Improvement of the cloning linker of the bacterial expression vector pEX

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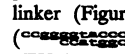
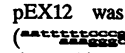
The pEX plasmids are a family of expression vectors for use in *E. coli* (1). Expression of cloned genes (as *cro-lacZ* fusion proteins) is controlled by the cI857 repressor gene. These vectors offer a number of clear advantages over other expression vectors: 1. Easy induction of gene expression (2 hours of incubation at 42 °C). 2. A very high and consistent level of expression with virtually any open reading frame (up to 1.6 kb). 3. The extreme resistance of the expression product to proteolytic cleavage. 4. A convenient protocol for direct immuno-screening of colonies. For these reasons the vectors have been widely used eg. for the construction of cDNA libraries and for epitope mapping. However, cloning in these vectors is sometimes hampered by the limited number of unique restriction enzyme sites. We constructed pEX11-13 to increase the number of restriction endonuclease sites present in the cloning linker (Figure 1). pEX11 and pEX13 were made by ligation of a synthetic ds-DNA molecule () in pEX1 x *Xma* I x *Bam*H I and pEX3, respectively. pEX12 was contructed by the ligation of an other synthetic ds-DNA oligonucleotide () in pEX2 x *Eco*R I x *Bam*H I. pEX12 has no *Spe* I site since this would result in an in-frame stop codon.

The following protocols are used with these vectors: Recombinant plasmids are introduced into the cells via the $CaCl_2$ transformation procedure, performing the heat shock at 37 °C (≈1x10^5 transformants/μg pEX DNA), or by electroporation (≈1x10^7 transformants/μg). As pEX host strain we use *E. coli* pop 2136 (constructed by dr Raibaud, Institut Pasteur, Paris, France). Recovery and selective growth (100 ampicillin μg/ml LB) is at 30 °C. To isolate pEX fusion proteins, cultures are diluted 50-fold in LB-ampicillin and grown at 30°C in 5 ml until an A_{600} of about 0.25. Transient expression of inserted DNA-fragments is induced by incubation at 42°C for 120 min. Subsequently, cells are spun down and lysed in 250 μl 15% (w/v) sucrose, 50 mM Tris-HCl (pH 8.0), 0.12% (v/v) Triton X-100. After sonication for 15 min, the insoluble hybrid protein is spun down (30 s, 16,000 g) and the viscous supernatant removed. The pellets are resuspended in 250 μl 50 mM Tris-HCl, pH 8.0, 0.12% Triton X-100, 10 mM EDTA, sonicated and spun again, and finally stored at -20°C in 100 μl of the same buffer. This procedure results in samples consisting >95% of hybrid-protein at a concentration of approximately 5 mg/ml, as estimated from SDS-PAGE (2). The pEX11-13 plasmids can be obtained from Biores b.v., P.O. Box 260, 3440 AG Woerden, The Netherlands.

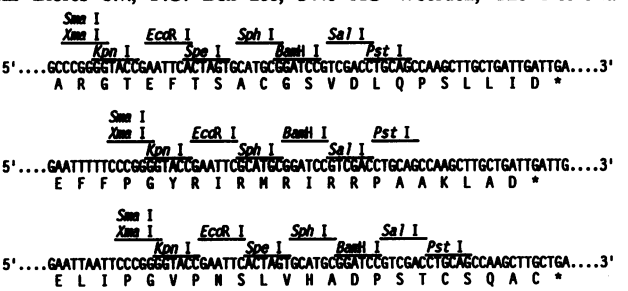


Figure 1. Unique restriction enzyme sites and reading frames of pEX11-13.

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