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Construction of random sheared fosmid library from Chinese cabbage and its use for *Brassica rapa* genome sequencing project

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Abstract

As a part of the Multinational Genome Sequencing Project of *Brassica rapa*, linkage group R9 and R3 were sequenced using a bacterial artificial chromosome (BAC) by BAC strategy. The current physical contigs are expected to cover approximately 90% euchromatins of both chromosomes. As the project progresses, BAC selection for sequence extension becomes more limited because BAC libraries are restriction enzyme-specific. To support the project, a random sheared fosmid library was constructed. The library consists of 97536 clones with average insert size of approximately 40 kb corresponding to seven genome equivalents, assuming a Chinese cabbage genome size of 550 Mb. The library was screened with primers designed at the end of sequences of nine points of scaffold gaps where BAC clones cannot be selected to extend the physical contigs. The selected positive clones were end-sequenced to check the overlap between the fosmid clones and the adjacent BAC clones. Nine fosmid clones were selected and fully sequenced. The sequences revealed two completed gap filling and seven sequence extensions, which can be used for further selection of BAC clones confirming that the fosmid library will facilitate the sequence completion of *B. rapa*.

Keywords: *Brassica rapa*; Chinese cabbage; Fosmid library; Genome sequencing; Physical contig

1. Introduction

Chinese cabbage (*Brassica rapa* ssp. *pekinensis*) is one of the most important vegetables in Korea and northeast Asia. It belongs to the genus *Brassica*, which consists of six species: three diploid species *B. rapa* (AA, 2n = 20), *B. nigra* (BB, 2n = 16) and *B. oleracea* (CC, 2n = 18), and three amphi-diploid species *B. juncea* (AABB, 2n = 36), *B. napus* (AACC, 2n = 38) and *B. carinata* (BBCC, 2n = 34) (U, 1935). The *Brassica* species are cultivated worldwide for various purposes including human diet, animal feed and oil production as well as being used medicinally as an anticancer compound (Fahey and Talalay, 1995; Higdon et al., 2007).

B. rapa includes a variety of important vegetables crops such as Chinese cabbage, Pakchoi, turnip, broccoletto and sarson and has favorable genetic and genomic attributes. Appropriately, *B. rapa* with a haploid genome size of 529 Mb (Johnston et al., 2005) was prioritized for sequencing by a multinational collaboration and the *Brassica rapa* Genome Sequencing Project (BrGSP) was launched for complete genome sequencing of *B. rapa* ssp. *pekinensis* inbred line 'Chiifu' by the Multinational Brassica Genome Sequencing project (MBGP; http://www.brassica.info and http://www.brassicagenome.org) and the Korea Brassica Genome Project (KBGP; http://www.brassica-rapa.org). As a part of the project, we are responsible for sequencing linkage groups R9 and R3.

Initially three large-insert bacterial artificial chromosome (BAC) libraries, KBrH (*Hin*d III), KBrB (*Bam*H I) and KBrS (*Sau*3A I), which provide more than 34-fold genome coverage were constructed for full sequencing of *B. rapa* ssp. *pekinensis* cv.

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Chiifu (Park et al., 2005; Yang et al., 2005; http://www.brassica-rapa.org/BGP/). Most of the BAC clones from the KBrH and KBrB libraries and a few BAC clones from the KBrS library were fingerprinted (Mun et al., 2008) and both ends of all BAC clones from the three BAC libraries were sequenced as proposed (Love et al., 2005; Hong et al., 2006; Mun et al., 2008) to create a genome-wide physical BAC contig map of the *B. rapa* genome (Mun et al., 2008). This has provided 242 seed BACs, which are being extended to connect the physical BAC contigs to each other using a BAC-by-BAC shotgun sequencing strategy (Mun et al., 2008). Information of genetic linkage maps of *B. rapa* (Kim et al., 2006; Choi et al., 2007; http://www.brassica-rapa.org/) and comparative analysis between BAC end sequences of *B. rapa* and sequence of the extensively studied model plant *Arabidopsis thaliana* supports the selection of BAC clones for BAC extension as *Arabidopsis* is thought ideal for comparative genomics with *Brassica* species (Park et al., 2005; Yang et al., 2005, 2006; Hong et al., 2006; Kim et al., 2007).

The sequencing of R9 and R3 generated 21 and 27 sequence scaffolds covering approximately 23 Mb and 26 Mb, respectively (Mun et al., 2008). These sequencing efforts have progressed further as a part of the MBGP. The BAC-by-BAC strategy utilized to date and the current physical contigs are expected to cover approximately 90% of the euchromatin regions of both chromosomes (unpublished results). As the project progresses to completion, BAC selection for sequence extension becomes increasingly limited, because the BAC libraries are restriction enzyme-specific. Development of another library, therefore, became a priority.

The present report describes the construction and characterization of a random sheared fosmid library to support and to complete the sequencing of *B. rapa* R9 and R3 as it was also used to finish rice (*Oryza sativa*) genome sequence (Ammiraju et al., 2005).

2. Materials and methods

2.1. Fosmid library construction

Plant material was grown in the greenhouse from seeds of Chinese cabbage, Chiifu (*B. rapa*). Four-week-old plants were harvested and stored at −80 °C. Frozen leaf tissue was used to prepare high molecule DNA using a modification of a previously described method (Liu and Whittier, 1994; Chalhoub et al., 2004). DNA was sheared by repetitive pipetting (100 times). The sheared DNA (approximately 20 μg) was end-repaired using a blunt end repair kit (Epicentre, Madison, WI, USA) following the manufacturer's instructions. The sheared and end-repaired DNA was size-separated on a 1% low-melting agarose gel using clamped homogeneous electric field (CHEF) pulsed-field gel (PFG) electrophoresis (Bio-Rad, Hercules, CA, USA) at 5−15 s, 120°, 18 h and 6 V/cm in 0.5× TBE buffer. The PFG midrange marker I (New England Biolabs, Beverly, MA, USA) was used for proper size-fraction. DNA fragments (33.5−63.5 kb) were excised from the gel and electro-eluted using a previously described method (Strong et al., 1997). Dialysis tubing (Spectrapor 2; Spectropor, Rancho Dominguez, CA, USA) was pretreated by heating membranes (7−10 cm strips) at 90 °C in a large volume of 10 mmol/L sodium bicarbonate ($NaHCO_3$) and 1 mmol/L EDTA for 30 min and rinsed three times in deionized water. The treated membranes were stored at 4 °C in 1 mM EDTA. Before use, the membranes were rinsed in 1× TAE buffer. For electro-elution, the gel slice containing DNA fragments was placed lengthwise immediately after being size-excised into a pretreated dialysis tube. One end of the tube was sealed with a dialysis clip, the gel slice was positioned to one side of the dialysis tube, 500 μL of sterile cold 1× TAE buffer was added, the air bubble was discarded carefully from the tube and the other end of the tube was sealed with a dialysis clip. An electrophoresis chamber filled with 1× TAE buffer was placed on ice and the dialysis tube containing the gel slice was placed in the pre-cooled chamber. Electro-elution was performed at 5 V/cm for 2 h and the polarity was reversed for 1 min to disassociate DNA from the side of the membrane. The assembly was removed carefully from the chamber and the eluted DNA was collected using a wide-bore tip.

DNA ligation was performed using Fosmid Library Production Kit (EpiCentre, Madison, WI, USA). The eluted DNA was ligated to pEpiFOS vector for 2 h at room temperature. The ligation reaction was inactivated at 70 °C for 10 min and then packaged into MaxPlax Lambda Packaging Extracts. The packaged fosmid clones were transfected into *Escherichia coli* EPI100-T1^R and incubated at 37 °C for 20 min. The transfected cells were plated on LB agar Q-trays (Genetix, Boston, MA, USA) containing 12.5 μg/mL chloramphenicol. Colonies were picked and transferred to wells of 384-well microtiter plates (Genetix) containing freezing broth medium (1% tryptone, 0.5% yeast extract, 0.5% NaCl, 0.63% K_2HPO_4, 0.045% sodium citrate, 0.009% $MgSO_4$, 0.09% $(NH_4)_2SO_4$, 0.18% KH_2PO_4, 4.4% glycerol and 12.5 μg/mL chloramphenicol) using Q-pix (Genetix), incubated at 37 °C overnight and stored at −80 °C.

2.2. Fosmid clone insert sizing

To determine the insert size of the fosmid clones, a total of 100 fosmid clones randomly picked before constructing a complete set of the fosmid library were analyzed. The 100 fosmid clones were cultured in 3 mL of LB medium containing 12.5 μL/mL chloramphenicol at 37 °C overnight. Plasmid DNA was isolated using a method simplified from the Plasmid Midi Kit (Qiagen, Valencia, CA, USA) and digested with *Not*I for 3 h to release the insert DNA from the vector. Digested DNA was separated on a 1% agarose gel using CHEF gel electrophoresis (Bio-Rad) at 5−15 s, 120°, 15 h and 6 V/cm in 0.5× TBE buffer.

2.2.1. Physical gap refinement and fosmid library screening

A total of 16 sets of primers were designed from the sequences of the flanking ends of nine physical gaps on R9, R3 and R10 that include two telomeric gaps (Tables 1 and 2, Fig. 1). The position of the *A. thaliana* sequence showing co-linearity with BAC sequence of *B. rapa* was considered as the

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