Survey sequencing of soybean elucidates the genome structure, composition and identifies novel repeats

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Abstract. In order to expand our knowledge of the soybean genome and to create a useful DNA repeat sequence database, over 24,000 DNA fragments from a soybean [\textit{Glycine max} (L.) Merr.] cv. Williams 82 genomic shotgun library were sequenced. Additional sequences came from over 29,000 bacterial artificial chromosome (BAC) end sequences derived from a BstI library of the cv. Williams 82 genome. Analysis of these sequences identified 348 different DNA repeats, many of which appear to be novel. To extend the utility of the work, a pilot study was also conducted using methylation filtration to estimate the hypomethylated, soybean gene space. A comparison between 8366 sequences obtained from a filtered library and 23,788 from an unfiltered library indicate a gene-enrichment of \(\sim 3.2\)-fold in the hypomethylated sequences. Given the 1.1-Gb soybean genome, our analysis predicts a \(\sim 343\)-Mb hypomethylated, gene-rich space.

Introduction

Soybean (\textit{Glycine max}) is the most valuable legume crop, with numerous nutritional and industrial uses because of its unique seed chemical position. Over 85 million metric tonnes of soybeans were produced in the US on \(>30\) million ha in 2004, with an estimated annual economic value exceeding US \$17 billion, second only to maize, and approximately twice that of wheat and ten times that of rice (http://www.nass.usda.gov/index.asp; verified 13 June 2006). While genomics is having a profound effect on plant biology, its direct impact on major crop species such as soybean remains limited. A primary difficulty is that the genomes of major crop species are large and complex, being as much as 50 times larger than that of the model plant \textit{Arabidopsis thaliana}. For example, the soybean genome at 1.12 billion bp (Arumuganathan and Earle 1991) remains a formidable challenge for genome sequencing using current technologies. Nonetheless, the scientific community clearly sees the need for sequencing of crop genomes. Indeed, the legume community recently chose soybean as the reference species for the phaseolid legumes, which comprise many of the major legume crops, and recommended sequencing the soybean genome (Gepts et al. 2005).

Soybean is a paleopolyploid with \(2n=40\) (Goldblatt 1981). Therefore, it is expected that any given gene will be present approximately four times in the genome. Indeed, Shoemaker et al. (1996) found that 60% of the time, hybridisation of random clones to soybean genomic

Abbreviations used: BAC, bacterial artificial chromosome; EST, expressed sequence tag; FISH, fluorescent in situ hybridisation; FP, filter power; gDNA, genomic DNA; Mb, megabases; MF, methylation filtration, methylation filtering; mRNA, microRNA; SSR, simple sequence repeats; UF, unfiltered; WGS, whole-genome shotgun.
soybean genome was (Singh and Hymowitz 1988). This analysis indicated that the soybean has been reported based upon pachytene analysis and that the V alhedian repeat (102 bp) with 82.6% sequence similarity to STR120 is found in 5000–10 000 copies. Lin et al. (1997) identified by Morgante et al. (2002) that the chromosomes had largely euchromatic arms. Estimates of the SB92 repeat to four or five locations within the genome, genome (Singh and Hymowitz 1988). This analysis indicated that the soybean genome was ~35% heterochromatic but several of the chromosomes had largely euchromatic arms.

Relatively few specific soybean repetitive sequences have been reported. A 120-bp soybean repeat (STR120) was identified by Morgante et al. (1997) and estimated to exist in 5000–10 000 copies. Lin et al. (2005) identified the STR102 repeat (102 bp) with 82.6% sequence similarity to STR120. Vahedian et al. (1995) used FISH to localise the 92-bp SB92 repeat to four or five locations within the genome, including two associated with the centromere. Estimates suggested that this one repeat represented ~0.9% of the genome (~1 × 10^6 bp). This high copy number but limited distribution suggests that the SB92 repeat must be localised in megabase-sized regions. Lin et al. (2005) found the STR102 repeat in clusters up to ~435.6 kb in length.

Transposable DNAs comprise a significant proportion of the repetitive DNA found in eukaryotic genomes. Vodkin et al. (1983) identified the first transposable element in soybean (termed Tgm). Seven different classes of the Tgm transposon were identified ranging in size from 1.6 to >12 kb (Rhodes and Vodkin 1988); however, the copy number of this transposon was not determined. A mariner-like transposon (Soymar1) was estimated to be present up to ~10 000 copies per haploid genome, with the largest member of this class being 3.5 kb (Jarvik and Lark 1998). Soybean retrotransposons include the gypsy/Tyl-like retroelement, Calypso (Wright and Veyts 2002), similar to the Arabidopsis element Athila4 found in the centromere, and a copia/Tyl-like retroelement (SIRE1-1, Laten and Morris 1993). These retroelements vary in size from 11 to 14 kb and are duplicated a few hundred times in the soybean genome. Lin et al. (2005) found both SIRE1 and Calypso-like elements in soybean BAC clones mapping to centromeres.

Compared with crop plants, the Arabidopsis genome is relatively gene-rich with an average gene density of 20–25 genes per 100 kb. These genes are relatively evenly distributed across the genome (Barakat et al. 1998). In contrast, estimates for large cereal genomes suggest that the gene-rich portion may only comprise 10–20% of the genome. Fewer studies have been done with soybean. DNA methylation in plants is associated with silent, heterochromatic DNA that in large cereal genomes is rich in transposable elements (Benettner 1996). Marek et al. (2001) compared 2000 soybean BAC-end sequences either selected based on RFLPs from methylation-sensitive restriction enzymes or based on the presence of SSRs (simple sequence repeats). The conclusions of this study were that the RFLP-selected BACs had 50% less repetitive sequences with a similar enrichment in genic sequences. Other data also suggest that hypomethylated, gene-rich regions exist in the soybean genome. For example, using a similar strategy, Mudge et al. (2004) selected BAC clones using PstI-generated RFLP probes. PstI is a methylation-sensitive enzyme and restriction sites would be expected in regions of hypomethylation. The probes used in this study identified BAC clones from only 24% of the genome, giving an estimate of the gene space of ~264 Mbp (i.e. 1.1 billion bp × 0.24). Only a few soybean BAC clones have been completely sequenced. Analysis of one such region (330 kb) revealed sequence containing few repetitive sequences and a gene density of ~1 gene per 5 kb (Foster-Hartnett et al. 2002). It is clear that more needs to be known about the soybean genome before an effective strategy for genomic sequencing can be developed. Collectively, the current data suggest a soybean genome containing hypomethylated, gene-rich segments with the hypermethylated DNA regions largely confined to islands of repetitive sequence found in pericentromeric regions.

In order to expand our knowledge of the soybean genome and to develop a useful DNA repeat sequence database, we sequenced over 24 000 DNA fragments from a shotgun genomic library of soybean cv Williams 82. Also included in our analysis were over 29 000 BAC-end sequences from a BstYI library of Williams 82 DNA (J Tomkins unpubl. data). This cultivar has been chosen as the primary model by the soybean community (Stacey et al. 2004). In order to derive more information from this effort and to explore a possible means for determining the genetic sequence of the soybean genome, we also conducted a pilot study using methylation filtration (MF). This is a very simple and robust method for enriching for hypomethylated, gene-rich sequences in complex plant genomes (Rabinowicz et al. 1999). Briefly, genomic DNA is used to transform an E. coli strain that preferentially cleaves methylated DNA resulted in three or more detectable bands, with over 90% detecting more than two bands. The soybean genome also exhibits evidence of two duplication events, one ~15 million years ago and another ~40 million years ago (Schlueter et al. 2004; Shoomaker et al. 2006). The latter likely predates the division of the galegoid and phaseoloid lineages.

DNA–DNA renaturation studies suggested that ~40–60% of the soybean genome sequence is repetitive (Goldberg 1978; Gurlay et al. 1979). Recently, Lin et al. (2005) used fluorescent in situ hybridisation (FISH) to explore the distribution of repetitive sequences in the soybean genome. These data supported the notion that the soybean repeats are largely localised to the pericentromeric regions resulting in largely euchromatic chromosome arms. Digestion of repetitive sequences with a methylation-sensitive enzyme (HpaII) suggested the centromeric repeats were methylated. These results are consistent with earlier studies. For example, one study, analysing the nature of BAC-end sequences, suggested that selected clones were either repeat-rich or gene-rich but some interspersion of such sequences was also found (Marek et al. 2001). A complete karyotype of BAC clones from only 24% of the genome, giving an estimate of the gene space of ~264 Mbp (i.e. 1.1 billion bp × 0.24). Only a few soybean BAC clones have been completely sequenced. Analysis of one such region (330 kb) revealed sequence containing few repetitive sequences and a gene density of ~1 gene per 5 kb (Foster-Hartnett et al. 2002). It is clear that more needs to be known about the soybean genome before an effective strategy for genomic sequencing can be developed. Collectively, the current data suggest a soybean genome containing hypomethylated, gene-rich segments with the hypermethylated DNA regions largely confined to islands of repetitive sequence found in pericentromeric regions.

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DNA sequences. Consequently, only hypomethylated DNA inserts ‘survive’ the cloning process. The MF strategy greatly reduces the time and cost of gene identification in plants by filtering out methylated repetitive elements while retaining hypomethylated gene fragments. MF has been successfully applied to genomes of more than a dozen plant species across the plant kingdom including monocots, dicots, gymnosperms, and even moss, a non-vascular plant (Rabinowicz et al. 2005). The data from soybean suggests that the MF method results in a ~3.2-fold gene-enrichment of an estimated ~343-Mb hypomethylated gene space.

Materials and methods

Genethresher\® library construction

Nuclear DNA was obtained from 4–6-week-old greenhouse-grown soybean seedlings from\® Glycine max (L.) Merr. cv. Williams 82. Shearing of nuclear DNA was performed using either a nuclease (Ci\-Us, Inc., Bedford, MA), or Hydrosheer (GeneMachines, San Carlos, CA). Sheared fragments were end-repaired with a variety of enzymes including Mungbean Nuclease, T4 DNA Polymerase, Klenow fragment, and T4 Polynucleotide kinase. End-repaired fragments were size-selected on an agarose gel and DNA fragments ranging from 0.7 to 1.5 kb were extracted and ligated to dephosphorylated, BioStar-digested pOT2 vector which was used to construct both methylation filtered (MF, Genethresher\®) and unfiltered (UF) libraries. Ligation reactions were transformed into MceI+ and MceI− strains of Escherichia coli for generation of filtered and unfiltered libraries, respectively. Recombinant clones were picked using a Genetic Q-bot robot (Research Genetics, Cathehdal, CA) and stored individually in 384-well microtiter plates.

DNA sequencing

The subclone libraries were quality tested and, once passed, they entered the production-sequence queue at Genome Sequencing Center, Washington University. Libraries were plated, and colonies resulting after overnight growth were harvested by robotic picker (OP-rix) that array subclones in 384-well microtiter trays. These trays hold glycerol-containing media and provide both a source of DNA for sequencing as well as an archive. Subclone DNAs were purifed by using a robotic assembly line and paramagnetic particle-based separation technology (CCS Packard, Inc., Torrence, CA; Hawkins et al. 1987; Clifton et al. 2004) ABI DyeTerminator (Applied Biosystems, Foster City, CA) reactions were used for sequencing. The reactions were performed in a 384-well format and were assembled using a BiomekFX robot. Thermocycling reaction times were as previously reported (Lander et al. 2001). The reaction products were loaded onto 3730xl DNA sequencers, and as sequence reads were completed, data were automatically processed and recorded in an Oracle database. For all of the above activities, sample processing and tracking were facilitated by a bar-code system that also is linked to the Oracle database. The reads were base called using the ABI KB software. Traces were submitted to the Trace Archive division of GenBank, and the reads were sent to the GSS section of GenBank. Accession numbers are noted below.

Database curation and filter power calculation

A first pass definition-line curation of publicly available sequence databases was done to eliminate obvious transposon sequences that would hamper subsequent analyses by virtue of inflating the true ‘gene’ content of the given database. The Arabidopsis protein set, which was used for the gene enrichment calculations and assessment of cross-genome annotation potential, was downloaded from the NCBI (ftp://ftp.ncbi.nih.gov/genomes/Arabidopsis_thaliana/CHR/\#\#.faa). The files were dated 23 May 2003 and contained 28,981 sequences (12,112,846 total letters). Repeats were removed from this dataset if the definition line met both of the following two criteria:

1. matched the case-insensitive regular expression \(.*\) with \(.*\) matches from MF sequences are compared to the proportion of matches in UF sequences over a range of Expectation values (E-values) from 1e-5 to 1e-20, such that all matches better than the given E-value are tabulated (Table 1). For soybean, the genome size is estimated at 1.1 Mb (Arumuganathan and Earle 1991). Dividing the genome size by the median 2.2 FP provides an estimate of a 343-Mb sampled space.

Collapsing read pairs

Read pairs from each nuclear clone were assembled using Phrap (+ \(\ast\) nucmatch 17 \(\ast\) nucscore 40 \(\ast\) forcelevel 1) The resulting contigs were trimmed for quality and vector. Read pairs that did not collapse were trimmed for sequence and vector. These sequence sets are hereto referred to as the nuclear collapsed set.

Repeat analysis with RepeatMasker

Repetitive elements were identified in the nuclear collapsed set for filtered and unfiltered sequences using RepeatMasker (AFA Smit, R Hubley, P Green RepeatMasker at http://repeatmasker.org, verified 6 June 2006) with the M. reesii repeat libraries (Bedell et al. 2000) and a collection of plant repeat databases from TIGR. Version 2 of the Brassicaceae, Fabaceae, Solanaceae repeat libraries were downloaded from TIGR. In addition, the TIGR cereal repeat database, dated 11 July 2003 was downloaded from ftp://ftp.tigr.org/pub/data/TIGR_Plan_repeats/ and contained 11,061 repeat entries. This collection of plant repeat was supplemented with the soybean sequences of the retrotransposon Calypso (AF186182–AF186186, AF378062–AF378073), repeat SB92(U11026), and repeat STR120 (U26697, U26698, U26700, U26701). RepeatMasker was run with the following parameters: \(\ast\) nolow. De novo repetitive sequence identification

De novo repetitive sequence identification was performed on the RepeatMasker masked nuclear collapsed sets for filtered and unfiltered sequences (see above). The masked sequences were split on 20 or more masked nucleotides (N5) and each segment was given...
Table 1. Distribution of soybean sequence repeats (%)

Genomic sequences for the various organisms were analysed for repeat content with RepeatMasker. The fraction for each repeat class is shown. The ‘mixed’ classes are those repeat features that are made up of overlapping sequences of two or more sub-classes. WGS, soybean whole-genome shotgun sequences; BAC ends, soybean sequences derived from BAC ends.

<table>
<thead>
<tr>
<th>Class</th>
<th>Sub-class</th>
<th>Glycine max WGS</th>
<th>Glycine max BAC ends</th>
<th>Lotus japonicus</th>
<th>Medicago truncatula</th>
<th>Arabidopsis thaliana</th>
</tr>
</thead>
<tbody>
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<td>Retrotransposons</td>
<td>Ty1-copia</td>
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<td>4.86</td>
<td>1.67</td>
<td>0.78</td>
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<td>0.44</td>
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<td>2.23</td>
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<tr>
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<td>0.68</td>
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<td>34.42</td>
<td>8.29</td>
<td>9.04</td>
<td>5.81</td>
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</table>

a unique identifier. The sequences were analysed with RECON (Bao and Eddy 2002a, b) for de novo repetitive sequence identification. The computation of RECON involved the following steps.

1. Run BLAST using each sequence against the database, with the expectation value threshold 1e–30.
2. Apply the MSPCollect tool of RECON, which converts each BLAST output into an MSP file. In the MSP file, each pairwise MSP result reported by BLAST is turned into a one-line summary in a certain format, containing variables of ‘score’, ‘identical percentage’, ‘sequence start position of query’, ‘sequence end position of query’, ‘query name’, ‘sequence start position of subject’, ‘sequence end position of subject’, and ‘subject name’.
3. Filter MSP files through three steps: (a) remove repeat sequences that are less than 50 bp; (b) remove those sequences with frequency of less than 10; (c) remove more lines of self-hits (i.e. query sequence is the same as the subject name) in the MSP file.
4. Run RECON using the remaining MSP file. RECON outputs all the repetitive sequences, but it does not provide a representative sequence for a group of sequences that are repetitive from each other. We then developed and ran a C-shell script that incorporates ClustalW (Thompson et al. 1994) for all the elements in a repeat family to obtain the aligned sequences. A consensus sequence was built for the family, where for each column in the alignment, we took the letter with largest occurring frequency in a column as its representative. This consensus sequence is considered to be the representative repeat sequence for this whole family.

Novel repeat copy number

Novel repeats were compared to the original nuclear collapsed dataset from unfiltered sequences using BLAST. Matches below 95% identity and under 50 bp were discarded. The total base pairs matched was tallied for each repeat and used to determine copy number.

Results

Reducing the soybean genome to the hypomethylated, gene-rich space

Methylation filtered libraries were constructed from soybean nuclear DNA in host strains of bacteria that restrict methylated DNA (Rabinowicz et al. 1999). Of 10,751 attempts from the MF library, 8,632 were successful, 8,366 of which were considered of nuclear origin based on comparison with chloroplast, mitochondriald, viral and bacterial databases. As a control and a tool for assessment of the whole genome composition, the same DNA ligations were propagated in host strains that do not restrict methylated DNA (unfiltered libraries, UF). The soybean whole genome shotgun, or unfiltered (UF), sequences comprise 26,108 attempts, of which 24,224 were considered successful and 23,788 were nuclear.

To calculate the genome space sampled by GeneThresher® technology, a method that relies on gene enrichment was used. The gene-enrichment method works by assuming that genes are enriched in the MF libraries proportional to the reduction in genome size. For example, if the genome is reduced by 3-fold, then gene discovery should occur 3-fold faster in MF whole-genome shotgun libraries.

The gene-enrichment factor is called Filter Power (FP) and FP can be used to derive the sampled genome space by dividing it into the size of the whole genome (G). We calculated the soybean FP using a subset of our filtered and unfiltered sequences compared with a curated database...
of known genes over a range of BLAST E-values (1e-5 to 1e-20). The FP is between 2.7 and 3.5 with a median value of 3.2. By dividing this range of FP values into the 1.1-Gb soybean genome, the sampled genome is estimated to be between 314 and 407 Mb with a median of 343 Mb (Fig. 1). The MF dataset consists of a nuclear coverage, after collapsing read pairs, of 3.66 Mb which is approximately a 0.01× coverage of the sampled space.

Gene ontology analysis
To determine whether there is an apparent bias in the enrichment for genes using MF, an analysis of the gene ontology terms for sequences with significant similarity (with E-value less than 1e–8) to an *Arabidopsis* protein (see Materials and methods) was performed for both the filtered and unfiltered sequences. As seen in Fig. 2, there was no significant difference between the genes enriched through methylation filtration (FGM) and through whole-genome shotgun (UGM).

Simple sequence repeats
Simple sequence repeats are stretches of DNA with simple sequence pattern repetitions, usually in the form of di-, tri-, or tetra-nucleotide expansions such as (CA)n, (CAG)n, or (GATA)n. These stretches of DNA are useful for genetic marker analysis because they are unstable and often are polymorphic between closely related individuals (Cordeiro *et al.* 2001; Klein *et al.* 2003). Overall, there was a higher density of SSRs in methylation-filtered soybean sequences with one SSR per 10.0 kb in MF, compared with one SSR per 15.3 kb in UF. Additionally, the SSRs obtained from MF are more likely to be gene-associated.

GC-rich trinucleotide SSRs (TNR) in monocots have been shown to be preferentially associated with coding regions (McCouch *et al.* 2002; Morgante *et al.* 2002). For soybean, there were 37 out of 739 (5%) GC-rich SSRs in UF v. 29 of 378 (7.7%) in MF, which is not a statistically significant difference.

Repetitive sequences in soybean
Estimates of the gene space by MF suggest that ~70% of the soybean genome is composed of methylated DNA. To determine the extent to which the soybean genome is repetitive, the unfiltered soybean sequences were masked using a compilation of plant repeats from TIGR with RepeatMasker (Table 1) (see Materials and methods). As a basis for comparison, 20,000 GenBank GSS sequences of the legumes *Lotus japonicus* and *Medicago truncatula*, as well as 20,000 random reads from the *Arabidopsis thaliana* genome, were also masked after contaminating sequences were removed. The results are shown in Table 1. Excluding the novel soybean repeats (see below), the most abundant class of repeats for soybean were retrotransposons (14.97%) and this also appeared true for *M. truncatula* and *L. japonicus*. Counting all the known repeat sequences, at least 25% of the soybean genome is repetitive. In addition, we included 29,117 soybean BAC-end sequences (with GenBank accession numbers from CZ498303 to CZ527432) of soybean in our repeat analysis. The relative distribution of repeats found in the BAC-end sequences was roughly equivalent to that found in the whole-genome shotgun sequences (Table 1).

The reduction of repeat sequences was calculated by comparing the fraction of repeat-masked sequences from unfiltered and filtered libraries with respect to the whole genome. Thus, the calculation becomes: Fraction repeat-masked Unfiltered / (Fraction repeat-masked Filtered / Filter Power). MF significantly reduced the known repetitive fraction by ~12-fold (see Table 2). Reduction of various repeat classes was not uniform. For example, ribosomal repeats were reduced by more than 40-fold, while DNA transposons were reduced roughly 8-fold. Such differences may be, in part, due to differential methylation status of these repeat classes as was observed when MF was applied to maize (Whitelaw *et al.* 2003) and sorghum (Bedell *et al.* 2005). Differences in reduction may also be due to the GC content of different repeat classes.

In summary, 235 representative repeats were identified from the unfiltered and filtered libraries of soybean. After using them as a filter, we identified 113 additional representative repeats for the 29,117 BAC-end sequences of soybean, i.e. 348 representative repeats in total were identified for soybean. These repeats can be found at...
Table 2. Frequency of repeat classes in filtered and unfiltered soybean libraries

<table>
<thead>
<tr>
<th>Class</th>
<th>Sub-class</th>
<th>Unfiltered soybean (%)</th>
<th>Filtered soybean (%)</th>
<th>Reduction factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retrotransposons</td>
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<td>0.13</td>
<td>3.32</td>
</tr>
<tr>
<td></td>
<td>Telomeric</td>
<td>0.04</td>
<td>0.08</td>
<td>1.61</td>
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<td></td>
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<td>0.02</td>
<td>40.76</td>
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<tr>
<td></td>
<td>SB92</td>
<td>0.98</td>
<td>0.14</td>
<td>22.33</td>
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<tr>
<td></td>
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<td>0.96</td>
<td>0.18</td>
<td>16.9</td>
</tr>
<tr>
<td></td>
<td>Soy novel</td>
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<td>5.18</td>
<td>10.36</td>
</tr>
<tr>
<td>Mixed</td>
<td></td>
<td>4.63</td>
<td>0.67</td>
<td>21.15</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>42.26</td>
<td>10.73</td>
<td>2.6</td>
</tr>
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</table>

http://www.soybeangenome.org/ (verified 6 June 2006). The distribution of the repeats v. their length is shown in Fig. 3A, while a histogram for the number of copies of repeats within the dataset is shown in Fig. 3B. It is worthwhile mentioning that some of the identified repeats are very long; 26 of them are longer than 500bp. Assuming that our sampling was random, the relative copy number shown should be representative of the whole genome. This analysis estimated that repetitive sequences make up ~10% of the genespace of soybean (data not shown).

Novel repeats in soybean

Results in Table 1 indicate that only approximately 5% of the Arabidopsis genome contains repetitive DNA. This value does not agree with other estimates of the repeat content of Arabidopsis (The Arabidopsis Genome Initiative 2000). This under-estimated value of repeat content is due to the lack of curated repeats. The TIGR Fabaceae repeat library contains 404 sequences of which only 14 are curated retrotransposons, the most abundant class of known repeats in soybean (see above). To further curate repeats in soybean, a de novo approach to repeat identification was taken using unfiltered, filtered, combined filtered and unfiltered soybean sequences, and BAC-end sequences (see Materials and
21 masked and novel sequences appear to be fairly specific for soybean. and 5.18% of the filtered soybean sequence. These soybean sequence was determined to contain these repeats leaving 346 (>1) similar to a known repeat (with E-value less than 1e–8), distribution of the number of repeats is large and complex with a high proportion of repetitive sequences, respectively, were masked. Putative repeats were screened for coding regions by comparison to an Arabidopsis protein database. They were also screened for known repeats with RepeatMasker. Of the 348 repeats derived from this analysis that were not coding for a known gene, two (≤1%) can be classified as similar to a known repeat (with E-value less than 1e–8), leaving 346 (>1%) as novel, unclassified repeats. Using these repeats with RepeatMasker, 16.78% of the unfiltered soybean sequence was determined to contain these repeats and 5.18% of the filtered soybean sequence. These novel sequences appear to be fairly specific for soybean. As seen in Table 1, only 0.08 and 0.41% of L. japonicus and M. truncatula sequences, respectively, were masked. Of the novel sequences, only 15 masked L. japonicus and 21 masked M. truncatula.

Discussion
The soybean genome, like most important crop genomes, is large and complex with a high proportion of repetitive elements (Stacey et al. 2004). Our results confirm this composition of the genome and also extend the analysis to the epigenetic component. The 1.1-Gb genome contains 40% identifiable repetitive elements with a hypomethylated gene space of ~340 Mb. The hypomethylated fraction contains relatively few repeats and is enriched for genes by more than 3-fold.

We have identified a total of 348 repetitive elements by analysis of whole-genome shotgun (WGS) and BAC-end sequences. This represents the only public repeat database available for use in masking repeats during analysis of soybean genomic sequence (Holmes 2002). Given that the TIGR Fabaceae repeat library is relatively small (404) and only a small fraction (14 of 404) represent retrotransposons, these sequences represent a significant contribution to our knowledge of legume DNA repeats. A significant fraction (~17%) appears to be soybean specific, as they are not found in Medicago truncatula, Lotus japonicus, or Arabidopsis. Methylation filtration is clearly an effective method for removing repetitive DNA. Analysis of the repeats found in the filtered libraries estimated that only around 10% of the hypomethylated, gene-rich segments of the genome are repetitive.

Early DNA-DNA renaturation studies suggested that 40–60% of the soybean genome sequence is repetitive (Goldberg 1978; Gurley et al. 1979). It has also been shown that more than 35% of the genome is made up of heterochromatin (Singh and Hymowitz 1988). Our estimate that the soybean gene space is ~342 Mb (31%) is roughly consistent with these earlier estimates. Additionally, analysis of eight gene-rich soybean BACs deposited in GenBank indicates that they are remarkably free of identifiable repeats. Using the most current plant repeat database from TIGR, supplemented with our current set of newly identified soybean-specific repeats, these eight BACs (i.e. GenBank Accessions AC166090, AC152056, AC166330, AC166092, AC166091, AC144537, AY262686, AF541963) average less than 5% repetitive elements with a range of 1.8–8.7%. This suggests that ~95% of the euchromatic BAC DNA will be accessible to MF clones. Therefore, the MF clones should supplement euchromatic, gene-rich BAC sequencing to a similar extent as WGS, allowing very extensive assemblies across the length of the BAC, similar to what was done to finish the rat genome (Gibbs et al. 2004). In maize, skim sequencing from BAC clones at less than 1× coverage combined with a deep coverage through gene enrichment is predicted to generate a high quality sequence map for a fraction of the cost of whole-genome sequencing (Martienssen et al. 2004). This will be even more efficient in soybean, given the paucity of repetitive elements in the euchromatic, gene-rich portion.

For the method development, we improved the procedure of RECON for repeat identification. In particular, we applied...
ClustalW to systematically retrieve consensus sequences from the multiple alignment of potential repetitive sequences. Such an approach allowed us to more accurately identify repeat representatives. Nevertheless, it should be noted that our procedure based on RECON cannot guarantee to identify all possible repeats. Some repeats with a weak pattern may be missed.

Accession numbers

The soybean GeneThresher® sequences are deposited in the Genome Survey Sequence (GSS) division of GenBank under accession: CL866625–CL874567, CL874569–CL876819, CL876829–CL877015, CL884614. The unfiltered sequences are deposited under accessions CL876820–CL876828, CL877016–CL884613, CL884615–CL900625. Soybean BAC-end sequences are deposited in the Genome Survey Sequence (GSS) division of GenBank under accessions: CZ493303–CZ527432.

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