

a plasmid pGPTV-HB containing genes for hygromycin resistance (HygR) and Bar. Transgenic plants showing in vitro resistance to 50 mg/l hygromycin and 10 mg/l bialaphos were obtained by using a two-step selection/regeneration procedure. Transformation efficiency of rice about 30% which was as high as reported in dicotyledons. Progenies(T₁ generation) derived from primary transformant of 17 lines segregated with a 3 resistant : 1 sensitive ratio in medium containing hygromycin and bialaphos. Stable integration of bar gene into chromosomal DNA was proven by Southern blot analysis of genomic DNA isolated from T₂ progenies. Transgenic plants(T₃) grown in the field were resistant to bialaphos (Basta) at a dosage lethal to wild type plants. These results show that the Bar and HygR gene have proven to be a useful selectable marker for the transformation of rice plants and for the production of herbicide-resistant plants.

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BAC CLONING AND LARGE BAC DNA TRANSFORMATION TECHNOLOGIES

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BAC (bacterial artificial chromosome) cloning has served an important role in agricultural and mammalian genomics since its introduction in 1992. BAC libraries are currently in use or under development for virtually every important genome. The primary reasons BACs are so useful is that they can maintain large DNA inserts (up to 350 kb) in *E. coli*. and are amenable to virtually all of the sophisticated molecular biology techniques developed over the last 20 years for small 2-10 kb plasmids. I have been constructing BAC libraries of a variety of organisms including rice, *Arabidopsis*, cotton, citrus, *Ashbya*, and human in the last several years. Recently, I have begun constructing a series of BAC libraries with a much larger insert size (182 - 202 kb) from human (http://www.tree.caltech.edu/lib_status.html). The larger insert BAC library will provide significant improvement to applications in physical mapping, positional cloning, and DNA sequencing.

DNA transfer into plants has been accomplished by several methods including *Agrobacterium*-mediated transformation, biolistic bombardment, and microinjection. BAC vectors have been engineered for transformations of large DNA inserts into plant genomes. A pBACwich system has been developed to achieve site-directed integration of DNA into the genome (Choi et al., unpublished work). A 150 kb cotton BAC DNA was transferred into a specific *lox* site in tobacco by biolistic bombardment and Cre-*lox* site specific recombination. These results open up a number of new possibilities for plant molecular biology and genetic engineering of plants. These systems will streamline positional cloning and the transfer of desirable traits into plants.