

The CopyControl™ PCR Cloning Kits Enable Faster, More Reliable Cloning of Any PCR Product Up to 15 Kb - Even If It Has High A-T, G-C or Repetitive Sequences, or Encodes a Toxic Peptide

Many researchers attempting to clone PCR products have encountered problems such as clone instability, deletions, rearrangements, mutations, and even loss of clones. Likely causes include: (1) intermolecular recombination events; (2) segment deletions, particularly if the PCR product has high A-T, or G-C content or repetitive sequences; and (3) expression of toxic or detrimental genes in PCR products cloned in high-copy vectors, either from their own promoters or by run-on transcription from vector promoters.

EPICENTRE's new CopyControl™ PCR Cloning Kits solve these problems. The unique CopyControl™ pCC1™ Vector has both a single-copy F-factor replicon and a tightly-controlled inducible high-copy origin of replication ("oriV"). The pCC1 Vector enables researchers to clone all PCR products at a single copy per cell in order to greatly reduce the possibility of sequence deletion, rearrangement, or the accumulation of lethal amounts of encoded and expressed protein. Once the CopyControl PCR clones are selected, they can be induced to high copy number (10 – 50+ copies per cell) in order to obtain high yields of DNA for sequencing, *in vitro* transcription, or other applications. Cloning of PCR products and screening of the clones can be completed in less than 24 hours.

Single-Copy Cloning Reduces the Risk of Sequence Deletions, Rearrangements and Cloning "Artifacts"

Often, the complete sequence of a PCR product is not known prior to cloning. Thus, researchers run the risk of analyzing

clones that, unknown to them, have undergone deletions or rearrangements during propagation in a high-copy vector. By cloning the PCR product in the CopyControl pCC1 Vector and growing the clones at single copy, researchers are assured of the stability of the cloned inserts and of the completeness and accuracy of sequence data.

Up to 15-Kb PCR or RT-PCR Products Obtained Using Any Thermostable Polymerase Can Be Cloned and Screened for Inserts in 24 Hours or Less

CopyControl PCR Cloning Kits employ a blunt-end cloning process (Figure 1) that converts the PCR product produced using any thermostable polymerase to blunt-ended, 5'-phosphorylated DNA for efficient ligation into the provided Blunt Cloning-Ready pCC1 Vector. The kit is ideal for cloning PCR products generated using the FailSafe™ PCR System (see center insert), but is also effective for cloning PCR products produced using proof-reading polymerases like *Pfu*, without the need to 3'-A tail as required with some PCR cloning methods. Additionally, CopyControl PCR Cloning Kits enable cloning of PCR products up to 15 Kb without the need for prior column or gel purification required by other PCR cloning procedures. Finally, the kits incorporate EPICENTRE's novel Colony Fast-Screen™ methodology for screening colonies for inserts in 1 hour or less, without minipreps or restriction digests (Figure 2).

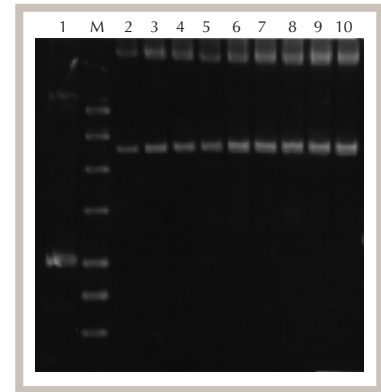


Figure 2. PCR clones can be screened in less than 1 hour without growing cultures or restriction digests. Very small portions from 9 white colonies resulting from cloning of a 5 Kb PCR product were randomly picked from a plate and processed using the EpiLyse™ and EpiBlue™ components of the CopyControl™ PCR Cloning Kit. The 5 Kb insert size of each clone was confirmed by agarose gel electrophoresis. M, DNA size ladder; Lane 1, 8 Kb supercoiled DNA marker; Lanes 2 - 10, randomly chosen clones. Total time including running the gel was approximately 60 minutes.

www.epicentre.com/ccpccr.asp

CopyControl™ PCR Cloning Kits are available with either TransformMax™ EPI300™ Electrocompetent *E. coli* or TransformMax™ EPI300™ Chemically Competent *E. coli* Cells.

CopyControl™ PCR Cloning Kit with TransformMax™ EPI300™ Chemically Competent *E. coli*

CCPCR1CC 20 Cloning Reactions

CopyControl™ PCR Cloning Kit with TransformMax™ EPI300™ Electrocompetent *E. coli*

CCEPCR1 20 Cloning Reactions

Contents:

CopyControl™ pCC1™ (Blunt Cloning-Ready) Vector, PCR Precipitation Solution, PCR End-Repair Enzyme Mix, PCR Cloning Buffer, Fast-Link™ DNA Ligase, EpiLyse™ Solution, EpiBlue™ Solution, Control PCR Product, Supercoiled DNA Marker, and Sterile Water.

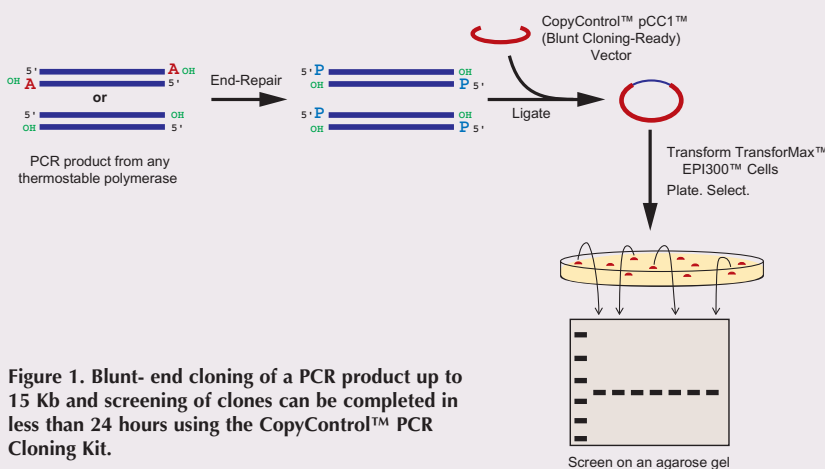


Figure 1. Blunt- end cloning of a PCR product up to 15 Kb and screening of clones can be completed in less than 24 hours using the CopyControl™ PCR Cloning Kit.