

flashBACULTRA™ Superior Expression and Yield

flashBACULTRA™

flashBAC™ is an expression system that allows the production of multiple recombinant viruses in a one-step process [1]. This technology has greatly de-skilled the process of making recombinant viruses and increased the throughput of recombinant protein production. It also has a chitinase gene deletion (chiA) that greatly improves the movement of recombinant proteins through the cellular secretory pathway.

flashBACGOLD™ is a further improvement on this system, by the removal of both

chiA and a protease (v-cath) from the virus genome [2]. These modifications are primarily designed to facilitate the movement of recombinant proteins through the cell's secretory pathway and to prevent their degradation once they are released into the culture media.

flashBACULTRA™ has taken this technology a step further, by the removal of three more virus genes (p10, p74 and p26) from the flashBACULTRA™ genome.

Technology Overview

P10 is a 10 kDa protein, expressed concurrently with polyhedrin (polh) late in infection and is nonessential in cell culture [3]. Both p10 and polh promoters share a 12-nucleotide consensus sequence [4] containing the transcription initiation ATAAG motif. P10 is activated a few hours before polh [5] and has been demonstrated to compete with polh at a transcriptional level [6]. However, inhibition and deletion of the p10 promoter has been shown to result in increased polh-controlled protein production [3, 7] and polh mRNA levels [6]. P10 also associates with occlusion bodies (OBs) [8] and is believed to mediate nuclear disintegration late in infection as its disruption has been shown to prevent OB release [7]. Recent work has also shown that it forms extensive cytoskeleton-associated or cytoskeletal-

like structures in the nucleus and cytoplasm, potentially de-stabilizing the cells cytoskeleton [8] and further depleting cellular resources.

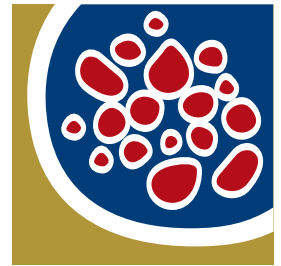
Deletion of p10 increases polh activity providing more recombinant protein, increases nuclear and cellular stability, ensuring a longer timeframe for protein expression and removes a major competitor for limiting cellular resources.

P74 is non-essential in cell culture but is essential for oral infectivity of occlusion-derived virus (ODV) in the host where it plays a role in midgut attachment and fusion [9]. Deletion of p74 has been shown to have no effect on virus production in vitro [10].

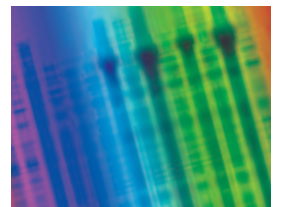
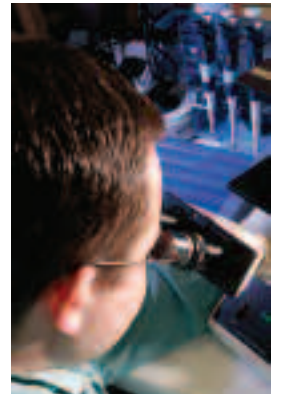
Deletion of p74 further increases the biosafety profile of recombinant

the science of baculovirus expression™

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baculoviruses in the environment, making them unable to traverse the insect gut wall.

P26 is an early gene that codes for a 240-amino acid polypeptide of unknown function [11] and has the same 5' terminus as p10 [12]. Deletion of the 3'-end of p26 and fusion to lacZ or p10 have previously been shown to have no effect on virus replication in vitro [6, 13].

Deletion of p10, p74 and p26 removes an unnecessary genetic burden from the recombinant virus genome, providing a more efficient baculovirus expression vector.

Advantages

- Increased recombinant protein transcription from the polh promoter
- More efficient transport through the endoplasmic reticulum (ER) and secretory pathway
- Increased cellular stability and longevity
- Increased recombinant protein secretion
- Increased recombinant protein yield
- Reduced proteolysis and degradation
- Increased recombinant protein quality

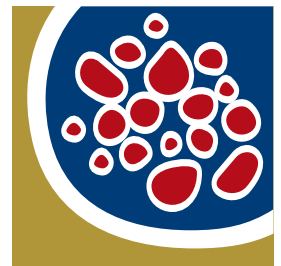
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flashBACULTRA™ Enhanced yields for difficult to express proteins

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