Construction and characterization of a bacterial artificial chromosome library of peach*

WANG Qian (王 倩), ZHANG Kaichun (张开春), QU Xueping (曲雪萍), JIA Jianhang (贾建航), LI Chuanyou (李传友), JIN Demin (金德敏) and WANG Bin (王 斌)

(Institute of Genetics, Chinese Academy of Sciences, Beijing 100101, China)

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Abstract This report briefly describes the construction and characterization of a peach [*prunus persica* (L.) Batch] Var. *Jingyu* bacterial artificial chromosome (BAC) library. The variety *Jingyu* has many important agronomic characters of stone fruits, and it is a main parent in Chinese peach breeding. After cloning of the high molecular weight peach DNA into pBeloBAC 11, we obtained over 22 000 recombinant clones. The BAC library has an average insert size of 95 kb and represents approximately 7 times peach haploid genome equivalents. After being screened with two randomly amplified polymorphic DNA markers, W4 and P20, which are linked to yellow flesh and nectarine genes of peach respectively, ten positive clones have been detected. This library is very useful for map-based cloning of peach genes and physical mapping of peach genome.

Keywords: **bacterial artificial chromosome, genomic library construction, *Prunus persica*.**

Peach [*Prunus persica* (L.) Batch] is a member of the *Rosaceae* family and one kind of the most important fruit trees in the world. With comparatively small size of genome ($2C = 5.8 \times 10^8$bp, $2n = 16$)[1], rather high self-compatibility and relatively short generation time (only 2—3 years for juvenile stage), peach is becoming the best genetically characterized among species of *Prunus* genus and a model species for identifying and isolating agricultural important genes in perennial fruit tree species[2].

Recent mapping studies using molecular markers have provided several linkage maps of peach. Many qualitative and quantitative agronomic characters have been mapped on those maps and some closely linked DNA markers have been acquired[3]. These researches have established fundamentals for map-based cloning (MBC) of peach genes, while the genomic library with large insert becomes a limit for MBC of peach genes.

Taking the advantages over YAC by higher cloning efficiency, lower chimerism and easier cloning screening and manipulation, bacterial artificial chromosome (BAC) is becoming a predominant MBC system[4,5]. To facilitate map-based cloning of peach genes and genomic physical mapping, we constructed and characterized a peach *Jingyu* BAC library in this study.

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1 Materials and method

1.1 Material

The leaves of young shoots of *persica* Var. *Jingyu* were collected from a garden in Beijing, China.

1.2 BAC library construction

Megabase size DNA was prepared from *Jingyu* nuclei embedded in agarose plugs as described by Wang's method[1)]. Size-selection of a *Hind* Ⅲ partial digest was carried out in a 1% agarose gel at 4.5V/cm with a 60—90s pulse for 18 h at 11℃. Electroeluted from the gel, the fragments with desirable size were ligated to dephosphorylated pBeloBAC11. Ligation mixture was used to transform the competent cells of *E. coli* DH10B by electroporation using an ECM600 electroporater machine (BTX, USA). The transformed cells were resuspended in 1 mL SOC medium, incubated at 37℃ for 1 h and then plated onto LB agar containing 12.5 μg/mL of chloramphenical (CM), 50 μg/mL of 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-gal) and 25 μg/mL of isopropylthio-β-D-galactose (IPTG). White clones were transferred to microtiler plates containing LB freezing buffer[5], incubated at 37℃ for 24 h and then stored at −80℃.

1.3 Recombinant BAC DNA analysis

BACs containing *Jingyu* DNA were isolated from 5 mL overnight culture using standard alkaline lysis procedures. The BAC DNA was digested with *Not* Ⅰ to free the inserted DNA fragment from the vector. The digested DNA was separated by pulse field gel electrophoresis (PFGE) in a 1% agarose gel in 0.5× TBE buffer at 11℃ using a Bio-Rad CHEF Mapper set at 6V/cm with a linear pulse time ramping from 5 s to 15 s for 13 h.

1.4 BAC library screening

The 384 × 3 × 3 BAC clones were inoculated on Hybond N^+ filters using a Biomek 2000 Robotics Workstation (Beckman). After being incubated for 12—18 h at 37℃, the clones were lysed and DNA fixed to filters using the method of Nezelic et al.[6] Prehybridization and filter washing were preformed as described by Woo et al.[7]. Two randomly amplified polymorphic (RAPD) markers, W4 and P20, which linked to yellow flesh and nectarine genes of peach respectively, were labeled using the random primer synthesis procedure.

2 Results and discussion

High quality and yield of DNA are required when constructing a genomic library with large insert DNA. Since peach leaves are rich in polysaccharides, we moderately modified the protocol of Fu et al.[8] and obtained high molecular weight (HMW) DNA larger than 2Mb. There was no contamination of small molecular weight DNA and cell debris, which made these HMW DNA readily accessible

1) WANG, Q. et al., An effective method for fruit trees high molecular weight DNA preparation, High Technique Letter, 2000, No.4.

by restriction enzymes. When the HMW DNA digested by *Hin*d III for 5 min, most fragments were in the desirable size ranging from 100 kb to 500 kb (figure 1).

Through optimizing the cloning procedure the HMW DNA of peach was successfully cloned into the BAC vector, pBeloBAC11, and over 22 000 recombinant clones were obtained. The average insert size of this library is 95 kb with individual clones ranging from 40 kb to 180 kb in size (fig. 2). Since the haploid genome of peach is 290 000 kb in size, the BAC library we constructed represents approximately 7 times peach haploid genome equivalents with an over 99% probability of isolating a specific genomic region.

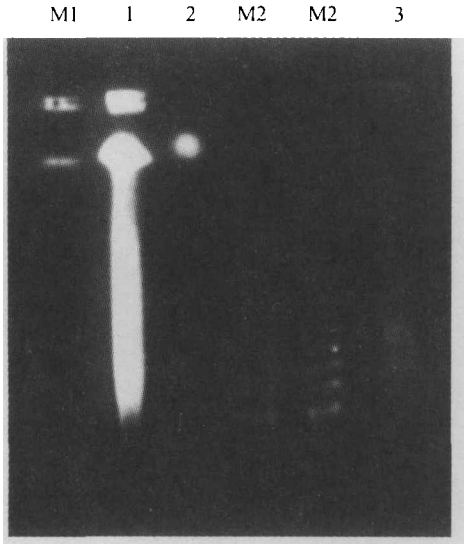

Fig. 1. PFGE pattern of peach DNA. M1, Yeast chromosome PF marker; M2, lambda concatamer (48.5 kb); 1, HMW DNA of peach; 2, HMW DNA of peach purified by PFGE; 3, peach HMW DNA digested with *Hin*d III.

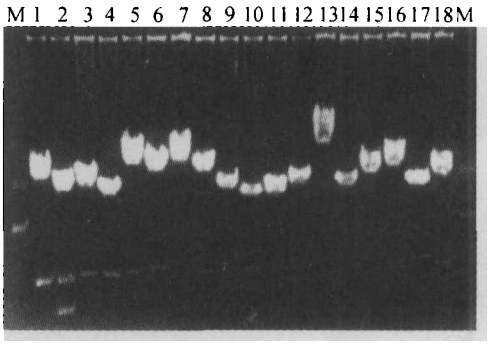

Fig. 2. Analysis of peach BAC clones by PFGE. M, lambda concatamer (48.5 kb); lanes 1—18, peach BAC clones digested with *Not*I.

To make evaluation the BAC library was screened with two molecular markers, W4 and P20, which are linked to yellow flesh and nectarine genes respectively. Ten positive clones were identified (data not shown) and their fingerprints are going to be used to assemble contigs.

The peach Jinyu is the main parent in Chinese peach breeding, which has many important agronomic traits of stonefruits. Owing to its high stability, easiness of isolation, high yield and large size, the peach BAC library is suitable for many peach genes screening, physical mapping and genome sequencing.

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