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Rapid recombinant protein production from piggyBac transposon-mediated stable CHO cell pools

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ABSTRACT

Heterogeneous populations of stably transfected cells (cell pools) can serve for the rapid production of moderate amounts of recombinant proteins. Here, we propose the use of the piggyBac (PB) transposon system to improve the productivity and long-term stability of cell pools derived from Chinese hamster ovary (CHO) cells. PB is a naturally occurring genetic element that has been engineered to facilitate the integration of a transgene into the genome of the host cell. In this report PB-derived cell pools were generated after 10 days of selection with puromycin. The resulting cell pools had volumetric productivities that were 3–4 times higher than those achieved with cell pools generated by conventional plasmid transfection even though the number of integrated transgene copies per cell was similar in the two populations. In 14-day batch cultures, protein levels up to 600 and 800 mg/L were obtained for an Fc-fusion protein and a monoclonal antibody, respectively, at volumetric scales up to 1 L. In general, the volumetric protein yield from cell pools remained constant for up to 3 months in the absence of selection. In conclusion, transfection of CHO cells with the PB transposon system is a simple, efficient, and reproducible approach to the generation of cell pools for the rapid production of recombinant proteins.

1. Introduction

Chinese hamster ovary (CHO) cells are the major host for the manufacture of recombinant therapeutic proteins by stable gene expression (Wurm, 2004). However, stable cell line development is a time-consuming process taking several months to complete (Browne and Al-Rubeai, 2007; Matasci et al., 2008; Wurm, 2004). For rapid access to recombinant proteins, transient gene expression (TGE) with suspension-adapted mammalian cells is often employed (Geisse and Voedisch, 2012; Hacker et al., 2013; Pham et al., 2006). Recent improvements in TGE in CHO, through host cell engineering and bioprocess optimization, have resulted in recombinant protein yields up to 2 g/L (Cain et al., 2013; Daramola et al., 2014; Rajendra et al., 2011). However, the relatively large amount of plasmid DNA required for transfection and its cost, together with the difficulty of handling large liquid volumes are limitations for TGE at volumetric scales above a few liters.

An alternative approach to the rapid production of moderate amounts of protein is through the generation of cell pools or bulk cultures. These are heterogeneous populations of recombinant cells obtained by gene transfer and genetic selection but without subsequent cell cloning steps (Fan et al., 2013; Li et al., 2013; Ye et al., 2010). The main reasons for using cell pools over TGE are the need for only a small amount of plasmid DNA and the ease of volumetric scale-up by dilution (Fan et al., 2013; Li et al., 2013; Ye et al., 2010). Cell pools also have an advantage over clonal cell lines because of shorter and less costly development times (Ye et al., 2010). A major drawback of cell pools is the heterogeneity resulting from a cell population having a range of growth rates and recombinant protein expression levels. Consequently, the volumetric productivity of cell pools is usually lower than that for clonal cell lines recovered from the same population and it may not remain constant over time in culture (Fan et al., 2013; Ye et al., 2010).

Transposons are naturally occurring mobile genetic elements that can be used in a modified form to facilitate the integration of transgenes into the mammalian cell genome (Izsvak and Ivics, 2004; Meir et al., 2011; Wilson et al., 2007). Engineered

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transposable elements have been used to generate stably transfected CHO- and human embryonic kidney (HEK293)-derived cell lines for protein production (Alattia et al., 2013; Li et al., 2013; Matasci et al., 2011), and they have also served as gene transfer vectors for applications in gene therapy and gene discovery (Ding et al., 2005; Ivics et al., 1997; Mossine et al., 2013; Tsukiyama et al., 2011; Wu et al., 2006). piggyBac (PB), a class II transposable element originally derived from the cabbage looper moth, is one of the several transposons modified to function in mammalian cells (Fraser et al., 1996; Meir et al., 2011; Wilson et al., 2007). The PB transposase (PBase) is known to have a preference for targeting transcriptionally active regions of the host genome. The integration sites have been found to be well-distributed in the genome but no integration clusters were observed even though a few apparent hot-spots of integration have been identified in several human cell types including HEK-293 (Huang et al., 2010; Meir et al., 2011, 2013; Wilson et al., 2007). However, the pattern of PB-mediated integration in CHO cells is not known.

The PB transposon system has been used to generate stable CHO cell lines at a significantly higher frequency and with higher volumetric recombinant protein productivity than by conventional plasmid transfection (Matasci et al., 2011). The system consists of one plasmid (helper vector) for the transient expression of the PBase gene and a second plasmid (donor vector) carrying the 5′ and 3′ inverted repeat elements (5′IR and 3′IR) delimiting the ends of the artificial transposon (Kahlig et al., 2009; Meir et al., 2011). One or more transgenes and the accompanying transcriptional regulatory elements can be introduced between the two IR elements. Following co-transfection of the dual vector system, transiently expressed PBase catalyzes the excision of the artificial transposon from the donor vector and mediates its integration into the host genome. PBase has been reported to be capable of mobilizing very large DNA molecules, such as bacterial artificial chromosomes (Li et al., 2011; Rostovskaya et al., 2012). However, the optimal transposon size may be below 14 kbp, since the frequency of transposition decreases as the size of the artificial transposon increases beyond this size (Alattia et al., 2013; Ding et al., 2005; Lacoste et al., 2009; Li et al., 2013; Meir et al., 2011; Wang et al., 2014).

Here, we take advantage of PB-based gene delivery to generate cell pools for recombinant protein production with CHO DG44 cells as the host. We succeeded in producing tumor necrosis factor receptor-Fc fusion protein (TNFR:Fc) and a monoclonal antibody at scales of up to 1 L, with volumetric yields up to 800 mg/L in 14-day batch cultures. Most of the pools had a constant volumetric productivity for up to 3 months in the absence of selection. Thus, PB-mediated gene delivery offers a rapid and efficient method for the production of recombinant proteins from cell pools.

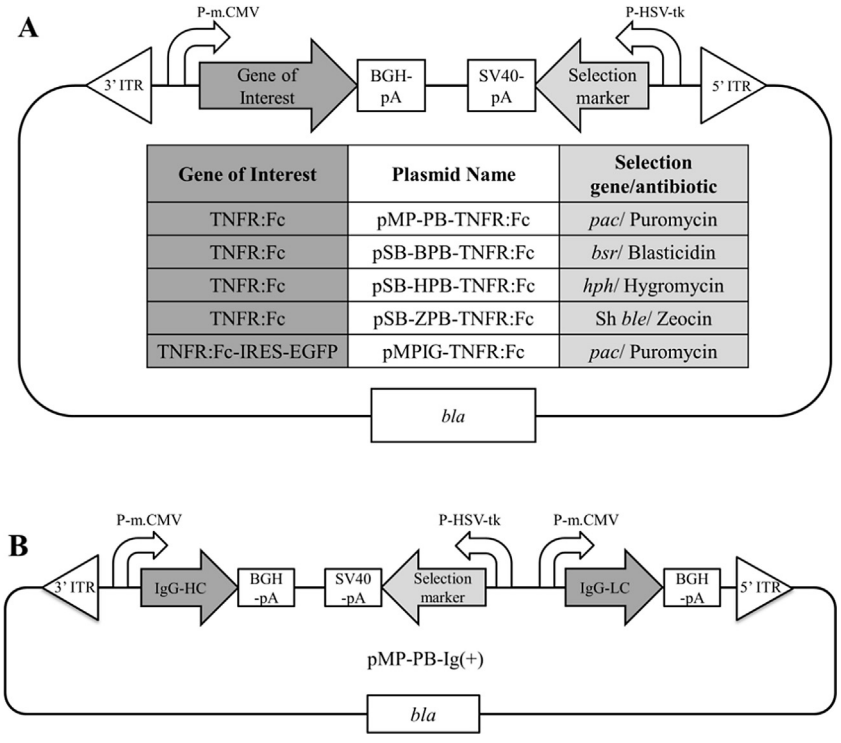
2. Materials and methods

2.1. Routine cell culture

Suspension-adapted CHO DG44 cells were grown in ProCHO5 medium (Lonza AG, Verviers, Belgium) supplemented with 13.6 mg/L hypoxanthine, 3.84 mg/L thymidine, and 4 mM glutamine (SAFC Biosciences, St. Louis, MO) by orbital shaking in glass bottles as previously described (Muller et al., 2005). The cells were routinely inoculated in ProCHO5 twice per week at a density of 3×10^5 cells/mL. Cell number and viability were assessed manually by the Trypan Blue exclusion method.

2.2. Plasmids

The donor vector pMPIG-TNFR:Fc (Fig. 1A) and the helper vector pmPBase have been described previously (Matasci et al., 2011). The donor vector has a bicistronic organization for the co-expression of



Gene of Interest	Plasmid Name	Selection gene/antibiotic
TNFR:Fc	pMP-PB-TNFR:Fc	*pac*/ Puromycin
TNFR:Fc	pSB-BPB-TNFR:Fc	*bsr*/ Blasticidin
TNFR:Fc	pSB-HPB-TNFR:Fc	*hph*/ Hygromycin
TNFR:Fc	pSB-ZPB-TNFR:Fc	Sh *ble*/ Zeocin
TNFR:Fc-IRES-EGFP	pMPIG-TNFR:Fc	*pac*/ Puromycin

Fig. 1. Schematic diagrams of PB donor plasmids. (A) The diagrams of several donor vectors having the same plasmid backbone are shown. For each plasmid, the name, gene of interest, and the selection gene are indicated. (B) Diagram of donor vector pMP-PB-Ig(+) having three separate expression cassettes within the artificial transposon. Abbreviations: internal ribosome entry site (IRES), enhanced green fluorescent protein gene (EGFP), tumor necrosis factor receptor Fc fusion gene (TNFR:Fc), bovine growth hormone polyadenylation element (BGH-pA), SV40 polyadenylation element (SV40-pA), mouse cytomegalovirus promoter (P-mCMV), herpes simplex virus-1 thymidine kinase promoter (P-HSV-tk), inverted terminal repeat (ITR), beta-lactamase gene (*bla*), antibody light chain gene (IgG-LC), antibody heavy chain gene (IgG-HC), and puromycin N-acetyl-transferase gene (*pac*).