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Identification of a novel endogenous regulatory element in Chinese hamster ovary cells by promoter trap

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ABSTRACT

The objective of this study was to identify and isolate endogenous promoters in Chinese hamster ovary (CHO) cells using a promoter trap approach. A promoter-less vector harboring a green fluorescent protein (GFP)-hygromycin resistance gene cassette was designed and transfected into CHO cells. Putative promoters were identified by selecting for GFP$^+$ clones under hygromycin selection. Genomic DNA from these clones was then digested and self-ligated to give rise to a plasmid carrying the putative promoter sequence as well as elements for replication in *E. coli*. Functional promoter sequences were subsequently identified by screening the recovered plasmids for their ability to drive GFP expression upon re-transfection into CHO cells. One of the fragments isolated through this approach was found to drive gene expression in two different reporter systems. Further dissection of the fragment led to the identification of a 156-bp element that was four-fold more active than the full-length fragment and 66% as active as the SV-40 promoter. Thus, promoter trap represents an effective strategy for identifying endogenous regulatory regions that can potentially be incorporated into expression vectors to augment expression of recombinant biopharmaceuticals.

1. Introduction

Most biopharmaceuticals today are produced in mammalian cells transfected with an expression vector that drives constitutive and high level expression of the recombinant protein (Wurm, 2004). Chinese hamster ovary (CHO) cells are one of the most commonly used cell lines in the commercial production of recombinant protein therapeutics, including monoclonal antibodies. Increased demand for these therapeutics has bolstered efforts to augment cell line productivity through improvements in expression technology and optimization of process conditions (Birch and Racher, 2006; Wurm, 2004).

A well-designed expression vector is the first step toward achieving high production of recombinant proteins (Ludwig, 2006). Vector systems for stable cell line generation typically employ strong viral promoters and regulatory elements, such as those derived from cytomegalovirus (CMV) and simian virus 40 (SV40), to drive transgene expression in mammalian cells. However, despite their ability to drive high level expression of the recombinant protein, viral promoters tend to suffer from several limitations. For instance, massive overexpression of transgenes from viral promoters can lead to cellular stress and apoptosis, and transgenes driven by viral promoters are well-documented to be prone to epigenetic silencing (Kim et al., 2011; Papadakis et al., 2004; Yang et al., 2010). Such unfavorable side effects could potentially be circumvented by substituting endogenous CHO regulatory elements into the current vector system. This would allow for more stable expression of recombinant proteins that is desirable for large-scale manufacturing and in a manner that is more adapted to the cellular capacity. One example has been the successful use of regulatory regions associated with the highly expressed CHO gene, Chinese hamster elongation factor-1α (CHEF1). CHEF1 vectors were able to drive higher recombinant protein levels than CMV-based vectors when transfected into both CHO and non-CHO mammalian cell lines (Running Deer and Allison, 2004).

The recent sequencing of the CHO genome has been a significant breakthrough to CHO cell engineering efforts and the bioprocess research community as a whole (Xu et al., 2011). However, gene annotations are still in progress, and annotation of CHO regulatory regions is still in its infancy. Thus, previous efforts at identifying novel endogenous CHO regulatory elements have utilized genomic library approaches, a method that involves shotgun cloning of genomic DNA fragments into a promoter-less

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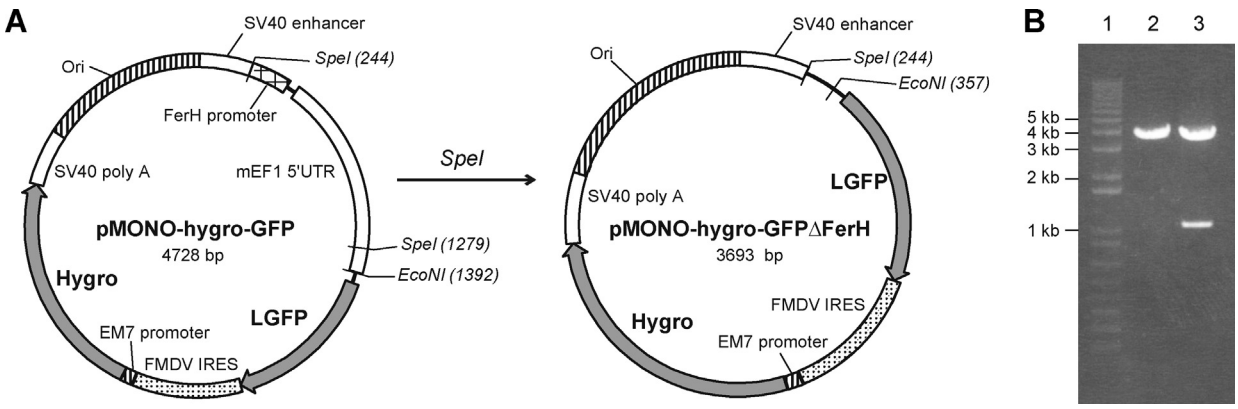

Fig. 1. Generation of the promoter-less vector. (A) The FerH promoter and mEF1 5′ UTR were removed from the commercially-available pMONO-hygro-GFP vector by SpeI digestion. (B) Absence of the FerH promoter was confirmed by vector digestion with SpeI. Lane 1, 1 kb DNA ladder; lane 2, pMONO-hygro-GFP-ΔFerH digested with SpeI; lane 3, original pMONO-hygro-GFP vector digested with SpeI. SV40, simian virus 40; poly A, polyadenylation sequence; FerH, ferritin heavy chain; mEF1 5′ UTR, mouse elongation factor 1 5′ untranslated region; LGFP, variant of the jellyfish green fluorescent protein; FMDV IRES, Foot and Mouth Disease Virus internal ribosomal entry site; EM7, synthetic bacterial promoter derived from the bacteriophage T7 promoter; Hygro, hygromycin; Ori, origin of replication.

reporter vector and transfecting this genomic library into cells. Individual clones exhibiting reporter activity (such as antibiotic resistance) are recovered, and the original genomic DNA fragments are isolated and sequenced (Pontiller et al., 2008, 2010). However, this approach is very labor-intensive and can yield potentially confounding results. Other methods include screening the flanking regions of highly expressed genes obtained from CHO microarray data for potential promoter candidates; this approach, however, limits identification to known genes (Thaisuchat et al., 2011).

In this study, we employed a promoter trap approach to identify endogenous promoter regions in the CHO genome. CHO cells were transfected with a promoter-less vector carrying a green fluorescent protein (GFP)-hygromycin resistance gene cassette. Using GFP expression as a read-out for integration of the promoter trap vector adjacent to a transcriptionally active endogenous promoter, several genomic fragments were recovered from GFP$^+$ clones. One fragment in particular was capable of driving gene expression in two different reporter systems. Further mapping of the fragment led to the identification of a 156-bp element that was four-fold

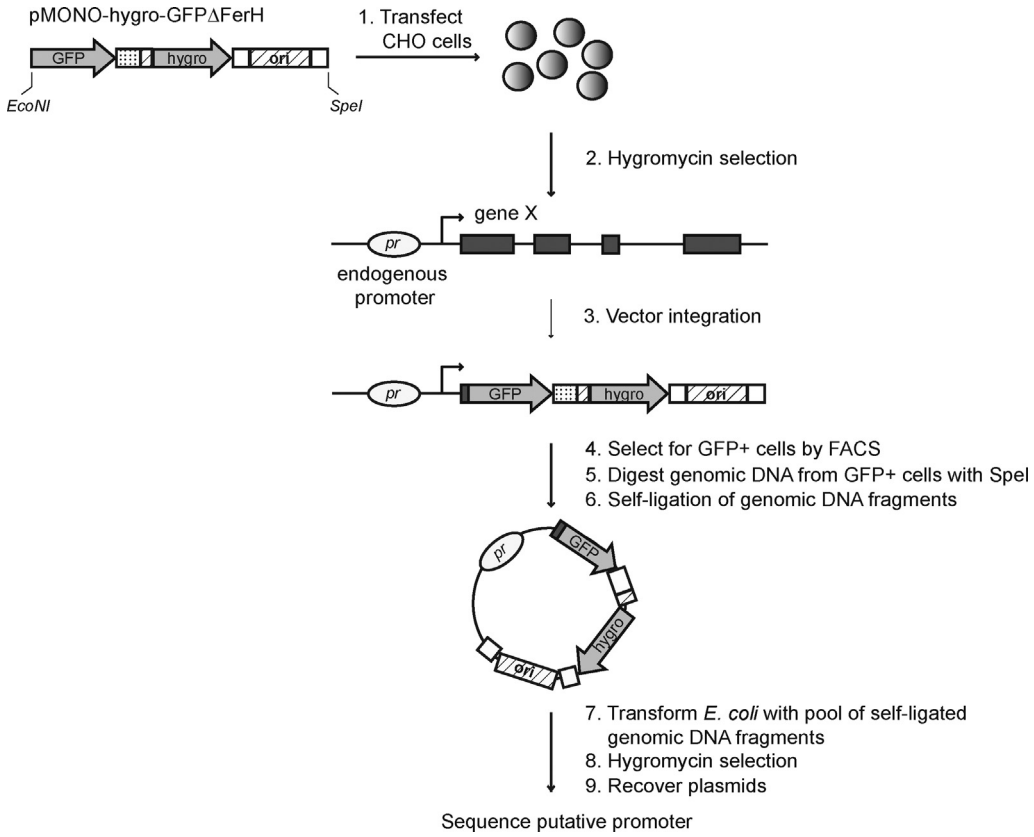

Fig. 2. Schematic representation of promoter trap strategy. The EcoNI-linearized promoter-less vector was transfected into CHO cells. Cells were grown under hygromycin selection to select for transfectants in which the vector had integrated into the host genome. Cells in which GFP expression is driven by endogenous promoters became GFP$^+$, and cells expressing the highest level of GFP were sorted by FACS. Genomic DNA was isolated from GFP$^+$ clones, digested with SpeI, and allowed to self-ligate. Self-ligated genomic DNA fragments from each clone were transformed into *E. coli*, and plasmids containing putative CHO promoters were recovered and sequenced.