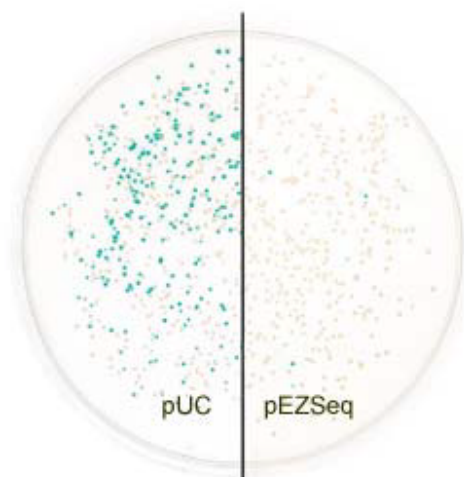


Blue/White Cloning Without The Blues

The pEZSeq™ Blunt Cloning Kit is optimized for the highest efficiency of blunt end cloning and sequencing using a blue/white vector. Three important features differentiate the pEZSeq kit from alternatives.

99% Recombinant clones

pEZSeq is supplied with blunt, dephosphorylated ends. It is the only commercial preparation of a blue/white screening vector that gives 99% recombinant colonies. In stark contrast, common pUC based vectors often produce as many as 30-60% non-recombinant blue colonies (see Figure to the right). The low background of blue colonies with the pEZSeq kit virtually eliminates robotic picking errors and greatly simplifies manual clone selection. For cDNA and shotgun library construction, the low background also allows transformants to be plated without blue/white screening, which may reduce cloning bias by decreasing the level of vector-driven transcription into the insert (using Lucigen's E. cloni® 10GF' strain; see below). In addition, strong transcription terminators are present on either side of the lacZ gene to reduce bias against inserts with promoter activity.

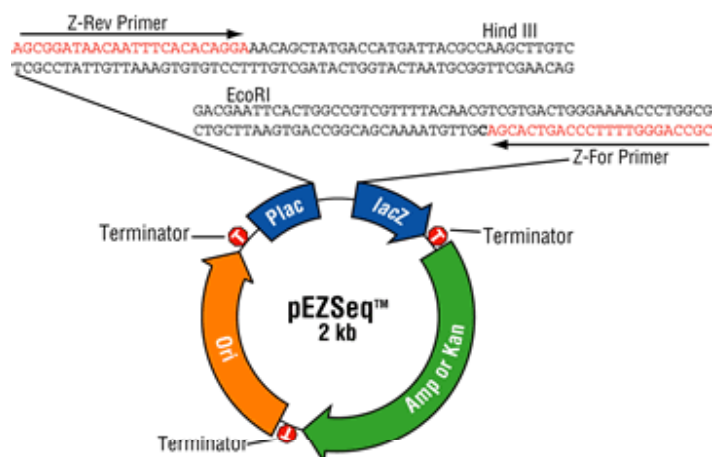


More insert sequence data

pEZSeq is designed with a minimal length of DNA between the cloning site and the primer binding sites (see Figure below). The resulting sequence reads therefore contain less vector DNA and up to 5-10% more insert sequence. The additional data can reduce the number of clones needed to assemble a contig and can add millions of extra bases to large-scale projects.

Transposon compatible micro vector

Like the other CloneSmart® vectors, pEZSeq is small (2056 bp) and contains little extraneous DNA. Nearly all of the vector sequence is essential for viability, so unwanted transposition events are minimized. This micro vector design also facilitates cloning larger inserts and simplifies subsequent manipulations. For example, large inserts can be rapidly sequenced with transposon insertional sequencing.



Transformation and Plating

pEZSeq may be transformed into appropriate host strains according to standard protocols. For blue/white selection, transformants should be plated on agarose plates containing ampicillin plus X-GAL/IPTG. After ligation and transformation, recombinants are easily detected as white colonies, and any non-recombinant clones will be blue.

Applications

- **Shotgun Sequencing** Sequencing large DNA regions, from several kilobases to megabases, is best carried out by a random shotgun approach. pEZSeq simplifies shotgun cloning and sequencing by significantly reducing the level of empty vector. And, like the other CloneSmart vectors, pEZSeq reduces bias in shotgun libraries by blocking transcription initiated by the insert.
- **PCR Cloning** The pEZSeq Blunt Cloning Kit provides a fast and simple protocol for cloning blunt PCR products. High-fidelity polymerases with proof-reading activity, such as Vent® (Tli) or Pfu, generate blunt DNA products that are ideal for cloning into pEZSeq. An aliquot of the PCR product (typically 5µl) is kinased briefly and ligated to the pEZSeq vector. Alternately, phosphorylated primers may be used in the PCR reaction to allow immediate cloning into pEZSeq. PCR products amplified by polymerases lacking proofreading activity, such as Taq, Tth, or Tfl, contain a single nucleotide overhanging on the 3' terminus. For cloning into pEZSeq, treatment with the PCR Terminator® reagents is sufficient to generate blunt ends. The fragment must also have 5' phosphate groups, which can be incorporated onto the primers prior to performing the PCR or can be added to the PCR product by kinase.
- **cDNA Cloning** Following first and second strand synthesis, a specific cDNA fragment can be ligated directly into the blunt site of pEZSeq. This vector is also suitable for construction of cDNA libraries. The fraction of clones with inserts can be visualized by blue/white screening.

Host Strains

The pEZSeq Blunt Cloning Kit includes either E. cloni® 10G or 10GF'. The 10GF' strain has the same genotype as and high transformation efficiency as 10G, but it harbors the F' episome. Both strains can be used for cloning methylated DNA (e.g., genomic DNA). The lac promoter of pEZSeq is constitutively active in 10G cells, so IPTG is not required for the blue/white screen. The lacIq allele on the F' of 10GF' efficiently represses the lac promoter, and the user has the option of adding IPTG to use the blue/white screen to assess cloning efficiency, or omitting IPTG induction to reduce cloning bias that may be created by high expression of the inserts.

Specifications

A typical library generated with the CloneSmart Library Construction Kit contains 1,000,000 single insert clones. This library is adequate for 2,000,000 sequencing reactions or about 1.2 billion bases of sequence data.

[pEZSeq Blunt Cloning Kit Manual](#)

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