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BACTERIAL ARTIFICIAL CHROMOSOME: A DISTINCT VECTOR

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ABSTRACT

Bacterial artificial chromosomes (BAC) are one of the most important research tools being significantly proven to efficiently hold up to 350 kb of DNA to be cloned. The development of BAC has been proven an ambitious success story for human genome project, in genomic DNA libraries and physical map construction for genomic sequencing. The salient feature of *Escherichia coli* derived F-factor is most basic requirement for BAC, which helps in maintenance of DNA clones and easy manipulation of cloned DNA. The following paper accounts details on BAC.

KEYWORDS: Bacterial Artificial Chromosome, DNA Libraries, Physical Map, Genome Sequencing

INTRODUCTION

Natural Chromosomes (Chromosome)

A threadlike linear strand of DNA and associated proteins in the nucleus of eukaryotic cells that carries the genes and functions in the transmission of hereditary information.

Bacterial chromosomes, (plasmids) which are circular, have a single site at which DNA replication originates, and attachment to the cell membrane results in segregation. Artificial bacterial chromosomes (BACs) mimic this using appropriate origin sequences.

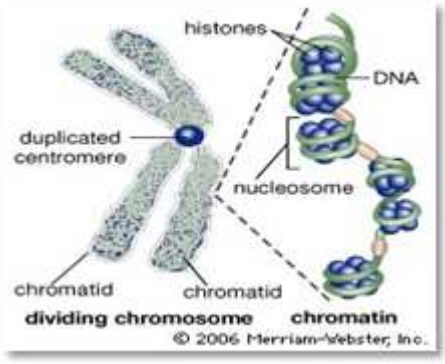

Figure 1

Functions

- Replication origin (many per chromosome) functions in interphase to duplicate DNA.
- Telomeres, (two per chromosome) which consist of DNA and protein, are located at the ends of chromosomes, protecting them from damage.
- Centromeres (one per chromosome) are specialized regions of DNA that are essential for the proper control of

chromosome distribution during cell division. *i.e* functions in mitosis for chromosome segregation.

Artificial Chromosomes

These are the customized constructs from laboratory that consist of DNA sequences which execute vital functions of natural chromosomes. They are use to incorporate and control novel DNA in cell, to learn about chromosome functions and genome mapping studies. (In short these are type of cloning vector that has some features of true chromosomes and is used to clone large fragments of DNA.) They are very similar to a natural chromosome but difference is that, It carries only the genetic information that are engineered into it.

Characteristics of Artificial Chromosomes

- ORI- A sequence that allows for the propagation of itself in the host.
- MCS- An insertion site for the foreign DNA also called a multiple cloning site that can be cut by several restriction enzymes. *i.e Recognition* site for restriction enzymes.
- SELECTABLE MARKER- A method for selection of the host cells that contain the insert DNA of interest. This is most often done through the use of selectable markers for drug resistance.

Types of Artificial Chromosome

- Bacterial artificial chromosome (BAC)
- P1 derived artificial chromosome (PAC)
- Yeast artificial chromosome (YAC)
- Human artificial chromosome (HAC)
- Mammalian artificial chromosome (MAC)

Bacterial Artificial Chromosome (BAC)

These are DNA construct, based on fertility plasmid (or F plasmid), used in transformation and cloning studies. They act as parent host, which guide genes for cloning. With the aim of amplifying gene, DNA sequence is extracted from preferred source using restriction enzymes to cut vector and target DNA and adhere into host bacterium. It has significant property of BAC to carry more genetic content then other carrier vectors. Over past many years scientists have developed many advanced vectors for altering genetic contents of bacteria. These are created with the help modifying phages (those viruses which infect bacterial cells only) or plasmids. And thus they are plasmid based vectors. Basically plasmids are extra chromosomal DNA part, freely floating rings in many bacteria. Mainly BAC are inserted into bacteria through process gene gun or so called electroporation method.

First artificial chromosome was designed by Hiroaki Shizuya in 1992 at California Institute of Technology by altering plasmid F factor. F factor is naturally used by bacteria in translocation of DNA from one cell to other during stress conditions to increase its survival chances and genetic variability. Normally BAC can carry large number of genes having thousand base pairs or many genes at a time. Many BAC libraries are prepared and retained by different universities, industries and government sectors. Additionally BAC also contain many additional features for easier access such as

luminescent genes which glow on successful transformation for easier identification, antibody resistance genes which destroy all other bacteria except the one which carry it. As of fact of fast reproducibility nature BACs are also used to clone multiple copies of specific gene sequences to obtain high copy number for healthier investigation of genome of organisms which slowly grows. This strategy has paced up disease related research by permitting insight into rapid detection of effectual antibacterial and antiviral drugs. It has also added in effective evaluation and production of sequences used in genetic manipulations in other organism, for various industries and research purpose.

BAC vectors generally have ability to carry upto 350 kb of DNA and also consist of different tools for proper work execution such as origin of replication; antibiotic resistance genes and a suitable place to insert clone DNA. Thus they are helpful in studying larger genes, quite a gene at time or whole viral genomes. By means of these vectors it was easier to cut down number of clones for human genome coverage six times tentatively that is to 50 million from 1.8 billion.

Gene Components

- oriS, repE – it is responsible origin of replication and for replication and regulation of copy number of F plasmid.
- parA and parB – they are mainly responsible in partitioning of F plasmid DNA to daughter cells at time of splitting up and guarantee stable maintenance of BAC.
- Selectable markers for antibiotic resistance, in some BACs they also contain lacZ at cloning site for blue/white screening purpose.
- T7 & Sp6 phage promoters are involved in transcription of inserted genes into BAC. (Gong S. *et al*., 2002)

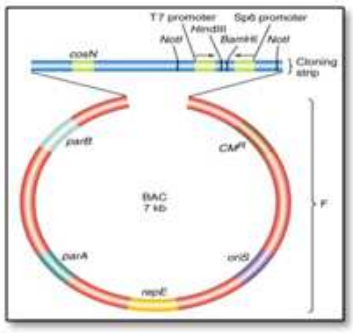

Figure 2: Structure of BAC Vector

Structure of a Bacterial Artificial Chromosome

- ***CM*^{R}** is a selectable marker for chloramphenicol resistance.
- ***oriS, repE, parA***, and ***parB*** are F factor genes for replication and regulation of copy number.
- ***cosN*** is the *cos* site from phage.
- ***Hin*dIII and *Bam*HI** are cloning sites at which foreign DNA is inserted.
- The two promoters are for transcribing the inserted fragment.
- The ***NotI*** sites are used for cutting out the inserted fragment.

General Procedure for Transforming and Selection of Transformed Cell

Scientists have made lot of changes in BACs for more specialized functions so to be of more use in various applications. Additionally with antibiotic resistance gene, *lacZ* a colur changing gene was added which enable the bacteria to turn colourless substance X-gal/IPTG into blue colour. This gene splits up when clone DNA get inserted into vector. So clearly distinguishes the proper transfection of bacteria. Other modification is use of gene *sacB,* encoding protein levan sucrose. Which turns sucrose into levan a toxic compound in bacteria? Likewise, bacteria grown with sucrose, will be lethal if sacb is not broken down by DNA insert. If system carries DNA insert it will broke up sacB and levancucrase won't be produced, so that bacteria will survive in sucrose media. Therefore will have transformed selected colonies only.(Peterson *et al*.,2000)

Advantages

- High clonal stability
- BAC can be transformed into E. coli very efficiently through electroporation, thus avoiding the packaging extract.
- Large carrying capacity(100–300 kb), high clonal stability
- Low rate of chimerism,
- Ease with which they can be handled

Disadvantages

- It lacks positive selection for clones containing inserts
- Very low yield of DNA

Some Modifications to BAC Vectors and Applications

- Researchers are investigating herpes virus, used BAC vector that are cultured in bacterial cells and when transferred in animal cell, release inserted DNA. With such instances it is easier to grow viruses for research purposes in adequate amount which are endemic for mammalian cell cultures and are normally complicated to maintain. Thus it is helpful for easier modification and studies each gene function.
- It has also impact on phylogenetic studies (*i.e* relationship of species to one another). In present era we have abundance of microbial species allover around the world which cannot be grown on cultures. BACs have permitted to have looked into such microbial DNA without even actually growing them, as DNA is kept inside easy to grow bacterial cultures.
- BACs are also in use to study pathogens and in designing new vaccines. Nowadays many of the pathogens are becoming resistant to all antibiotics in drugs. Here in BACs are playing a significant role in drug and antibiotic deiscovery in new environment. For instance developing enzymes which clean oil spills breed farm animals with healthier etc.
- They are also useful in modifications of modal and experimental animals via using wider variety of BACs. (Shizuya *et al*., 1992)

- Chromosome organization evaluation and manipulation, cytogentic genome mapping, chromosome based gene cloning, genome molecular organization, map based cloning of important genes, molecular physical mapping, marker based gene manipulation etc are some other applications too.

Contribution to Models of Disease

Some Inherited disease

- BACs at present are utilized at a greater extent in modeling genetic diseases.
- BACs are now used to some to an extent with mice in study of neurological disease like Alzheimer's disease or as in the case of aneuploidy related with Down syndrome.
- In investigation of specific oncogenes associated with cancers.
- Used for genetic studies because they accommodate much larger sequences without the risk of rearrangement, and therefore more stable than other types of cloning vectors.

CONCLUSIONS

BAC vector thus proves to be an efficient and suitable tool for mapping and genome study with worldwide application.

REFERENCES

1. Gong s,Yang X, Li C, and Heintz N., 2002, Highly Efficient Modification of Bacterial Artificial Chromosomes (BACs) Using Novel Shuttle Vectors Containing the R6K Origin of Replication, Cold Spring Harbor Laboratory, 12: 1992–1998.
2. Peterson D., J. Tomkins, D. Frisch, R. Wing, and A. Paterson., 2000, “Construction of plant bacterial artificial chromosome (BAC) libraries: an illustrated guide,” *Journal of Agricultural Genomics*, vol. 5, 1–100.
3. Shizuya, H., Birren, B., Kim, UJ., Valeria, M., Slepak, T., Tachiiri, Y., & Simon, M. 1992 Cloning and stable maintenance of 300-kilobase-pair fragments of human DNA in Escherichia coli using an F-factor-based vector. Proc. Natl. Acad. Sci. 89; 8794-8797.