

# CONSTRUCTION OF BAC CLONE SHOTGUN LIBRARIES FOR COMPARATIVE SEQUENCE ANALYSIS OF THE SHORT-ARMS OF CHROMOSOME 3 OF FOUR ORYZA SPECIES



## Abstract

To gain insight into the understanding of genome biology, that includes speciation, domestication, and gene regulation, a comparative genomics project was assembled within the *Oryza* genus. Specifically, this research compares homologous regions of the short arms of chromosome three from four of 24 *Oryza* species. These species represent diploid and polyploidy genomes: *O. glaberrima* AA, *O. punctata* BB, *O. officinalis* CC, and *O. minuta* BBCC.

Physical maps of Bacterial Artificial Chromosome (BAC) clone libraries from each species were aligned to *O. sativa* pseudomolecules and homologous region BAC clones were selected from each target species. From each BAC clone, which contained the targeted sequence, small insert sub-clone library was constructed, called BAC clone shotgun library. BAC clone shotgun libraries are used to accommodate the limitations of the current sequencing technology which is unable to produce sufficient sequence from large 130kb BAC inserts, but rather can process the smaller 2-3kb insert sequences of shotgun libraries.

To go from BACs to Shotguns, BAC DNA was isolated, sheared into random 2-8kb fragments and end-repaired. Following gel electrophoresis, a range of 2-6kb DNA was selected and purified. DNA was ligated into the high-copy vector, pBluescriptIIKS+, followed by electroporation into competent *E. coli* cells. Analysis of the transformants by insert size checking and sequence homology revealed the quality and utility of the BAC clone shotgun libraries. BAC subclone libraries were further sequenced to deeper coverage and assembled to determine the full sequence of a BAC clone.

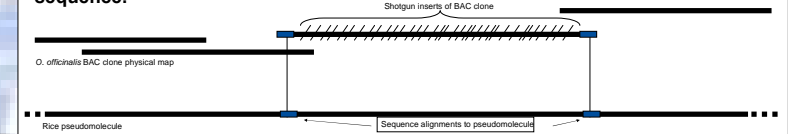


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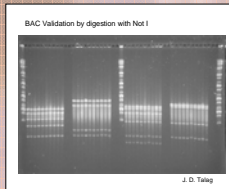
## THE TAKE HOME MESSAGE.....

Shotgun subclones of BAC clones—when sequenced—will elucidate the BAC sequence.



## SHOTGUN LIBRARY CONSTRUCTION: FROM BACS TO SHOTGUNS

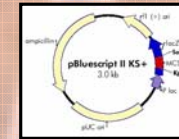
### Start of Procedure:



**VALIDATION:** This process examines the contamination and the identity of the obtained clone. It is done by comparing the NOT I digestion patterns of ten individual colonies. The clone is also submitted for BAC end sequencing and contrasted with that of the known end sequence.

**PRECIPITATION:** The sheared DNA is precipitated out of solution and purified.

**DNA END REPAIR:** The damaged ends resulting from the shearing process are repaired by adding complementary bases to the sticky ends via a combination of End-It Buffer, ATP, dNTPs, and End-It-Enzyme.



**LIGATION:** To recircularize the vector containing the insert DNA, the shotgun vector (pBluescript II KS+), buffer, and the enzyme (Ligase) are added to the selected DNA.

**ELECTROPHORATION:** This process transforms the plasmids containing the inserted DNA fragments into *E. coli* cells via an electromotive force applied to the bacteria.



**PLATING:** The incubated samples are plated on agar plates prepared with the appropriate antibiotic and growth media. The resulting blue and white colonies are counted. The obtained information is sent for robotic plating and picking.

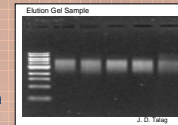
**PLASMID DNA ISOLATION:** Following validation, the bacterial membrane of the *E. coli* cell is lysed, in order to release the plasmid containing the desired insert.

**DNA SHEARING:** The prepared DNA is combined with a series of solutions and the samples are then injected into the Hydroshear machine which randomly shears the DNA into 2-8kb fragments.



**SIZE SELECTION:** To obtain the desired insert size, the end-repaired DNA is run through gel electrophoresis allowing the 2-6kb fragments to be identified and cut from the gel.

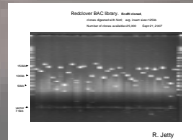
**ELUTION:** Elution is the process used to extract the size-selected DNA from the gel. This is done using several buffers and QIAEX II beads, which bind the DNA.



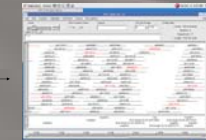
## GENOMICS OVERVIEW: FROM ORGANISM TO SEQUENCE



DNA is isolated from the desired organism—in this case *Oryza*.



The isolated DNA is then digested via restriction enzymes. The resulting 120-150kb fragments are then ligated into the BAC vector and stored in *E. coli* cells for amplification.



The BAC libraries are fingerprinted and end sequenced to produce a framework for genomic study.

### SHOTGUN LIBRARY CONSTRUCTION

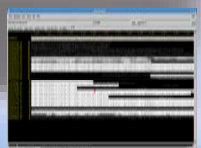
The BAC libraries are broken down into 2-3kb segments via a random shearing process. The resultant sheared DNA is ligated into the shotgun vector and again transformed into *E. coli* cells. The resultant libraries are termed *shotgun libraries*.



Breaking down the BAC clones into smaller subclones allows the BAC sequence to be determined. This process involves the complementary addition of fluorescently tagged ddNTPs to the subclone DNA.



Web display showing the production of shotgun libraries for comparative analysis of the rice species.



Shotgun sequences are piled up in CONSED. Consensus is the BAC sequence.