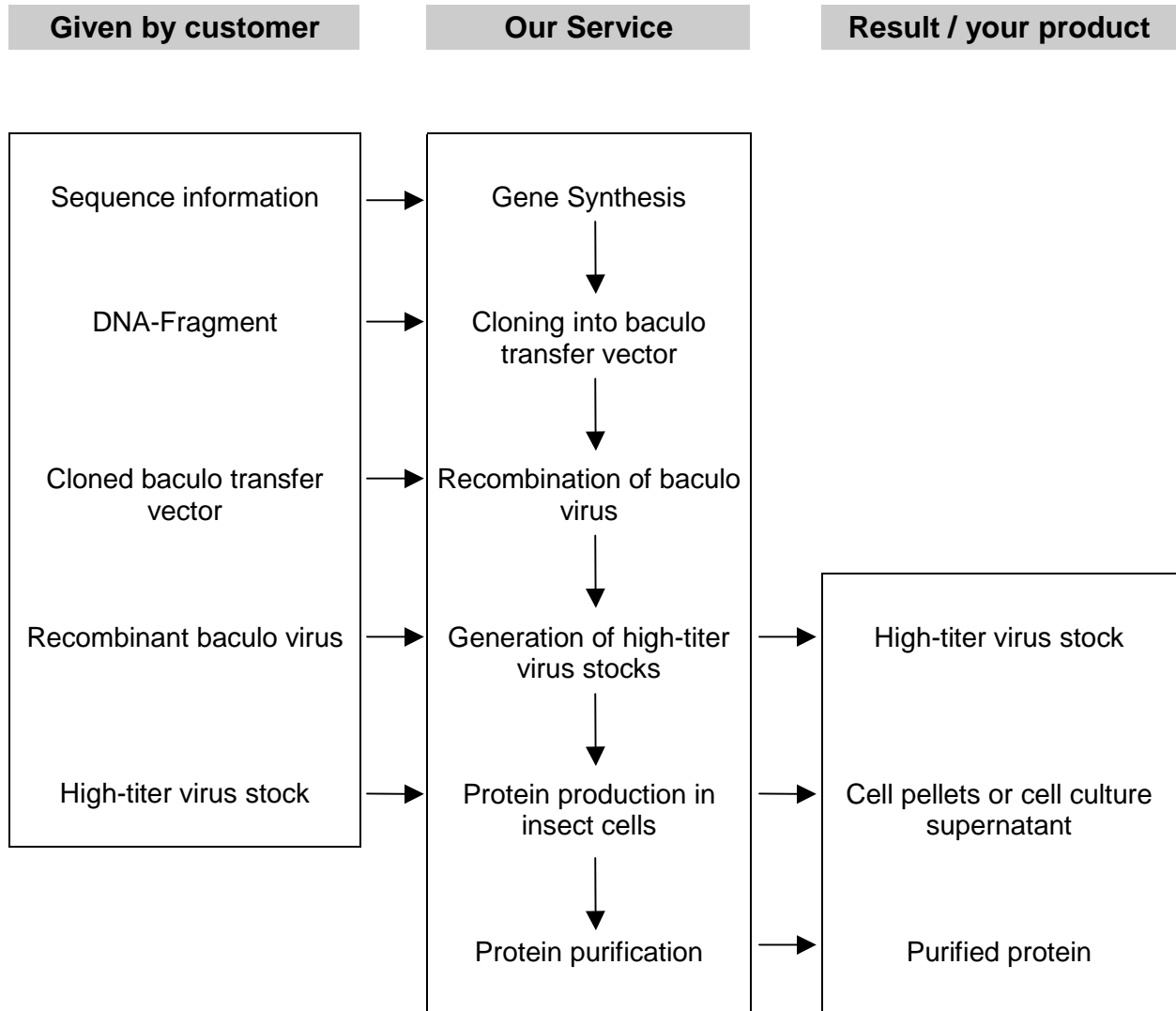


Baculo Virus Service



Baculo Virus Service

Gene Synthesis

Cloning of a gene from genomic DNA or cDNA is time consuming, tedious and a source for mistakes. By a close cooperation Genaxxon can offer the synthesis of genes with 100% sequence accuracy. Coding sequences from 100bp up to complete genes with more than 10 kb can be synthesised according to your needs. This way a targeted amino acid exchange in the recombinant protein can be performed very easily.

(Time schedule: 1 – 3 weeks)

Cloning of baculo transfer vector

The coding gene sequence will be cloned into a baculo transfer vector by Genaxxon using an approved and optimised vector system.

A DNA fragment, that has to be provided by the customer, should be available as an insert in a cloning vector. The coding sequence will be amplified or isolated after restriction digest. On request we will add Tags or sequences to the gene sequence usable for the purification or the detection of the protein, as protease cutting or signal recognition sites.

The newly constructed transfer vector will be purified by ion exchange chromatography and the quality of the preparation checked.

The identity of the insert will be verified by sequencing **and** an appropriate restriction digest.

For reference reasons we will provide our customers with 10 ug of the purified vector. The rest of the DNA will be used for the construction of recombinant baculo viruses.

(Time schedule: 1 – 2 weeks)

Recombination of baculo viruses

The baculo viruses will be reconstructed/recombined from the baculo transfer vector and viral DNA using insect cells as a host. If customers provide the transfer vector, the DNA should be purified by ion exchange chromatography and checked that the vector will be compatible with the virus used.

Adherent growing Sf9 cells will be transfected by lipofection with the transfer vector together with linear DNA from the Autographa californica Nuclear Polyhedrosis virus (AcNPV). The recombinant baculo virus from the cell culture supernatant will be harvested and 10 virus clones will be isolated and amplified by subsequent plaque purification.

Sf9 cells will be infected with the virus stock to check expression efficiency of the recombinant protein. Identification of the expressed protein will be checked by immuno-Western blot, for which an appropriate antibody should be available from customer. 5 successfully recombined viruses will be chosen for further work.

(Time schedule: 1 – 2 weeks)

Baculo Virus Service

Generation of high titer virus stocks

For identifying the most efficient virus the titer of all 5 chosen virus stocks has to be determined. For doing that the 5 viruses will be used for a test infection at the same MOI (multiplicity of infection) to check the expression strength of all 5 clones. From the 5 clones the most effective will be chosen for the further work.

The virus will be amplified by subsequent infection of Sf9 cells until a minimum titer of 1×10^9 pfu/ml (plaque forming unit) has been achieved. Depending on the planned production scale virus stocks of 100 ml up to several litre can be produced.

High titer virus stock will be sterile filtered, split into 50 ml aliquots and delivered as cool shipment.

(Time schedule: 2 – 3 weeks)

Protein production in insect cells

Before starting with the production of recombinant proteins, all relevant infection parameters will be checked for optimal results to guarantee highest levels (yields) of soluble recombinant protein. We will perform subsequent tests varying and optimising especially MOI and the time between infection and harvesting of the infected cells.

For the production of recombinant proteins we use Sf9 cells that have been adapted for growth in suspension. The cells are grown in serum free media (produced by ourselves), especially suitable for easier isolation and purification of proteins from the cell supernatant.

Cells are cultivated/grown under continuous control of the whole production process from 100 ml scale in 100 ml Spinner flasks up to 20 litre bioreactors. Infection with the high-titer virus stock is performed while the cells are in their logarithmic growth phase. According the before optimised production time, cells, respective the cell supernatant will be harvested and the recombinant protein will be checked by Immuno-Western Blot. Customers will get the cells as deep frozen pellet on dry ice. The cell culture supernatant will be delivered as sterile filtered, cooled concentrate.

(Time schedule: 2 – 4 weeks)

Protein purification

The recombinant protein can be purified from cells or cell culture supernatants. Cells will be disintegrated under mild conditions. Cell culture supernatants containing secreted protein will be concentrated by cross-flow methods. The subsequent purification will be performed by FPLC, using protocols from customer or establishing an optimised purification protocol for each protein. For the purification we will use different purification methods and chromatographic columns, according the features/properties of the protein. Success and yield of the purification will be documented by SDS-PAGE with subsequent Coomassie staining. Customers get the proteins in aliquots as requested

(Time schedule: 1 – 3 weeks)

Baculo Virus Service

Price list

Catalog No.	Service description		Price (Euro)
P2022.0000	Primer, design and synthesis (1 pair, up to 35 bases)	primer pair	89,00
P2016.0000	Subcloning of customer construct into baculo transfer vector <ul style="list-style-type: none"> • Isolation of coding DNA fragment • Cloning into baculo transfer vector • Production and purification of vector • Sequencing and restriction digest 	clone	1699,00 plus costs for sequencing and primer
P2023.0000	Verification sequencing (< 300 bp)	clone	44,00
P2024.0000	Verification sequencing (> 300 bp)	bp	0,16/bp
P2017.0000	Construction of recombinant Baculo virus <ul style="list-style-type: none"> • Transfection of Sf9 cells • Plaque isolation and purification of 10 clones • Expression control • Choice of 5 clones 	clone	3600,00
P2018.0100	Generation of high titer stock (100 ml) <ul style="list-style-type: none"> • Titer determination of 5 clones • Comparison of protein expression • Choosing most effective clone • Amplification of clone • Production of virus stock • Sterile filtration and mycoplasma test 	100 ml	800,00
P2018.0500	Generation of high titer stock (500 ml)	500 ml	1500,00
P2018.1000	Generation of high titer stock (1 L)	1 L	2300,00
P2019.0000	MOI Assay of protein production	Set-up	790,00
P2020.0500	Protein production from rec. Baculo virus <ul style="list-style-type: none"> • Optimisation of infection parameter • Upscaling of Sf9 cells • Suspension culture in serum free medium • Infection with high titer virus stock • Harvesting • Expression control and pelleting 	500 ml	820,00
P2020.1000	Protein production from rec. Baculo virus	1 L	1750,00
P2020.5000	Protein production from rec. Baculo virus	5 L	3500,00
P2020.1020	Protein production from rec. Baculo virus	20 L	7800,00
P2025.0010	Transient protein expression in insect cells	10 ml	2990,00
P2027.0000	Gene modification prior to subcloning	Set-up	On request

For more information, please contact Genaxxon at: www.genaxxon.com