# Construction of a genetic linkage map of *Brassica rapa* ssp. *pekinensis* using a CKRI population

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## ABSTRACT

Genetic map and integration with physical map are useful information for the genome sequencing project. An initial reference linkage map of *B. rapa* has already been reported using 78 CKDH lines because of the merits of a doubled haploid (DH) population such as repeatable and larger heritability of quantitative characters within short period. But, DH population might have tissue culture artificial effects and reduce genetic variations.

To generate a higher resolution genetic map, we used 190 recombinant inbred lines (CKRI). This population was derived from same parental lines with CKDH and fixed for 8 recombination events. To construct the map, first, out of the markers that were already mapped in CKDH, only the fully sequenced BAC originated markers were transferred to the CKRI map. And then, to saturate the map additionally, we have designed 1954 SSR primers from BAC-end sequences and surveyed the polymorphism. Among these SSR markers, 1898 primers were successfully amplified and 444 primers represented the polymorphisms between parental lines, and the genotyping using those polymorphic markers into CKRI population are currently under progress. Up to now, 300 polymorphic primers were genotyped in CKRI population. Construction of linkage map using 307 SSR markers gave into 10 linkage groups designated as A01–A10 with a total length of 985.6 cM with an average distance of 3.2 cM between two adjacent loci. The length of the linkage groups ranged from 51.8 to 134.1 cM. And a physical map were integrated to the genetic linkage map using webFPC software. This integrated map was comprised of 154 FPC professed contigs and 63 singletons which have been already anchored on the genetic map.

As the CKRI linkage map was saturated with more BACs derived SSR markers, the physical map will get a higher coverage of the genome. They will be more useful for QTL analysis, the map-based cloning of tightly linked genes, comparative genome analysis and the integration of physical maps with genetic maps for the Multinational *Brassica rapa* Genome Sequencing Project.

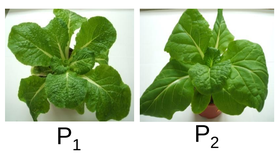
## MATERIAL & METHODS

### ◆ Plant materials

- **Parents line**

  $P_1$ : *B. rapa* ssp. *pekinensis* inbred lines, Chiifu 401-42

  $P_2$ : *B. rapa* ssp. *pekinensis* inbred lines, Kenshin 402-43



$P_1$ $P_2$

- **Mapping population**

  $F_8$ population (190 individuals) derived from two *B. rapa* ssp. *pekinensis* inbred lines, Chiifu-401-42 and Kenshin-402-43 (CK).

### ◆ Development of molecular markers

- **SSR motif survey**
  - Public available sequences of BACs of *B. rapa* ssp. *pekinensis* were collected from GeneBank.
  - 749 SSR primers were designed from 367 sequenced BACs.
  - 1954 SSR primers were designed from BAC-end sequences.
- **Primer design program** : Primer3

### ◆ Map construction

- **Linkage analysis**
  - Analysis program: JoinMap ver. 4.0
  - Map distance calculation: Kosambi's method
  - Analysis parameter : LOD 2.0 ~ 3.5
- **Integration of physical map with genetic map**
  - Analysis program: webFPC v2.1 program

## RESULT & DISCUSSION

### 1. Development of SSR markers

We have designed 1954 SSR primers from BAC-end sequences and surveyed the polymorphism. Among these SSR markers, 1898 primers suc cessfully amplified and 444 primers represented the polymorphisms between parental lines, 300 primers showed the size matching of PCR amplicons with t he BACs used for designing of primer and Chiifu. Genotyping using these pri mers is on progress with population.

**Table 1.** Summary of the primer survey results

| Sequence resources | No. of | | | |
|---|---|---|---|---|
| | Total primer assayed | Polymorphism (%) | Non amplified (%) | BAC anchored |
| BAC-end | 1618 | 371(22.9) | 55 (3.4) | 269 |
| Fully sequenced BAC | 336 | 73(21.7) | 1 (0.3) | 64 |
| Total | 1954 | 444(22.7) | 56 (2.9) | 333 |

### 2. Linkage group assembly and distribution of markers

A total of 307 loci, including 159 BAC anchoring SSR markers already mapped in CKDH were successfully transferred to CKRI and 146 new developed BAC derived SSR markers, were assigned into 10 linkage groups with LOD values of 2.0–3.5 (Table 2). The genetic map had a total length of 985.6 cM, with an average distance of 3.2 cM between two loci. The whole *B. rapa* genome could be assembled into ten (A01–A10) linkage groups (Fig. 1). The length of the linkage groups ranged from 51.8 to 134.1 cM for A04 and A03, respectively. The number of markers in the 10 linkage groups ranged from 10 for A04 to 57 for A03.

**Table 2.** Summary of genotyping data

| Marker ID | Sequence resource | No. of | |
|---|---|---|---|
| | | genotyping with population | markers mapped |
| cnu_m000* | Seed BAC | 116 | 101 |
| nia_m000* | Seed BAC | 66 | 58 |
| BBSRC_m000 | Seed BAC | 49 | 35 |
| BRPGM_m0000 | BAC end | 145 | 111 |
| | Total | 376 | 307 |

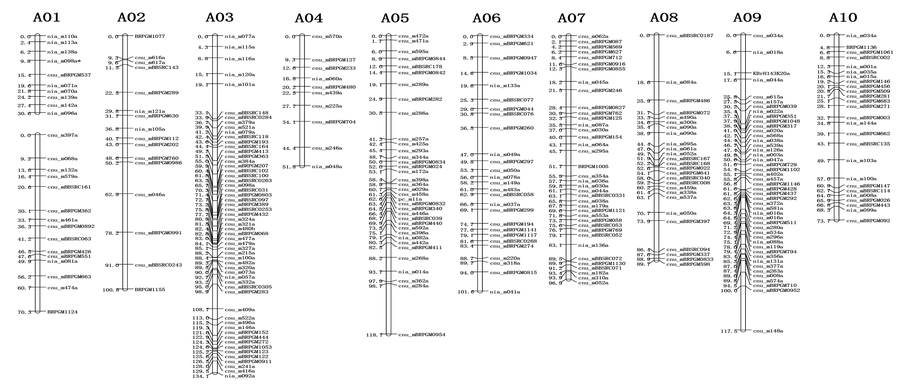



**Fig.1** Generation of map. The map was constructed from 190 CKRI plants derived from two *B. rapa* ssp. *pekinensis* inbred lines, Chiifu-401-42 and Kenshin-402-43(CK) using 307 SSRs. Positions of loci are given in cM on the left side. The 10 LGs were designated as A01–A10.

### 3. Integration of physical map

These BAC based primers included in this map enable us to do the integration of the physical map. The physical map was integrated with the genetic map generated in this study using webFPC v2.1 program (Fig.2). A total of 154 FPC professed contigs and 63 singletons were aligned in the 10 linkage groups. Finally, 15942 BAC clones distributed in the 10 linkage groups, ranged from 207 for A04 to 6322 for A07.

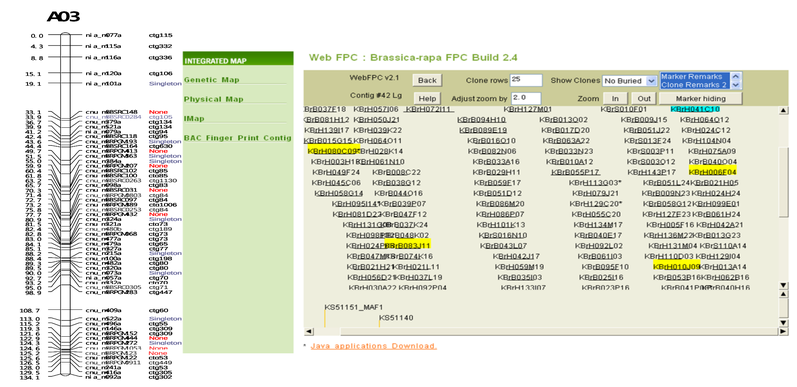



**Fig.2** Integration of physical map with genetic linkage map. The contig ID has been listed beside the linkage map corresponding to each of the markers which include in this linkage map. And the right side is a view of the web FPC software and contig42 containing all positive BAC clones.

### 4. Further study

- To saturate the map, more primers derived from BAC-end sequences will be developed and added into the linkage map.
- To construct a higher density linkage map of *Brassica rapa,* the CKRI genetic linkage map will be integrated with the CKDH linkage map.
- To understand genomic structure difference, genome wide comparative map will be constructed within *Brassicace*.

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