

Vectors used for genomic, cDNA, and subcloning

General definition

Cloning Vector - intentionally designed artificial DNA construct used by molecular biologists to amplify selected pieces of DNA inserted into the construct; examples include plasmid, phage, phagemid, cosmid, fosmid, yeast artificial chromosome (YAC) and bacterial artificial chromosome (BAC). Cloning vectors minimally contain an origin of replication, selectable marker gene (e.g., ampicillin resistance gene), and multiple cloning site containing unique restriction enzyme sites; other useful features may also be present.

Plasmid: A small circular DNA molecule found in bacteria that replicates independently of the chromosome. Plasmids are used as cloning vectors. Example: PUC19, ColE1 origin of replication, high copy number, ampicillin resistance gene, 500-5000 kbp inserts. Low copy number plasmid vectors such as pHOS (TIGR) and pSmart (Lucigen) series are thought to increase the randomness of small insert libraries.

Phage: A virus that infects bacterial hosts and may be utilized to introduce genes. Phages are/were widely used as cloning and expression vectors. Examples: M13, lambda phage. The **M13** vectors are derivatives of the single-stranded, male-specific filamentous DNA bacteriophage M13. Double-stranded circular DNA (replicative form, or RF) can be isolated from cells by standard plasmid preparation techniques and used for cloning, while the single-stranded viral DNA (+ strand) can be isolated from phage particles collected from culture medium. **Lambda** phage has a linear 45 kbp chromosome, in which 9-23 kbp can be replaced with DNA to be cloned. **P1** bacteriophage can hold more DNA than lambda and is the basis of PAC vectors.

Phagemid: A phage-plasmid vector able to replicate as single- or double-stranded DNA. Phagemids can be induced to produce phage particles containing single-stranded DNA. Example: pBluescript

series (Stratagene), which contains a filamentous f1 phage intergenic region including the origin of replication.

Cosmid: Artificially constructed cloning vector containing the *cos* gene of phage lambda. Cosmids can be packaged in lambda phage particles for infection into *E. coli*; this permits cloning of larger DNA fragments (up to 45 kb) than can be introduced into bacterial hosts in plasmid vectors. Cosmids and cosmid recombinants replicate as plasmids. Likely to be less stable than plasmids because of large insert and high copy number.

Fosmid: vector containing the single copy *E. coli* F-factor replicon, developed as an improved method for constructing libraries of cosmid-sized (approximately 40 Kb) clones. The stability of inserts cloned into fosmid vectors has been shown to be substantially greater than in high copy vectors. Copy control fosmids, e.g. pCC1fos, contain both the *E. coli* F-factor replicon and the oriV high-copy origin of replication, thus providing the user the clone stability afforded by single-copy fosmid cloning and the high yields of DNA that can be realized from cosmid clones.

Artificial chromosomes:

YACs: yeast artificial chromosomes (replicate in yeast)

BACs: bacterial artificial chromosomes (replicate in *E. coli*); contain the *E. coli* F-factor single-copy origin of replication.

PACs: DNA constructs that are derived from the DNA of P1 bacteriophage. They can carry large amounts (about 100-300 kbp) of other sequence.