CONSTRUCTION AND CHARACTERIZATION OF A COMMON BEAN BAC LIBRARY

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Abstract
A bacterial artificial chromosome (BAC) genomic DNA library of the HR45 common bean line (*Phaseolus vulgaris* L.), which is highly resistant to common bacterial blight of common beans incited by *Xanthomonas axonopodis* pv. *phasoli* (Xap), was constructed and preliminary characterization was done. The library consists of 33024 clones arrayed in 86 384-well microtiter plates. Based on a genome size of 588MBP for *P. vulgaris* L, the library would cover 5 fold of the bean genome. The library is stable, all clones analyzed to date have inserts and the average size of the insert is 100kb.

Methods
Leaf nuclei were isolated by differential centrifugation and embedded in plugs of low melting temperature agarose (TAMU, 2002). This high molecular weight nuclear DNA was partially digested with Hind III (Folkertsma et al., 1999), size selected twice by pulsed-field gel electrophoresis, electroeluted from the gel (Strong et al. 1997) and ligated into the vector pIndigoBAC -5 (Epicentre). The ligation mixture was electroporated into TransforMax EC100 Electrocompetent *E.coli* cells (Epicentre). 33,024 individual clones were picked and grown in 384 well (capacity 120µl/well) microtiter plates in 90µl of modified LB medium containing 4.4 % glycerol. A total of three copies of this library were stored at -80° C.

Results and Discussion
Not I digestion of 100 randomly picked clones indicated that all of the clones analyzed have an insert and the insert size ranges from 30 kb to 200 kb (Fig. 1) with an average insert size of 100 kb (Fig 2.). Because leaf nuclei were used as the source for high-molecular-weight DNA, contamination of the library with organelle sequences should be low. Stability analysis of clones with larger inserts from cultures of generation one and 100 with restriction enzymes has shown that the large insert library is stable in the *E coli* host. In addition, the use of Hind III for this library would complement the previously reported BAC libraries for generating overlapping clones.
This library will facilitate analysis of the physical organization of the bean genome and positional cloning of genes and QTLs associated with various traits such as disease resistance. Detailed analysis of this BAC library is still underway.

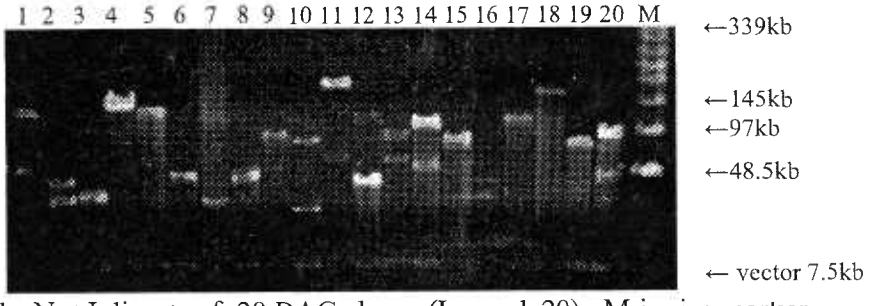

Figure 1: Not I digests of 20 BAC clones (Lanes 1-20). M is size marker.

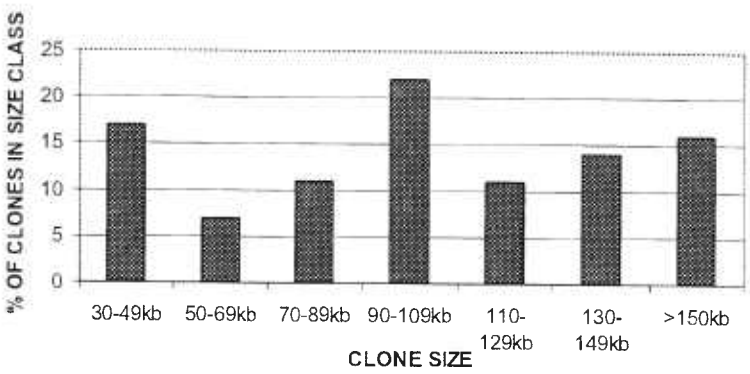

Figure 2: Size distribution of inserts

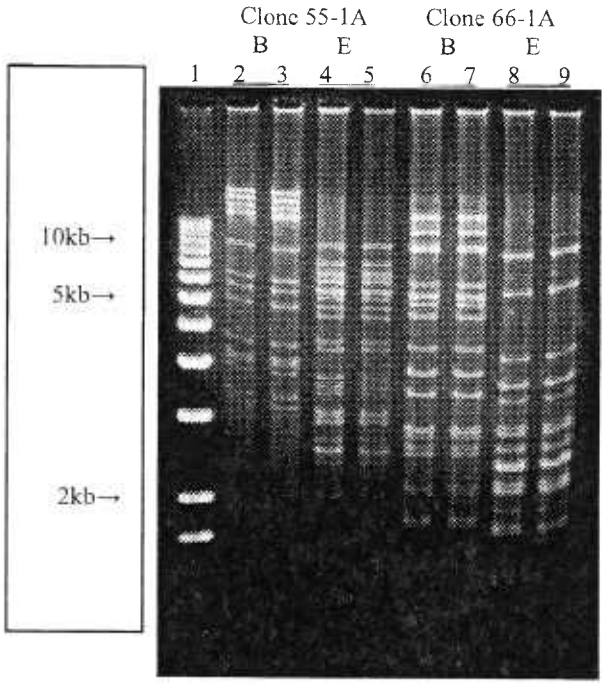

Figure 3. Stability Test. Lane 1 is a size marker. Lanes 2-5 are clone 55-1A. Lanes 6-9 are clone 66-1A. Lanes 2,3,6,7 were digested with Bam HI and 4,5,8,9 were digested with Eco RI. The first of each pair is generation one and the second is generation 100.

References

Folkertsma, R.T. et al. 1999. Molecular Breeding, 5, 197-207.
Strong, S.J. et al. 1997. Nucleic Acids Research, Vol 25, No. 19, 3959-3961.
Texas A& M BAC Center, BAC Library Protocols (1), 2002