Engineered gene for *Escherichia coli* alkaline phosphatase for the construction of translational fusions

Johann Kohl, Florian Rüker*, Gottfried Himmler, Diethard Mattanovich and Hermann Katinger
Institut für Angewandte Mikrobiologie, Universität für Bodenkultur, Peter Jordanstrasse 82, A-1190 Wien, Austria

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Conjugates between different proteins are widely used in all fields of biochemistry. Recently, the possibility of joining proteins on the level of their genes has become more and more important and useful. One of the most attractive marker enzymes used for such conjugates is *Escherichia coli* alkaline phosphatase (EcphoA). Using oligonucleotide-directed mutagenesis, we have introduced the indicated restriction sites into the EcphoA gene (1) in order to facilitate the production of translational gene fusions (figure 1). Mutation I has produced a BamHI and an NlaIV site in front of the GTG start codon, Mutation II sites for SmaI, XmaI, HgICl and NlaIV at the boundary between the leader and the coding region, and Mutation III sites for EcoRV and XbaI at the end of the coding region.

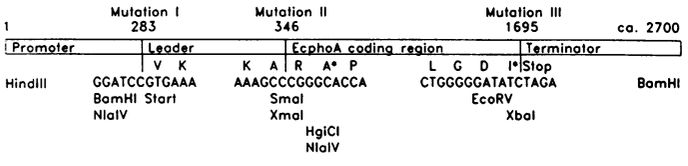

Figure 1. For legend see text. Numbers refer to nucleotide positions in the EcphoA gene (1). Amino acids that differ relative to the EcphoA wild type are marked with an asterisk.

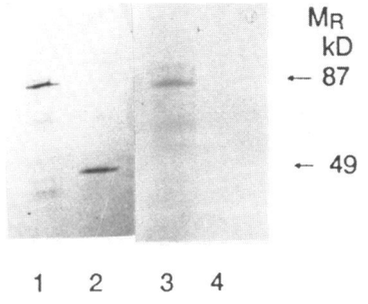

Figure 2. For legend, see text.

Foreign genes can be inserted into the restriction sites at Mutation II or into the EcoRV site at Mutation III. In order to increase flexibility and to minimize disturbance in the EcphoA gene, we have not inserted multiple cloning sites, but prefer to subclone structural genes first into separate polylinker-plasmids if necessary, and then to introduce those genes into the EcphoA coding region. The BamHI, NlaIV and the XbaI sites serve for excision of the complete gene if other regulatory signals than the EcphoA promotor and terminator are to be used. The GTG start codon can be replaced by an ATG start codon by insertion of a 6-base NcoI linker at the Klenow-treated BamHI site or by subcloning the gene into expression vectors which already contain an ATG start codon (e.g. pKK233-2).

We have fused this engineered gene with the gene coding for the coat protein region of Plum Pox Virus (CP-PPV). The 1154bp CP-PPV gene was released from the plasmid pPPVNAT65 (2) with HindIII and HaeIII and treated with S1 nuclease to remove the protruding HindIII ends. This fragment was inserted into the EcoRV site at the 3′ end of the engineered EcphoA gene, the fusion gene was cut out with BamHI and partial NlaIV and, after filling in with Klenow, inserted into the blunt ended EcoRI site of the expression vector pKK223-3 (Pharmacia) to yield the final plasmid pKK-phoACP. This plasmid codes for a fusion protein of approx. 87kD. As a control, an analogous expression plasmid was constructed with the unfused EcphoA gene as an insert (pKK-phoA), yielding a protein of 49.5kD. Figure 2 shows the phosphatase activity of both, fused and unfused gene product on a Western blot (lane 1 and 2), as well as the specific reaction of the fusion protein with anti-PPV serum conjugated with peroxidase (lane 3; lane 4: unfused EcphoA, no reaction).

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* To whom correspondence should be addressed