Construction of an Euchromatin Enriched Genomic DNA library in wheat and development of STS marker sets

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ABSTRACT

To develop the EEG (Euchromatin Enriched Genomic) DNA library of wheat, we used the Mcr A and Mcr BC system in DH5 alpha bacteria cell line. About three thosand EEG colonies have been constructed by using junk DNA exclusion. Among the colonies, we analyzed the genetic information of five hundred using blast searches of NCBI and GRAMENE web sites. More than two hundred STS primer sets have been developed using sequencing data of selected colonies. Twenty-five percent of designed primer sets have been shown to be polymorphic in wheat germplasm using six endonucleases. These primers could be useful for specific allele tagging in mapping populations and germplasm and for the study of functional genomics of wheat

INTRODUCTION

There are several molecular marker types available for identifying germplasm and for analyzing genetic mapping populations. RAPD, ISSR and AFLP markers are made from undefined elements and SSR and STS markers are made from defined elements. Molecular markers should be improved for 1) specific allele tagging which are derived from euchromatin, 2) labor and cost effectiveness which are based on agarose gel and 3) having allele descriptions which has a bar-code style which does not demands detailed band size information. The objectives of construction of the EEG DNA library is making DNA probes for 1) germplasm discrimination, for 2) linkage map construction that could show ready-to-use polymorphisms, for 3) genetic dissection with linkage map based reliable marker systems and for 4) allele refining that could detect the latent polymorphism (SNPs) and association analysis between marker allele types and phenotypic data sets. The results of this study could be used for discrimination of wheat germplasm and for detecting specific alleles in mapping populations.

MATERIALS AND METHODS

Construction of EEG Library

The reference DNA of wheat in this study was 'Keumkang', a Korean variety. The DNA was extracted by the CTAB method. The DNA was digested with restriction enzyme, *Spe* I and was ligated with pGEM7zf(+) vector treated with *Xba* I. The restriction enzymes, *Xba* I and *Spe* I have compatible ends and the restriction site of *Xba* I is located on MCS of pGEM7zf(+) vector. The ligated vector was transformed into a *mcr*A and *mcr*BC active *E.coli* cell line which has methylated cytosine restriction system A, BC. To increase the efficiency of transformation, the electroporation mediated transformation method was tried.

Sequencing , obtaining the information of colonies and design the STS primers

Selected colonies which had more than 700bp of PCR product were sequenced and the sequence data was searched using the NCBI and GRAMENE web site to get additional genetic information. With the result of sequencing data of individual colonies, we designed specific primers using the Primer 3 program.

Polymorphism with Six Endonuclease

With the designed STS primers, PCR was undertaken with thirty wheat lines. Each PCR product was digested with six endonucleases, *Alu* I, *Hae* III, *Hinf* I, *Rsa* I, *Tsp*50a and *Taq* I. The digested PCR product was electrphoresed on 1% agarose gels.

RESULTS AND DISCUSSION

Analysis of clones and design of STS primers

After transformation of restricted DNA, we have acquired 3,041 clones. Among those cloned, we selected 1,115 clones to be sequenced as colony PCR suggested that the insert was more than 700bp. The analysis of sequencing data showed 80% of sequenced clones had genetic information. We designed the STS primers based on sequence data and about 37% of designed primers were successful.

Polymorphism with STS primer and restriction enzyme in wheat germplasm

One hundred fifty-seven STS primers derived from wheat colony sequence that had genetic information were selected. While most primers amplification showed no polymorphism in the wheat germplasm, when treated with six restriction enzymes, the digested amplicon showed polymorphism (Fig.2). The database of primer and restriction enzyme is under construction.

Table 1. Number of tested clones and primers

No. of cultured clones	No. of sequenced clones	No. of analyzed clones	No. of designed primers	No. of successful primers	No. of primers showing polymorphism
3,041	1,115	897	648	246	107

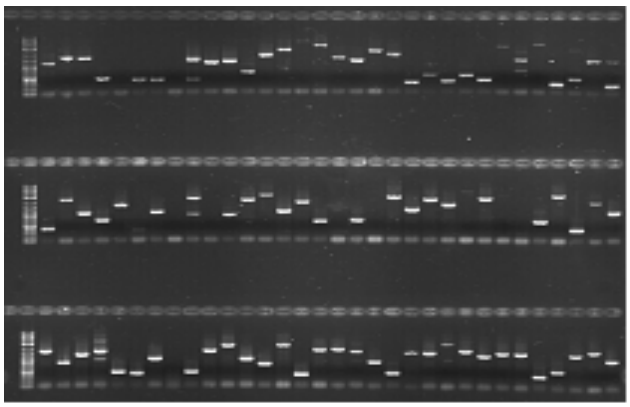
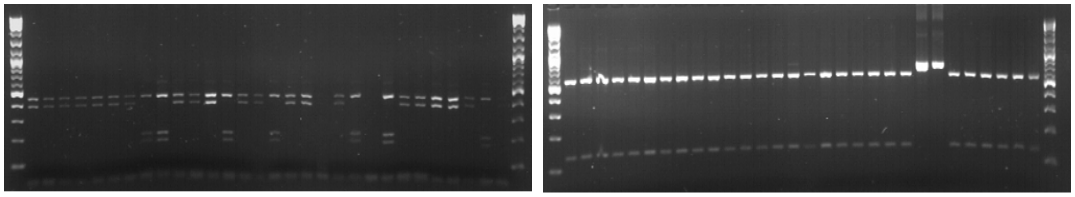


Fig.1. The PCR product of selected white colonies



(A) (B)

Fig.2. Polymorphism in wheat germplasm with gene based STS primer and restriction enzyme digestion
(A) : KG3 primer + Taq I enzyme, (B) : KG26 primer + Hae III enzyme