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Optimisation of insect cell growth in deep-well blocks: development of a high-throughput insect cell expression screen

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Abstract

This report describes a method to culture insects cells in 24 deep-well blocks for the routine small-scale optimisation of baculovirus-mediated protein expression experiments. Miniaturisation of this process provides the necessary reduction in terms of resource allocation, reagents, and labour to allow extensive and rapid optimisation of expression conditions, with the concomitant reduction in lead-time before commencement of large-scale bioreactor experiments. This therefore greatly simplifies the optimisation process and allows the use of liquid handling robotics in much of the initial optimisation stages of the process, thereby greatly increasing the throughput of the laboratory. We present several examples of the use of deep-well block expression studies in the optimisation of therapeutically relevant protein targets. We also discuss how the enhanced throughput offered by this approach can be adapted to robotic handling systems and the implications this has on the capacity to conduct multi-parallel protein expression studies.

Keywords: Baculovirus; Deep-well block protein expression; High-throughput

Baculovirus protein expression is a eukaryotic based expression system, and thus utilises many of the protein modification, processing and transport systems present in higher eukaryotic cells. Since it is a helper-independent virus, it can be propagated to very high titres and easily adapted to suspension culture. This makes it possible to generate large amounts of recombinant protein with relative ease [1,2]. Baculovirus mediated insect expression has therefore become one of the most popular systems for the production of large quantities of recombinant protein required for structural and functional study of therapeutically relevant bio-molecules.

Typically, before large-scale bioreactor expression can commence, a myriad of optimisation experiments are required, as the target protein may not necessarily provide large quantities of soluble material in the first instance. These experiments may include varying the time of infection (48–96 h), the volume of virus added to the viable cell number (expressed as multiplicity of infection) or the analysis of protein fusion tags to enhance solubility, stability, and simplify purification protocols. Given that optimisation is traditionally performed in suspension cultures at the 100 mL–1 L scale prior to a final bioreactor run, this situation can rapidly lead to a drain on resources in terms of equipment, labour, and reagents. Indeed, for these very reasons, extensive optimisation of expression conditions is often not possible in instances where functional protein expression requires the co-expression of one or more co-factors or subunits using two or more viruses, such as with G-protein coupled receptors.

To address these issues, we describe here a method to culture insect cells in 24 deep-well blocks to conduct optimisation studies. Miniaturisation of this process provides the necessary reduction in terms of resource

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allocation, reagents, and labour to allow extensive and rapid optimisation of expression conditions. Moreover, with minor adaptations to the molecular cloning and recombinant virus production strategy this strategy allows the use of liquid handling robotics in much of the initial optimisation stages of the process, thereby greatly increasing the potential throughput of the laboratory. We present several examples of the use of deep-well block expression studies in the optimisation of therapeutically relevant protein targets. We also discuss how the enhanced throughput offered by this approach can be adapted to robotic handling systems and the implications this has on the capacity to conduct multi-parallel protein expression studies.

Materials and methods

Molecular biology

Generation of GFP pFastBac plasmid

A 716 bp DNA fragment containing the entire open reading frame of GFP[1] (GenBank Accession No. U17997) was amplified from the plasmid pGFP (BD Biosciences) by PCR using gene specific primers containing *Xho*I and *Kpn*I flanking sites, and sub-cloned into the p10 multiple cloning site (MCS) of pFastBac Dual (Invitrogen) to create a GFP co-expression plasmid compatible with the Bac-to-Bac expression system as previously described [3].

Generation of GST-PI3-K/p85 pFastBac DUAL baculovirus expression vectors

A 1214 bp DNA fragment containing a 50 kDa SH2-IS-SH2 fragment (Asn323-Arg724) of the human p85α (GenBank Accession No. Mb1906) was amplified from human brain cDNA (BD Biosciences) by PCR using gene specific primers containing *Xho*I and *Kpn*I restriction enzyme sites, and ligated into pFastBac DUAL to create p50 pFastBac DUAL. 3933 and 3209 bp DNA fragments containing the entire open reading frames of the bovine PI3-K p110α isoform (GenBank Accession No. NM_006219) and human PI3-K p110β (GenBank Accession No. NM_006219) fused to GST fusion tags were then amplified by standard PCR using gene specific oligonucleotides containing *Spe*I and *Hin*dIII restriction enzyme sites, and ligated into p50 pFastBac DUAL to create the co-expression plasmids bovGST-PI3-Kα/p85 pFastBac DUAL and huGST-PI3-Kβ/p85 pFastBac DUAL, respectively. Positive recombinant plasmids containing both GST-PI3-K p110α or p110β and the truncated p85 adaptor proteins were confirmed by restriction digest analysis and DNA sequence verification (Solvias AG). Baculovirus expressing a GST tagged form of human PI3-Kγ was a kind gift from Matthias Wymann (University of Fribourg).

Generation of $A_{2A}AR$ and $G_{S\alpha 2}$ FastBac1 plasmids

Human adenosine A_{2A} receptor (huA_{2A}AR; GenBank Accession No. NM_000675) and the gene encoding the human $G_{s\alpha 2}$ G-protein subunit (GenBank Accession No. X04408), containing flanking *Nhe*I and *Kpn*I and *Sal*I and *Xba*I restriction enzymes sites, respectively, were amplified by PCR, and sub-cloned into the corresponding restriction endonuclease sites of pFastBac to create A_{2A}AR pFastBac and $G_{s\alpha 2}$ pFastBac, and sequence verified (Solvias AG).

Generation of a $G_{\beta 4}/G_{\gamma 2}$ pFastBac Dual plasmid

The human $G_{\beta 4}$ subunit insert was sub-cloned from a $G_{\beta 4}$/pCR2.1 TOPO (GenBank Accession No. AF300648) construct into the *Bam*HI and *Eco*RI sites of pFastBac Dual and the $G_{\gamma 2}$ subunit (GenBank Accession No. AF493870) was sub-cloned into the *Xho*I and *Nhe*I sites of the same pFastBac Dual plasmid to create a single expression plasmid, $G_{\beta 4}/G_{\gamma 2}$ pFastBac Dual. Positive recombinant plasmids containing both $G_{\beta 4}$ and $G_{\gamma 2}$ proteins were confirmed by restriction digest analysis and DNA sequence verification (Solvias AG).

Virus propagation and titre determination

Single virus studies (GFP or PI3-K/p85)

Expression plasmids containing either GFP-pFastBac, bovGST-PI3-Kα/p85 pFastbac DUAL or huGST-PI3-Kβ/p85 pFastBac DUAL were transposed into DH10Bac competent cells following the manufacturer's instructions and plated onto Luria plates containing 50 μg/mL kanamycin, 7 μg/mL gentamicin, 10 μg/mL tetracycline, 100 μg/mL X-gal, and 40 μg/mL IPTG. Following incubation at 37 °C for 48 h several positive recombinant bacmids were picked and viral DNA was isolated from 10 mL overnight Luria cultures containing the antibiotics kanamycin (50 μg/mL), gentamicin (7 μg/mL), and tetracycline (10 μg/mL). Recombinant bacmids confirmed by PCR were transfected into a 2 mL adherent *Sf*21 cell culture volume (9×10^5 cells) using the cationic liposome Cellfectin (Invitrogen) and cultured for 96 h at 27C in Sf-900 II SFM medium supplemented with 50 μg/mL gentamicin. Viral supernatants were then harvested and amplified to generate large, high titre viral stocks expressing GFP; bovGST-PI3-Kα/p85 and huGST-PI3-Kβ/p85, respectively.

[1] *Abbreviations used:* DTT, dithiothreitol; GFP, green fluorescent protein; HT, high-throughput; MOI, multiplicity of infection; *Sf*, *Spodoptera frugiperda*; SDS–PAGE, sodium dodecyl sulphate–polyacrylamide gel electrophoresis; TTBS, 20 mM Tris, pH 7.5, 150 mM NaCl, and 0.1% (v/v) Tween 20.