Selective cloning of a defined number of tandem DNA repeats in *Escherichia coli*

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Repetitive DNA sequences play an important role in biology, especially at the level of eucaryotic gene transcription control. Their construction, assembly and cloning by conventional means or by PCR derived methods is usually very cumbersome. This method is based on the association of a small DNA tag with the sequence to be polymerised. The multimeric sequence is constructed in a stepwise manner, the tag allowing for simple in vivo selection of the \((n+1)\) polymer containing vector at each step. Thus, \(n\) rounds of cloning generate a family of vectors, each harbouring a defined number of ordered tandem copies of the sequence of interest, ranging from 1 to \(n\). Contrary to the multimerization methods based on PCR, this method is independent of the size of the sequence to be polymerised. It is, in fact, especially well suited for the moderate polymerisation of short DNA sequences such as regulatory proteins binding sites, or the preparation of small size DNA ladders.

The outline of the method is as follows (Fig. 1). The sequence to be polymerised is first cloned between two compatible ends restriction sites in a cloning vector (sites A and B), generating vector 1X. Then, a small cassette encompassing an amber suppressor tRNA gene with its promoter and transcription terminator is cloned next to it (between sites C and D), to obtain vector 1X+S. This is used to transform an amber mutated strain, such as, for example, XAC-1 (1). The 2X+S vector is obtained by cutting out the 1X+S fragment with restriction enzymes A and D, and cloning it between sites B and D of vector 1X. Growth of the transformants on minimal medium ensures that only the correct construct is selected for.

In the same manner, the 3X+S vector is obtained by cloning the 2X+S A/D fragment into the 1X vector between the B and the D sites and selecting the transformants for the presence of the suppressor gene. The 3X+S vector will thus contain three adjacent copies of the sequence of interest, in the same orientation, between two unique restriction sites A and B, as the sites between the copies are destroyed by the cloning procedure and the asymmetrical cloning ensures that only one orientation of the sequence is inserted. Each time the cloning step is repeated, one unit of the sequence is added to the polymerised sequence. With a recombination deficient amber mutated strain as a host for the successive transformations, the limit to the number of copies of the sequence of interest is only given by the size of the plasmid. If the \(nX+S\) containing vector is destroyed by additional restriction cuts when preparing the \(nX+S\) fragment, then no purification of this fragment is required before cloning, and the successive rounds of cloning are very simple.

A 42 bp core sequence of the RRE has been defined as the minimal element required to bind HIV-1 regulatory protein Rev (2). In order to study the binding of Rev with this target sequence, we constructed a family of vectors harbouring one to six copies of this 42 bp core RRE sequence. This was easily achieved by cloning the 42 bp synthetic sequence between the *SalI* and *XhoI* restriction sites.

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Polymerisation of a 42 bp ‘minimal’ RRE sequence. Oligonucleotides 5′-TCGACTATGGGCGCAGCGTCAATGACGCTGACGGTACAGGCC-3′ and 5′-TCGAGGCTGATCCGAGGTCAATGACGCTGACGGTACAGGCCCA-3′ were synthesised on an Applied Biosystem 380A. They were purified on Nensorb columns (DuPont) and annealed in 100 mM NaCl. The resulting 42 bp fragment was cloned between the SalI and XhoI sites of pBluescript (Stratagene). A PvuII/HindIII fragment from pGFIB1-phe was then cloned in the 1X vector cut by SmaI and HindIII, generating the 1X+S vector. A XbaI–SalI fragment from this vector (containing one copy of the RRE sequence and the suppressor tRNA gene) was then cloned into a XbaI/XhoI cut 1X vector (containing one copy of the RRE sequence). The resulting 2X+S vector was then used as a source of a XbaI–SalI fragment for the next round of cloning in the 1X vector to generate the 3X+S vector. Iterative cloning led to vectors containing one to six tandem copies of the minimal RRE sequence. These vectors were cut with SalI and XhoI and the DNA was separated on a 8–25% acrylamide gradient gel (Phastgel, Pharmacia). The gel was then silver stained. Lane 1, 123 bp size marker (Pharmacia); lane 2, RRE 1X (42 bp); lane 3, RRE 2X (84 bp); lane 4, RRE 3X (126 bp); lane 5, RRE 6X (252 bp).

sites of pBluescript, and adding the tRNA_CUA_Phe gene from pGFIB1-phe (3) next to it between the XbaI and the HindIII sites.

The successive nX+S fragments were then obtained with Xbal/Xhol restriction cuts and cloned into the Xbal/SalI sites of vector 1X (Fig. 2).

One major advantage of this polymerisation method is that it is sequence and size independent. It can therefore be used to obtain short polymeric sequences derived from bacterial operator sequences or eucaryotic UAS for example. This can be very useful for DNA binding protein purification or band-shift assays. The orientation of the copies can be predefined in order to obtain direct or inverted repeats. The DNA tag, a suppressor tRNA gene cassette, is large enough to be easily detected by standard restriction analysis of the constructs, but its small size does not overload the vector. Although the insert gets harder to clone as the polymerised sequence increases in size, the suppression provides a very effective selection for the correct constructs in strains with an adequate genetic background.

Two or more different sequences can be combined in this protocol to build a more complex repetitive motif. This enables to assemble a gene coding for a protein where for example several copies of a repeated peptide motif are linked by a spacer sequence. Moreover, the suppressor tRNA gene has been successfully used as a DNA tag for other difficult cloning experiments.

REFERENCES