

BigEasy® Long PCR Cloning Kit

Easy cloning of amplicons up to 30 kb

Description

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. There are two different versions of the kit that 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.

Non-proofreaders (Taq, Tfl, Tth, etc.)

The BigEasy Long PCR Kit for Non-Proofreading DNA Polymerases is based on 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. The unique combination of a G-tailed inserts DNA and C-tailed vector is the basis for GC Cloning (patents pending).

Proofreaders (pfu, Vent®, etc.)

Proofreading DNA polymerases (and mixtures of proofreading and non-proofreading polymerases) produce primarily blunt-ended PCR products. The pJAZZ-OK Blunt vector is for cloning long PCR products from these enzymes.

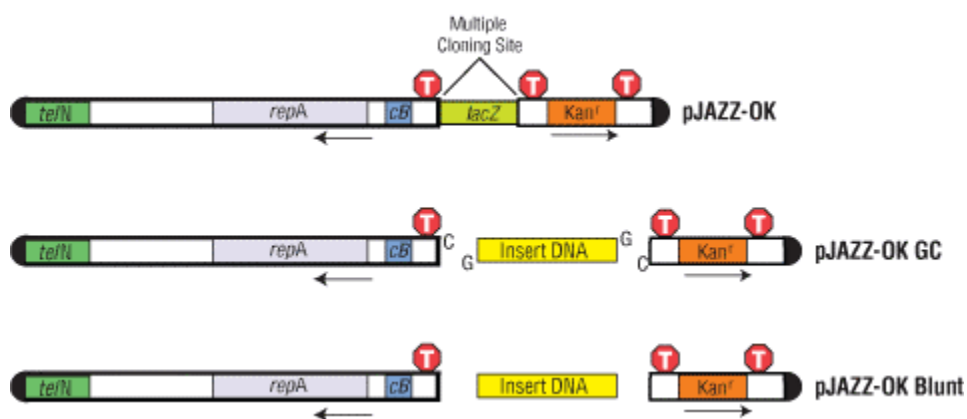


Figure 1. Processing and ligation of the pJAZZ-OK GC and Blunt vectors. Top panel – Before processing, the pJAZZ-OK vector (12.9 kb) contains a *lacZ* stuffer fragment. Middle panel – After processing, the vector has 3'-C tails and dephosphorylated ends. The left arm is 10 kb and the right arm is 2.2 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. *tefN*, protelomerase gene; *repA*, replication factor and origin of replication; *cB*, regulator of copy number; *Kan^r*, kanamycin resistance gene. Approximate position of transcription terminators (T) are indicated.

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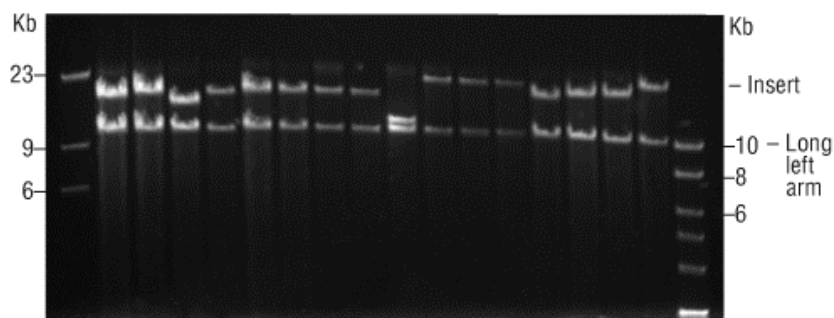


Figure 2. Gel image of large PCR products cloned into BigEasy Long PCR Cloning Kit. Right arm ran off gel.

Advantages

- Perfect for long PCRs, large cDNAs, and 10-30 kb libraries.
- Highest stability cloning system known.
- Get every gene & sequence.
- No insert size or content bias.

BigEasy-TSA Genotype:

F- *mcrA* Δ (*mrr-hsdRMS-mcrBC*) *f80dlacZ* Δ M15 Δ *lacX74* *endA1* *recA1**araD139* Δ (*ara, leu*)7697 *galU*
galK *rpsL* *nupG* l- *tonA* AmpR *sopAB* *telN* *anta*

Note:

Lucigen's BigEasy-TSA Electrocompetent Cells must be used for high efficiency transformation with pJAZZ-OK ligation reactions. These cells yield > 4 X 10¹⁰ cfu/ μ g of supercoiled control plasmid.

[Click here for BigEasy Long PCR Cloning Kits Manual](#)

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