATAGGGGTGGGCACCTTAACCCGATATCCGAAGTGGCCACCCGAAACCCGATCCGAAA : 228
          *      200     *      220     *      240     *      260     *      280     *
Tayou2 (p) : AAACCGAACCCGAATCCGAACCGAAGTAGCAAAATACCCGAACCGGTATTGAAATAGGAGAGATTGGATACCCGAACCCGAACCGGATATACCCGAA : 388
Duooshi (p) : ----- : -
          *      300     *      320     *      340     *      360     *      380     *
Tayou2 (p) : CCGAATGGATATCCGAAGATAACCGAATAGTATAATTAACTTATGTTTCTAGTTTATATCCCTCCATTTATATAAATATTTATATATGATAG : 485
Duooshi (p) : ----- : -
          *      400     *      420     *      440     *      460     *      480     *
Tayou2 (p) : TACACATAATTTAAGTTCATATGATATACATCAATTCGGAAAATAATGATTTGCTACTCACTTAAATGCAATGCAAGTTTTTATTTCAAATAAT : 582
Duooshi (p) : ----- : -
          *      500     *      520     *      540     *      560     *      580     *
Tayou2 (p) : AACAAAAGTTATATCCAAAATTAABAAAAAATAACTAAATTAGTGTCTTTTAGTTTAAAAATGTTATGTCRAATCTATTAACCATCAATCTAT : 679
Duooshi (p) : ----- : -
          *      600     *      620     *      640     *      660     *      680     *
Tayou2 (p) : TAAAAATAAAAAATAGTTACTTAAAGTTATATTTTAAATACAAATAAACCTGAGAAAAGAAAATTTAATATTTTTTTTCAAATCTAAATATCCG : 776
Duooshi (p) : ----- : -
          *      700     *      720     *      740     *      760     *      780     *
Tayou2 (p) : AACCCGATCCGAAATTAACCGAATCCGAACCTAAATTAACCCGAACCCGACCCGAGTACAGAAATPACCCGAACCCGATAGACCTCTATACCGAAAT : 873
Duooshi (p) : ----- : -
          *      780     *      800     *      820     *      840     *      860     *
Tayou2 (p) : CCGAAAATCCGAAATTAACCCGACCCGAAACCCGATCCGAAACCCGACCCGACCCGATAGACCTCTATACCGAAATTAACCCGATAGACCTCTAT : 970
Duooshi (p) : ----- : 268
          *      880     *      900     *      920     *      940     *      960     *
Tayou2 (p) : TGAGATGACAAAGACCGCGATAGAAAGAAAGAAACCAAGCAGCTATAGCAGGGAACGTAAGTAAAGTCTGCTATCATCTATTAACGGTCAAGTTC : 1067
Duooshi (p) : TGAGATGACAAAGACCGCGATAGAAAGAAAGAAACCAAGCAGCTATAGCAGGGAACGTAAGTAAAGTCTGCTATCATCTATTAACGGTCAAGTTC : 365
          *      980     *      1000    *      1020    *      1040    *      1060    *
Tayou2 (p) : AGATCATAGACATTTAATAGCGTTTCTTTGTCCTTACATTTTAAACCGCGTGAAGATATCCATTTATTTGATATATATAGCCAAAAGATAT : 1164
Duooshi (p) : AGATCATAGACATTTAATAGCGTTTCTTTGTCCTTACATTTTAAACCGCGTGAAGATATCCATTTATTTGATATATATAGCCAAAAGATAT : 462
          *      1080    *      1100    *      1120    *      1140    *      1160    *
Tayou2 (p) : ACTTCTCGATTTACATCTGTCAGAACATTTAAACAAGAAAGAACACATCAGGACCTTACCGGTTTTCGATCTGTCAGTCTGCGGCTGCT : 1261
Duooshi (p) : ACTTCTCGATTTACATCTGTCAGAACATTTAAACAAGAAAGAACACATCAGGACCTTACCGGTTTTCGATCTGTCAGTCTGCGGCTGCT : 559
          *      1180    *      1200    *      1220    *      1240    *      1260    *
Tayou2 (p) : AACCAATTAACCTTCGCTAAAACCTGAAAAGACTATGCTAAAAGTTTCTTTAGTATGTTTACTCTGCACATATAGCGTGGATTATGGGTTT : 1358
Duooshi (p) : AACCAATTAACCTTCGCTAAAACCTGAAAAGACTATGCTAAAAGTTTCTTTAGTATGTTTACTCTGCACATATAGCGTGGATTATGGGTTT : 656
          *      1280    *      1300    *      1320    *      1340    *      1360    *
Tayou2 (p) : GCAATACAAATTAAGCACAATCGTTTAGTTACCCGCAAAAATATCATGATTCCTCTAAAATCACAATGATTAATGACATTTACAAAATTTTGTGA : 1455
Duooshi (p) : GCAATACAAATTAAGCACAATCGTTTAGTTACCCGCAAAAATATCATGATTCCTCTAAAATCACAATGATTAATGACATTTACAAAATTTTGTGA : 753
          *      1380    *      1400    *      1420    *      1440    *      1460    *
Tayou2 (p) : TTATTACTCGGAATAATCAAAGAAAATATATTTGAGCACATTAACA AAAATGGAAGTTTGAAGTATGTGACCTAAAGCCAAATGAAAGACTG : 1552
Duooshi (p) : TTATTACTCGGAATAATCAAAGAAAATATATTTGAGCACATTAACA AAAATGGAAGTTTGAAGTATGTGACCTAAAGCCAAATGAAAGACTG : 850
          *      1460    *      1480    *      1500    *      1520    *      1540    *
Tayou2 (p) : ACACCTTCATTTGACCAACAGTCTAGTTCGTATCTCAAGCTGGATCTCCCTTTACCCGTTTATATCCGATGATAATTTCAAAAATCAACTCGGATA : 1649
Duooshi (p) : ACACCTTCATTTGACCAACAGTCTAGTTCGTATCTCAAGCTGGATCTCCCTTTACCCGTTTATATCCGATGATAATTTCAAAAATCAACTCGGATA : 947
          *      1560    *      1580    *      1600    *      1620    *      1640    *
Tayou2 (p) : AGTAAACATCAAAAATTAACAAGATACATTTGGAGAAAAGAAAGGTTTTTATTCGGCCATTTACTAGAGGTATATTTATCTCATTCAAAGCTCA : 1746
Duooshi (p) : AGTAAACATCAAAAATTAACAAGATACATTTGGAGAAAAGAAAGGTTTTTATTCGGCCATTTACTAGAGGTATATTTATCTCATTCAAAGCTCA : 1044
          *      1660    *      1680    *      1700    *      1720    *      1740    *
Tayou2 (p) : TATAAAAAAGATTTTAAAGTAAAATATCTCTCTCAAAGCTTACGAGTATTTAGAACAGTACCACACTAGGATCTCTCTCTTCACTCTCTGATAA : 1843
Duooshi (p) : TATAAAAAAGATTTTAAAGTAAAATATCTCTCTCAAAGCTTACGAGTATTTAGAACAGTACCACACTAGGATCTCTCTCTTCACTCTCTGATAA : 1141
          *      1760    *      1780    *      1800    *      1820    *      1840    *
Tayou2 (p) : TATGCCATTGIGTGATTTGTCATATATCTATATGTACCTCCATCGGATAAGACTCAGGCTAACCTCTTATCTCTCAAAGAAAGACAGCTTTTAA : 1940
Duooshi (p) : TATGCCATTGIGTGATTTGTCATATATCTATATGTACCTCCATCGGATAAGACTCAGGCTAACCTCTTATCTCTCAAAGAAAGACAGCTTTTAA : 1238
          *      1860    *      1880    *      1900    *      1920    *      1940    *
Tayou2 (p) : TAAAA : 1945
Duooshi (p) : TAAAA : 1243
          TAAAA
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