needed to improve the amount of protein expression and empty capsid assembly in insect cells.

There is thus a need for an improved method for producing empty virus capsids which produces an immunogenic, stable product at a reasonable yield.

SUMMARY OF ASPECTS OF THE INVENTION

The present inventors have surprisingly found that the reason for the low yield which has historically been associated with FMDV empty capsid production is that the level of 3C protease is too high in the host cell, which causes toxicity. In order to produce empty capsid particles at high yield, it is necessary to balance the expression and/or activity of the 3C protease, so that it is expressed/active enough to cleave the capsid protein precursor but not expressed/active at levels which induce toxicity in the host cell.

Thus, in a first aspect, the present invention provides a construct which, when expressed in a host cell, is capable of producing empty picornaviral capsids, the construct comprising:

(i) a nucleotide sequence encoding a capsid precursor protein;

(ii) a nucleotide sequence encoding a protease capable of cleaving the capsid precursor protein; and

(iii) a control element which controls the expression of the protease

such that, when the construct is present in the host cell, the control element causes the protease to be expressed at a level sufficient to cleave the capsid precursor protein, but not sufficient to induce significant toxicity in the host cell;

wherein the control element is a retrovirus frameshift site.

Also disclosed is, a construct which, when expressed in a host cell, is capable of producing empty virus capsids, the construct comprising:

(i) a nucleotide sequence encoding a capsid precursor protein;

(ii) a nucleotide sequence encoding a protease capable of cleaving the capsid precursor protein; and

(iii) a control element which controls the expression of the protease such that, when the construct is present in the host cell, the control element causes the protease to be expressed at a level sufficient to cleave the capsid precursor protein, but not sufficient to induce significant toxicity in the host cell.

The control element may be derivable from a retrovirus, in particular the control element may be a retrovirus frameshift site, such as the HIV-1 frameshift site. The HIV-1 frameshift site causes a frameshift in about 5% of the mRNAs translated.

The frameshift site may be located between the nucleotide sequence encoding the capsid precursor protein and the nucleotide sequence encoding the protease, such that when the construct is translated:

(1) if the ribosome does not undergo a frameshift it produces a truncated product (which comprises the capsid precursor protein but not the protease); but

(2) if the ribosome does undergo a frameshift, both the capsid precursor protein and the protease are translated.

In the construct of ~~the first aspect of the invention~~one of the examples of the patent disclosure, the activity of the protease may also be reduced. For example, the protease may include at least one mutation which reduces its capsid precursor protein cleaving activity.

The protease (e.g. mutant protease) may have approximately 3-fold lower capsid precursor protein cleaving activity that the wild-type protease.

The empty capsids produced by a host cell comprising a construct according to the first aspect of the invention may be picornavirus capsids, such as Foot and Mouth Disease Virus (FMDV) capsids.

In order to produce empty FMDV capsids, the precursor protein may be P1 and the protease may be 3C. In order to reduce the activity of the 3C protease, it may comprise a mutation, for example at Cys142.

In a second aspect, the present invention provides a vector comprising a construct according to the first aspect of the invention. The vector may, for example, be a baculovirus transfer vector; a DNA vector, plasmid or a viral vector.

In a third aspect, the present invention provides a host cell comprising a construct according to the first aspect of the invention. In a first embodiment, the host cell may be capable of producing a vector in accordance with the second aspect of the invention. In a second embodiment, the host cell may be capable of producing empty virus capsids.

The host cell may, for example be an insect cell or a mammalian cell.

Further aspects of the invention relate to:

(i) a method for producing empty virus capsids by expressing a construct according to the first aspect of the invention in a host cell and harvesting empty virus capsids produced by the host cell;

(ii) a method for the production of a vaccine, by producing empty virus capsids by such a method and incorporating the empty virus capsids in a vaccine;

~~(iii) a method of treating and/or preventing a disease in a subject, by expressing a construct according the first aspect of the invention in the subject such that empty capsids are produced *in vivo*;~~

~~(iv)~~(iii) a construct according to the first aspect of the invention or a vector according to the second aspect of the invention for use in the prevention and/or treatment of a disease;

~~(v)~~(iv) the use of construct according to the first aspect of the invention, or a vector according to the second aspect of the invention in the manufacture of a medicament for the prevention and/or treatment of a disease;

(v) the use of a retroviral control element to control the expression of a picornavirus protein, wherein the retroviral control element is a frameshift site and the picornavirus protein is a protease; and

(vi) a construct which comprises a nucleotide sequence encoding a picornavirus protein under the control of a retroviral control element, wherein the picornavirus protein is a protease and the retroviral control element is a frameshift site.

Also disclosed are:

~~(vi)~~(i) the use of a retroviral control element to control the expression of a picornavirus protein; ~~and~~

~~(vii)~~(ii) a construct which comprises a nucleotide sequence encoding a picornavirus protein under the control of a retroviral control element; and

(iii) a method of treating and/or preventing a disease in a subject, by expressing a construct according the first aspect of the invention in the subject such that empty capsids are produced *in vivo*;

"Empty capsid"-based vaccines are advantageous over traditional inactivated whole-pathogen vaccines (e.g. for FMD) because that do not require high-security containment facilities for manufacture, thus alleviating any risk of virus "escape". They are also non-infectious, easy to manipulate and (in view of the present invention) easy and inexpensive to prepare.