Eukaryotic vectors for protein expression

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INTRODUCTION

Expression vectors are actually the plasmids that allow the expression of the foreign DNA.

Organization and expression of the eukaryotic genome are nowadays studied in vivo as it gives us the live telecast of working with eukaryotic cells.

There are different eukaryotic vectors that can be brought into use for studying the expression of the eukaryotic genome.

But there are some vectors which are commonly used such as yeast, animal and plant. In expression vectors it is actually the sequences more commonly called regulatory sequences that allow finished products that is protein to be obtained by means of common pathway of transcription followed by translation of the genes.

Most of the drugs containing protein products manufactured by the pharmaceutical industry are made by using expression vectors only.

Appropriate selection of vector for maintaining it within the host is an important part of any expression system.

A promoter within an expression system can be either regulated or constituted (unregulated) one. Stable proteins are obtained if the promoter is constituted but the protein of interest that is, desired one can be obtained by making use of vector containing regulated promoter.

Also most optimal combination of cell density and specific protein can be obtained in short time by making use of regulated promoter.

One can easily isolate as well as purify a protein if it is exported outside the cell. Protein modifications are required for proteins produced by recombinant technology are available only in eukaryotic cells.

Common host for eukaryotic expression includes yeast, insect cells and mammalian cells.

Eukaryotic expression vectors are similar to prokaryotic expression vectors by many ways such as promoter, transcription, transcription and translation signal sequences. Shuttle vectors are first propagated in bacteria and then transferred to eukaryotic cells for expression as it contains prokaryotic sequences.

YEAST CELLS

Yeast can be grown easily on both small scale and large scale and it is also considered as a safe organism. Therefore it is used in pharmaceuticals for use in human without any approval from the government. It mostly secretes protein in a very small quantity but subsequently engineered to produce recombinant proteins which can be purified easily.

VECTORS FOR EXPRESSION OF PROTEINS IN " YEAST"

Different varieties of vectors are use for use with yeast and so they are classified into three main classes:

1. Plasmid vectors like yeast shuttle vectors.
2. Vectors that integrate into the yeast chromosome but this approach is not used mostly as it gives just the single copy of the cloned gene and is also lost in large scale production.

3. Yeast artificial chromosomes- it is however not so convenient for use as expression vectors.

Highest level of expressions can be obtained by yeast episomal plasmid but they are unstable in large cultures.

Insulin, blood coagulation factors, several growth factors and several virus proteins are now produced using *Saccharomyces cerevesiae.*

(i)Yeast Episomal Plasmids (YEps)

They are first constructed by Beggs in 1978 using a naturally occurring yeast plasmid. It is 2µm long that is 6. 3 kb and is found in many strain of *Saccharomyces cerevesiae* and has no known function. There are 50-100 copies per cell which basepairs to two unique regions each with a pair of genes transcribed from a divergent promoter. The plasmid may replicated autonomously or integrate with the chromosome. They have been extensively used in the production of either intra- extracellular heterologous proteins. They form the basis of several cloning techniques. It has transformation frequency of 10^{3} -10^{5} transformants per µg DNA. They are actually fragment of yeast nuclear DNA and E . coli vector pMB9. There are two stages of preparation

1. Vector pMB9 and 2 µm is cut with EcoRI and then ligated
2. Nuclear yeast DNA digested with Pst-I is inserted in yeast hybrid.

The advantages are as follows:

1. HCN (50-100).
2. High transformation frequency.
3. High stability.

4. Very useful for studying complementation.
5. Readily recovered from yeast.

The disadvantages are as follows:

1. Recombinant vectors have been developed from this plasmid but are unstable.
2. Novel recombinants are generated in vivo by recombination with endogenous 2µm plasmid.

(ii) Yeast Integrative Plasmid (YIps)

It is bacterial plasmid that can insert itself into DNA of one of the yeast chromosome. Genes integrated into yeast chromosomes are less liable to be lost by the cell as it divides than are the genes on the plasmid. Although transformation efficiency of yeast integrative plasmid is low and the copy number is one, it has proved to be useful in yeast genetics. Yeast integrative are used for placing DNA segments within yeast genome. They can be replicated and maintained in E . coli but not in yeast. It has a transformation frequency of 10^{4} transformants per µg DNA.

The advantages are as follows:

1. Gives most stable maintenance of cloned genes.
2. It behaves as an ordinary genetic marker.
3. Useful for surrogate genetics of yeast like to introduce deletions, inversions etc.

The disadvantages are as follows:

1. Low transformation frequency.

2. Chromosomal DNA needs to be cut with restriction endonuclease for recovering yeast but it doesn't cleave original vector containing cloned gene.

(iii) Yeast Replicative Plasmid (YRps)

These plasmids were constructed by Struhl et al in 1979. It consists of 1. 4 Kb yeast DNA fragment containing the trp1 yeast gene inserted into EcoRI site of pBR322. They remain as independent plasmids and do not integrate. Their copy number is 1-20 per cell. They carry autonomous replicating sequences (ARS) that allow them to replicate when the cell divides. These vectors have chromosomal replication origins and give rise to high frequency of transformants that is 10^{4} μg DNA. The resulting transformants are highly unstable.

The advantages are as follows:

1. Can be easily recovered from yeast.
2. It has high copy number.
3. It has high transformation frequency.
4. Very useful for complementation studies.
5. It can be integrated into the chromosome.

The disadvantages are as follows:

Instability of transformants

(iv) Yeast Centromere Plasmids (YCps)

These are the plasmids that contain sequences around the centromere region of chromosomes and chromosomal replication region is similar to yeast replicative plasmids that is it possess autonomous replicating

sequences (ARS). It has a transformation frequency of 10^4 transformants per µg DNA They shows three characteristics of chromosomes in yeast cells and they are as follows:

- They are mitotically stable in absence of selective pressure
- They segregate in Mendelian manner during meiosis
- They are found in LCN that is, 1-2/cell in the host.

Advantage-they can be stably maintained.

Disadvantagesare as follows:

1. It has low copy number
2. Wild type yeast cells are force to maintain multiple Yeast centromere plasmids bearing independent selectable markers, the cells grow and cell viability is reduced indicating effect from presence of excess chromosome.

(v) Yeast Artificial Chromosome (YAC)

All the autonomous vectors like YEp, YIp, YCp, YRp exist in yeast as circular DNA molecules, thus none of these resemble normal yeast chromosome that have a linear structure. Also the ends of all chromosomes of yeast have telomeres as that of linear eukaryotic chromosomes. It has two telomeres one of the left and other on the right and thus prevents degradation and are required for chromosomal replication. Origin of replication that is “ ori” site on the plasmid is the site where the DNA replication begins. The presence of yeast centromere helps in proper segregation of chromosome. Chromosome won’t get pulled into new cells during cell division without the absence of centromere regions. Selectable markers are also present in some yeast

artificial chromosomes. They are present as single copy per cell. In actual, they are hybrids of bacterial plasmid DNA and yeast DNA. They are grown in yeast.

Advantages are as follows:

1. Have large carrying capacity
2. It is highly stable
3. It is very efficient because it mimics the chromosome as it has a sequence that functions as origin of DNA replication, centromeric and telomeric sequences.
4. Large genes such as that for muscular dystrophy can be cloned in linear manner.

Disadvantages are as follows:

1. It is an inefficient system
2. Very few clones can be prepared per µg of DNA
3. Resolution is difficult once introduced into yeast cell
4. Cannot be mapped by standard techniques.