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HOMOPOLYMERTAILING

A Techique of Homopolytailing offers the production of sticky end on a blunt ended a molecules.

A homopolymer is simply a polymer in which all the sub unit are same.

A DNA strand can be maid up eg entirely of deoxyguamicine or poly (dc) tailing involves the enzyme.

Terminal deoxynucleotide transferease to add a series of nucleotides on the 3' prime- OH termine of a DNA molecule since this reaction carried out in presence of just one deoxynucleotide. It is called **Homopolymer tail.**

To be able to ligate together to tailed molecules the Homopolymers must be complete generallt and frequently poly dG tails are attached to vector poly de to the DNA which is to be glow base pairing between the two will occur when the DNA mole are next. If the poly dG and poly dc tails are not exactly the same length then the base paired recombinate will have links as well as this continiously repair is therefore required using DNA polymerase to fill in the nicks followed by DNA ligase to join the segments by phosphodiester bonds this repair reaction result in the formation of complete clone DNA in a vector.

BAC (Bacterial Artificial Chromosomes)

The BAC vector was first constructed in 1992 and was based on **E.coli** factor F. The factor is involved in an extra chromosomal element.

BAC vectors have the origin of replication (Oris) of E.coli f factor, which allows a strict copy number control and stably maintains 1 to 2 copies of the vector per cell. The low copy number of BACs helps maintain the DNA inserts without any change arising from recombination between the copies of DNA inserts that is likely to happen in case of multicopy vectors.

The low copy number also avoids any count selection that may arise due to an expression of the cloned genes.

BACs were created because of the problems faces with YACs

The original BAV vector, PBAC OBC was based on a mini F plasmid, PMB 01331 which varied following genes.

i) Oris and rep E genes that are essential for self replication.
ii) Par A and Par B genes which maintain copy number to one or two per **E.Coli** genome.
iii) Multiple cloning sites blanked by universal promoters TT and SP6.
iv) Cos N and 10 x p sites to permit linearization of the plasmid for convenient restriction mapping.
v) A chloramphenicol resistance gene for negative selection of non transformed bacteria.

This vector is capable of maintaining insert up to 300 Kbp long.

- Modification of the original BAC vector are p B alo BAC II, BAC e 3.6 and PEc BAC.
- BACs are the vectors that can carry much larger fragments of DNA than cosmids because they do not have packaging constrants.
- P Belo BAC II and PEC BAC I are the most widely used derivatives of PBAC108 L.

The original cloning site is replaced with a lac z gene carrying a multiple cloning site.

P Belo BAC II has two ECORI site one in the lac 2 gener and one in the EMR gene where as PEC BAC I has only the ECORI site in the lac z gene.

Further improvements to BACs have been made by replacing the lac z gene with the sac B gene.

Insertional in activation of Sac B permits growth of host cell on sucrose containing media i.e. positive selection for inserts site for the insertion of transposons has also been included. Maximum DNA inserts possible with different cloning vectors.

Advantages

BCAs over other vectors BACs are stable more user friendly than YACs . BACs do not face the problem of chimerism i.e. variation in cloned DNA caused by recombination. Suitable for most applications in the analysis of large genomes.

BACs are able to maintain even the normally unstable genomic DNA in to stable state such as genemes of Archaea and mammals which are normally found in high copy numbers.

Disadvantages

Some what laborious construction BAC libraries as in vitro manipulations such as digestion etc have to be performed in agarose plugs to avoid shearing of the large DNA molecules.