

## BigEasy<sup>®</sup> v2.0 Linear Cloning System

Easily clone large DNAs up to 30 kb.  
Now Available...pJAZZ<sup>®</sup>-OK (Kanamycin-resistant) Vector

Lucigen's new BigEasy v2.0 Linear Cloning System makes it a breeze to clone any DNA up to 30 kb. Gaps and finishing costs are dramatically reduced. You get the sequences of key genes and entire genomes much faster, due to the revolutionary pJAZZ<sup>®</sup> vector in the BigEasy Kit. Unlike all other plasmid vectors, the pJAZZ vector is linear, rather than circular, offering much greater insert stability and true bias-free cloning.

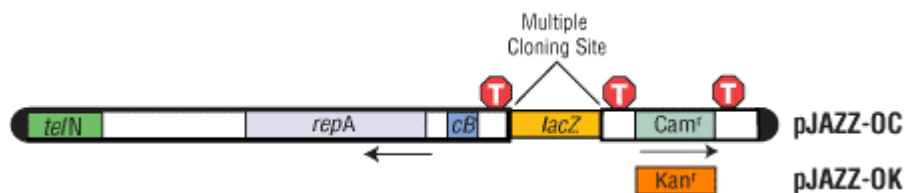
The BigEasy Cloning Kit is based on a novel linear cloning vector (patents pending) derived from the linear phage N15 of E. coli. Due to its unique replication system, the pJAZZ vector behaves like a linear plasmid in BigEasy-TSA<sup>™</sup> Electrocompetent Cells. However, the pJAZZ vector has an unprecedented ability to maintain DNAs that are otherwise unclonable. Because the pJAZZ vector is linear, the ends of the plasmid can rotate freely. Therefore, it is not under torsional stress, and cloned sequences are much more stable. In contrast, conventional circular plasmids become supercoiled in the host cell. This supercoiling induces torsional stress in the circular plasmid DNA, which causes structural instability of AT-rich sequences or inverted repeats. In addition, the pJAZZ vector incorporates Lucigen's CloneSmart<sup>®</sup> technology for transcription-free cloning (U.S. Pat. 6,709,861), which further reduces instability or loss of insert DNA.

The BigEasy Kit is ideal for constructing bias-free, large insert genomic libraries, or for cloning difficult DNA of any size up to 30 kb. This Kit is particularly useful when the target DNA may contain regions that are unclonable in conventional BAC or other vectors, including high AT content, toxic genes, strong promoters, or nucleotide repeats.

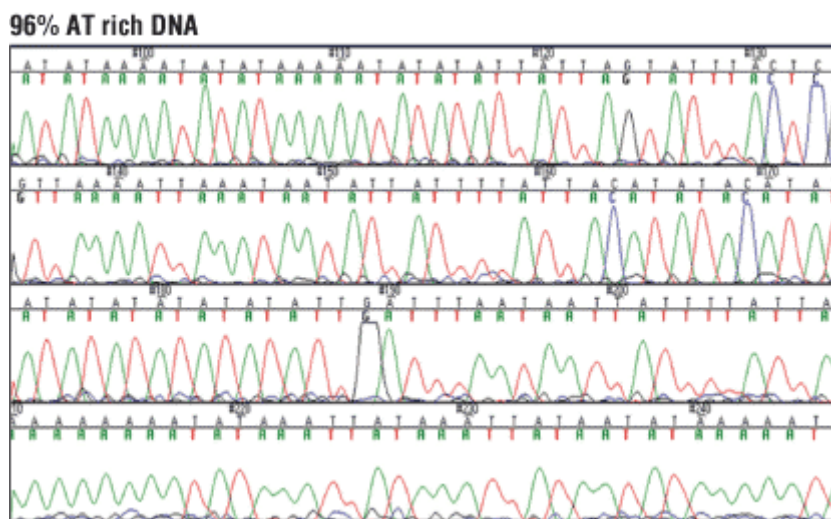
In the second generation BigEasy v2.0 System, the new vector (pJAZZ-OC or pJAZZ-OK) is smaller and fewer antibiotic genes are involved. In addition, the new host strain (TSA<sup>™</sup>) has no other endogenous plasmids, for easier genomic amplification applications.

### BigEasy v2.0 Advantages

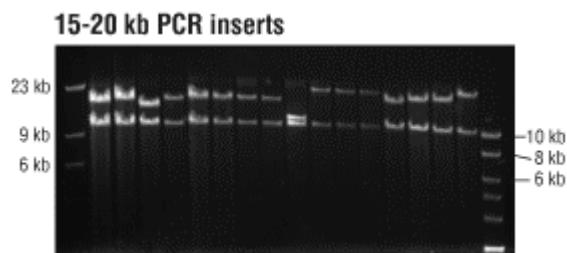
- **Perfect for large cDNAs, and 10-30 kb libraries.** Genomes are cloned without the need for fosmids.
- **Highest stability cloning system known.** The linear pJAZZ vectors avoid insert instability caused by supercoiling in conventional circular vectors. Added stability is achieved with Lucigen's patented CloneSmart<sup>®</sup> transcription terminators and promoter-free insertion site in the pJAZZ vectors, which prevent transcription/translation of toxic or deleterious sequences. (Figure 1).
- **Get every gene & sequence.** Clone large inserts from even the toughest DNAs, including those with extensive nucleotide repeats and extremely high AT content (Figures 2-6).
- **No insert size or content bias.** All DNAs are cloned with equal ease.
- **Convenient and easy to use.** The ready-to-use pJAZZ vectors are dephosphorylated and pre-cut at NotI or SmaI (blunt) sites. Backgrounds are very low, and an inducible origin of replication provides high DNA yields. Standard methods are used for transformation and for isolation of plasmid DNA. BigEasy v2.0 kits contain all needed reagents for ligation and transformation.
- **Choice of Antibiotic Resistance.** The pJAZZ vector is available with Chloramphenicol Resistance (-OC), or Kanamycin Resistance (-OK).
- **Clone and manipulate large operons**



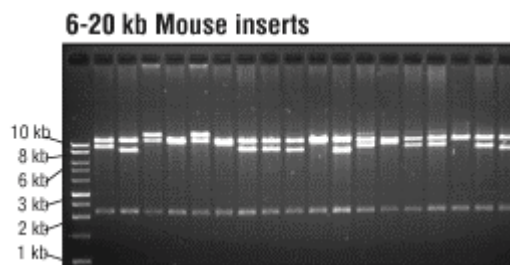
**Figure 1.** Schematic diagram of the pJAZZ-OC and pJAZZ-OK linear vectors. RepA, replication factor and low copy origin of replication (~2-4 per cell; inducible 5-10 fold); Camr, chloramphenicol resistance gene; Kanr, kanamycin resistant gene; telN, protelomerase gene; cB, replication regulator. Approximate position of transcription terminators (T) are indicated.



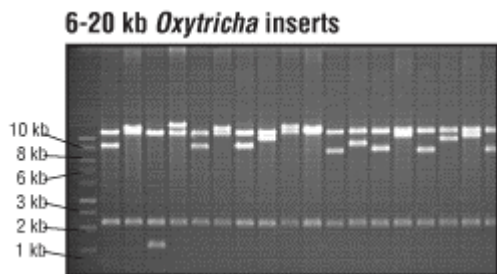
**Figure 2.** Sequence trace of a *Piromyces* clone from Figure 6, showing extremely high (96%) AT content.



**Figure 3.** Various 15-20 kb PCR amplification products cloned into the pJAZZ® linear vector.



**Figure 4.** Mouse genomic DNA sheared to 6-20 kb and cloned into the pJAZZ linear vector.



**Figure 5.** *Oxytricha trifallax* genomic DNA (75-85% AT) sheared to 6-20 kb and cloned into the pJAZZ linear vector.



**Figure 6.** *Piromyces* (85% AT) cloned in the pJAZZ vector. This DNA was unclonable in all other vectors.



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For maximum transformation efficiency, use of Lucigen's BigEasy TSA Electrocompetent Cells is required. BigEasy-TSA Cells ( $>4 \times 10^{10}$  cfu/ $\mu$ g) allow the highest transformation efficiency and inducible copy number amplification with this vector. The BigEasy strain is derived from Lucigen's E. cloni® 10G. These cells give high yield and high quality plasmid DNA due to the endA1 and recA1 mutations. They contain the mcr and mrr mutations, allowing methylated genomic DNA that has been isolated directly from mammalian or plant cells to be cloned without deletions or rearrangements.

The pJAZZ vectors contain a low copy, inducible origin of replication. The copy number is  $\sim 2-4$ /cell prior to induction; it is increased by approximately 5-10 fold in BigEasy-TSA cells by addition of the Induction Solution provided in the BigEasy Kit. Standard alkaline lysis methods of plasmid preparation are effective for isolation of linear pJAZZ clones. For most clones, Induction Solution can be added to the culture medium before use. Overnight induction will yield approximately 5-20  $\mu$ g of linear plasmid DNA per 1 ml culture. Without induction, the pJAZZ vector yields 0.5-2  $\mu$ g per ml of culture. Approximately 150-400 ng of recombinant plasmid is sufficient for sequencing, with the higher range of template required for larger inserts. Standard protocols for cycle sequencing work well for the pJAZZ vectors. The BigEasy Kit includes sequencing primers.

[Click here for - BigEasy v2.0 Linear Cloning System Manual](#)

[Click here for - BigEasy-TSA Electrocompetent Cell Manual](#)