Application Forum

Linear Plasmid Vector for Cloning “Unclonable” DNA

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Cloning large PCR products and other difficult DNA fragments is dramatically improved using Lucigen’s novel linear plasmid vector. The BigEasy® Linear Cloning Kit, containing the pJAZZ® linear vector, ensures unprecedented stability of inserts in *Escherichia coli*. The ends of the vector are free to rotate during replication, so the inserted DNA is not subject to torsional stress caused by supercoiling. Transcriptional terminators flank the cloning site, preventing transcriptional interference between the vector and insert.

Regions of potential secondary structure are significantly more stable in this linear, non-transcribed state. AT-rich PCR amplicons of >20 kb were readily cloned into the pJAZZ vector, whereas similarly AT-rich regions as short as 2 kb were unstable in pUC plasmids (Figure 1, A and B).

Highly repetitive DNAs are also stable in the pJAZZ vector. These inserts have been cloned and maintained through numerous rounds of sub-culturing. Up to 300 copies (~1 kb)

of the GCC repeat of the Fragile X locus were stable in the pJAZZ vector. While they could be cloned into a transcription-free pSMART® vector, they could not be maintained. No clones were obtained at all in pUC19 (Figure 2A). Other highly repetitive DNAs were unclonable in all circular plasmids tested. However, the pJAZZ linear vector produced a library of full-length clones. The sequence of one such clone is shown (Figure 2B).

The pJAZZ vector is particularly well suited to library construction and genomic analysis. The lack of cloning bias greatly simplifies sequence assembly and finishing. Cloning is performed by standard ligation and electroporation, and linear plasmid DNA from transformants is isolated by standard alkaline lysis preparation.

Additional information and data are available at www.lucigen.com.

Figure 1

A) *L. helveticus* DNA in the pJAZZ vector

B) AT-rich inserts are unstable in pUC19

Figure 2

A) GCC-Repeat insert is stable in pJAZZ-OC

B) Representative Sequence of cDNA repeat insert