

Development of High Density Integrated Reference Genetic Linkage Map for Multinational *Brassica rapa* Genome Sequencing Project

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Abstract

A high density integrated map of *B. rapa* was constructed based on two mapping populations, 'Chiifu x Kenshin' and 'Chiifu x RcBr'. The integrated map contains 1017 markers covering 1262.0 cM length with an average of 1.24 cM between two loci. A total of 155 SSR anchoring 102 new BACs and 146 intron polymorphic markers were mapped in the integrated map which would be helpful in aligning the sequenced BACs in ongoing multinational *B. rapa* Genome sequencing project (MBrGP). Further, high degree of co-linearity between A genome linkage groups were observed by integration of *B. rapa* consensus map with that of 10 A genome linkage groups of *B. juncea*. It suggested conservation of chromosomal regions between *B. rapa* and *B. juncea* even though the two species evolved independently after their divergence. Thus, the sequencing information of *B. rapa* would be helpful for *B. juncea* breeding and the already identified gene blocks and known QTLs information in *B. juncea* could be transferred to *B. rapa* species.

Introduction

Chinese cabbage inbred line Chiifu-401-42 was selected as the representative model for Brassica A genome sequencing by the Multinational Brassica Genome Project (MBrGP) in 2003 (Yang et al., 2005). As its ongoing, amounts of BAC specific SSR markers were developed and it gave an opportunity to develop integrated genetic, physical and sequence-based maps of *B. rapa*. Kim et al. (2009) updated the version I reference map of Choi et al. (2007) and could align 188 seed BACs in 10 *B. rapa* linkage groups using BAC anchored SSR markers. However, the number of BAC anchored SSRs markers were very less to cover the whole genome of *B. rapa* in all the maps for MBrGP.

In our study we have mapped additional BAC sequences derived SSR markers and constructed a high density integrated linkage map of *B. rapa* for MBrGP, using two populations with Chiifu-401-42 as common parent.

Materials and methods

• Mapping population

CKDH population consisting of 78 doubled haploid lines from F₁ crossing by 'Chiifu-401-42' and 'Kenshin'.

F₂ population (190 individuals) derived from self-pollination of an F₁ individual originated from a cross between 'Chiifu-401-42' and 'RcBr-IBM-218-DH1'.



• Molecular markers

The SSR markers were developed based on BAC-end sequence of *B. rapa* ssp. *pekinensis* inbred lines, Chiifu-401-42 and were used to construct reference map of CKDH and CKRI population before.

IP (Intron polymorphic) markers developed by Panjabi et al. (2008) were also used to genotype CKDH population and CRF₂ population.

• **Linkage map analysis:** Joinmap 4.0 was used to construct individual map and generate integration map by "Combine the Groups for Map Integration" function.

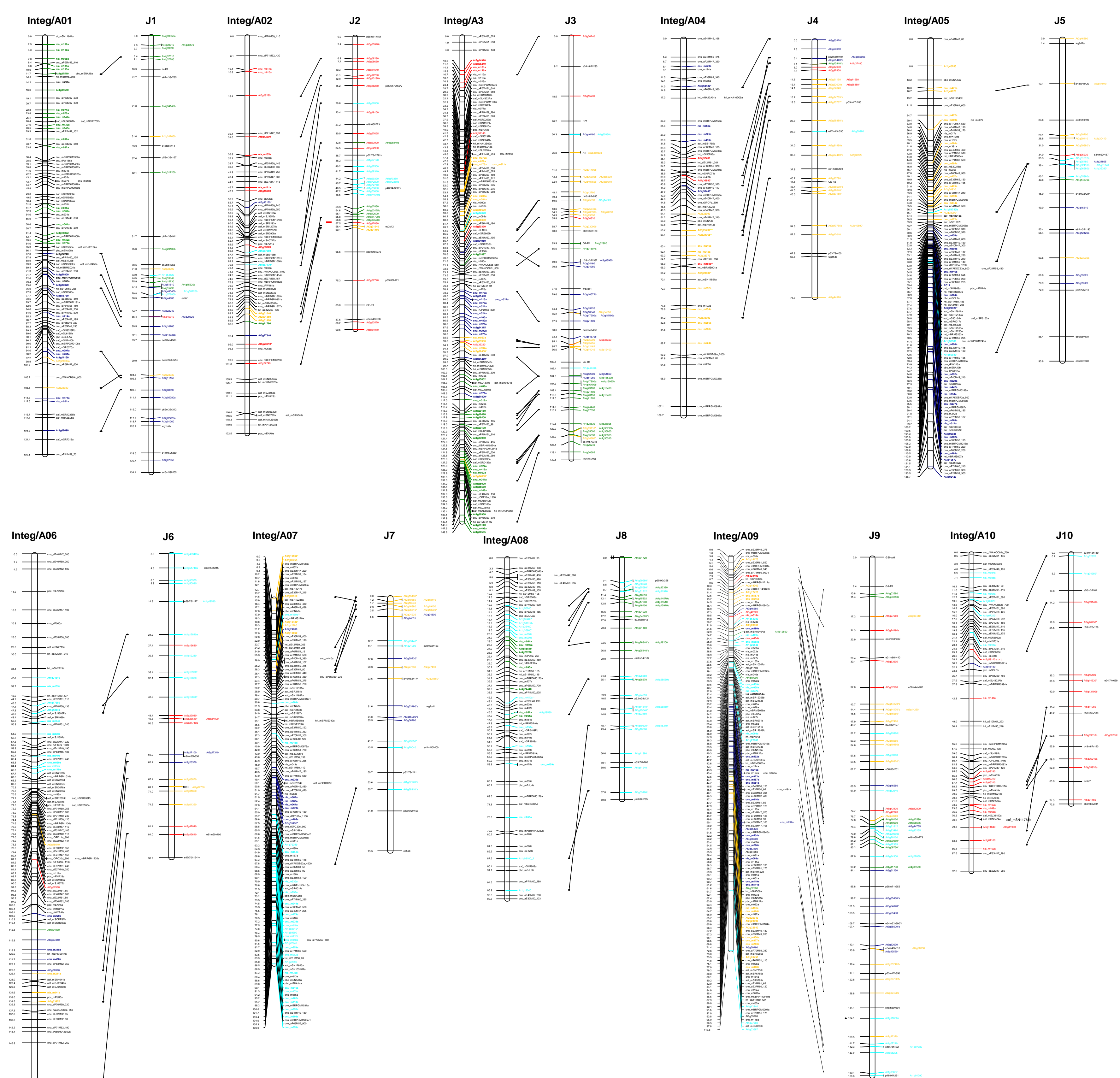
Result

- The CKDH updated map increased a length of 51.8 cM making the total genome coverage 1175.1 cM after 95 additional IP markers were mapped.
- A new map based on CRF₂ population was constructed including 250 BAC end SSR markers and 68 IP markers covering 1105 cM length.
- Based on 113 common markers between these two maps, we generated an integrated map including 1017 markers covering 1262 cM total length. A total of 155 SSRs anchoring 102 new BACs were mapped after integration and 100 IP markers were mapped to 10 homologous linkage groups of *B. rapa* corresponding to A genome linkage group of *B. juncea*. It showed co-linear between these two genomes and suggested conservation of chromosomal region of these two species.

Table 1. Summary of characters of CKDH, CRF₂ and integrated map of *B. rapa*

Linkage group	No. total markers			No. BAC derived SSR markers					New BACs ^a	No. IP markers			Total length (cM)			Average distance (cM)		
	CKDH	CRF ₂	Integ.	CKDH	CRF ₂	Common	Integ.	CKDH		CRF ₂	Common	Integ.	CKDH	CRF ₂	Integ.	CKDH	CRF ₂	Integ.
A01	73	35	96	21	28	10	39	12	6	7	2	11	140.0	110.8	129.1	1.92	3.17	1.34
A02	50	24	68	5	14	2	17	11	8	10	4	14	121.2	88.1	122.5	2.42	3.67	1.80
A03	119	38	145	34	26	12	48	12	16	12	0	28	123.3	140.9	149.8	1.03	3.7	1.03
A04	43	23	61	11	18	5	24	9	5	5	0	10	93.1	100.7	109.7	2.17	4.38	1.80
A05	103	35	122	25	28	14	39	11	6	7	2	11	135.3	136.5	138.7	1.31	3.90	1.14
A06	94	14	100	13	11	7	17	3	8	3	1	10	155.2	76.1	158.5	1.65	5.44	1.59
A07	110	42	135	24	39	15	48	13	12	3	2	13	98.3	111.7	106.9	0.89	2.66	0.79
A08	53	27	75	11	21	4	28	11	6	6	1	11	91.9	91.8	99.3	1.73	3.40	1.32
A09	112	64	151	37	55	21	69	15	21	9	2	28	123.2	162.7	154.9	1.10	2.54	1.03
A10	56	16	64	9	10	5	14	5	7	6	3	10	93.6	85.7	92.6	1.67	5.36	1.45
Total	813	318	1017	190	250	95	343	102	95	68	17	146	1175.1	1105	1262.0	1.45	3.47	1.24

^a the number of BACs not anchored in CKDH map of Kim et al. (2009)



References

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