pMAL-c2(Novagen) in E. coli. Unique 1,536 bps iap gene of Listeria grayi was amplified with specific primer using polymerase chain reaction(PCR) and then the amplified gene was cloned with expression vector pMAL-c2. Recombinated pMAL-iap/grayi was induced with IPTG and overexpressed recombinant p60 that fused with maltose binding protein(MBP) in E. coli strain DH5  $\alpha$  F'. Optimum concentration of IPTG induction time were estimated 0.5mM and 4hrs respectively. Overexpressed p60/grayi was analysed by SDS-PAGE and was purified by amylose resin based affinity chromatography. Purified recombinant p60 protein will be useful in rapid detection and production of monoclonal antibody against Listeria grayi.

## H802

## Selective Isolation of Mammalian Genes by TAR cloning

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Mammalian genome analysis has been advanced considerably by the development of YAC and BAC cloning system. These traditional methods of isolation of a specific gene from a YAC or BAC genomic library have typically involved a long and laborious process of identification of the region of interest among thousands random YAC or BAC clones. Using the recently developed TAR cloning technique in S. cerevisiae, which allows entire genes and large chromosomal regions to be specifically and accurately isolated from total genomic DNA. In spite of this usefulness of TAR cloning, the frequency of capture of the recombinant insert was less than 1% of transformants. To improve the frequency of positive clones, non-homologous end-joining pathway is

essential for repair of specific classes of double strand breaks termini in cells of S. cerevisiae. To know the effects of the NHEJ in homologous recombination of TAR cloning, several isogenic strains with defects in NHEJ pathway genes are examined in TAR cloning. To examine the essential region of targeting Hook, we mutagenized each 20 bp of the 60 bp of Hook and examined the frequency of the homologous recombination in TAR cloning using of the SV40 model system.