Lucigen®
Simplifying Genomics

Expresso® CMV system: effortless mammalian expression cloning

The Expresso® systems dramatically increase the speed and efficiency of target gene cloning and protein expression. With Expressioneering™, PCR products are cloned instantly and directionally into pre-processed mammalian expression vectors without sample cleanup or enzyme treatment.

Lucigen has developed the Expresso Cloning and Expression System for fast and efficient cloning and recombinant protein production. The Expresso kits use an *in vivo* recombinational cloning strategy, called Expressioneering Technology, to permit the fastest possible turnaround from gene sequence to expression clone (**Fig. 1**). Expressioneering resembles ligase-independent cloning (LIC) in that it depends on the addition of short sequences at both ends of a target gene by PCR. These added sequences provide complementarity to the ends of the vector flanking the cloning site, allowing recombination *in vivo*. However, Expressioneering circumvents the need to generate single-stranded DNA ends using the exonuclease activity of T4 DNA polymerase that is inherent to LIC methodology.

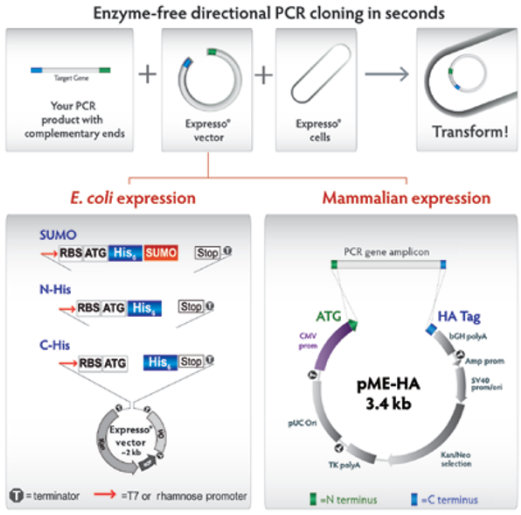

Figure 1 | Expressioneering technology for efficient, high-speed cloning and protein expression. PCR product of the target gene containing 18-bp overlap with the vector ends is mixed with the appropriate Expresso vector and *E. cloni*® 10G cells. Heat-shock transformation followed by selection on kanamycin agar plates yields more than 90% correct recombinant clones.

Saurabh Sen, Heather Sternard, Eric Steinmetz & Ronald Godiska

Lucigen Corporation, Middleton, Wisconsin, USA. Correspondence should be addressed to S.S. (ssen@lucigen.com).

Instant cloning with Expressioneering Technology

To clone by Expressioneering, an aliquot (typically 1 µl) of unpurified PCR product is mixed with the pre-processed Expresso vector and immediately transformed into the chemically competent *Escherichia coli* cells provided. Recombination between the vector and PCR product within the host cells precisely fuses the target gene to the vector in the proper orientation. The design and preparation of the Expresso system vectors ensure minimal background transformation with nonrecombinant clones. For most genes, more than 90% of colonies will have the target gene correctly inserted into the vector.

Elimination of the requirement for PCR product cleanup and enzyme treatment not only saves the cost of enzymes and multiple incubation and sample handling steps but also simplifies the design of expression clones. Seamless fusion to the vector eliminates undesirable amino acids encoded by restriction sites or scars left by site-specific recombination sites. End points of the target protein can be precisely designed. Additional sequences, such as short fusion tags or protease cleavage sites, can be introduced via primer design.

The power of Expressioneering has been validated for hundreds of proteins using the Expresso T7 and Rhamnose kits for bacterial expression. Lucigen has now developed the Expresso CMV system to bring this technology to mammalian cell culture. The Expresso CMV system features a pre-processed, cloning-ready vector, pME-HA, which contains the strong constitutive cytomegalovirus (CMV) promoter and a hemagglutinin (HA) epitope tag for routine expression and detection in most mammalian cell lines. A bovine growth hormone (bGH) polyadenylation signal (polyA) is included in the vector. At 3.4 kb, the pME-HA vector is considerably smaller than most other commercially available mammalian expression vectors. This smaller size facilitates the cloning of larger inserts, as well as downstream manipulations such as site-directed mutagenesis. CloneSmart® transcription terminators (T) prevent transcription into or out of the vector backbone to increase stability of clones. Cloning is performed using Lucigen's high-efficiency chemically competent E. cloni® 10G cells, allowing recovery of plasmid DNA of high quality and yield for transfection into mammalian cells.

APPLICATION NOTES

Recombinant protein expression with Expresso CMV system

The Expresso CMV system has been used to seamlessly clone various genes for robust expression of recombinant proteins in several mammalian cells. Expression from the pME-HA vector is equivalent to or better than the most commonly used commercial vector, pcDNA3.1 (**Fig. 2**). This result demonstrates the superiority of using a minimal vector, which subsequently improves the efficiency of transfection and expression.

G protein–coupled receptors (GPCRs) are among the most challenging yet most sought-after drug targets for the pharmaceutical industry. We have successfully cloned several GPCRs in the pME-HA vector. More than 94% of the clones obtained were found to be correct. Expression of the receptors has been confirmed by western blot, and receptor functionality is evidenced by ligand binding activity (**Fig. 3a,b**).

The pME-HA vector is compatible with commonly used cell lines and commercially available transfection reagents. The vector incorporates a dual selection marker (kanamycin resistance in bacteria and neomycin/G418 resistance in mammalian cells), which will allow selection of stable transformants that constitutively express recombinant protein from the CMV enhancer-promoter.

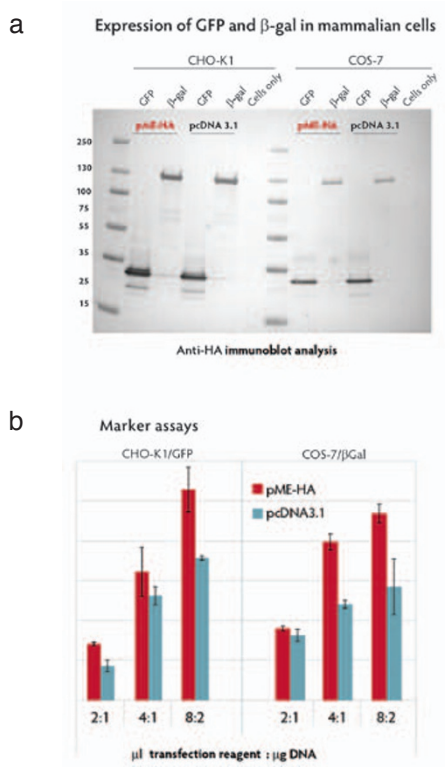

Figure 2 | Recombinant protein expression using the Expresso CMV system: comparison of pME-HA to pcDNA3.1. (**a**) The β-galactosidase gene (3.1 kb) and the GFP gene (0.8 kb) were cloned into pME-HA or pcDNA3.1 vectors and transfected into CHO-K1 or COS-7 cells. Expression of β-galactosidase (β-gal) and GFP in CHO and COS cells was confirmed 24 h after transfection by anti-HA western blots. (**b**) GFP expression was quantified by fluorimetry, and β-gal activity was assayed enzymatically. The pME-HA vector showed similar or slightly higher expression compared to the widely used vector pcDNA3.1.

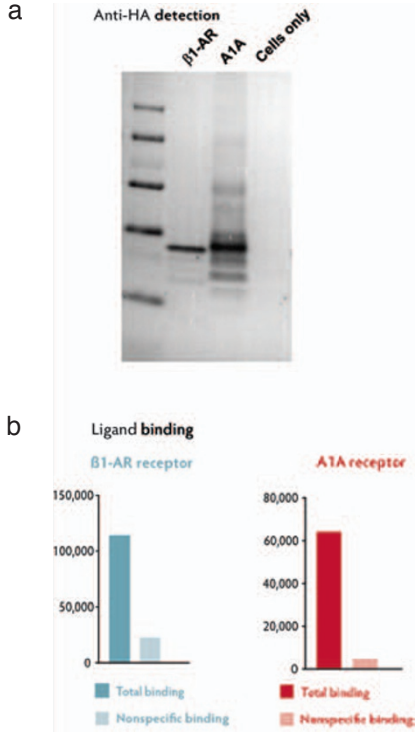

Figure 3 | Expression of GPCRs using the Expresso CMV system. (**a**) β1-adrenergic receptor and adenosine A1A receptor were cloned into pME-HA vector and transfected into CHO-K1 cells. Expression of the GPCRs was confirmed by anti-HA western blots. (**b**) Significant binding of appropriate ^{3}H-labeled ligands was observed. Similar results were obtained in COS cells (data not shown).

Use of Expresso CMV for high-throughput applications

Because of the simplicity of Expressioneering Technology, the Expresso CMV system is uniquely suited for cloning and expression in a high-throughput (96-well) format. The high efficiency, low background and fixed orientation of the inserts allow a streamlined workflow for expression analysis in mammalian cell culture. For example, one can grow the bacterial transformation mix directly in liquid medium (under antibiotic selection) for preparation of DNA for transfection into mammalian cells. Typically, more than 90% of bacterial transformants will contain the correctly cloned gene of interest.

The convenient pre-processed vector, which eliminates multiple enzyme treatment and cleanup steps, and the high percentage of positive clones will prove beneficial to researchers, whether studying a handful of proteins or undertaking large-scale, high-throughput screening. The Expresso CMV system is available in five- and ten-reaction kits for bench-scale cloning and expression trials or as a custom product in a 96-well format for large-scale screening.

ACKNOWLEDGMENTS

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