A ubiquitous mammalian expression vector, pHMG, based on a housekeeping gene promoter

Claudie Gautier, Majid Mehtali and Richard Lathe

LGME-CNRS and U184-INSERM, 11 rue Humann, 67085 Strasbourg, France
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Housekeeping genes are highly conserved between species and are expressed in all cell types. The mouse HMGCR (Hydroxy-methylglutaryl-Coenzyme A Reductase) promoter was used to construct a cassette vector: pHMG (figure 1). pHMG derives from pPolyIII-i (1), a plasmid comprising an origin of replication and a beta-lactamase gene (ampicillin resistance). The BglII site was removed by filling in and religation (pCL640) and a new polylinker (NotI-BamHI-EcoRV-SalI-KpnI-XhoI-NotI, top strand sequence dGGCCGCAGATCTGGA-TCCGATATCGTCGACGGTACCCTCGAGGC) was inserted between the two NotI sites. The SV40 polyadenylation signal on a KpnI-SalI fragment (MM & RL, unpub.) was inserted between the KpnI-XhoI sites. Finally, pHMG (also termed pCL642) was generated by introducing a mouse genomic BamHI fragment (MM & RL, unpub.) corresponding to the promoter, the first exon (non translated) and the first intron of HMGCR.

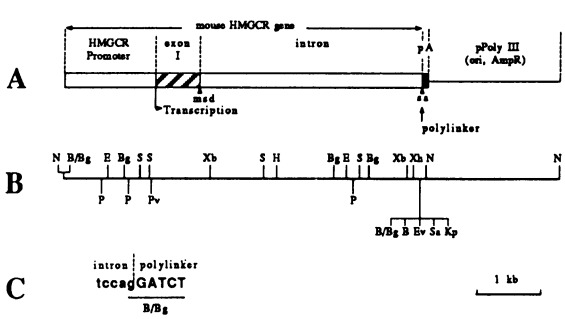

Figure.1. Structure of pHMG.
A. HMGCR promoter region, first exon (cross-hatched) and major intron; pA (solid box), polyadenylation signals; msd, major HMGCR splice donor site; sa, invariant slice acceptor site.
B. Restriction sites are B, BamHI; Bg, BglII; E, EcoRI; Ev, EcoRV; H, HindIII; K, KpnI; N, NotI; P, PstI; Pv, PvuII; S, SmaI; Sa, SalI; Xb, XbaI; Xh, XhoI; Bg/B, a BamHI/BglII fusion site; Xh/Sa, a XhoI-SalI fusion site. **C.** Overlapping BamHI site with the splice acceptor site in the HMGCR gene.

To test the efficiency of the promoter, we introduced the firefly luciferase gene (2) into pHMG. This construct was cotransfected into FR3T3ras (a rat fibroblast cell line transformed by the ras oncogene) with pSV2-CAT (3) in which the Chloramphenicol Acetyl Transferase (CAT) gene is driven by the SV40 early promoter. Luciferase activity (normalized to CAT activity) was 96% of that obtained upon transfection with pRSV-L (2). Further pHMG constructs containng CAT were also analyzed and here the expression level was approximately 104% of that obtained with pSV2CAT. Other cell lines tested (mice LMTK fibroblasts and a human hepatic cell line Hep-G2) gave similar levels of expression.

Transgenic mice lines established with pHMG-CAT (derived from a precursor construct of pHMG) presented a ubiquitous pattern of CAT expression (tail, spleen, liver, intestine, kidney, heart, brain, stomach, born marrow, skin, muscle, pancreas, lung, thymus, salivary gland,testicle) (MM & RL, unpub.). Major transcript sizes (for the CAT fusion gene) were 1.6, 1.9 and 2.2 kb, as predicted from the major processing patterns of the homologous hamster gene (4).

The pHMG vector may be of wide application in the expression of cloned genes regardless of species or differention state of the host cell.

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