

## ABSTRACT

To transfect mammalian cell cultures, a DNA fragment must be cloned into an *E. coli*-mammalian shuttle vector. We have developed a minimal mammalian expression vector, pME-HA, for simplified cloning and robust expression of proteins in mammalian cells.

The pME-HA vector utilizes "Expresso" recombinational cloning to provide rapid, precise, and directional insertion of fragments into the cloning site. The small size of the vector (3.4 kb) and minimal amount of bacterial DNA sequence supports higher transfection efficiency and simplifies subsequent manipulations. The vector has been used to clone and express several marker proteins, as well as clinically relevant G-Protein Coupled Receptors (GPCRs).

## Expresso Cloning

The Expresso CMV Cloning and Expression System uses Expressioneering™ Technology for instant, enzyme-free, directional cloning, with no cloning "scar". Homologous recombination occurs in the bacterial host to seamlessly clone PCR-amplified DNA into the pMA-HA expression vector without the need for purification or enzymatic treatment of the PCR product (Figure 1).

### Expressioneering™ Technology



Figure 1. Three steps required for Expresso Cloning.

- 1) Amplify the target gene with primers that contain 18-bp overlap with vector ends.
- 2) Mix the unpurified PCR product with the pME-HA vector preparation, and add the DNAs directly to *E. coli* 10G cells.
- 3) Transform by heat shock. Select for recombinants on kanamycin agar plates.

## PCR primer design

Expresso cloning requires that the PCR product contain at least 18 bp of homology to the pME-HA vector termini. The PCR primers are therefore designed with the vector-homologous portion on their 5' ends, followed by regions that will anneal to the gene of interest.

### Vector Prep:

```

CMV Promoter   Kozak Start   HA Tag   Stop
ATAAAGAGGAGATA CCACC ATG   Y P Y D V P D Y A   *
TAT CCG TAT GAC GTG CCC GAC TAT GCC TAA...
TATTCTCTCTAT GTGG TAC   ATA GGC ATA CTG CAC GGG CTG ATA CGG ATT...
  
```

### PCR product:

```

Forward Primer →
5' - GAAGGAGATA CCACC ATG   Gene of Interest   (TAT) CCG TAT GAC GTG CCC
CTTCTCTAT GTGG TAC           (ATA) GGC ATA CTG CAC GGG-5'
Reverse Primer ←
  
```

## pME-HA Vector

The pME-HA vector contains the CMV promoter to drive constitutive high-level expression in mammalian cells. The kan/neo gene provides selection in bacteria or in mammalian cultures. Non-essential regions have been removed from the vector.

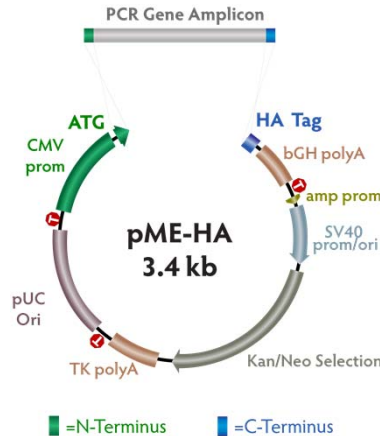


Figure 2. The pME-HA vector. Ampicillin and SV40 promoters drive expression of Kan/Neo resistance in bacteria and mammalian cells. The HA Tag (heme agglutinin peptide) can be appended to recombinant proteins to facilitate antibody binding for affinity purification or Western blotting.

CloneSmart® transcription terminators (T) prevent transcription into or out of the vector backbone, to increase stability of clones.

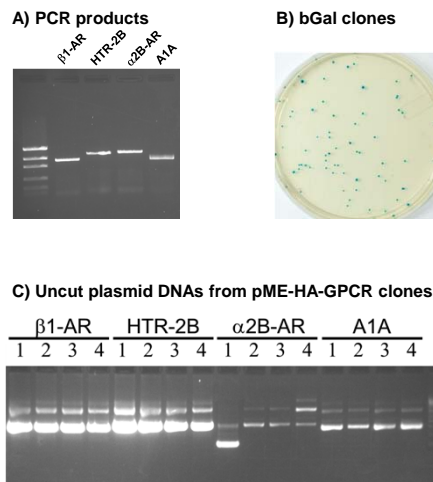
## Highly Efficient Cloning

Several GPCR genes (Panel A), as well as the beta-Galactosidase gene ("bGal", 3.1 kb; not shown) were amplified by PCR for Expresso cloning into the pME-HA vector.

Two ul of unpurified PCR was mixed with the pME-HA vector prep and immediately transformed into *E. coli* 10G Cells by heat shock. Each transformation yielded 300-1000 cfu.

Nearly all the pME-HA-bGal clones contained the correct insert, resulting in blue colonies on X-Gal agar (Panel B).

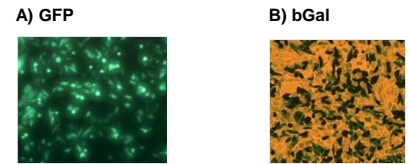
Four colonies from each of the pME-HA-GPCR transformation reactions were randomly selected for plasmid isolation; all but one contained the expected insert (Panel C). Mini-preps yielded 1-5 ug of plasmid DNA per ml of bacterial culture.



## Robust Mammalian Cell Expression

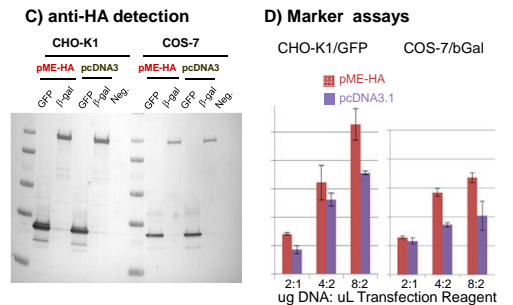
### Visual Detection

The bGal gene (3.1 kb) and GFP gene (0.8 kb) were cloned into pME-HA or pcDNA3.1 vectors and transfected into CHO-K1 or COS-7 cells. After 24 hours, beta-galactosidase activity was visualized by staining, and GFP expression was detected by UV fluorescence. Expression from the pME-HA vector was observed in ~50-80% of the cells (Panels A and B, CHO-K1).



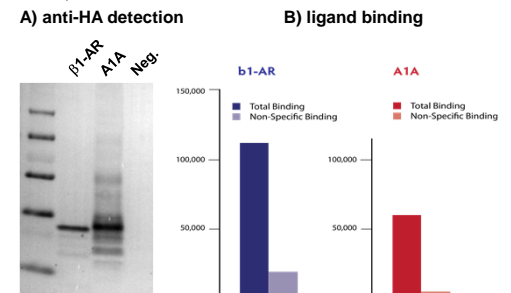
### Western Detection and Activity Assays

Crude lysates were prepared at 24 hours post-transfection and fractionated by SDS-PAGE. Western blots were reacted with an antibody against the HA peptide (Sigma), followed by a secondary antibody conjugated to horse radish peroxidase (Sigma) (Panel C). GFP expression was quantitated by fluorimetry, and bGal activity was assayed enzymatically (Panel D). The pME-HA vector showed similar or slightly higher expression than the widely-used pcDNA3.1.



## Expression of GPCRs

Crude lysates were prepared from the GPCR transfectants of as described above. Expression of GPCRs in CHO cells was confirmed by anti-HA Western blots (Panel A). Significant binding of radioactive ligands to cell lysates was observed (Panel B). Similar results were obtained in COS cells (not shown).



## CONCLUSIONS

The pME-HA vector provides rapid, precise, and efficient cloning, with robust protein expression:

- Fast: 5-second, directional cloning with no enzymatic incubations or vector prep.
- Precise: Lack of cloning "scars" results in precise sequence junctions and correct reading frames.
- Small: The small vector size yields higher transfection efficiency and easier downstream manipulation.
- Efficient mammalian transfection. The pME-HA vector is efficiently transfected into and expressed in mammalian cells.

### Acknowledgements

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