An expression vector system for stable expression of oncogenes

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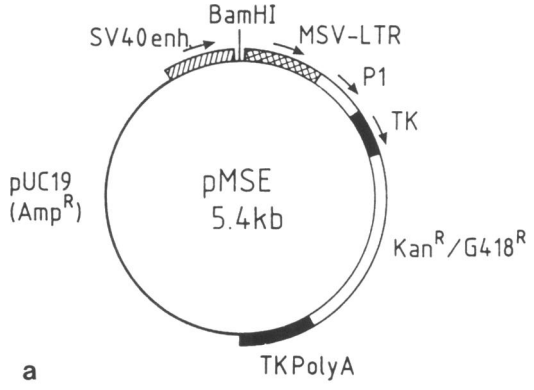
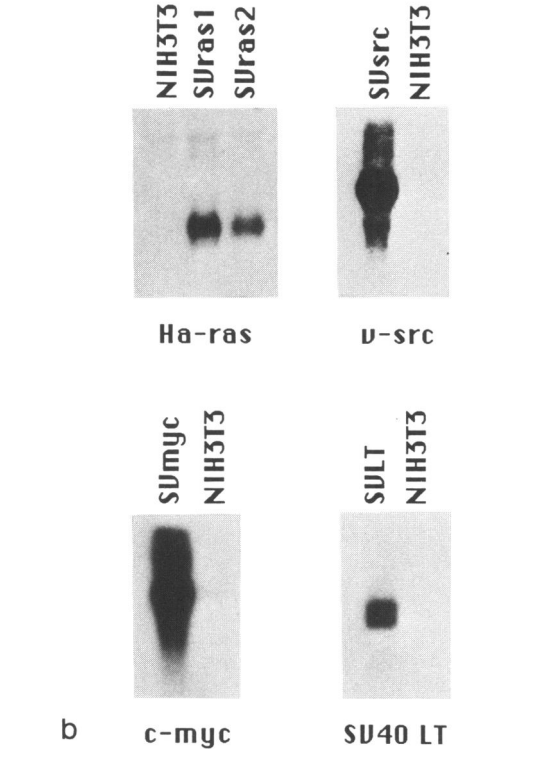
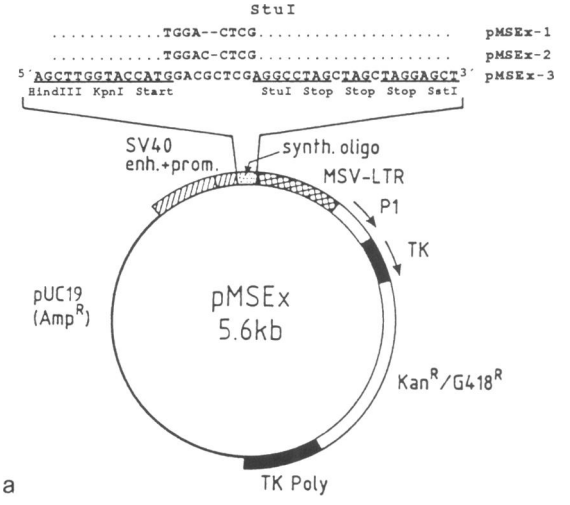
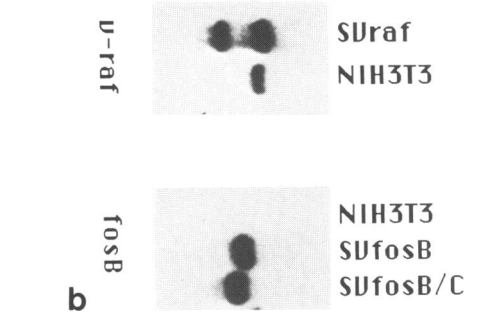
A series of mammalian expression vectors was constructed for the stable integration and high constitutive expression of cellular and viral oncogenes. Based on pUC19, the vectors contain several elements (Fig. 1a). These are a SV40 early promoter fragment containing the enhancer core sequences (1), a unique cloning site followed by a retroviral MSV-LTR providing a transcription termination signal and serving as additional enhancer of a hybrid Tn5 KmR/G418R gene under the control of the thymidine kinase (TK)-promoter (derived from pHS272; 3). The vectors can thus be used as bacterial/mammalian shuttle plasmids. The SV40 promoter has a 3 bp deletion at the BglI site within the origin of replication to prevent autonomous replication in the presence of SV40 large T-antigen (2). In addition, the pMSEx vectors contain a synthetic oligonucleotide providing a translation initiation signal (4) followed by a StuI site in three different positions (pMSEx1−3, see Fig. 2a) and stop signals in all reading frames. The StuI sites may prove useful for cloning blunt end cDNA fragments without addition of linkers.

The following oncogenes were inserted into pMSPE: SV40LT,









c-*myc*, Ha-*ras*, c-*jun*, v-*sis*, v-*src*, v-*abl*, v-*fms*, v-*erb*A. In the same manner, a c-*jun*- and *fos*B cDNA fragment, a chimeric *fos*B/v-*fos* gene and a v-*raf* fragment were cloned into the respective pMSEx vectors.

Expression of the inserted genes was analysed by a focus assay on mouse NIH3T3 and rat 208F fibroblasts, in the case of cells transfected with constructs expressing SV40LT, *fos*B and c-*jun* also by indirect immunofluorescence (data not shown). In focus assays, more than 500 foci per μg of DNA were obtained with constructs expressing either Ha-*ras*EJ, SV40LT, v-*src* or *fos*B. Expression was also analysed by probing RNA of stably transfected NIH3T3, 208F or HaCaT (5) cells. Fig. 1b and 2b show examples of an RNA analysis of cell lines obtained after transfecting NIH3T3 cells with the oncogenes indicated, which were cloned either in pMSPE (Fig. 1b) or in pMSEx (Fig. 2b). mRNA of the expected size could be detected at high levels in all cases analysed.

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