

## NEW BigEasy® Long PCR Cloning Kit

- Easy cloning of amplicons up to 30 kb
- Highest stability cloning system known
- Get every gene and sequence
- No insert size bias
- Inducible copy number for high DNA yields

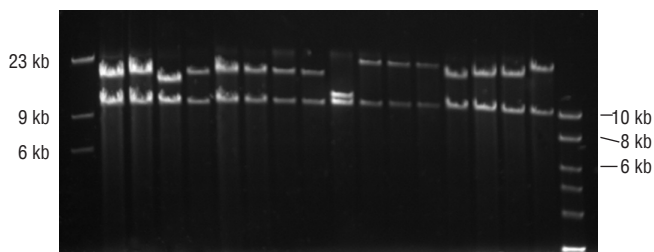
The BigEasy Long PCR Cloning Kits contain everything needed to efficiently clone PCR products up to 30 kb into an unbiased, high-fidelity cloning vector (Figure 1). The two different versions of the kit are compatible with either proofreading or non-proofreading PCR polymerases. They can also be used to clone any blunt or G-tailed DNA up to 30 kb. These vectors incorporate Lucigen's exclusive CloneSmart® transcription-free cloning technology to reduce bias and maximize cloning efficiency.

### Proofreading PCR polymerases (Pfu, Vent®, etc.)

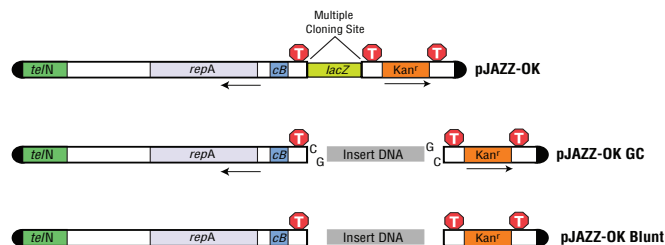
Proofreading DNA polymerases (and mixtures of proofreading and non-proofreading polymerases) produce primarily blunt-ended PCR products. The BigEasy Long PCR Kit with the ultra high-stability, linear pJAZZ®-OK Blunt vector is for cloning long PCR products from these enzymes (Figure 2).

### Non-proofreading PCR polymerases (Taq, Tfl, Tth, etc.)

The BigEasy Long PCR Kit with the ultra high stability, linear pJAZZ-OK GC vector utilizes a newly discovered attribute of non-proofreading DNA polymerases: these enzymes can add a single 3'-G residue to the ends of DNA molecules. The 3'-G tailing occurs during PCR with a non-proofreading polymerase. The pJAZZ-OK GC vector (for non-proofreaders) contains a single 3'-C overhang, which is compatible with the 3'-G overhang generated by the non-proofreading polymerase (Figure 2). The unique combination of a G-tailed insert and C-tailed vector is the basis for GC Cloning (patents pending).



**Figure 1.** Large PCR products (15-25 kb) cloned into BigEasy Long PCR Cloning Kit. Bands from left vector arms are at 10 kb. The bands from the right vector arm ran off the gel.



**Figure 2.** Processing and ligation of the pJAZZ-OK GC and Blunt vectors. Top panel – Before processing, the pJAZZ-OK vector (12.9 kb) contains a *lacZ* stuffer fragment. Middle panel – After processing, the vector has 3'-C tails and dephosphorylated ends. The left arm is 10 kb and the right arm is 2.2 kb. G-tailed insert DNA is ligated to vector arms. Or Bottom panel – after processing, the vector has blunt dephosphorylated ends and blunt insert DNA is ligated to vector arms. *telN*, protelomerase gene; *repA*, replication factor and origin of replication; *cBI*, regulator of copy number; *Kan<sup>r</sup>*, kanamycin resistance gene. Approximate positions of transcription terminators (T) are indicated.

**Note:** Lucigen's BigEasy-TSA™ Electrocompetent Cells must be used for high efficiency transformation and copy number induction with pJAZZ vectors. These cells yield  $\geq 4 \times 10^{10}$  cfu/ $\mu$ g of supercoiled control plasmid.

**BigEasy-TSA Genotype:** F- *mcrA*  $\Delta$ (*mrr-hsdRMS-mcrBC*)  $\phi$ 80*dlacZ* $\Delta$ M15*lacX74 endA1 recA1 araD139*  $\Delta$ (*ara, leu*)7697 *galJ gaK rpsL nupG* $\lambda$ - *tonA Amp<sup>r</sup> sopAB telN antA*

### ORDER INFORMATION

Each BigEasy Long PCR Cloning Kit contains: pre-cut pJAZZ-OK vector (Blunt or C-tailed overhang), CloneSmart DNA Ligase, CloneDirect™ 10X Ligation Buffer, PCR Control Template and Primers, Sequencing Primers, T4 Polynucleotide Kinase, 10X Primer Kinase Buffer, BigEasy-TSA Electrocompetent Cells, Positive Control Plasmid, Recovery Medium, Arabinose Induction Solution, and complete protocols.

Product	Size	Cat. No.
BigEasy Long PCR Cloning Kit (pJAZZ-OK Blunt) For proofreaders	5 rxns	43054-1
	10 rxns	43054-2
	20 rxns	43054-3
BigEasy Long PCR Cloning Kit (pJAZZ-OK GC) For non-proofreaders	5 rxns	43066-1
	10 rxns	43066-2
	20 rxns	43066-3

CloneSmart Technology is covered by U.S. Patent 6,709,861, and other patents issued or pending in the U.S. and other countries and assigned to Lucigen. Inducible Copy Amplification is covered by U.S. Patent 5,874,259, and other patents issued or pending in the U.S. and other countries and licensed to Lucigen. Vent is a trademark of New England Biolabs, Inc.