Claims

Claim 1. An insect cell comprising no more than two nucleic acid construct consisting of:

i) a first expression cassette comprising a first promoter operably linked to a nucleotide sequence encoding an mRNA, translation of which in the cell produces at least one of parvoviral Rep 78 and 68 proteins;

ii) a second expression cassette comprising a second promoter operably linked to a nucleotide sequence encoding an mRNA, translation of which in the cell produces at least one of parvoviral Rep 52 and 40 proteins;

iii) a third expression cassette comprising a third promoter operably linked to a nucleotide sequence encoding parvoviral VP1, VP2, and VP3 capsid proteins; and,

iv) a nucleotide sequence comprising a transgene that is flanked by at least one parvoviral inverted terminal repeat sequence, wherein the nucleotide sequence comprising the transgene flanked by the parvoviral inverted terminal repeat sequence is present on a second nucleic acid construct,

wherein, at least one of the first and second expression cassette are present on a first nucleic acid construct with the third expression cassette, and

wherein, upon transfection of the cell with the first and second nucleic acid constructs, the first promoter is active before the second and third promoters.

Claim 2. An insect cell according to claim 1, wherein the second nucleic acid construct further comprises a fourth expression cassette comprising a fourth promoter operably linked to a nucleotide sequence encoding parvoviral VP1, VP2, and VP3 capsid proteins, wherein the first promoter is active before the second, third and fourth promoters

Claim 3. An insect cell according to claim 1, wherein the third and fourth promoters are identical, and wherein optionally, the parvoviral VP1, VP2, and VP3 capsid proteins encoded by the nucleotide sequences in the third and fourth expression cassettes are identical.

Claim 4. An insect cell according to claim 3, wherein the at least one of parvoviral Rep 78 and 68 proteins and the at least one of parvoviral Rep 52 and 40 proteins comprise a common amino acid sequence comprising the amino acid sequence from the second amino acid to the most C-terminal amino acid of the at least one of parvoviral Rep 52 and 40 proteins, wherein the common amino acid sequences of the at least one of parvoviral Rep 78 and 68 proteins and the at least one of parvoviral Rep 52 and 40 proteins are at least 90% identical, and wherein the nucleotide sequence encoding the common amino acid sequence of the at least one of parvoviral Rep 78 and 68 proteins and the nucleotide sequence encoding the common amino acid sequences of the at least one of parvoviral Rep 52 and 40 proteins are less than 90% identical.

Claim 5. An insect cell according to claim 4, wherein the common amino acid sequences of the at least one of parvoviral Rep 78 and 68 proteins and the at least one of parvoviral Rep 52 and 40 proteins are at least 99% identical, preferably 100% identical.

Claim 6. An insect cell according to claim 4 or 5, wherein the nucleotide sequence encoding the common amino acid sequence of the at least one of parvoviral Rep 78 and 68 proteins has an improved codon usage bias for the cell as compared to the nucleotide sequence encoding the common amino acid sequences of the at least one of parvoviral Rep 52 and 40, or wherein the nucleotide sequence encoding the common amino acid sequence of the at least one of parvoviral Rep 52 and 40 proteins has an improved codon usage bias for the cell as compared to the nucleotide sequence encoding the common amino acid sequences of the at least one of parvoviral Rep 78 and 68 proteins, wherein preferably, the difference in codon adaptation index between the nucleotide sequences coding for the common amino acid sequences in the at least one of parvoviral Rep 78 and 68 proteins and the at least one of parvoviral Rep 52 and 40 proteins is at least 0.2.

Claim 7. An insect cell according to any one of the preceding claims, wherein the first promoter is a constitutive promoter.

Claim 8. An insect cell according to any one of the preceding claims, wherein at least one of the second, third and fourth promoters is an inducible promoter.

Claim 9. An insect cell according to claim 8, wherein the inducible promoter is a viral promoter that is induced at least 24 hours after transfection or infection of the cell with the virus.

Claim 10. An insect cell according to any one of the preceding claims, wherein at least one of the first and second nucleic acid construct is stably integrated in the genome of the insect cell.

Claim 11. An insect cell according to any one of the preceding claims, wherein at least one the first and second nucleic acid construct is an insect cell-compatible vector, preferably a baculoviral vector.

Claim 12. An insect cell according to claim 11, wherein:

a) the first promoter is selected from a deltaEl promoter and an El promoter; and,

b) the second, third and fourth promoters are selected from a polH promoter and a p10 promoter.

Claim 13. An insect cell according to claim 11 or 12, wherein at least one expression cassette comprises at least one baculovirus enhancer element and/or at least one ecdysone responsive element, wherein preferable the enhancer element is selected from the group consisting of hr1, hr2, hr2.09, hr3, hr4, hr4b and hr5.

Claim 14. An insect cell according to any one of the preceding claims, wherein the nucleotide sequence encoding an mRNA, translation of which in the cell produces only at least one of parvoviral Rep 78 and 68 proteins, comprises an intact parvoviral p19 promoter.

Claim 15. An insect cell according to any one of the preceding claims, wherein the at least one of parvoviral Rep 78 and 68 proteins, the at least one of parvoviral Rep 52 and 40 proteins, the parvoviral VP1, VP2, and VP3 capsid proteins and the at least one parvoviral inverted terminal repeat sequence are from an adeno associated virus (AAV).

Claim 16. An insect cell according to any one of claims 4 to 15, wherein the first nucleic acid construct is DuoBac CapRep6 (SEQ ID NO. 10) and the second nucleic acid construct is DuoBac CapTrans1 (SEQ ID NO. 12), and wherein preferably the first and second constructs are present in a 3 : 1 molar ratio.

Claim 17. A method for producing a recombinant parvoviral virion in a cell comprising the steps of:

a) culturing an insect cell as defined in any one of claims 1 - 16 under conditions such that recombinant parvoviral virion is produced; and,

b) recovery of the recombinant parvoviral virion,

wherein no more than two nucleic acid constructs are employed in the insect cell for the production of the recombinant parvoviral vector.

Claim 18. A method according to claim 17, wherein the parvoviral virion is an AAV virion.

Claim 19. A method according to claim 17 or 18, wherein recovery of the recombinant parvoviral virion in step b) comprises at least one of affinity-purification of the virion using an immobilised anti-parvoviral antibody, preferably a single chain camelid antibody, or filtration over a filter having a nominal pore size of 30 - 70 nm.

Claim 20. A first nucleic acid construct as defined in any one of claims 1 - 15.

Claim 21. A second nucleic acid construct as defined in any one of claims 1 - 15.

Claim 22. A kit of parts comprising at least a first nucleic acid construct as defined in any one of claims 1 - 15 and a second nucleic acid construct as defined in any one of claims 1 - 15.