pSH4: a mammalian cDNA expression vector

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In our continued efforts to develop an *in vivo* functional assay for isolation of cDNAs encoding transacting regulatory protein factors (1), we have constructed a mammalian cDNA expression vector for cloning cDNAs directionally. This vector, pSH4, is a composite vector derived from Okayama-Berg cDNA expression vectors pLI and pcDVI and retains all the important features of it (2) {details of construction are available on request}. The directional cloning involves the generation of *Hind*III sites at the 3' termini and *Eco*RI sites at the 5' termini of cDNA molecules on the ligation of the oligonucleotide pGCTTGAATTCAAGC (3). The cDNAs, when cloned in pSH4 at the *Eco*RI and *Hind*III sites, have the 5' end near the regulatory region and the 3' end towards poly A addition signal (Fig. 1). This procedure of directional cDNA cloning is technically far simpler and more rapid than the existing protocols (4). In pSH4, the regulatory regions are derived from Okayama-Berg cDNA expression vector, pL1. An intron containing donor and acceptor splice sites from SV40 16S and 19S late mRNAs is present upstream of the cDNA cloning site. While one splicing event allows for the use of initiation codon of cDNA, alternative splicing will permit translation from the AUG of the vector sequence, to synthesize the fusion protein, even from a truncated cDNA. An intron between the transcription initiation site and initiating AUG allows the splicing of the transcript even if polyadenylation occurs within the cDNA sequence. Also, it has been observed that SV40 small t-antigen intron at the 3' end of cDNA sometimes leads to cryptic splicing if 5' splicing donor sites are present in the cDNA (2, 5, 6). The vector can be linearized using *Sal*I and *Nor*I. The insert cDNA can be cut out from this vector by *Bam*HI. The presence of SV40 origin of replication will allow pSH4 to replicate in Cos cells and other permissive cells on provision of T-antigen.

A cDNA library from the lymphoblastoid cell line (JY) has been constructed in this vector using *E. coli* DH10B (*mcrA*⁻, *mcrB*⁻ and *mrr*⁻) host which prevents the reduction in transformation efficiency due to methylation of cytosine residues generated by *A*luI methylase (7). The pSH4 expression vector has been successfully used to express an almost full length cDNA of the HLA-B7 in J27 cell line.

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pSH4KX is a representative cDNA clone. Wavy line indicates the cDNA insert. K = Kanamycin resistance gene, S = *Sal*I, N = *Nor*I, B = *Bam*HI.

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