

Baculovirus-Based Gene Libraries

Field of the Invention

This invention relates to baculovirus-based gene libraries.

Background of the Invention

5 Viral vectors are the most effective gene transfer vectors currently used in gene therapy. However, a problem is the relatively small size of foreign DNA fragments they can carry. Baculoviruses have proved to be safe and efficient gene transfer vectors, and they are able to carry considerably larger (>50kb) insertional DNA-fragments than the traditional viral vectors.

10 Summary of Invention

The present invention is based on the use of an *attAEori* element in creating baculovirus-based gene libraries.

Desired genomic fragments are converted to circular form and the *attAEori* element integrated into the genomic circle pool to create entry clones.

15 The entry clone, now containing the two *att*-sites (*attL1* and *attL2*), is cloned into a particular transfer plasmid, e.g. by bacteriophage lambda cloning. The transfer plasmid, containing the target DNA cassette, is further transferred into the baculovirus genome, called a bacmid, along with part of the transfer plasmid. The site-specific integration of the target DNA-cassette into bacmid
20 can occur by Tn7-mediated transposition. Baculoviruses are produced by transfecting insect cells with this recombinant bacmid-DNA.

Existing results indicate that this method works well with plasmids sizes up to 33.5 kb, and it is to be expected that it can be used with larger plasmids. It is thus possible to transfer different libraries to baculovirus format by means
25 of the invention.

Description of the Drawings

Fig. 1 shows the *attL* Adaptor Element containing attachment sites from the bacteriophage Mu.

Fig. 2 shows the p alt Aesys transposon vector.

30 Fig. 3 shows an overview of the different aspects of the *attAe* system.

Description of Preferred Embodiments

The new *attAEori* (*attAE* with an origin of replication) system provides a means to study how large DNA fragments baculoviruses can carry, e.g. up to 50, 100, 200 or 500 kb. Conversion is based on the usage of a simple and flexible
5 *attL1* & *attL2* adapter element (*attAE*, Fig. 1), which can be incorporated into desired target DNA by various means, e.g. using conventional RE-cloning techniques (ref. 8) or taking advantage of transposase or integrase-based recombinational cloning systems (refs. 3, 4, 6, 9).

The desired genomic DNA is treated with a suitable restriction enzyme,
10 such as *NotI*, an 8-cutter which generates average fragment sizes of 100kb. The fragments may then be isolated and purified and converted to circular form.

The *attAEori* element may be integrated into the genomic circular DNA by a number of methods. One such method is to ligate the *attAEori* element directly into linearised genomic DNA (i.e. before it is converted to circular form).

15 The preferred method is to use the MuA Transposase reaction.

In order to take advantage of the bacteriophage Mu (ref. 3) in an *in vitro* random transposition reaction, catalyzed by Mu transposase protein, the *attAEori* is constructed into a transposon vector. The resulting vector is named for *pattAEsys* (Fig. 2). In the first reaction, mediated by Mu transposon, the
20 *attAEori* will be integrated into target DNA in the random fashion yielding numerous different insertion clones, which can be selected using antibiotics such as kanamycin. The simple Mu reaction will thus create high diversity of bacteriophage lambda recombination system compatible entry clones (ref. 4). In a preferred embodiment, these clones can be converted effectively into
25 baculoviruses by pBVboostFG system (ref. 1).

The two attachment sites of the bacteriophage lambda-based recombinational cloning system (*attL1* and *attL2*), needed for the LR reaction [*attL* (entry clones) X *attR* (destination vector-like pBVboostFG)] and boosted baculovirus preparation, are cloned into the transposon vector using appropriate
30 restriction enzymes. The *attL1* element is cloned into 486 and the *attL2* into

1653 site of the transposon vector, respectively, to flank the kanamycin resistance gene.

The *attL1* is cloned between *EcoRI* and *EcoRV* sites of the transposon vector. For the *attL2*, a linker is used and cloned into the *BamHI*-site of the
5 vector, into which the *attL2*-site is then inserted. The *attL2* is cloned between the linker's *NcoI* and *PstI* -sites.

BglII-sites are used to free the *attAE* from the *pattAEsys* plasmid before the transposition reaction. The transposition reaction is performed with *MuA*-transposase, and the clones containing both target DNA and *attAEori* are
10 screened using kanamycin selection. Any desired covalently closed DNA can be used as a target. Plasmids of 3 - 33.5 Kbp have been used successfully as model targets.

The library of entry clones may be amplified by transformation of suitable *E. coli* or other host cells.

15 The amplified entry clones, containing now the two *att*-sites (*attL1* and *attL2*) of *attAE*, can be converted compatible to pBVboost system (ref. 1) using pBVboostFG tri (Fig. 3). The cloning is performed using bacteriophage lambda site-specific recombination system (ref. 4). In the LR reaction the *attL1* and *attL2* sites, that now flank the desired target DNA, react with *attR1* and *attR2*
20 sites of pBVboostFG tri. The *attL1* and *attL2* reacts only with *attR1* and *attR2*, respectively. The recombination of these sites in the conversion reaction yields expression clones (donor vectors), which contains the desired target DNA in the form compatible with pBVboost system (ref. 1), allowing easy generation of baculoviruses with negligible background.

25 In order to produce baculoviruses, the part of the pBVboostFG tri transfer plasmid containing now the desired DNA is first converted into baculovirus genome (bacmid). This was performed by using the pBVboost Baculovirus Expression System (ref. 1). Site-specific integration of the target DNA-cassette into bacmid occurs by Tn7-mediated transposition under optimized selection
30 protocol. Recombinant bacmids can be generated at high frequencies with a negligible background.

Baculoviruses may be produced by transfecting insect cells with recombinant bacmid-DNA. Virus production can be verified by immunoblotting using an antibody against the baculovirus major envelope glycoprotein gp64.

The results indicate that the attAEori system performs well with plasmids
5 sizes up to 33.5 Kbp, and tests with larger plasmids are in progress. The results provide a proof of principle that the attAEori system can be used as a fast and straightforward baculovirus library construction method by incorporating attAEori into desired target DNA by transposition (MuA Transposition reaction), RE-cloning (e.g. genomic DNA) or other means (e.g. site-specific recombination).
10 Figure 3 gives an overview of the different aspects of the attAE system.

References

The following references are incorporated herein by reference.

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CLAIMS

1. A nucleotide molecule comprising a selectable gene flanked by *attL1* and *attL2* attachment sites of a bacteriophage and additionally comprising an origin of replication.
- 5 2. A molecule according to claim 1, wherein the bacteriophage is Mu.
3. An entry clone comprising a molecule according to claim 1 or 2 and foreign DNA.
4. Use of an entry clone according to claim 3, to create a baculovirus-based gene library.