

# Vectors Used in Gene Manipulation- A Retrospective

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## Abstract

"A vector is a DNA molecule that is capable of replication in a host organism, and can act as a carrier molecule for the transfer of genes into the host. The biology of gene cloning is concerned with the selection and use of a suitable vector, a living system or host in which the vector can be propagated. Based on literature survey a general view is given on vectors used in gene manipulation. Cloning vectors like plasmid, (insertion) vector, (replacement) vector, cosmid, phagemids, M13 vector, YAC, BAC and PAC are mainly focused. Expression vector and shuttle vector are also reviewed. Vectors for cloning in higher plants like Ti plasmids, Ri plasmids and vectors for animals cells like SV40, adenovirus, bovine papilloma virus, pox viral vectors are also included. This communication will be helpful for the reader working with different types of vectors used in gene cloning and expression.

**Key words:** Vector, cloning vectors, expression vectors, vectors for plants and animals.

## Introduction

A vector is an autonomously replicating DNA into which a foreign DNA is inserted for transfer or propagation in an organism. In general, a vector should have an autonomous

specialized and designed to perform a specific function. Characteristics of vectors that make it useful for molecular cloning are listed in the Table 1. In this article vectors have been classified into cloning vectors, expression vectors, vectors for higher plants and vectors

for animal cells. The main aim of writing this manuscript is to let the reader aware of the vectors used in gene cloning and expression.

## Cloning vectors

Cloning vector - a DNA molecule that carries foreign DNA into a host cell, replicates inside a bacterial (or yeast) cell and produces many copies of itself and the foreign DNA. The cloned genes in these vectors are not expected to express themselves at transcription or translation level. These vectors are used for creating genomic libraries or for preparing the probes or in genetic engineering experiments or other basic studies. Selection of cloning vectors depends on the objective of cloning experiment, ease of working, knowledge existing about the vector, suitability and reliability. A variety of small, autonomously replicating molecules are used as cloning vectors (Voet *et al.*, 1999). Vectors that are commonly used in cloning experiments are presented in the Table 2.

## Plasmids as vectors

Plasmids are circular DNA molecules that lead an independent existence in the bacterial cells. They are naturally occurring, extra chromosomal DNA fragments that are stably inherited from one generation to another generation in an extra chromosomal state. The incorporation of DNA fragments into plasmid

Table: 1 Characteristics of vector used in molecular cloning.

S.No	Characteristics of vector
1.	Ability to self replicate.
2.	Origin of replication.
3.	One and often multiple, restriction enzyme recognition site.
4.	Selectable characteristics so that the transformed cells may be recognized from untransformed cells.
5.	Promoter along with regulatory control.
6.	Less than 10 Kb in size.

replicating system which is independent of cell cycle, so that many copies can be made. Vectors along with foreign DNAs can be introduced in appropriate host cell and are maintained for study and expression. In this communication, based on literature survey, information has been gathered on cloning vectors viz, plasmid, (Insertion) vector, (replacement) vector, cosmid, phagemids, M13 vector, YAC, BAC and PAC vectors. Much focus has been given on expression vector, shuttle vector and a number of other vectors used in plant and animals.

A huge array of different types of vector is available today, with many being highly

Table: 2 Commonly used vectors used in molecular biology.

S.No	Vectors	Important uses of vectors	Maximum insert size in kb	Examples
1.	Plasmid	General DNA manipulation	10-20	$P^{BR322, PUC18}$
2.	(Insertion)	Construction of cDNA libraries	~10	$gt11$
3.	(Replacement)	Construction of genomic libraries	~23	ZAP, EMBL4
4.	Cosmid	Construction of genomic libraries	~44	$P^{JBB}$
5.	Phagemid	General DNA manipulation <i>Invitro</i> mutagenesis	10-20	$P^{Bluescript}$
6.	M13	DNA sequencing <i>Invitro</i> mutagenesis	8-9	M13mp18
7.	BAC	Construction of genomic libraries	130-150	$P^{BAC108L}$
8.	YAC	Construction of genomic libraries	1000-2000	$P^{YAC4}$
9.	PAC	Construction of genomic libraries	75-90	$P^{Ad10SacBII}$

vectors not only allow foreign DNA to be replicated in cloned cells for later isolation and identification, but can also be designed so that cells transcribe and translate this DNA into protein (Strickberger, 2004). They are widely distributed throughout the prokaryotes and range in size from approximately 1500bp to over 300kbp. Common plasmid vectors are 2-4kb in length and capable of carrying 15 kb of foreign DNA (Hartwell *et al.*, 2004). Most plasmids exist as closed circular double stranded DNA molecules that often confer a particular phenotype on to the bacterial cells in which they are replicated. Few types of plasmids are also able to replicate by inserting themselves into the bacterial chromosomes which are called episomes. Basic properties of plasmid are listed in the Table 3. They can

be classified as low copy number (<10) or high copy number (>20).

Low copy number plasmids tend to exhibit stringent control of DNA replication with replication of p<sup>DNA</sup> closely tied to host cell chromosomal DNA replication. High copy number plasmids are termed as relaxed plasmids, with DNA replication not depend on host cell chromosomal DNA replication (Nicholl, 2002). Choice of vectors with low or high copy depends on the objective of the cloning. If the target is to clone and express a gene for synthesis of particular protein or secondary metabolites in bacterial system for higher production, high copy number is preferred. But in transformation experiments, low copy plasmids are automatic choice (Satya, 2007).

vectors, clones are not colonies of cells, but plaques formed when a phage clears out a hole in a lawn of bacteria (Weaver, 2005). Bacteriophage is a genetically complex but very extensively studied virus of *E.coli*. The DNA of phage is a linear duplex molecule of about 48.5kbp. As with plasmid vectors, improved phage vector derivatives have been developed. There have been several aims, which are listed in the Table 5. About one third of genome is non essential and can be replaced with foreign DNA. DNA is packaged into infectious phage particles only if it is in between 40000 and 53000bp long, a constraint that can be used to ensure packaging of recombinant DNA only (Nelson and Cox, 2007). Engineered vector of are of two major types that is insertion vectors and replacement vectors (Channarayappa, 2006).

Table: 3 Important properties of plasmids.

S.No	Basic properties of plasmid
1.	Plasmids are replicon that are stably inherited in an extrachromosomal state.
2.	Exist as double stranded circular DNA molecule.
3.	Posses origin of replication.
4.	Selectable marker that enable them to be detected.
5.	Must have at least one unique restriction site, to enable DNA to get inserted into it.

be classified into two groups namely conjugative plasmid and non conjugative plasmid. Conjugative plasmids can initiate their own transfer between bacteria by the process of conjugation, which requires functions specified by the *tra* (transfer) and *mob* (mobilizing) regions carried on the plasmid. Nonconjugative plasmids are not self transmissible, but may be mobilized by a conjugation proficient plasmid if their *mob* region is functional.

A further classification is based on the number of copies of the plasmid found in the host cell, a feature known as copy number. Depending on the frequency common plasmid vectors

The most useful classification of naturally occurring plasmids are based on the main characteristics coded by the plasmid genes. The five main types of plasmid according to this classification are listed in the Table 4 (Brown, 2006).

### Phage as vectors

Bacteriophages are natural vectors that transduce bacterial DNA from one cell to another. Phage vectors have a natural advantage over plasmids that is they infect cells much more efficiently than plasmids transformed cells, so the yield of clones with phage vectors is usually higher. With phage

### (insertion) vector

With an insertion vector, a large segment of the non-essential region has been deleted, and two arms ligated together. Insertion phage vector has single restriction site which is used for insertion of foreign DNA. The size of DNA fragment that an individual vector can carry depends of course on the extent to which the non-essential region has been deleted. Smaller foreign DNA can be packed here. The example of (insertion) vector is gt10 and Charon 16A.

gt10 is a 43 kb double stranded DNA and can clone fragments of up to 7kb. It can carry up to 8kb of new DNA, inserted into a unique EcoRI site located in the CI gene. This vector gives clear plaques. Insertional activation of this gene means that recombinants are distinguished as clear rather than turbid plaques. In Charon 16A up to 9kb foreign DNA can be cloned. Charon 16, with which insertion of new DNA into a unique EcoRI site inactivates the lacZ gene carried by the vector. Recombinants produces clear, rather than blue, plaques on X-gal agar.

### (replacement) vector

A replacement vector has two restriction sites which flank a region known as stuffer fragment. Often the replaceable fragment carries additional restriction sites that can be used to cut it up into small pieces, so that its own insertion during a cloning experiment is very unlikely. Replacement vectors are

Table: 4 Classification of naturally occurring plasmid on the basis of main characteristics coded by the plasmid genes.

S.No	Types of plasmid	Description about plasmid
1.	Fertility/F plasmids	Carry <i>tra</i> genes which has the ability to promote conjugal transfer of plasmid.
2.	Resistant or 'R' plasmid	Carry genes conferring on the host bacterium resistance to one or more antibacterial agents, such as chloramphenicol, ampicillin and mercury.
3.	Col plasmids	Code for colicins-proteins that kills other bacteris; e.g. ColE1 of <i>E.Coli</i> .
4.	Degradative plasmids	Allow the host bacterium to metabolize unusual molecules such as toluene and salicylic acid.
5.	Virulence plasmids	Confer pathogenicity on the host bacterium; e.g. Ti plasmids of <i>Agrobacterium tumefaciens</i> , which induce crown gall disease on dicotyledonous plants.

Table: 5 Main aims of phage vectors.

S.No	Aims of improved phage vector
1.	To increase the capacity of foreign DNA fragments, preferably for fragments generated by any one of several restriction enzymes.
2.	To devise methods for positively selecting recombinant formation.
3.	To allow RNA probes to be conveniently prepared by transcription of the foreign DNA insert: this facilitates the screening of libraries in chromosome walking procedure.
4.	To develop vectors for the insertion of eukaryotic cDNA such that expression of the cDNA in the form of fusion polypeptide with -galactosidase is driven in <i>E.coli</i> : this form of expression vector is useful in antibody screening.

generally designed to carry large pieces of DNA than insertional vector can handle. The example of replacement vectors are EMBL4, Charon 40 and WES. B<sup>1</sup>. EMBL4 can accommodate up to 9-23 kb of foreign DNA by replacing a segment flanked by pairs of EcoRI, BamHI and SalI sites. Charon 40 can take upto 9-22 kb of foreign DNA. In WES. B<sup>1</sup> two EcoRI sites flank the replacement fragment, and recombinant selection is solely on the basis of size. Insert upto 15 kb can be easily cloned (Brown, 2006).

### Cosmids

Cosmids were first developed in 1978 by Barbara Hohn and John Collins. They are plasmids that contain phage *cos* site (Collins and Bruning, 1978). The first part of their name, “*cos*” comes from the fact that cosmid contains the cohesive ends, or *cos* site of normal. These ends are essential for packaging the DNA into phage heads. The last part of their name “*mid*” indicates that cosmid carry a plasmid origin of replication like the one found in the P<sup>BR322</sup> plasmid (Zubey *et al.*, 1995). As plasmids, cosmids contain an origin of replication and selectable marker. They also possess a unique restriction enzyme recognition site into which DNA fragments can be ligated. After the packaging reaction has occurred, the newly formed particles are used to infect *E.coli* cells. The DNA is injected into the bacterium like normal DNA and circularizes through complementation of the *cos* ends. The circularized DNA will, however, be maintained in the *E.coli* as a plasmid. Therefore selection of transformants is made on the basis of antibiotic resistance and bacterial colonies (rather than plaque) will form that contain the recombinant cosmid (Reece, 2004).

With a cosmid vector of 5kb, we demand the insetion of 32-47 kb of foreign DNA which is

much more than a phage vector can accommodate. In order to clone foreign DNA into cosmid vector, cosmid DNA is first made to linearise by cutting it with appropriate restriction enzyme. Then it is treated with the calf intestine phosphate to remove phosphate group (5') at its ends so as to prevent recircularization of cosmid DNA. Advantage of using cosmid vector is that larger DNA can be cloned than what is possible with phage or plasmid. As larger inserts are possible, genomic library can be created which is composed of fewer clones to be screened. Efficiency of cosmid is high enough to produce a complete genomic library of 10<sup>6</sup> - 10<sup>7</sup> clones from a mere 1 ug of insert. Genomic libraries of Drosophila, mouse and several other organisms have been produced with cosmid vectors (Jogdand, 2006).

### Phagemids

Phagemids are plasmids that contain f1 phage origin of replication for the production of single stranded DNA. They are generally small plasmids so that they have the ability to accept larger DNA inserts than M13 based vectors. They are originally developed in the year 1980s, when it was found that the insertion of the f1 origin of replication could be cloned into p<sup>BR322</sup> to drive the production of single stranded DNA (Dotto and Horiuchi,

1981; Dotto *et al.*, 1981). The f1 replication origin was not sufficient to direct single-stranded DNA production, but if a bacterium carrying a phagemid was superinfected with a functional wild type M13 or f1 helper phage, then the production of single stranded phagemid DNA would occur. Phagemids have several attractive features that overcomes problems commonly encountered with cloning in bacteriophage (Sambrook and Russel, 2001) and are listed in the table 6.

### M13 vector

M13 is a filamentous phage. Its DNA molecule is much smaller than the genome. It is 6407 nucleotides in length, circular and consists entirely of single stranded DNA. The genome is less than 10kb in size, well within the range for a potential vector. Upon infection of *E. coli*, the DNA replicates initially as a double stranded molecule by subsequently producing single stranded virions for infection of further bacterial cells (Wilsons and Walker, 2005). Double stranded replicative form of the M13 genome behaves very much like a plasmid and can be treated as such for experimental purposes. Genes cloned with an M13 based vector can be obtained in the form of single stranded DNA. M13 phage is used in Sanger's method of DNA sequencing.

### BAC

Bacterial artificial chromosomes (BACs) are engineered version of F plasmids (Shizuya *et al.*, 1992). They are capable of carrying approximately 200 kbp of inserted DNA sequence. The F-factor origin of replication (*Oris*) maintains their level at approximately one copy per cell. In addition to *Oris*, BACs contain four factor genes required for replication and maintenance of copy number,

Table: 6 Attractive features of phagemids that overcome problems commonly encountered with cloning in bacteriophage

S.No	Attractive features of phagemids
1.	A positive selectable marker that can be used to select bacteria transformed by the phagemid.
2.	Higher yield of double stranded DNA.
3.	Elimination of the time consuming process of subcloning DNA fragments from plasmids to filamentous bacteriophage vectors.
4.	A significant reduction in the frequency and extent of deletions and rearrangements in single stranded DNA.
5.	The ability to allow segments of DNA several kilobases in length to be isolated in single stranded form.

*repE, parA, parB anf parC*. In addition to the F-factor genes, pBeloBac11 also contain a selectable antibiotic resistance marker (CAM<sup>R</sup>) and *lacZ*<sup>1</sup> gene harbouring a multiple cloning site for the blue-white screening of BACs containing inserts (Kim *et al.*, 1996). Additionally, the BAC contains a *cos* site (*cosN*) and *loxP*site. These sites are used for specific cleavage of the insert containing BAC during restriction mapping. The *cosN* site can be cleaved using terminase (Rackwitz *et al.*, 1985), while the *lox P* site can be cleaved by the Cre protein in the presence of an oligonucleotide to the *loxP* sequence (Abremski *et al.*, 1983). The first BAC vector, PBAC108L lacked a selectable marker for recombinants. Thus clones with inserts had to be identified by colony hybridization. Two widely used BAC vectors P<sup>BeloBAC11</sup> and P<sup>ECBAC1</sup> are derivatives of P<sup>BAC108L</sup>.

BACs are often used to sequence the genome of organisms in genome projects, for example the Human Genome Project. A short piece of the organism's DNA is amplified as an insert in BACs, and then sequenced. Finally, the sequenced parts are rearranged in silico, resulting in the genomic sequence of the organism.

BACs are capable of maintaining human and plant genomic fragments of greater than 300kb for over 100 generation with high degree of stability (Woo *et al.*, 1994). They have lower level of chimerism (Hartl *et al.*, 1994; Sternberg, 1994)). They are now being utilized to a greater extent in modeling genetic diseases, often alongside transgenic mice. BACs have been useful in this field as

complex genes, may have several regulatory sequences upstream of the encoding sequence, including various promoter sequences that will govern a gene's expression level. The above vectors have been used to some degree of success with mice when studying neurological diseases such as Alzheimer's disease or as in the case of aneuploidy associated with Down syndrome. There have also been instances when they have been used to study specific oncogenes associated with cancers. BACs can also be utilized to detect genes or large sequences of interest and then used to map them onto the human chromosome using BAC arrays. They are preferred for these kinds of genetic studies because they accommodate much larger sequences without the risk of rearrangement, and are therefore more stable than other types of cloning vectors.

### YAC

Yeast artificial chromosome (YAC) vectors allow the cloning, within yeast cells, of fragments of foreign genomic DNA that can approach 500kbp in size. These vectors contains several elements of typical yeast chromosomes including a yeast centromere (CEN<sub>4</sub>), yeast autonomously replicating sequence (ARS1), yeast telomere (TEL), genes for YAC selection in yeast, bacterial replicating origin and a bacterial selectable marker.

CEN<sub>4</sub> is specified by a 125 bp DNA segment. The consensus sequence consist of three elements that is a 78-86 bp region with more

than 90% AT residues, flanked by a conserved sequence on one side and a short consensus sequence on the other. Yeast ARS elements are essentially origin of replication that function in yeast cells autonomously .

Telomeres are the specific sequences that is 5'-TGTGGGTGTGGTG-3' that are present at the end of the chromosomes in multiple copies and are necessary for replication and chromosome maintenance. The vector has a functional copy of URA3, a gene involved in uracil biosynthesis, and also a TRP1, a gene involved in tryptophan biosynthesis, that allow selection of yeast cells that have taken up the vector.

Yeast expression vectors, such as YACs, YIPs (yeast integrating plasmid), and YEps (yeast episomal plasmid), are extremely useful because one can get eukaryotic protein products with posttranslational modifications as yeasts are themselves eukaryotic cells. However, YACs have been found to be more unstable than BACs, producing chimeric effects. Before the advent of the Human Genome Project, YACs and BACs were used to map sections of DNA of interest when hunting for specific genes. Various difficulties associated while working with YACs are listed in the Table 7. Inserts as large as 1000kb can be cloed into YAC vectors (Berg *et al.*, 1995).

### PACs

To overcome some of the problems associated with cosmid or YAC systems, a method for cloning and packaging DNA fragments using a bacteriophage PI system has been developed that offers the ability to clone large genomic DNA fragments of between 70-95 kbp in size. PI bacteriophage has a much larger genome than phage (110-115bp).

Vectors have been designed with the essential replication components of PI incorporated into plasmid. Upon infecting *E.coli*, bacteriophage PI may either express lytic functions, producing 100-200 new bacteriophage particles and lysing the infected bacterium. PI phage has two replication regions that is one to control lytic DNA replication and other to maintain the plasmid during non-lytic growth. Phage PI uses a 'head full' packaging strategy and can accommodate a total DNA length of approximately 110-115kbp.

Table: 7 Problems linked while handling with YAC vector.

S.No	Difficulties associated while working with YAC
1.	Very large DNA molecules are very fragile and prone to breakage, leading to problem of rearrangement.
2.	High rate of loss of the entire YAC during mitotic growth.
3.	Difficult to separate YAC from the other host chromosomes because of their similar size. Separation requires sophisticated pulse-field gel electrophoresis (PFGE).
4.	Yield of DNA is not high when the YAC is isolated from yeast cells.
5.	Clones tend to be unstable, with their foreign DNA inserts often being deleted.

Table 8: Important characteristics features of expression vector.

S.No	Essential features of expression vector
1.	Origin of replication is functional in target host cell.
2.	Availability of antibiotic resistance genes or other genetic selection mechanism.
3.	Promoter (Strong or weak as per suitability) along with regulatory control.
4.	Restriction site immediately downstream from the promoter.
5.	Unique restriction site for cloning located in a position where the inserted cDNA sequence can be expressed effectively.

## Expression vectors

Expression vectors are vectors that allow one to construct gene fusions that replace native promoter of a gene with another promoter. Expression vectors allow the expression of cloned gene, to give the product (protein). This can be achieved through the use of promoters and expression cassettes and regulatory genes (sequences). These vectors are used for transformation to generate transgenic plants, animals or microbes where cloned gene expressed to give the product. Commercial production of cloned gene may also be achieved by high expression using these vectors. The essential features of expression vector are summarized in the Table 8. For expression of cloned gene in plants or animals only plant specific or animals specific promoters work. Expression vectors may be used to isolate specific cDNAs (Watson *et al.*, 1992). They are required if one wants to prepare RNA probes from the cloned genes or to purify large amount of gene products (Primrose *et al.*, 2001). Several expression vectors are available to the *Saccharomyces* researcher and can be obtained from colleagues or from commercial sources. The ADH1 promoter is commonly used for high level constitutive expression in glucose-grown cells. GAL1 and GAL10 are induced to very high level in galactose grown cells but expression is dramatically repressed by growth on glucose (Michels, 2002). An expression system developed by Mumberg *et al.* (1995) allows the constitutive production of a gene product over a 1000 fold range.

## Shuttle vectors

Shuttle vector is that which can replicate in the cells of more than one organism. Transfer of genes between unrelated species is one of the requirements of biotechnology. Broad host range vector exist in gram negative bacteria and Streptomyces naturally. A shuttle vector however may be required having necessary replicon for maintenance in different combinations in unrelated hosts. These vectors have great importance in the genetic manipulations of industrially important species. Shuttle vectors can exploit gene manipulation procedures of different hosts. Now a days the above vector which function in a given set of species or host are commercially available. For example, one type of shuttle vector is used to clone the genes in *E.coli* and yeast and another type of

shuttle vector is used for *E.coli* and animal cells, eg SV40 plasmid vector. The first shuttle vector that became popular and introduced the idea of such interchangeable vectors is the one that could be used in both *E.coli* and yeast (*Saccharomyces cerevisiae*). It contains ori of both species and a number of marker each for the two hosts. A cloning site is of course provided ( Khan and Khanum, 2004).

## Vectors for cloning in higher plants

Research is going on for availability of suitable vectors for cloning in plant cells. Vector system should permit, gene cloning in organisms such as *E.coli* so that the recombinant molecules can be readily constructed and manipulated into these hosts prior to introduction into host plant cells. Genetic vehicles may be derived from naturally occurring plant vectors such as Ti (tumor inducing) and Ri (root inducing) plasmids of *Agrobacterium*, plant viruses or viroids or artificial vectors (developed by utilizing components of plant genome).

## Ti Plasmids

Ti plasmids are large, circular double stranded DNA molecules of about 200kb, and like other bacterial plasmids, exists in *Agrobacterium* cells as independently replicating genetic units. Ti plasmids are maintained in *Agrobacterium* because a part of the plasmid DNA, called T-DNA carries the genes coding for the synthesis of unusual amino acids called opines (Watson *et al.*, 1992). T-DNA (15-30kb) is transferred to the plant cells during infection and becomes integrated into plant nuclear DNA, where it is expressed. It is responsible for induction and maintenance of the tumorous state and opines synthesis in the plant cell. Vir genes of Ti plasmid are required for mobilization of DNA from bacterium to plant cell. Features of Ti plasmid which make them attractive gene vectors are listed in the Table 9. A Ti plasmid mutant was made in which all the on-cogenic

functions of the T-DNA have been removed and replaced by pBR322. This Ti plasmid, pGV3850, still mediates efficient transfer and stabilization of its truncated T-DNA into infected plant cells. In addition to this, integration and expression of this minimal T-DNA in plant cells does not interfere with normal plant cell differentiation. A DNA fragment cloned in a pBR vector can be introduced in the pGV3850 T-region upon a single recombination event through the pBR322 region of pGV3850 producing a co-integrate useful for the transformation of plant cells. Based upon these properties, pGV3850 is proposed as an extremely versatile vector for the introduction of any foreign DNA of interest into plant cells (Zambryski *et al.*, 1983).

## Ri Plasmids

Ri plasmids of *Agrobacterium rhizogenes* which is causative agent of hairy root disease may also provide effective vectors. Host range of *Agrobacterium* genes is smaller than that of *Agrobacterium tumefaciens*. Ri plasmids are around 150kb in size. T-DNA of Ri plasmid is incorporated stably into genome of plant cell. It is technically easier to regenerate whole plants from hairy roots than from *Agrobacterium tumefaciens* transformed tissues. Vectors based Ri plasmids can be used for gene manipulation. The Ri TDNA region can be used for the construction of plant transformation vectors similar to the Ti-plasmid derived vectors (Vilas *et al.*, 1987).

## Vectors for animal cells

Replicons analogous to bacterial plasmids are not found in animal cells. Some viruses are used to develop the vectors for animal cells. Plasmids bearing the Epstein-Barr virus nuclear antigen (EBNA-1) and origin of replication (*oriP*) can be maintained within some primates and canine cell lines but not in rodents cell lines (Yates *et al.*, 1985). The process of DNA uptake may be either transient or stable.

Table: 9 Important features that made Ti plasmid as attractive gene vectors.

S.No	Features of Ti-plasmid vector
1.	Ti plasmid integrates into plant genome and is stably transmitted through division of mitosis and meiosis.
2.	Genes like nopaline synthase ( <i>nos</i> ) encoded by the T-DNA possess promoters that function in plant cells.
3.	Foreign DNA inserted into T-region is cotransfected and integrated into plant genome as part of the T-DNA.
4.	<i>Agrobacterium</i> has broad host range therefore T-DNA can be transferred to many cotyledonous plants.

### SV40

The first mammalian cell viral vector to be developed was based on the simian virus 40 (SV40) (Hamer and Leader, 1979). SV40 is a primate double stranded DNA tumor virus whose genome is 5243 bp in size. Genes are encoded on both strands of the genome such that they overlap each other. Virus has two life cycles depending upon host cell line employed. In permissive cells (Monkey cells) a productive lytic cycle occurs while in non permissive (rat or mouse cells) viral replication is blocked and host cells are transformed (no growth as monolayer but proliferate without substratum attachment). Recombinant SV40 vectors (rSV40) are good candidates for gene transfer, as they display some unique features: SV40 is a well-known virus, nonreplicative vectors are easy-to-make, and can be produced in titers of 10(12) IU/ml. They also efficiently transduce both resting and dividing cells, deliver persistent transgene expression to a wide range of cell types, and are nonimmunogenic. Present disadvantages of rSV40 vectors for gene therapy are a small cloning capacity and the possible risks related to random integration of the viral genome into the host genome (Vera and Fortes, 2004).

### Adenovirus

Adenovirus has 35 kb linear genome. Variants containing single cloning site and accommodating only 5% extra DNA can be used as vectors and can be propagated alone. If viral genes are deleted then recombinant vector can be propagated in helper cell lines only. Adenovirus can be used as vector for stable and transient expression. This virus bearing foreign DNA can be used to produce the foreign protein in many different cell types, but gene expression is usually transient because the viral DNA does not integrate into the host genome. The lack of integration may, however, be advantageous if adenoviral vectors are used in gene therapy. Most vectors derived from the adenoviral genome are replication deficient. Adenoviral vectors are useful because they are highly efficient at getting DNA into cells. They are capable of containing DNA inserts upto about 8kbp in size and can infect both replicating and differentiating cells. Additionally, since they do not integrate into the host genome, they cannot bring about mutagenic effects caused by random integration events. The

Table: 10 Companies synthesizing vectors			
S.No	Name of companies	S.No	Name of companies
1.	Invivogen	2.	EMD Biosciences
3.	Fermentas	4.	Bio-Medicine
5.	Promega	6.	Biocompare
7.	Sigma-Aldrich	8.	Icosagen
9.	Fisher Scientific	10.	Clontech
11.	GenScript	12.	Stratagene

Table: 11 Cloning and expression vectors.		
S.No	Name of vector	Company that provides the vector
1.	pGS-21a	GenScript
2.	pGen2.1	GenScript
3.	pDream2.1-cGFP	GenScript
4.	pDream2.1/MCS	GenScript
5.	pRNA-Luc/Neo	GenScript
6.	pUC18 plasmid DNA	GenScript
	pUC57 plasmid DNA	GenScript

Table: 12 Inducible siRNA expression vectors					
S.No	Vector	Promoter	Resistance	Marker	Company
1.	pRNATin-H1.2/Neo	H1	Neomycin	cGFP	GenScript
2.	pRNATin-H1.2/Hygro	H1	Hygromycin	cGFP	GenScript
3.	pRNAin-H1.2/Neo	H1	Neomycin	-	GenScript
4.	pRNATin-H1.2/Adeno	H1	Kanamycin	cGFP	GenScript
5.	pRNAin-H1.2/Shuttle	H1	Neomycin	-	GenScript
6.	pRNATin-H1.4/Retro	H1(Inducible)	Hygromycin	cGFP	GenScript
7.	pRNATin-H1.4/Lenti	H1(inducible)	Neomycin	cGFP	GenScript

Table: 13 Plasmidic SiRNA expression vectors.				
S.No	Vector	Promoter	Resistance	Company
1.	pRNA-U6.1/Neo	U6	Neomycin	GenScript
2.	pRNA-U6.1/Hygro	U6	Hygromycin	GenScript
3.	pRNA-U6.1/Zeo	U6	Zeomycin	GenScript
4.	pRNA-H1.1/Neo	H1	Neomycin	GenScript
5.	pRNA-H1.1/Hygro	H1	Hygromycin	GenScript
6.	pRNA-H1.1/Zeo	H1	Zeomycin	GenScript
7.	pRNA-CMV3.1/Neo	CMV	Neomycin	GenScript
8.	pRNA-CMV3.1/Hyg	CMV	Hygromycin	GenScript
9.	pRNA-CMV3.1/Puro	CMV	Puromycin	GenScript
10.	pRNAT-U6.1/Neo	U6	Neomycin	GenScript
11.	pRNAT-U6.1/Hygro	U6	Hygromycin	GenScript
12.	pRNAT-H1.1/Neo	H1	Neomycin	GenScript
13.	pRNAT-H1.1/Hygr	H1	Hygromycin	GenScript

disadvantage of adenoviral vector is that expression is transient since the viral DNA does not integrate into the host. These vectors are based on an extremely common human pathogen and *in vivo* delivery may be hampered by prior host immune response to one type of virus.

### Bovine papilloma virus

Bovine papilloma virus is of interest because its genome can be maintained

extrachromosomally (at 20-200 copies per cell) in transformed or tumour cells. Bovine papilloma virus (BPV-1) DNA replicates exclusively as an extrachromosomal molecule in virally induced tumors as well as in transformed mouse fibroblasts in culture. The complete viral genome or a 69% HindIII BamHI fragment thereof have been used as vectors to introduce cloned prokaryotic or eukaryotic genes into mammalian cells in culture. These recombinant molecules replicate as multicopy plasmids in stably transformed cells. This suggests that a broad potential exists for BPV-1 DNA-derived vectors (Matthias *et al.*, 1983).

### Pox viral vectors

Pox viruses such as vaccinia, have a very large DNA genome (187kb) and can accommodate large inserts of up to 35kb. No helper virus is required for propagation. A variety of foreign genes have been cloned in vaccinia including HTLVIII envelope protein, hepatitis B virus surface antigen, influenza virus, hemagglutinin, rabies virus glycoprotein etc. Vaccine production is being investigated using such expression.

### Vectors available commercially

With the advents of tools and technologies of molecular biology and biotechnology the naturally occurring vectors were manipulated to produce a variety of gene products with high yield. A number of companies are now

Table: 14 Viral siRNA vectors.					
S.No	Vector	Promoter	Resistance	Marker	Company
1.	pRNA-U6.1/Shuttle	U6	Neomycin	-	GenScript
2.	pRNA-H1.1/Shuttle	H1	Neomycin	-	GenScript
3.	pRNAT-H1.1/Shuttle	H1	Neomycin	cGFP	GenScript
4.	pRNA-H1.2/Shuttle	H1 (inducible)	Neomycin	-	GenScript
5.	pRNA-H1.1/Adeno	H1	Kanamycin	-	GenScript
6.	pRNAT-H1.1/Adeno	H1	Kanamycin	cGFP	GenScript
7.	pRNATin-H1.2/Adeno	H1 (inducible)	Kanamycin	cGFP	GenScript
8.	pRNAT-U6.2/Lenti	U6	Neomycin	cGFP	GenScript
9.	pRNATin-H1.4/Lenti	H1 (inducible)	Neomycin	cGFP	GenScript
10.	pRNA-H1.1/Retro	H1	Hygromycin	-	GenScript
11.	pRNAT-H1.4/Retro	H1	Hygromycin	cGFP	GenScript

available that produce improved qualities of vectors. Now a days the companies are preparing kit that contains the required material for gene cloning and expression. Various companies manufacturing vectors are listed in the Table 10. A number of cloning and expression vectors are listed in the Table 11. Various inducible siRNA expression vectors and plasmidic SiRNA expression vectors are listed in the Table 12 and 13 respectively. Moreover a number of viral siRNA vectors are also listed in the Table 14.

## Conclusion

Vectors are boon to researchers who are working in the field of gene expression. The quality and yield of gene product depends upon the efficiency of expression which in turn depends upon the selection and designing of vector. Designing of a particular vector required a complete manipulation of naturally occurring vector. This article is completely based on literature survey and summarizes cloning vector, expression vector, shuttle vector and also other vectors used in gene manipulation in plants and animals. This article will enhance the knowledge of reader regarding the vectors and will help those researchers who are working in the field of molecular biology. Moreover it will help the molecular biologists and biotechnologists to design vectors used in gene cloning and expression so to increase the quality and yield of gene product efficiently and also as per requirement.

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