Application Note

MCQ Gas Blender 100 Series Application

Modified atmosphere: enhancing insect cell cultures experimentation

Introduction

Vaccines, antibiotics an others essential medicines have always constituted basic necessities for our society. The need of these goods, greatly increased over the last several decades, leads the modern science towards new reliable and efficient drug production methods. The large-scale production of basic medicines relies on the capacity to synthesize adequate amounts of specific proteins, naturally expressed in their wild type form or appositely modified. One of the most widely used technique designed to enhance the proteins production, is the Heterologous Protein Expression (HPE).

MCQ Gas Blender 100 Series

HPE technique is base on the following principles:

- The cell type that naturally expresses the target protein is selected.
- The genetic material required to express the target protein is collected from the cell.
- The genetic material is experimentally transferred into a another cell type that does not normally express that protein.
- The cell type with the new inserted genetic material gains the ability to express the target protein.

The two cell types usually derive from different organisms, thus the term heterologous.

The expression of functional proteins in heterologous hosts is currently a cornerstone of modern biotechnology. Aside from the large-scale commercial applications, this techniques is an incredible useful tool for lab basic research, thus requiring constant improvements in speed, costs and effectiveness. A key step of the proteins production is the cell culture, during which the system atmosphere plays a crucial role. For this reason the MCQ proposes its Gas Blender 100 Series as a fundamental tool to control, study and improve the culture expression.

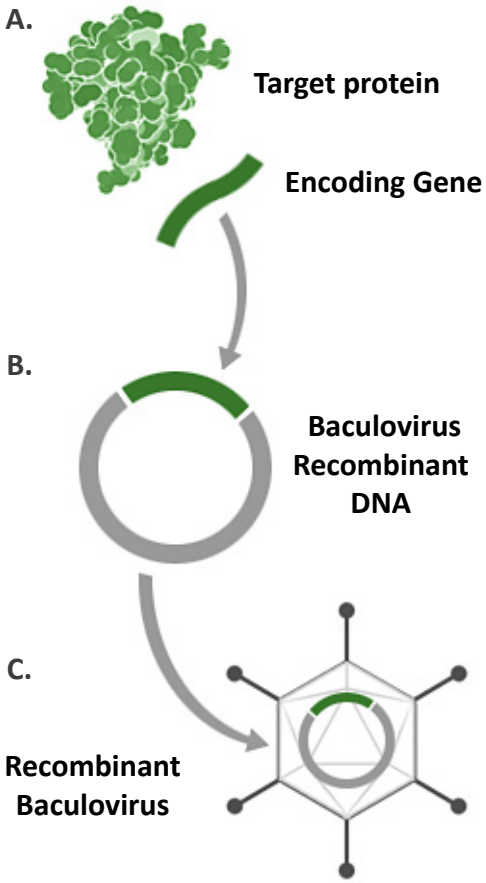
Baculovirus Expression Vector System

The modern HPE exploits many types of heterologous hosts. The most widely used expression systems are based on E. coli as the host cell. Yeats, molds and mushrooms (i.e. fungi) are another important class of hosts (more detailed information about fungi cultures can be found here [link]). A relatively new and promising approach to the expression system is instead based on insect cell hosts. Like bacteria and fungi, the expression capacity of insect cells is artificially modified to allow the culture to produce specific protein(s). The modification process takes place via the Baculovirus Expression Vector System (BEVS).

BEVS technique major steps can be summed up as follow:

- The small part of DNA filament (the gene) encoding the information required to express the target protein is transferred into the Baculovirus genome via a transfer vector. The transfer vector replaces a nonessential Baculovirus gene with the foreign gene, leaving the virus reproduction and infection capacities unchanged.

- The resulting Baculovirus (called recombinant) is used to infect insect cell cultures. The new genetic material is transferred into the host cells and after few hours the culture starts to express the desired protein.



BEVS Mechanism:

A. The target protein for the expression is selected and the related encoding gene is isolated.

B. A vector system is used to insert the gene inside the Baculovirus DNA.

C. The result is a recombinant baculovirus capable of transferring the modified DNA inside a host cell.

BEVS features

• Advantages

BEVS is widely used in research and scientific industrial communities for the production of high quality proteins, most of them originated form mammalian cells that are unsuitable to lab cultures. This technique offers many advantages over the other culture systems, including increased production rates, higher yields, products with improved solubility and the ability to synthesize proteins with posttranslational modifications (often identical to those that occur in mammalian cells).

The nature of the Baculovirus itself constitutes a great advantage for researchers. Baculoviridae family is one of the largest known groups of viruses, capable of infecting over 500 species of insect cells, and more recently, even few of mammalian cell lines. The standard BEVS procedure makes use of a specific Baculovirus, called Autographa Californica nuclear polyhedrosis virus (AcNPV), especially suitable for its narrow host range.

The AcNPV is known to infect only few lepidopteran (moth) families, and has been proved to be not infectious for mammalian cell lines. The use of this specific baculovirus in therefore not only highly selective for insect cell lines but also safe for humans.

• Disadvantages

Despite these potential advantages, BEVS suffers some of the peculiar downsides shared by any heterologous expression system. Differences in proteins expressed by mammalian and baculovirus infected insect cells have been described, and in some cases, overcome. Issues related with the infection cycle can affect the proteins folding, eventually leading to the formation of undesired aggregates. The expression rate for certain proteins can be considered inadequate and some posttranslational modification may occur uncontrollably or may not occur at all.

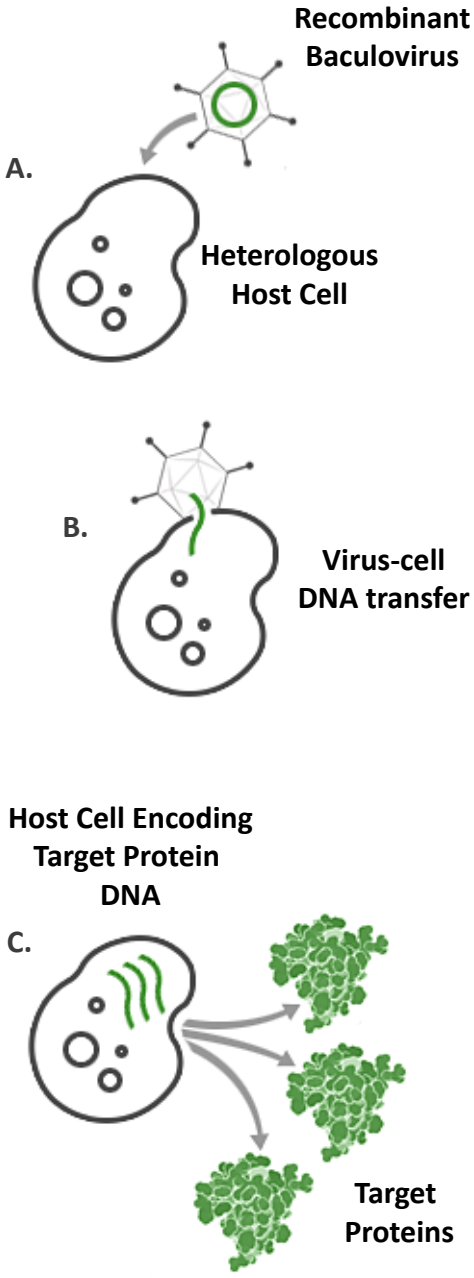
Insect Cell Culture

As said before, BEVS involves the use of a specific virus, capable of infecting only lepidopteran cells. The most widely used insect cells are the Sf9 and Sf21 cell lines. Both the cells lines are valuable choices, but Sf9 cells (that are a sub-clone of the Sf21 cells) are often preferred due to their faster growth rate and higher cell densities. Insect cells are suitable for both small and large-scale cultures. In case of small cultures, shaker or spinner culture flasks may provide adequate starting materials but for bigger productions, the growth of insect cells must be carried out in bioreactors and the culture is constantly monitor with advanced experimental equipment.

• Dissolved Oxygen

A proper monitoring and control over the working parameters is crucial for cell cultures. Nutrient composition of the culture medium, pH, temperature, moisture and other atmosphere parameters are all subjected to a constant process of optimization in order to improve the culture results. Insect cells grow trouble-less in ambient temperature (optimum from 25 to 27°C) and without any supplement of CO_2, but still the culture atmosphere plays a key role in the growth process, for one of the crucial parameters is the dissolved oxygen (DO).

Atmospheric oxygen tends spontaneously to dissolve into water until a saturation equilibrium is reached. For pure water being in



Protein Expression Mechanism:

A. Baculovirus containing the recombinant DNA is inoculated in the insect cell culture.

B. The infection allows the DNA to be tranferred to the cells.

C. Cells encoded with the recombinant DNA start to express the desired proteins.

contact with a pure oxygen atmosphere, the saturation value (i.e. the amount of dissolved oxygen) at 25°C and 1 bar is about 8 mg/l. Since the relation between atmospheric oxygen and dissolved oxygen is directly proportional, when pure oxygen is replaced by a lower oxygen atmosphere, the DO decreases accordingly. In case of pure water being in contact with air (21% of oxygen) the dissolved oxygen results in 21% of the saturation value (so 21% of 8 mg/l, about 1,7 mg/l).

The influence of dissolved oxygen on insect cell cultures is well documented in the scientific literature [1]. Many experimentations make use of standard air as culture atmosphere. In some cases this configuration can prove to be effective, but generally the lack a proper oxygen control is troublesome. The amount of DO can be either too high, causing a cell intolerance, or to low, causing cells starvation [2]. Both the effects are detrimental for cell growth rate and protein expression quality [3,4]. Working with an atmosphere composition control system grants the possibility to undertake advanced experimentations, in which culture's responses to different DO values can be checked with proper tests, allowing the researchers to find the optimal growth condition for each cell culture [5,6].

MCQ solution

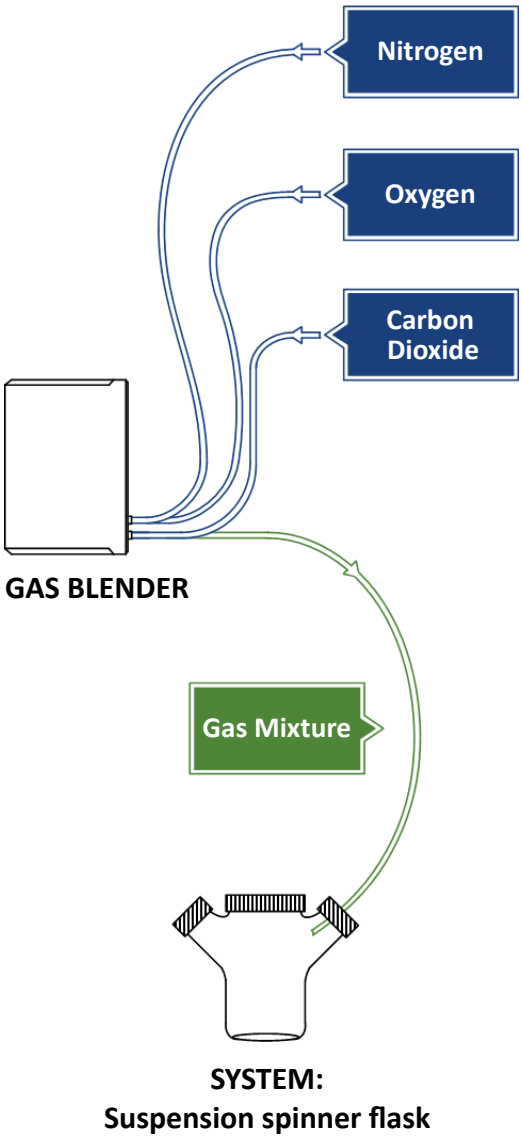
For all those applications in which a fine control over the atmosphere's composition is required, MCQ suggests the use of its Gas Blender 100 Series.

The Gas Blender 100 Series is the ideal instrument for precision gas mixtures preparations and dynamic gas mixtures applications. The Gas Blender 100 Series is a professional product designed to work with 3 components non aggressive gas mixtures. The instrument is calibrated on customer request and in case of need it's possible to work with different gas settings configurations through the use of conversion factor related to each gas media. The Gas Blender 100 Series main features are a high precision (1% accuracy for each channel), a high repeatability (0,16% of reading value) and the fastest response time for setpoint value changes (50 ms) available on the market.

Bundled with the instrument the MCQ provides the Gas Mixer Manager, a software (compatible with any Windows-operating desktop and laptop personal computer) that ensures an easy and intuitive way to manage mixtures dynamically.

Hardware configuration

An example of MCQ Gas Blender 100 Series hardware configuration is represented in the image alongside. The instrument works with dry, non-aggressive gases. The gas sources can be both pure or mixtures (in our example pure gases have been chosen for simplicity). The gas cylinders are connected to the instrument through 6 mm diameter tubes and a check valve is installed along each line as backflow prevention device. Each gas media is connected and controlled by a dedicated channel of the Gas Blender 100. Another 6 mm tube finally connects the instrument to the working system in which the experiment takes place. A PC is connected to the Gas Blender through a simple USB connection. All the instruments features and the gas mixture properties can then be manage with the Gas Mixer Manager software.



References

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Standard configuration for cell culture

Nitrogen, oxygen and carbon dioxide are blended togheter to create the appropriate gas mixture for the experiement. The mixture flows inside a flask in which a suspended cell line is cultured. The % of oxygen in the atmosphere of the flask directly affects the amount of dissolved oxygen inside the growth medium.