Vector (molecular biology)

From Wikipedia, the free encyclopedia

In molecular cloning, a **vector** is a DNA molecule used as a vehicle to artificially carry foreign genetic material into another cell, where it can be replicated and/or expressed. A vector containing foreign DNA is termed recombinant DNA. The four major types of vectors are plasmids, viral vectors, cosmids, and artificial chromosomes. Common to all engineered vectors are an origin of replication, a multicloning site, and a selectable marker.

The vector itself is generally a DNA sequence that consists of an insert (transgene) and a larger sequence that serves as the "backbone" of the vector. The purpose of a vector which transfers genetic information to another cell is typically to isolate, multiply, or express the insert in the target cell. Vectors called expression vectors (expression constructs) specifically are for the expression of the transgene in the target cell, and generally have a promoter sequence that drives expression of the transgene. Simpler vectors called transcription vectors are only capable of being but not translated: they can be replicated in a target cell but not expressed, unlike expression vectors. Transcription vectors are used to amplify their insert.

Insertion of a vector into the target cell is usually called transformation for bacterial cells, transfection for eukaryotic cells, although insertion of a viral vector is often called transduction.

Contents

Characteristics

Plasmids

Plasmids are double-stranded generally circular DNA sequences that are capable of automatically replicating in a host cell. Plasmid vectors minimalistically consist of an origin of replication that allows for semi-independent replication of the plasmid in the host and also the transgene insert. Modern plasmids generally have many more features, notably including a "multiple cloning site" which includes nucleotide overhangs for insertion of an insert, and multiple restriction enzyme consensus sites to either

side of the insert. In the case of plasmids utilized as transcription vectors, incubating bacteria with plasmids generates hundreds or thousands of copies of the vector within the bacteria in hours, and the vectors can be extracted from the bacteria, and the multiple cloning site can be cut by restriction enzymes to excise the hundredfold or thousandfold amplified insert. These plasmid transcription vectors characteristically lack crucial sequences that code for polyadenylation sequences and translation termination sequences in translated mRNAs, making protein expression from transcription vectors impossible. Plasmids may be conjugative/transmissible and non-conjugative:

- conjugative: mediate DNA transfer through conjugation and therefore spread rapidly among the bacterial cells of a population; e.g., F plasmid, many R and some col plasmids.
- nonconjugative- do not mediate DNA through conjugation, e.g., many R and col plasmids.

Viral vectors

Viral vectors are generally genetically engineered viruses carrying modified viral DNA or RNA that has been rendered noninfectious, but still contain viral promoters and also the transgene, thus allowing for translation of the transgene through a viral promoter. However, because viral vectors frequently are lacking infectious sequences, they require helper viruses or packaging lines for large-scale transfection. Viral vectors are often designed for permanent incorporation of the insert into the host genome, and thus leave distinct genetic markers in the host genome after incorporating the transgene. For example, retroviruses leave a characteristic retroviral integration pattern after insertion that is detectable and indicates that the viral vector has incorporated into the host genome.

Transcription

Transcription is a necessary component in all vectors: the premise of a vector is to multiply the insert (although expression vectors later also drive the translation of the multiplied insert). Thus, even stable expression is determined by stable transcription, which generally depends on promoters in the vector. However, expression vectors have a variety of expression patterns: constitutive (consistent expression) or inducible (expression only under certain conditions or chemicals). This expression is based on different promoter activities, not post-transcriptional activities. Thus, these two different types of expression vectors depend on different types of promoters.

Viral promoters are often used for constitutive expression in plasmids and in viral vectors because they normally force constant transcription in many cell lines and types reliably.

Inducible expression depends on promoters that respond to the induction conditions: for example, the murine mammary tumor virus promoter only initiates transcription after dexamethasone application and the *Drosophilia* heat shock promoter only initiates after high temperatures.

Expression

Expression vectors produce proteins through the transcription of the vector's insert followed by translation of the mRNA produced, they therefore require more components than the simpler transcription-only vectors. Expression in different host organism would require different elements, although they share similar requirements, for example a promoter for initiation of transcription, a ribosomal binding site for translation initiation, and termination signals.

Prokaryotes expression vector

- Promoter - commonly used inducible promoters are promoters derived from *lac* operon and the T7 promoter. A stronger promoter; Trp/Tryptophan Operon and Tac Promoter, a hybrid collection of both the Trp and Lac Operon promoters.
- Ribosome Binding Site (RBS) Follows the promoter, and promotes efficient translation of the protein of interest.
- Translation initiation site - Shine-Dalgarno sequence enclosed in the RBS, 8 base-pairs upstream of the AUG start codon.

Eukaryotes expression vector

Eukayrote expression vectors require sequences that encode for:

- Polyadenylation tail: Creates a polyadenylation tail at the end of the transcribed pre-mRNA that protects the mRNA from exonucleases and ensures transcriptional and translational termination: stabilizes mRNA production.
- Minimal UTR length: UTRs contain specific characteristics that may impede transcription or translation, and thus the shortest UTRs or none at all are encoded for in optimal expression vectors.
- Kozak sequence: Vectors should encode for a Kozak sequence in the mRNA, which assembles the ribosome for translation of the mRNA.

Features

Modern vectors contain essential components as well as other additional features:

- Origin of replication: Necessary for the replication and maintenance of the vector in the host cell.
- Promoter: Promoters are used to drive the transcription of the vector's transgene as well as the other genes in the vector such as the antibiotic resistance gene. Some cloning vectors need not have a promoter for the cloned insert but it is an essential component of expression vectors so that the cloned product may be expressed.
- Cloning site: This may be a multiple cloning site or other features that allow for the insertion of foreign DNA into the vector through ligation.
- Genetic markers: Genetic markers for viral vectors allow for confirmation that the vector has integrated with the host genomic DNA.
- Antibiotic resistance: Vectors with antibiotic-resistance open reading frames allow for survival of cells that have taken up the vector in growth media containing antibiotics through antibiotic selection.
- Epitope: Vector contains a sequence for a specific epitope that is incorporated into the expressed protein. Allows for antibody identification of cells expressing the target protein.
- Reporter genes: Some vectors may contain a reporter gene that allow for identification of plasmid that contains inserted DNA sequence. An example is *lacZ-α* which codes for the N-terminus

fragment of β-galactosidase, an enzyme that digests galactose. A multiple cloning site is located within *lacZ-α*, and an insert successfully ligated into the vector will disrupt the gene sequence, resulting in an inactive β-galactosidase. Cells containing vector with an insert may be identified using blue/white selection by growing cells in media containing an analogue of galactose (X-gal). Cells expressing β-galactosidase (therefore doesn't contain an insert) appear as blue colonies. White colonies would be selected as those that may contain an insert. Other commonly used reporters include green fluorescent protein and luciferase.

- Targeting sequence: Expression vectors may include encoding for a targeting sequence in the finished protein that directs the expressed protein to a specific organelle in the cell or specific location such as the periplasmic space of bacteria.
- Protein purification tags: Some expression vectors include proteins or peptide sequences that allows for easier purification of the expressed protein. Examples include polyhistidine-tag, glutathione-S-transferase, and maltose binding protein. Some of these tags may also allow for increased solubility of the target protein. The target protein is fused to the protein tag, but a protease cleavage site positioned in the polypeptide linker region between the protein and the tag allows the tag to be removed later.

See also

- Plasmid
- Viral vector
- Cloning vector
- Expression vector
- Minicircle
- Recombinant DNA
- Naked DNA
- Vector (epidemiology), an organism that transmits disease

References

- Freshney, Ian R. *Culture of Animal Cells: A manual of basic technique*. John Wiley & Sons, Inc., Hoboken, New Jersey. ISBN 978-0-471-45329-1

External links

- Waksman Scholars introduction to vectors (http://dwb.unl.edu/Teacher/NSF/C08/C08Links/mbclserver.rutgers.edu/~sofer/cloningvectors.html)
- A comparison of vectors in use for clinical gene transfer (http://journals.cambridge.org/fulltext_content/ERM/ERM1_11/S1462399499000691sup002.htm)

- Gene Transport Unit (http://www.fitbiotech.com/gtutechnology.html)

Retrieved from "http://en.wikipedia.org/w/index.php?title=Vector_(molecular_biology)&oldid=628883420"

Categories: Molecular biology
