Discerning the putative U and V chromosomes of *Saccharina japonica* (Phaeophyta) by cytogenetic mapping of sex-linked molecular markers

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Running title: Discerning the kelp sex chromosomes U and V

Supplementary TABLE 1. Nucleotide sequence of primers employed in the present study.

Primer	Sequence (from 5′ to 3′)	Product size (bp)	Annealing temperature (°C)	Reference
DNA cloning for female gametophyte markers				
f170-F	TGACCGCGCTGGTCTTGAGGATA	619	54	This study
f170-R	CACCTGATACAGCACCCCA			
f58-F1	CAGACCATTATCTGATGTAAACAATGACAG	1105	56	Zhang et al. 2018
f58-R1	TTTGGGCCTTGAGGGATC			
f58-F2	TACCTGGTATTTGATACCATCTGAC	505	62	This study
f58-R2	TTTGGGCCTTGAGGGATC			
DNA cloning for male gametophyte markers				
m1840-F	AGGACGGGAACCACTACGG	436	55	This study
m1840-R	CTGGCGTTTCCACTATGC			
m16-F	TACGATTCAGGCAACACACC	472	56	Zhang et al. 2018
m16-R	CTTCCAAACCTTCCCTAGCA			
qRT-PCR				
Qg1-F	CATTGGCGAGGTATAAGAGC	128	55	This study
Qg1-R	GGGAAGGACGAGAACAGAT			
Qg2-F	GACGAGGCGAAGGCAAAG	91	55	This study
Qg2-R	CGTTCGGCGTAACAATGAC			
Qg3-F	GGGATGACGCAAGAAACG	186	55	This study
Qg3-R	CAGCGGAAGCGATGTGAG			
Qg4-F	ATGCCGAACATAACATCCT	181	55	This study
Qg4-R	GCTGCTCCATTTCCTCTTG			
Q18S-F	TCGGACGGTTTTGTGGTG	191	55	This study
Q18S-R	CCTTCCTTGGATGTGGTAGCC			
DNA cloning for annotated genes				
g1-F1	ATGCCAGGAGAGCCTCAACC	593 for female; 571 for male	60	This study
g1-R1	CTGGATTTCGATGTCTGCTG			
g1-F2	TGATCCTCAACCTGACCCAAG	590 for female; 568 for male	60	This study
g1-R2	ACCCATCACAAAACACAC			

g2-F	ATGGCACAGCATAGCGCCAT	258 for female; 254 for male	58	This study
g2-R	CTACACCACCGCTCTCACC			
g3-F1	AGCAACAGCAACAGCCTTACCA	782 for female; 771 for male	60	This study
g3-R1	TTCTTGCGTCATCCCTCTT			
g3-F2	AAGAGGGATGACGCAAGAA	633 for female; 663 for male	60	This study
g3-R2	GTTGCCGGTTTCGAGAATG			
g4-F	ATGCCGAACATAACATCCTT	252 for female; Not available for male	58	This study
g4-R	TTAGTAACACATGAATCCTGA			

Supplementary TABLE 2. Blast searching results of the four markers used in the present study against the availably assembled genome of *Saccharina japonica*.

Genome source	Sex-linked genes or markers			
	SJ-f_000170	MSj68-58-2	SJ-13_001840[b]	MSj68-16-2[b]
Ye et al. 2015	NA[a]	Only matched to Scaffold220 (444,739 bp) with 52% (573/1,105 bp) sequence identity.	Only matched to Scaffold3074 (24,639 bp) with 100% sequence identity (633 bp)	Only matched to Scaffold1744 (87,786 bp) with 100% sequence identity (472 bp)
Shao et al. 2019	Only matched to Contig4871 (6,269 bp) with 100% sequence identity (639 bp)	Only matched to Contig3481 (188,527 bp) with 100% sequence identity (1,105 bp)	Only matched to Contig4772 (10,276 bp) with 100% sequence identity (633 bp)	Only matched to Contig2278 (161,232 bp) with 100% sequence identity (472 bp)
Fan et al. 2020	NA[a]	Thirty pseudo-chromosomes were matched, but the sequence identities were <50%	No pseudo-chromosome was matched, except Chr0 matched with 100% sequence identity (633 bp)	No pseudo-chromosome was matched, except Chr0 matched with 100% sequence identity (472 bp)

[a]NA, not available.
[b]Chr0, an artificial chromosome constructed by scaffolds that were not linked to other genetic linkages (Fan et al. 2020).

Supplementary TABLE 3. Summary of the original sequencing data of two BAC clones.

	BAC669-A11	BAC652-P6
Length (bp)	110,085	104,111
Bases	1,600,988,320	1,500,167,532
Min	51	55
Max	55,664	55,664
Average	9,463.89	8,722.63
N50	10,464	9,685
C+G content (%)	51.53	50.43
Ns%	0	0

Supplementary TABLE 4. Comparison between the sequenced inserts of two screened BAC clones and the assembled genome of *Saccharina japonica*.

BAC clone	Assembled gametophyte genome of *S. japonica* by Ye et al. (2015)		Assembled sporophyte genome of *S. japonica* by Shao et al. (2019)		Assembled gametophyte genome of *S. japonica* by Fan et al. (2020)	
BAC669-A11	matched genome scaffolds	% of coverage[a]	matched genome contigs	% of coverage[a]	matched pseudo-chromosomes	% of coverage[a]
	Scaffold1221	17.88	Contig1705	20.58	Chr0	53.26
	Scaffold1777	14.73	Contig676	18.86	Chr6	31.55
	Scaffold408	13.30	Contig2317	17.70	Chr2	27.11
	Scaffold1524	11.42	Contig408	17.39	Chr4	23.09
	Scaffold70	11.32	Contig416	15.47	Chr1	20.63
	Scaffold285	11.10	Contig1544	15.13	Chr14	20.19
	Scaffold2802	11.02	Contig195	14.46	Chr13	19.56
	Scaffold378	10.49	Contig586	12.57	Chr16	19.25
	Scaffold765	10.40	Contig611	12.13	Chr15	18.98
	Scaffold351	10.11	Contig584	11.96	Chr30	16.78
			Contig1816	11.76	Chr7	16.63
			Contig327	11.41	Chr18	14.63
			Contig856	11.29		
			Contig1669	11.21		
			Contig1183	11.19		
			Contig1831	11.15		
			Contig310	10.96		
			Contig3284	10.68		
			Contig296	10.18		
			Contig550	10.13		
			Contig3385	10.11		
			Contig331	10.07		
	Overall coverage[b]	55.69	Overall coverage[b]	82.59	Overall coverage[b]	94.13

BAC652-P6	matched genome scaffolds	% of coverage[a]	matched genome contigs	% of coverage[a]	matched pseudo-chromosomes	% of coverage[a]
	Scaffold513	11.09	Contig3481	79.78	Chr0	32.95
	Scaffold254	10.26	Contig676	13.72	Chr12	26.93
	Scaffold74	10.16	Contig1040	10.40	Chr6	26.03
			Contig1864	10.33	Chr11	22.68
			Contig1277	10.12	Chr2	18.40
					Chr14	17.83
					Chr9	15.17
					Chr21	13.88
					Chr20	10.68
	Overall coverage[b]	25.81	Overall coverage[b]	92.56	Overall coverage[b]	98.76

[a]Only those scaffolds, contigs, or pseudo-chromosomes with more than 10% coverage of the BAC clones were taken into consideration for calculation.

[b]Overall coverage was also directly generated from NCBI BlastX online.

Supplementary TABLE 5. Summary of repetitive elements in the sequences of the two BAC clones as predicted online by the RepeatMasker website.

Start	End	Repeat	Type
BAC669-A11 (110,085 nt)			
2,128	2,168	+ $(CAG)_{13}$	STR
2,629	2,647	+ $(G)_{19}$	STR
2,879	2,901	+ $(ACA)_{7}$	STR
4,453	4,478	+ $(GTAC)_{6}$	STR
6,334	6,391	+ $(AGCGCTC)_{8}$	STR
10,501	10,540	+ $(CGGCC)_{8}$	STR
15,785	15,867	+ $(ATGT)_{20}$	STR
23,667	23,726	+ $(T)_{60}$	STR
23,727	23,748	+ $(TTTG)_{5}$	STR
29,389	29,428	+ $(GTAC)_{10}$	STR
30,567	30,606	+ $(GTAC)_{10}$	STR
30,885	30,938	+ $(TGTA)_{13}$	STR
31,346	31,383	+ $(GCA)_{12}$	STR
32,338	32,393	+ $(ATA)_{18}$	STR
32,656	32,674	+ $(C)_{19}$	STR
33,916	33,947	+ $(TGTATG)_{5}$	STR
34,629	34,692	+ $(AACAAC)_{10}$	STR
36,101	36,149	+ $(GGAACCTCC)_{5}$	STR
37,121	37,156	+ $(CGTACT)_{6}$	STR
37,836	37,869	+ $(TATG)_{8}$	STR
38,383	38,405	+ $(T)_{23}$	STR
39,048	39,111	+ $(GCA)_{21}$	STR
39,112	39,159	+ $(GGCGGC)_{8}$	STR
40,019	40,041	+ $(TAAT)_{5}$	STR
41,044	41,072	+ $(TAATTTT)_{4}$	STR
49,589	49,632	+ $(GTAC)_{11}$	STR
55,574	55,601	+ $(CTCC)_{7}$	STR
56,282	56,306	+ $(ATTG)_{6}$	STR
57,869	57,895	+ $(T)_{27}$	STR
58,985	59,012	+ $(TGC)_{9}$	STR
59,026	59,153	+ $(CGAGTA)_{21}$	STR
59,999	60,064	+ $(AAC)_{22}$	STR
62,127	62,251	+ $(GCA)_{41}$	STR
62,254	62,345	+ $(CGG)_{30}$	STR
62,555	62,612	+ $(TGC)_{18}$	STR
62,789	62,841	+ $(CGCTTCG)_{7}$	STR
63,097	63,141	+ $(AGCGCGA)_{5}$	STR
63,374	63,420	+ $(GTATGT)_{8}$	STR
74,909	74,996	+ $(TACTGC)_{14}$	STR
77,103	77,141	+ $(CAG)_{13}$	STR
78,240	78,283	+ $(GT)_{22}$	STR
79,291	79,322	+ $(TTACG)_{6}$	STR
87,269	87,307	+ $(GTT)_{13}$	STR
88,706	88,737	+ $(TTACG)_{6}$	STR
92,644	92,670	+ $(GTTTT)_{5}$	STR
106,940	106,968	+ $(CACAG)_{6}$	STR

109,961	109,998	+ $(GCA)_{12}$	STR
8,225	8,282	– *Gypsy*-55_LMi-I	LTR/*Gypsy*
29,022	29,077	– F*Gypsy*	LTR/*Gypsy*
65,680	68,671	– *Gypsy*	LTR/*Gypsy*
79,761	81,450	– *Gypsy*	LTR/*Gypsy*
83,036	83,155	– *Gypsy*-8_AA-I	LTR/*Gypsy*
83,223	83,574	– *Gypsy*-10_ES-I	LTR/*Gypsy*
83,922	84,174	– *Gypsy*-10_ES-I	LTR/*Gypsy*
84,430	84,761	– *Gypsy*-10_ES-I	LTR/*Gypsy*
93,169	93,712	– *Gypsy*	LTR/*Gypsy*
94,122	94,367	– *Gypsy*	LTR/*Gypsy*
96,477	97,341	– *Gypsy*	LTR/*Gypsy*
24,654	25,053	– *Copia*-1_ES	LTR/*Copia*
25,675	27,129	– *Copia*-2_ES	LTR/*Copia*
44,775	44,832	– *Copia*-2_ES-I	LTR/*Copia*
45,207	46,218	– *Copia*_ES	LTR/*Copia*
46,842	48,219	– *Copia*_ES	LTR/*Copia*
71,243	72,741	– *Copia*_ES	LTR/*Copia*
73,300	74,071	– *Copia*-2_ES-I	LTR/*Copia*
8,909	8,995	– I-Jockey-5_DPer	LINE/I-Jockey
21,758	22,478	– FRTE_pol	LINE/RTE-BovB
31,580	31,618	– I-Jockey-1_DPer	LINE/I-Jockey
39,269	39,532	– CR1	LINE/CR1
61,909	62,027	– L1-Tx1	LINE/L1
69,730	70,025	– I-Jockey-2_DPer	LINE/I-Jockey
86,099	86,169	– L2-Tx2	LINE/L2
BAC652-P6 (104,111 nt)			
2,352	2,478	+ $(CACCAAG)_{10}$	STR
2,619	2,691	+ $(TGCTTGG)_{10}$	STR
3,369	3,445	+ $(CAGAAG)_{16}$	STR
13,403	13,429	+ $(AT)_{18}$	STR
17,300	17,322	+ $(G)_{23}$	STR
18,723	18,760	+ $(TGC)_{12}$	STR
19,964	20,000	+ $(CTGTG)_{7}$	STR
22,029	22,058	+ $(GTT)_{10}$	STR
27,691	27,727	+ $(CAG)_{13}$	STR
27,959	28,007	+ $(AGC)_{19}$	STR
29,097	29,137	+ $(CAG)_{13}$	STR
29,457	29,498	+ $(AC)_{21}$	STR
32,510	32,544	+ $(ACA)_{11}$	STR
32,986	33,052	+ $(TTGTCTT)_{16}$	STR
33,152	33,176	+ $(TGTT)_{6}$	STR
38,231	38,305	+ $(AGC)_{24}$	STR
38,470	38,501	+ $(GGGTT)_{6}$	STR
41,386	41,444	+ $(CTG)_{19}$	STR
42,161	42,188	+ $(GAAAA)_{6}$	STR
42,209	42,366	+ $(CGCTG)_{31}$	STR
46,523	46,572	+ $(AACCG)_{10}$	STR

46,661	46,862	+ $(CACCG)_{40}$	STR
47,377	47,433	+ $(CCGCAGC)_{8}$	STR
47,731	47,803	+ $(TATTTAT)_{10}$	STR
48,557	48,608	+ $(GGTTC)_{10}$	STR
49,096	49,142	+ $(TGTACTC)_{7}$	STR
51,553	51,596	+ $(CTG)_{14}$	STR
59,377	59,395	+ $(A)_{19}$	STR
63,499	63,542	+ $(CCGTC)_{9}$	STR
64,041	64,166	+ $(CCCA)_{31}$	STR
68,498	68,541	+ $(ACA)_{4}$	STR
74,404	74,439	+ $(CTA)_{12}$	STR
76,748	76,771	+ $(CTG)_{8}$	STR
76,977	77,020	+ $(CTA)_{14}$	STR
77,233	77,271	+ $(CTG)_{13}$	STR
79,083	79,128	+ $(CCCTGA)_{7}$	STR
79,885	79,942	+ $(TGGT)_{14}$	STR
80,063	80,138	+ $(GGTG)_{19}$	STR
80,940	80,957	+ $(T)_{18}$	STR
81,515	81,534	+ $(GCT)_{5}$	STR
88,314	88,357	+ $(GCT)_{14}$	STR
88,760	88,816	+ $(GCT)_{19}$	STR
89,507	89,547	+ $(TACGAG)_{7}$	STR
90,436	90,454	+ $(G)_{19}$	STR
91,447	91,483	+ $(GCT)_{12}$	STR
92,248	92,272	+ $(GCT)_{8}$	STR
93,862	93,887	+ $(GCA)_{8}$	STR
24,635	24,804	– ERV1	LTR/ERV1
89,620	89,677	– *Gypsy*-55_LMi-I	LTR/*Gypsy*
91,713	91,806	– *Gypsy*-1_AC-I	LTR/*Gypsy*
95,875	96,052	– *Gypsy*-3_ES-I	LTR/*Gypsy*
96,456	98,992	– *Gypsy*	LTR/*Gypsy*
99,245	100,946	– *Gyps*	LTR/*Gypsy*
101,139	101,939	– *Gypsy*	LTR/*Gypsy*
102,185	102,655	– *Gypsy*-5_ES-I	LTR/*Gypsy*
3,577	3,634	– L1-Tx1-3_PM	LINE/L1
32,367	32,431	– RT1	LINE/RT1
34,382	34,938	– RTE-BovB	LINE/RTE-BovB
40,702	40,797	– RTAg4	LINE/R1
53,325	53,403	– L1-Tx1-3_PM	LINE/L1
63,918	64,039	– I-Jockey-3_DGri	LINE/I-Jockey-3
78,536	78,770	– RTAg4	LINE/R1
93,198	93,289	– Ag-Jock-1	LINE/Ag-Jock-1
93,623	93,697	– I-Jockey-3_DGri	LINE/I-Jockey-3

STR: short tandem repeat
LTR: long terminal repeat
LINE: long interspersed nuclear element

Supplementary TABLE 6. Proportion of repetitive elements and coding sequence in the sequenced BAC clones.

	Proportion of LTR elements (Bases per 1 kb)	Proportion of LINE elements (Bases per 1 kb)	Proportion of coding sequence (%)
BAC669-A11	116.794	30.006	1.301
BAC652-P6	57.451	12.924	0.243
Mean	87.954	21.704	0.783

Supplementary TABLE 7. Nucleotide and deduced amino-acid sequences of the annotated genes from the two BAC clones of *Saccharina japonica* female gametophytes.

Annotated gene	The closest Blast match to the assembled genome by Ye et al. (2015)	The closest Blast match to the assembled genome by Shao et al. (2019)	Linkage	Source	DNA sequence[a]	Locus ID	cDNA sequence[a]	Deduced amino-acid sequence[b]
Gene 1	Scaffold2746	Contig4871	Female	BAC6669-A11	atgccaggagagcctcaacctgatcctcaacctgacccaagccaaccaggtcaacctccacgaactccagtgccgatgcccaccgtgggggaaggacgagaacagatggatgtaatgtcgaggttgctgaaagaccgcattctactgctgggacaagatgttaacgacgaggtcggaaatgtcctggtcgctcagctcttatacctcgccaatgacgaccccgaaaaagatatcacgctctacatcaactctcccggcggctcagtgtcggcgggcctggcgatatacgatactatgcaggtgacacaacagttcagtttttccaagtttccccatgggtgtcggtgttgaattgtcatgttcttgcgttttgatgactgaaattccgtctcatttcccttcctgaccggtgctgtatgcagttcattccgtgtgacgttgccacagtctgtttcggcatggcagcgtctatgggagccttttttgttgggagcaggaagccccggcaagcgcaagtctctcccgaattcccgaataatgattcatcaaccgctgggtggagcggctggtcaggcagcagacatcgaaatccaggtgtgttttgtgatgggttgtatcccagtacaggacacctaa	SJ-f_000080[c]	atgcccaccgtggggaaggacgagaacagatggatgtaatgtcgaggttgctgaaagaccgcattctactgctgggacaagatgttaacgacgaggtcggaaatttcattccgtgtgacgttgccacagtctgtttcggcatggcagcgtctatgggagccttttgttgggagcaggaagccccggcaagcgcaagtctctcccgaattcccgaataatgattcatcaaccgctgggtggagcggctggtcaggcagcagacatcgaaatccag	MPTVGEGREQMDVMSRLLKDRILLLGQDVNDEVGNFIPCDVATVCFGMAASMGAFLLGAGSPGKRKSLPNSRIMIHQPLGGAAGQAADIEIQ
			Male	PCR amplification	ggctataaacgcgaaacgcaacttgcgtacagacggaaaggaggcgtgttgttggggatccgatggaccgggacctgtgtgcctgtgaccgctgccgcaagaaagtgctgtctcacactttgcccagctgccgctgcaatgaagtttgccgttct	TRINITY_DN17336_c0_g3[d]	atgaagtttgccgttctatatttgttctttgcgtacgtggtgtcgcaaacttttgcgtttgcgcctgcg	MKFAVLYLFFAYVVSQTFAFAPAPPSS

atatttgttctttgcgtacgtggtgtcgcaaacttttgcgttt	cctccttcctcttggag WRAASR
gcgcctgcgcctccttcctcttggagagcagcgagtcgc	agcagcgagtcgcct LRSRTSV
ctgcgcagtcgcacgagtgttctgacgatgccaggaga	gcgcagtcgcacgag LTMPGEP
gcctcaacctgatcctcaacctgacccaagccaaccag	tgttctgacgatgccag QPDPQPD
gtcaacctccacgaactccagtgccgatgcccaccgtg	gagagcctcaacctga PSQPGQP
ggggaaggacgagaacagatggatgtaatgtcgaggtt	tcctcaacctgaccca PRTPVPM
gctgaaagaccgcattctactgctgggacaagatgttaa	agccaaccaggtcaa PTVGEG
cgacgaggtcggaaatgtcctggtcgctcagctcttata	cctccacgaactccag REQMDV
cctcgccaatgacgaccccgaaaaagatatcacgctcta	tgccgatgcccaccgt MSRLLK
catcaactctcccggcggctcagtgtcggcgggcctgg	gggggaaggacgag DRILLLG
cgatatacgatactatgcaggtgacacaacagttcagtttt	aacagatggatgtaat QDVNDE
tccaagtttccccatgggtgtcggtgttgaattgtcatgttc	gtcgaggttgctgaaa VGNVLV
ttgcgttttgatgactgaaattccgtctcatttccctttcattc	gaccgcattctactgct AQLLYL
cgtgtgacgttgccacagtctgtttcggcatggcagcgtc	gggacaagatgttaac ANDDPE
tatgggagccttttgttgggagcaggaagccccggcaa	gacgaggtcggaaat KDITLYI
gcgcaagtctctcccgaattcccgaataatgattcatcaa	gtcctggtcgctcagct NSPGGSV
ccgctgggtggagcggctggtcaggcagcagacatcg	cttatacctcgccaatg SAGLAIY
aaatccaggcgaaggaaattttgttcacgaaacgacttct	acgaccccgaaaaag DTMQFIP
taatggctacatgtcggagtacacggagcagccggttg	atatcacgctctacatc CDVATVC
gcaagatagaggaagataccgatcgagatttcttcatga	aactctcccggcggct FGMAAS
ctccacacgaggcgctggagtatgggctgatagatgaa	cagtgtcggcgggcct MGAFLL
gttatcaagaccaagaccagccatctcccgctcccccag	ggcgatatacgatact GAGSPG
atgcccttccttgagtgagggcctctttcaaggcttttgat	atgcagttcattccgtg KRKSLPN
cctctgacgcaattactcaggcttgaaaattatggaattcc	tgacgttgccacagtct SRIMIHQ
cacgatattttatttcgatacaacgtttgacttgtttttttagc	gtttcggcatggcagc PLGGAA
aggatagtgtgtgcgacaaccttgcctaataaagtttgttg	gtctatgggagccttttt GQAADIE
acgggaaggaggtttccccgatcactcgttgtggtacaa	gttgggagcaggaag IQAKEIL
cgttcacagatgcttccagtacctggaagttaagtaaacg	ccccggcaagcgcaa FTKRLLN
tatgttggagagaaccctgcaagtacttcgtatgctcgca	gtctctcccgaattccc GYMSEY
gtatttcgggtttgtatgctggggatacttccctactccca	gaataatgattcatcaa TEQPVG
gtgttttcggggtccgatactgaaactctctgtacttgagt	ccgctgggtggagcg KIEEDTD

					attttagagtggtgtactgcgggtatttcaattgctggcag tattttgtccgttgttttgctagtattgcgagtacttgcagttt caaaatactctctcaatagcgcagtatactcggaatgtgg agcatgcttcgaccatctgcgcaccgtttcgatattttcat ccttttgtttttgcagacaggtggtcccacgagtgggagtt ggagcaaattacttccggggaggggggaaaggagtac tttgagtacggacagtatttcagcaatatttacggcgagta gtcgcagtatttacgtggtttaatacttctgattactgcgga gtactgcaggtatttcggatttgtactcggggttctgtattg ctcatactcccagaactccaagtatttgggtgagcagtac tgctcatctcccagagtttgcagtatttagaccgtcagtac tgtaaaaccatccgtactcaagagtaatggaatgtaatca atacttggagtatactcggagtataaagtacaagggtgc gctccgcgggcagctattcgccgcccgccgctcttgctc aaggaagcggatagaacaaagtgtgaagcagacagag ggcagaggacgctcatgatgtcgtgcaatgggagactt gcaaatgccaagcgctgtgttgcaggataaactatgccg ggccttctcgtgatgttgtgcgcagaaggttaaaaa		gctggtcaggcagca gacatcgaaatccagg cgaaggaaattttgttc acgaaacgacttcttaa tggctacatgtcggag tacacggagcagccg gttggcaagatagagg aagataccgatcgaga tttcttcatgactccaca cgaggcgctggagtat gggctgatagatgaag ttatcaagaccaagac cagccatctcccgctc ccccagatgcccttcct tgagtga	RDFFMTP
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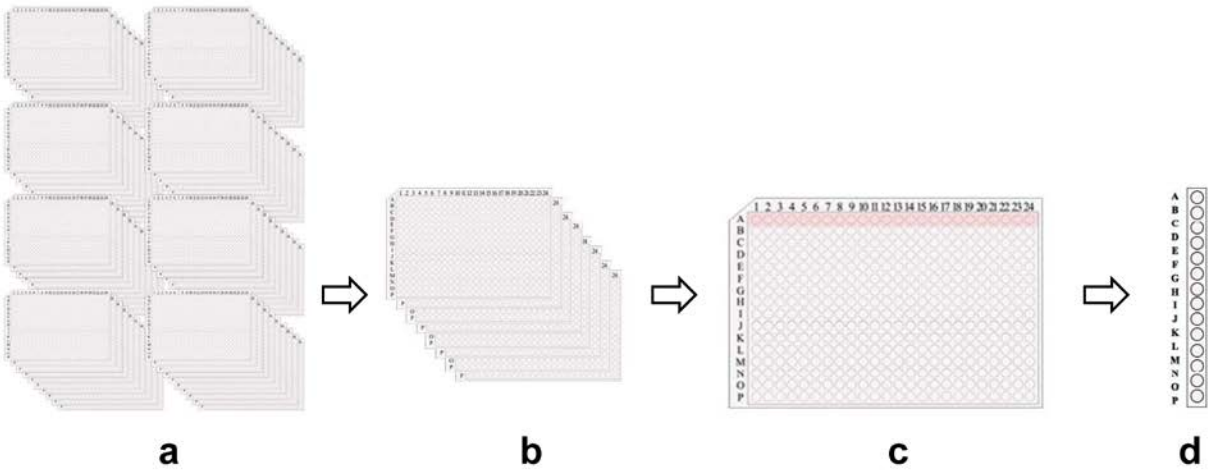
Gene 4	Scaffold1081	Contig3481	Female	BAC652-P6	atgccgaacataacatcctttgacgtttttccacccgtggt gtcattctccacattattttactcgctcgtgacccaggtgg ctcctcctgatcacccggcgggaacaacgtgggtgtgg gacgatgggtctggcgtggtgctgaaggctgcagaaaa agcacaagaggaaatggagcagcgcaaggccgagca aaaggaggaggttcgagcagtctgtctttttcgcttgtca ggattcatgtgttactaa	PCR amplification	atgccgaacataacat ccctttgacgtttttccac ccgtggtgtcattctcc acattattttactcgctc gtgacccaggtggctc ctcctgatcacccggc gggaacaacgtgggt gtgggacgatgggtct ggcgtggtgctgaag gctgcagaaaaagca caagaggaaatggag cagcgcaaggccgag caaaaggaggaggtt cgagcagtctgtcttttt cgcttgtcaggattcat gtgttactaa	MPNITSF DVFPPVV SFSTLFY SLVTQVA PPDHPAG TTWVWD DGSGVV LKAAEK AQEEME QRKAEQ KEEVRA VCLFRLS GFMCY
			Male	PCR amplification	Not available	PCR amplification	Not available	Not available

[a]Annotated gene was found by BAC clone sequencing and determined by transcriptome data (Ye et al., 2015), apart from those marked by superscript letters, also verified by PCR amplification from female and male genomic DNA samples. The underlined sequences denoted the intron ones.

[b]Amino-acid sequence was predicted by ORFfinder (https://www.ncbi.nlm.nih.gov/orffinder/).

[c]Data from Lipinska, A. P., Toda, N. R., Heesch, S., Peters, A. F., Cock, J. M., Coelho, S. M. 2017. Multiple gene movements into and out of haploid sex chromosomes. Genome Biol. 18, 104. doi: 10.1186/s13059-017-1201-7

[d]Transcriptome data from Bi, Y.-H., Li, J.-L., Zhou, Z.-G. (2018). Full-length mRNA sequencing in *Saccharina japonica* and identification of carbonic anhydrase genes. Aquaculture and Fisheries 4(2), 53-60. doi: 10.1016/j.aaf.2018.11.002



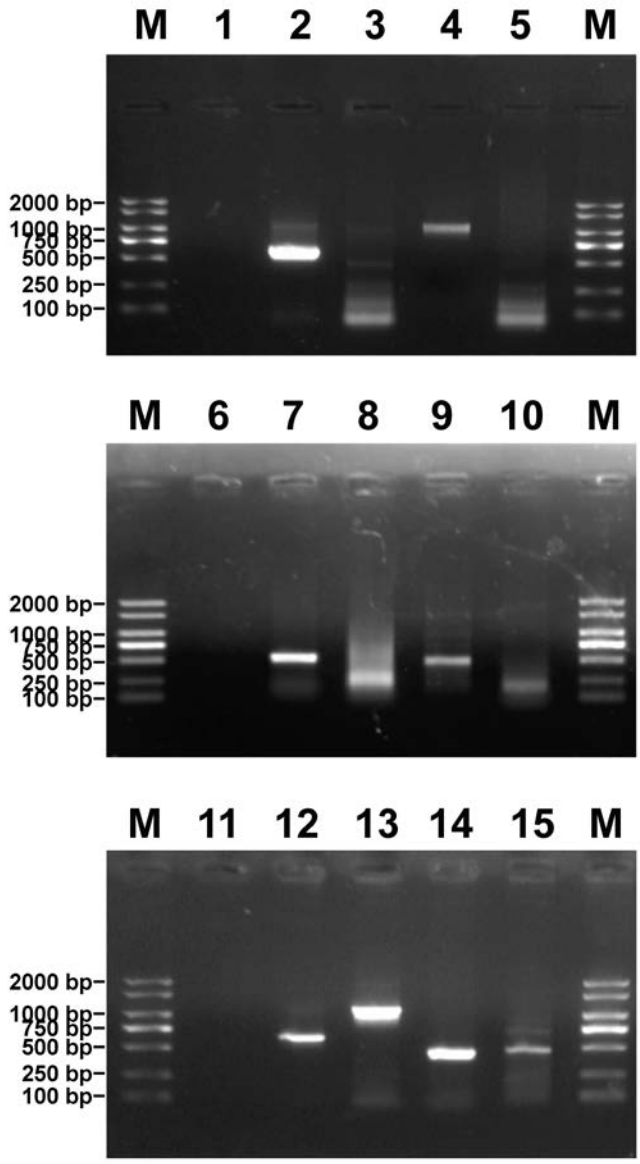
Supplementary FIGURE 1. Schematic representation of 3D screening pool for *S. japonica* female gametophyte genome BAC library.

a) Construct the primary pool of the BAC library: 12 channels pipette was used to construct the the primary pool in sequence, and merged each 10 384-well original plates into a new 384-well plate pool. The specific operation was as follows: Took out the 384-well plate stored at −80°C and melted at room temperature. Added 50 μL LB liquid medium per well (containing chloramphenicol resistance) in advance into a new 384-well plate, and drew about 2 μL bacterial solution from the constructed BAC library to the corresponding cloning pool. Finally, the 384-well plate was cultured in a shaker with 37°C for 6 h, and stored at −80°C for use.

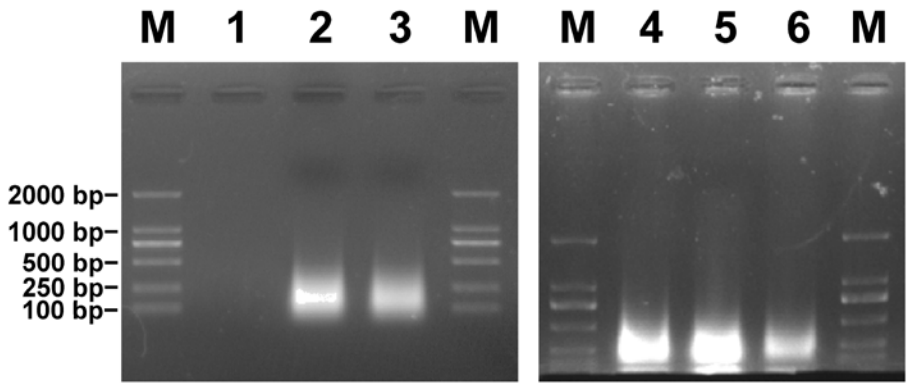
b) Construct the secondary pool of the BAC library: According to step one, combined the constructed eight primary pool plates into a new 384-well plate pool, and cultured in a shaker with 37°C and 180 rpm·min^{-1} for 6 h, then stored at −80°C for use.

c) Construct the third-level pool of the BAC library: Took a sterile 96 deep-well plate, added LB freezing liquid medium containing chloramphenicol (12.5 g·mL^{-1}) to it, 500 μL per well, and then added the constructed one the monoclonal bacteria liquid in each of the 24 wells in each horizontal row of the secondary pool plate were added to one well of the 96-well plate. Finally, marked the plate number and date of the constructed pool on the 96-well plate, incubated with 37°C and 180 rpm·min^{-1} for 6 h, then stored at −80°C for use.

d) Three-dimensional PCR screening of BAC library: Designed and synthesized specific primers according to the conservative regions of genes (Table 1), and used PCR amplification techniques to screen BAC library. Screened the third-level pools first, then screened the secondary and primary pools, and finally screened the original BAC plates. The positions of positive clones in the plate pool, row pool, and column pool were compared and screened to determine the corresponding specific position of the positive clone. Picked out the positive clones from the frozen BAC library, performed PCR identification and full-length sequencing of the BAC clones.

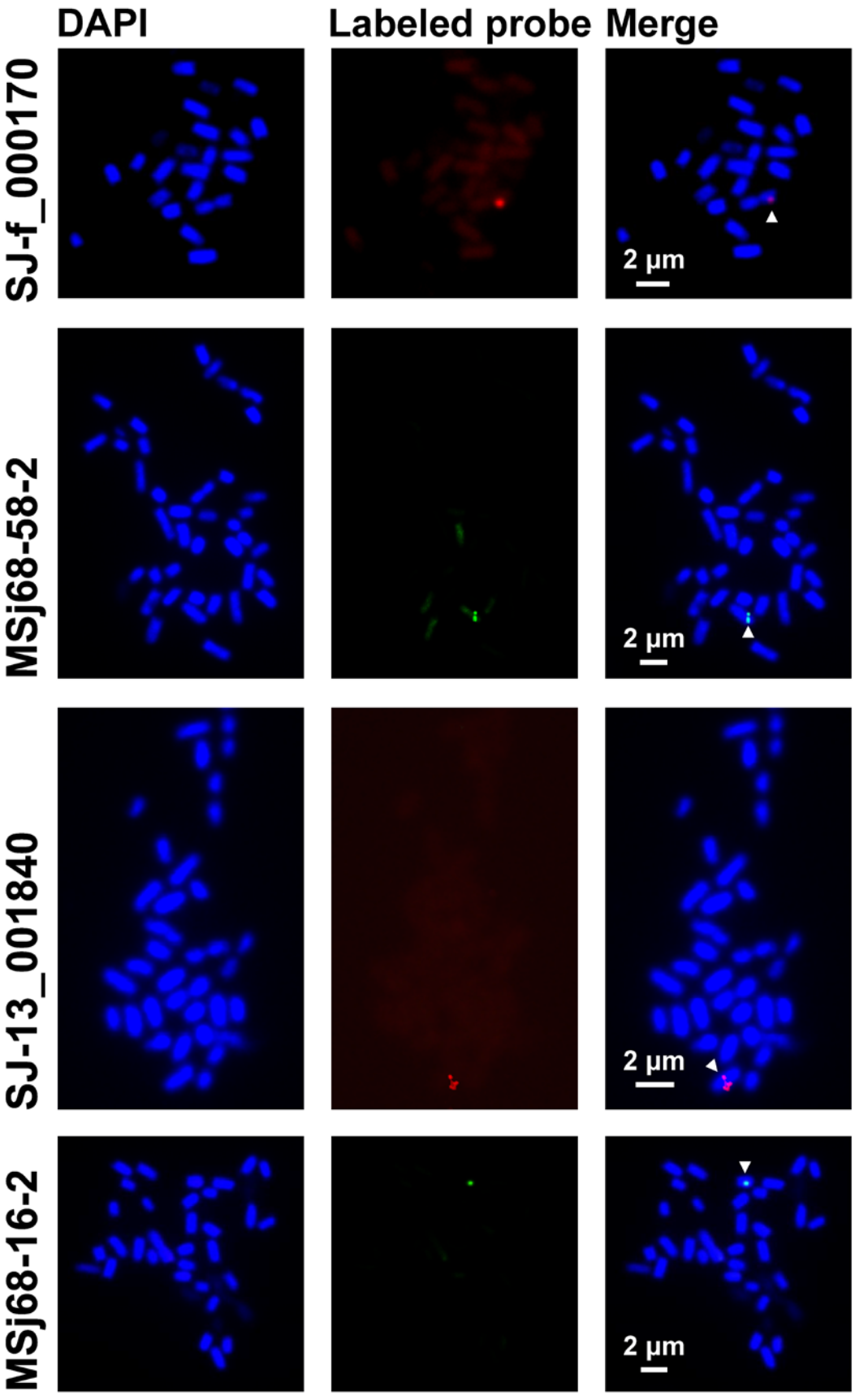


Supplementary FIGURE 2. Electrophoresis profiles of sex-linked marker products amplified from *Saccharina japonica* gametophytes and sporophytes.
Lanes 2, 3, and 12: sex-linked gene SJ-f_000170; Lanes 4, 5, and 13: sex-linked marker MSj68-58-2; Lanes 7, 8, and 14: sex-linked gene SJ-13_001840; Lanes 9, 10, and 15: sex-linked marker MSj68-16-2; Lanes 2, 4, 8, and 10: *Saccharina japonica* female gametophytes; Lanes 3, 5, 7, and 9: *S. japonica* male gametophytes; Lanes from 12 through 15: *S. japonica* sporophytes; Lanes 1, 6, and 11: controls with H_2O as template instead of genomic DNA; and Lane M: DL2000 Plus DNA Marker (Vazyme, Nanjing, China).



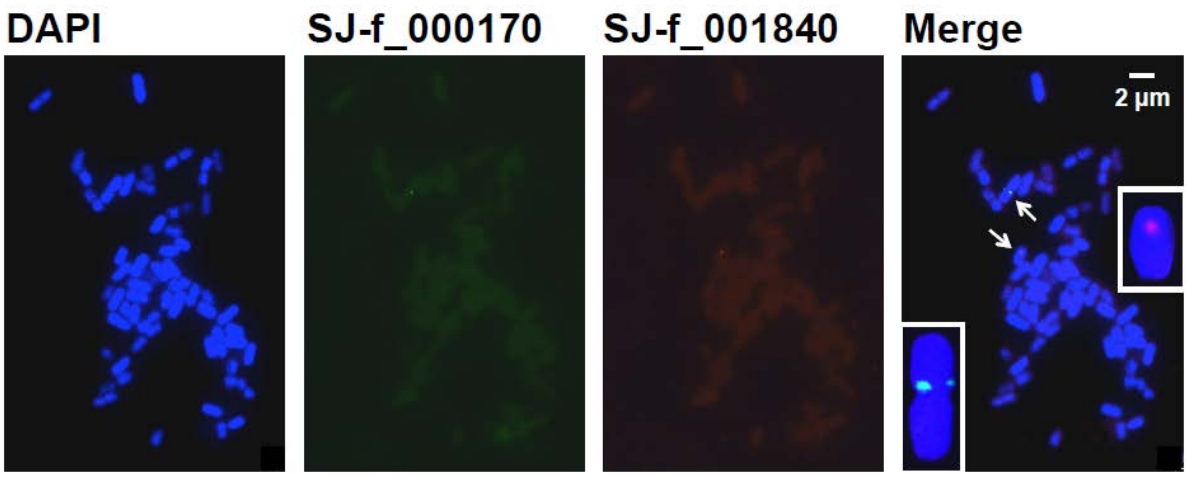
Supplementary FIGURE 3. Electrophoresis profiles of the labeled probes by nick translation.

Lane M: DL2000 DNA Marker (TaKaRa, Kyoto, Japan); Lane 1: control with H_2O as template instead of genomic DNA; Lane 2: labeled MSj68-58-2 (green); Lane 3: labeled SJ-f_000170 (red); Lane 4: labeled SJ-13_001840 (red); Lane 5: labeled MSj68-16-2 (green); and Lane 6: labeled SJ-f_000170 (green).

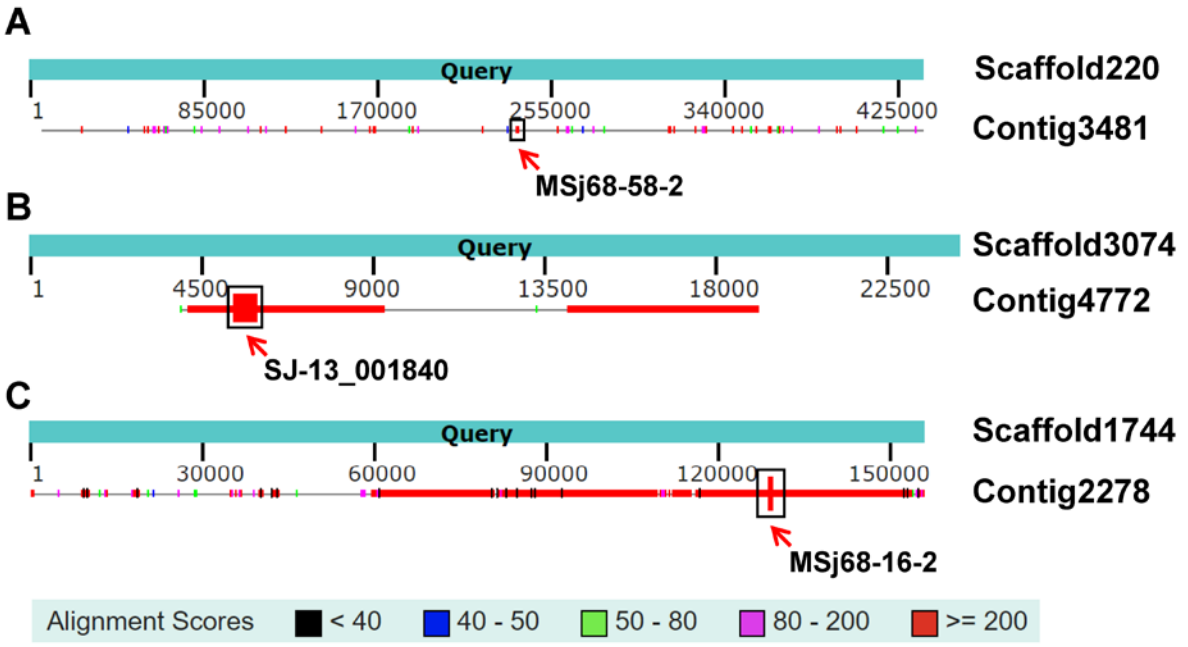


Supplementary FIGURE 4. FISH images showing hybridization sites of the four sex-linked markers on *Saccharina japonica* gametophyte chromosomes counterstained with DAPI (blue).

Hybridization signals of SJ-f_000170 marker (red) and MSj68-58-2 marker (green) on the chromosomes of *Saccharina japonica* female gametophytes are indicated by arrowheads. Hybridization signals SJ-13_001840 marker (red) and MSj68-16-2 marker (green) on the nuclei of *S. japonica* male gametophytes are also indicated by arrowheads. FISH signals of both MSj68-58-2 (green) and SJ-13_001840 (red) markers presented are conspicuously twin spots.

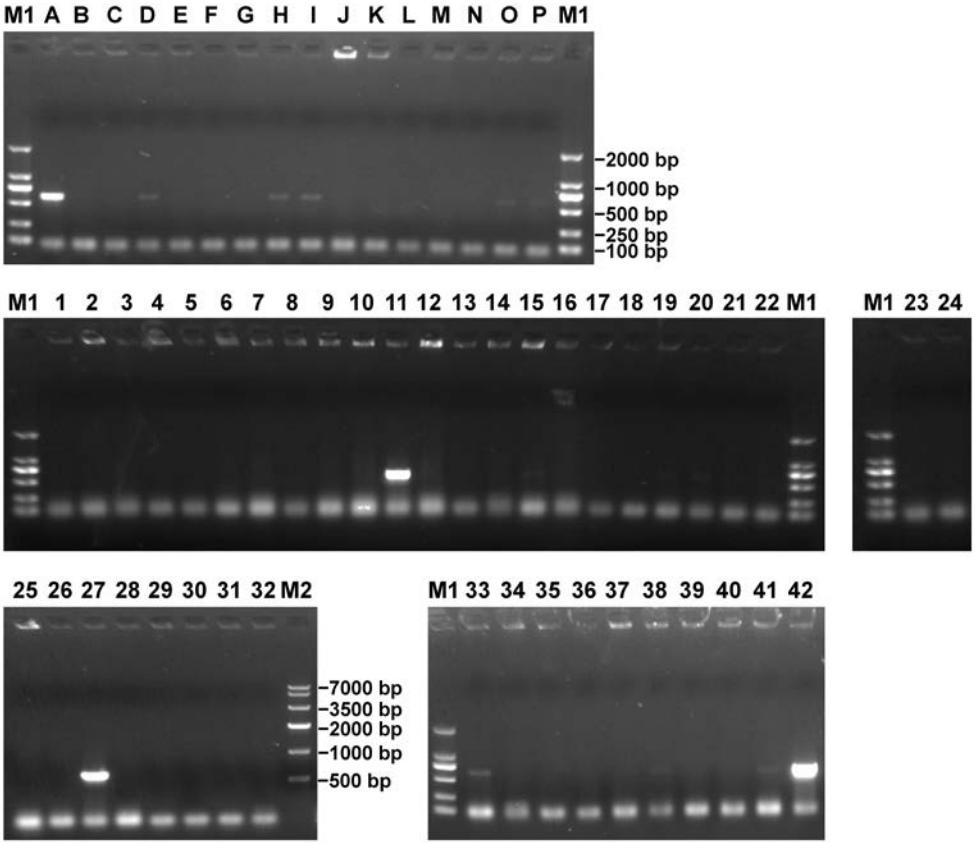


Supplementary FIGURE 5. Co-localization of two sex-linked markers SJ-13_001840 (red) and SJ-f_000170 (green) using dual-color FISH technique on *Saccharina japonica* sporophyte chromosomes which were counterstained with DAPI (blue).
Arrows denote the sites of hybridization signals on sporophyte chromosomes. The FISH signal of SJ-f_000170 (green) presented are unambiguously twin spots. The insets in the merged image clearly illustrate the signals are present on two different chromosomes in size.



Supplementary FIGURE 6. Alignment of the matched scaffolds and contigs which were retrieved from the assembled *Saccharina japonica* gametophyte genome (Ye et al., 2015) and sporophyte one (Shao et al., 2019), respectively, using each sex-linked marker as a query.

The arrows denote the relative position of each sex-linked marker used in the present study at the matched contigs and scaffolds. Alignment scores: the graphic is an overview of the database sequences aligned to the query sequence. These are represented horizontal bars colored coded by score and showing the extent of the alignment on the query sequence. Separate aligned regions on the same database sequence are connected by a grey line.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100 bp
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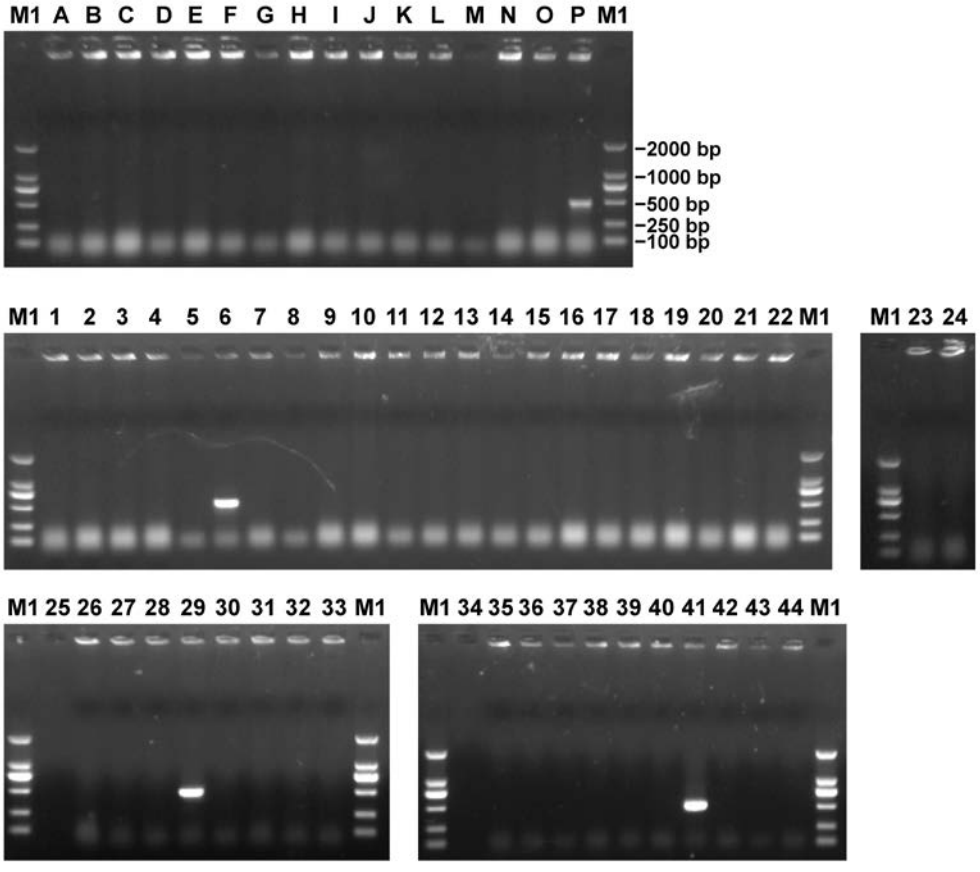
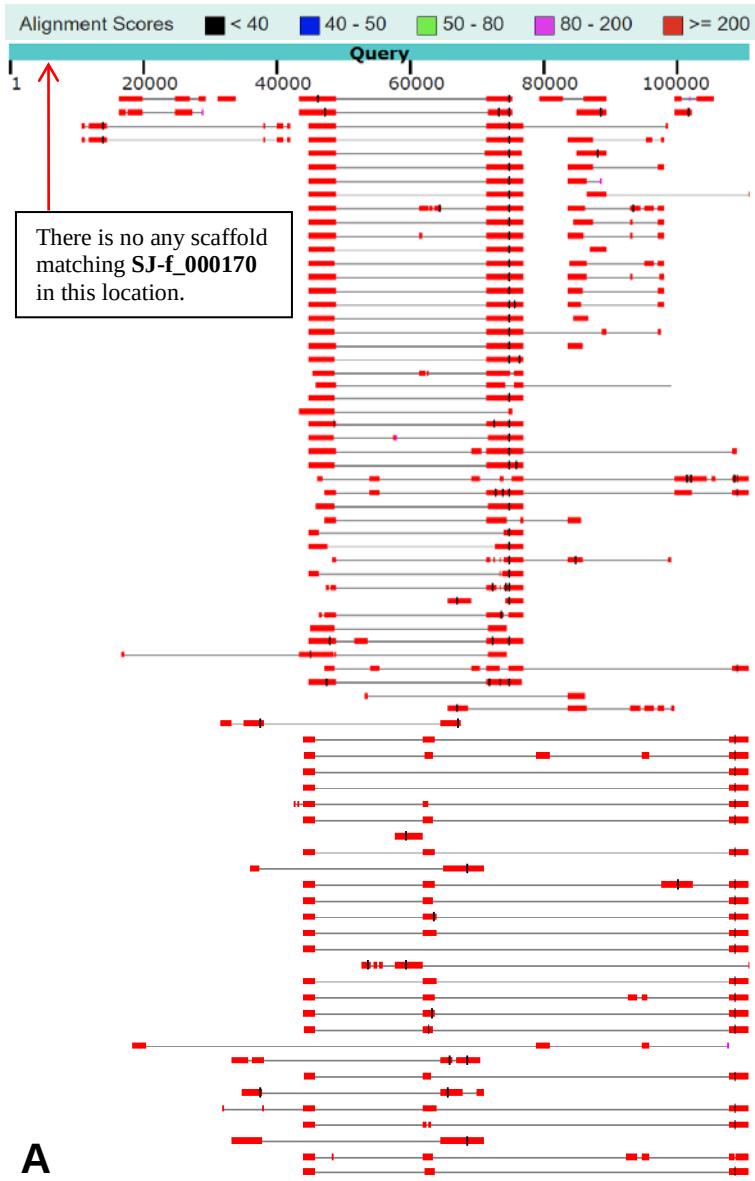
M1 23 24
25 26 27 28 29 30 31 32 M2
7000 bp
3500 bp
2000 bp
1000 bp
500 bp
M1 33 34 35 36 37 38 39 40 41 42

(a)


M1 A B C D E F G H I J K L M N O P M1
2000 bp
1000 bp
500 bp
250 bp
100 bp
M1 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 M1
M1 23 24
M1 25 26 27 28 29 30 31 32 33 M1
M1 34 35 36 37 38 39 40 41 42 43 44 M1

(b)

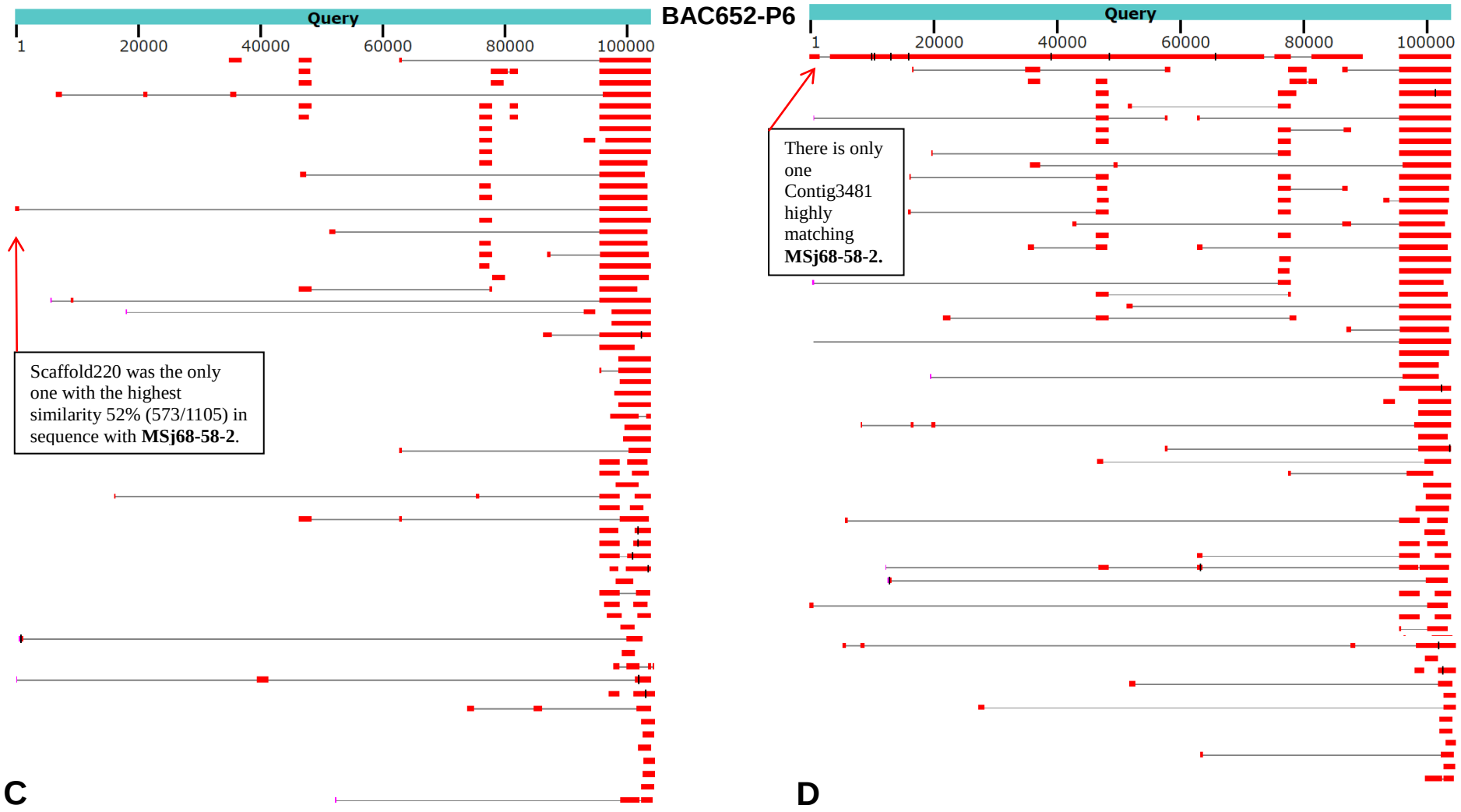
Supplementary FIGURE 7. Electrophoresis profiles of amplified products for screening of positive clones carrying the U-linked markers SJ-f_000170 (a) and MSj68-58-2 (b) from the constructed BAC libraries of *Saccharina japonica* female gametophytes.
Lane M1: DL2000 DNA Marker (TaKaRa, Kyoto, Japan); Lane M2: DNA Marker VI (Tiangen Biotech, Beijing, China); Lanes from A trough P: representing the longitudinal screening of the BAC secondary pool; Lanes from 1 through 24: representing the horizontal screening of the BAC secondary pool; Lanes from 25 through 32 (a) or 33 (b): representing the screening of the BAC primary pool; Lanes from 33 (a) or 34 (b) through 42 (a) or 44 (b): representing the screening of the original BAC library.



BAC669-A11

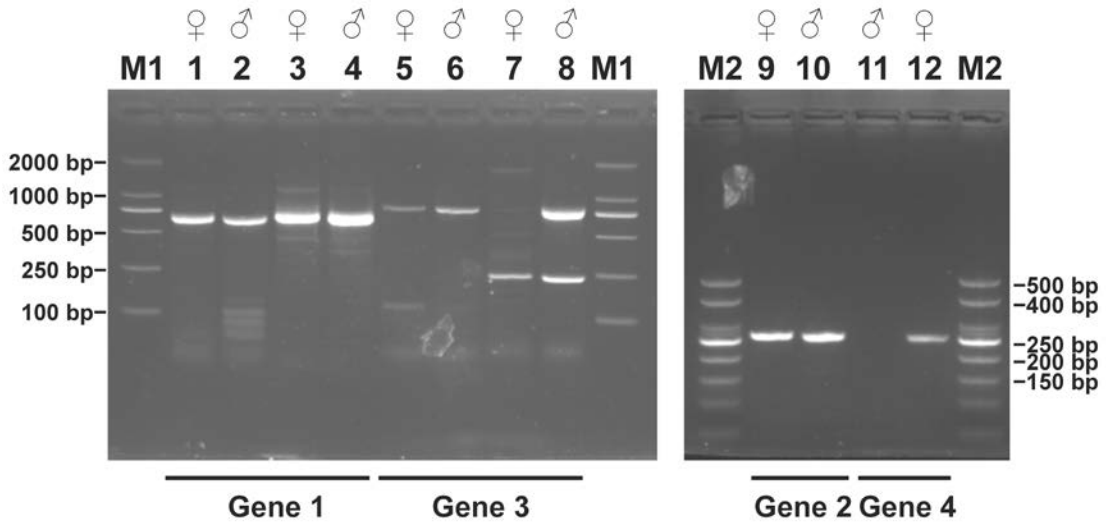
A

B

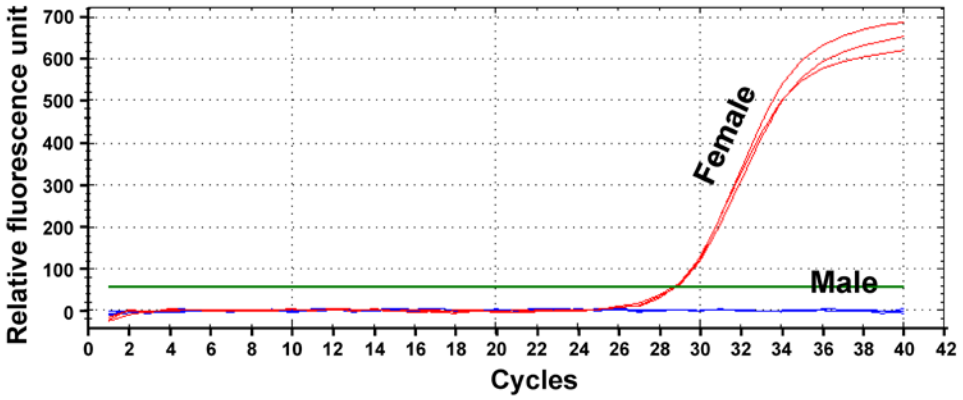


Supplementary FIGURE 8. Sequence comparison between each insert of the two screened BAC clones as a query and the matched scaffolds or contigs from the assembled gametophyte (A and C, Ye et al., 2015) or sporophyte genomes (B and D, Shao et al., 2019) of

***Saccharina japonica*.**

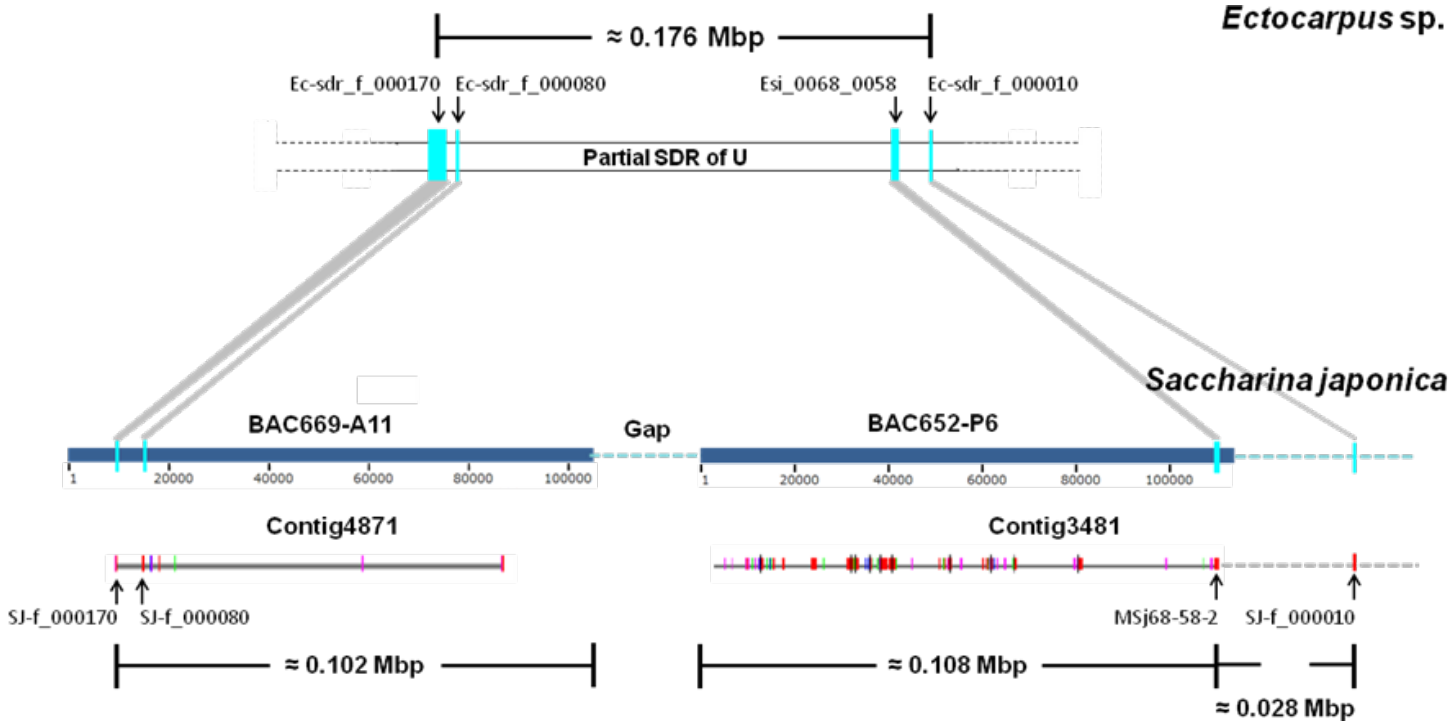


Supplementary FIGURE 9. Electrophoresis profiles of the four annotated genes amplified from genomic DNA of *Saccharina japonica* male and female gametophytes. Lanes 1 and 2: partial products of Gene 1 as amplified by the pair of primers g1-F1/R1; Lanes 3 and 4: partial products of Gene 1 as amplified by the pair of primers g1-F2/R2; Lanes 5 and 6: partial products of Gene 3 as amplified by the pair of primers g3-F1/R1; Lanes 7 and 8: partial products of Gene 3 as amplified by the pair of primers g3-F2/R2; Lanes 9 and 10: Gene 2 products as amplified by the pair of primers g2-F/R; Lanes 11 and 12: Gene 4 products as amplified by the pair of primers g4-F/R; M1 representing D2000 Marker (TaKaRa, Kyoto, Japan) while M2 representing DNA Ladder A Plus (Sangon, Shanghai, China).



Supplementary FIGURE 10. Amplification curve of annotated Gene 4 from the sequenced BAC clone BAC652-P6 as detected by quantitative real-time PCR (qRT-PCR).

Amplification curve was plotted by cycle number versus RFU, and three replicates were performed for each reference DNA sample.



Supplementary FIGURE 11. Comparisons of the genomic regions surrounding the genes SJ-f_000080, SJ-f_000170, SJ-f_000010, and marker MSj-68-58-2, which were syntenic to the Ec-sdr_f_000080, Ec-sdr_f_000170, Ec-sdr_f_00010, and Esi_0068_0058 in the U SDR of *Ectocarpus* sp. (Lipinska et al., 2015, 2017), respectively, in between the sequenced inserts of two BAC clones and the assembled genome of *Saccharina japonica* (Shao et al., 2019).

Alignment scores in each matched contig were the same as Supplementary Figures 6 and 8.