Claims

1. A combination of a nucleic acid molecule, which is also referred to as third nucleic acid molecule, and a nucleic acid molecule, which is also referred to as second nucleic acid molecule,

wherein the third nucleic acid molecule comprises

(1) a nucleic acid molecule comprising the following elements:

(a) a first part of a genome of an adenovirus, wherein the first part of a genome of an adenovirus comprises exactly one inverted terminal repeat of an adenovirus;

(b) a nucleotide sequence, preferably a genomic nucleotide sequence, or a transcription unit;

(c) a regulatory nucleic acid sequence which has a regulatory activity in a prokaryote;

(d) exactly one site-specific recombination site recombining in the presence of a corresponding recombinase;

(e) a nucleotide sequence providing for a negative selection marker;

(f) a bacterial nucleotide sequence unit comprising (i) bacterial nucleotide sequences for conditional replication and (ii) a nucleotide sequence providing for a positive selection marker; and

(g) a first restriction site; or

(2) a nucleic acid molecule comprising a nucleotide sequence according to SEQ ID NO: 6; or

(3) a heterologous nucleic acid molecule comprising a third nucleic acid molecule as defined in (1) contained in the organism deposited with the DSMZ under the Budapest treaty under accession number DSM 23754;

and

wherein the second nucleic acid molecule comprises

(1) a nucleic acid molecule comprising the following elements:

(a) a bacterial nucleotide sequence unit comprising
(i) bacterial nucleotide sequences for single copy replication, and
(ii) a nucleotide sequence providing for a selection marker;

(b) exactly one site-specific recombination site recombining in the presence of a corresponding recombinase;

(c) a second part of a genome of an adenovirus, wherein the second part of a genome of an adenovirus comprises exactly one inverted terminal repeat of an adenovirus; and

(d) a restriction site which is referred to as second restriction site; or

(2) a nucleic acid molecule comprising a nucleotide sequence according to SEQ ID NO: 2 and/or SEQ ID NO: 13 and/or SEQ ID NO: 14; or

(3) a heterologous nucleic acid molecule comprising a second nucleic acid molecule as defined in (1) contained in the organism deposited with the DSMZ under the Budapest treaty under accession number DSM 24298 and/or DSM 24299;

wherein the second nucleic acid molecule and the third nucleic acid molecule each and independently is either a linear molecule or a circular molecule, preferably the second nucleic acid molecule is a circular molecule and the third nucleic acid molecule is a circular molecule;

wherein the second nucleic acid molecule and the third nucleic acid molecule complement each other to form a complete viral genome; and

wherein the recombinase recombining the recombination site of the third nucleic acid molecule is the same as the recombinase recombining the recombination site of the second nucleic acid molecule.

2. The combination according to claim 1, wherein in the nucleic acid molecule of (1) the regulatory nucleic acid sequence which has a regulatory activity in a prokaryote, the site-specific recombination site and the nucleotide sequence providing for a negative selection marker are arranged in a 5’ -> 3’ direction.

3. The combination according to any one of claims 1 to 2, wherein the third nucleic acid molecule is a linear molecule, wherein elements (a) to (f), preferably upon cleavage of the circular molecule of the third nucleic acid molecule with the first restriction enzyme which recognizes and cleaves at the first restriction site, are arranged in a 5’ -> 3’ direction in the following sequence as follows:

1. the first part of a genome of an adenovirus;

2. the nucleotide sequence, preferably the genomic nucleotide sequence, or the transcription unit;

3. the regulatory nucleic acid sequence which has a regulatory activity in a prokaryote;

4. the site-specific recombination site recombining in the presence of a corresponding recombinase;

5. the nucleotide sequence providing for a negative selection marker; and

6. the bacterial nucleotide sequence unit comprising (i) bacterial nucleotide sequences for conditional replication and (ii) a nucleotide sequence providing for a positive selection marker.

4. The combination according to any one of claims 1 to 3, wherein the adenovirus is a human adenovirus and preferably the adenovirus is human adenovirus type 5, and more preferably the entire left end of adenovirus type 5 upstream of the TATA box of the E1 transcription unit, or one or several parts thereof.

5. The combination according to any one of claims 1 to 4, preferably claim 4, wherein the bacterial nucleotide sequences for conditional replication comprise an origin of replication, whereby preferably the origin of replication is the minimal origin of phage gR6K.

6. The combination according to any one of claims 1 to 5, preferably any one of claims 4 to 5, wherein the regulatory sequence which has a regulatory activity in a prokaryote is a sequence which directs expression of a nucleotide sequence in a prokaryote, preferably in a prokaryotic host cell.

7. The combination according to any one of claims 1 to 6, preferably any of claims 5 to 6, wherein the negative selection marker or the expression of the nucleotide sequence providing for a negative selection marker mediates or confers sensitivity to a selecting agent and/or a selecting condition.

8. The combination according to claim 7, wherein the nucleotide sequence providing for a negative selection marker is a gene selected from the group comprising the *galK*, *tetAR*, *pheS*, *thyA*, *lacy*, *ccdB* and *rpsL* gene.

9. A combination of a nucleic acid molecule which is also referred to as first nucleic acid molecule, and a nucleic acid molecule which is also referred to as second nucleic acid molecule,

wherein the first nucleic acid molecule comprises

(1) a nucleic acid molecule comprising the following elements:

(a) exactly one site-specific recombination site recombining in the presence of a corresponding recombinase;

(b) a bacterial nucleotide sequence unit comprising (i) bacterial nucleotide sequences for conditional replication and (ii) a nucleotide sequence providing for a first selection marker;

(c) a first part of a genome of an adenovirus, wherein the first part of a genome of an adenovirus comprises exactly one inverted terminal repeat of an adenovirus;

(d) a transcription unit; and

(e) a first restriction site; or

(2) a nucleic acid molecule comprising a nucleotide sequence according to SEQ ID NO:1 and/or SEQ ID No:15; or

(3) a heterologous nucleic acid molecule comprising a first nucleic acid molecule as defined in (1) contained in the organism deposited with the DSMZ according to the Budapest treaty under accession number DSM 23753;

and wherein the second nucleic acid molecule comprises

(1) a nucleic acid molecule comprising the following elements:

(a) a bacterial nucleotide sequence unit comprising
(i) bacterial nucleotide sequences for single copy replication, and
(ii) a nucleotide sequence providing for a selection marker;

(b) exactly one site-specific recombination site recombining in the presence of a corresponding recombinase;

(c) a second part of a genome of an adenovirus, wherein the second part of a genome of an adenovirus comprises exactly one inverted terminal repeat of an adenovirus; and

(d) a restriction site which is referred to as second restriction site; or

(2) a nucleic acid molecule comprising a nucleotide sequence according to SEQ ID NO: 2 and/or SEQ ID NO: 13 and/or SEQ ID NO: 14; or

(3) a heterologous nucleic acid molecule comprising a second nucleic acid molecule as defined in (1) contained in the organism deposited with the DSMZ under the Budapest treaty under accession number DSM 24298 and/or DSM 24299, wherein preferably the nucleic acid molecule contained in the organism is a heterologous nucleic acid molecule;

and wherein the first nucleic acid molecule and the second nucleic acid molecule each and independently is either a linear molecule or a circular molecule, preferably the first nucleic acid molecule is a circular molecule and the second nucleic acid molecule is a circular molecule;

wherein the first nucleic acid molecule and the second nucleic acid molecule complement each other to form a complete viral genome; and

wherein the recombinase recombining the recombination site of the first nucleic acid molecule is the same as the recombinase recombining the recombination site of the second nucleic acid molecule.

10. The combination according to claim 9, wherein the genome of an adenovirus of the first nucleic acid molecule is a human adenovirus genome, preferably a human adenovirus genome which is different from human adenovirus type 5 genome, more preferably the genome of an adenovirus of the first nucleic acid molecule is a human adenoviral type 19a genome.

11. The combination according to any one of claims 9 to 10, wherein the bacterial nucleotide sequences for conditional replication of the first nucleic acid molecule comprise an origin of replication.

12. The combination according to any one of claims 9 to 11, wherein the sequence providing for a first selection marker of the first nucleic acid molecule is a nucleic acid sequence coding for an enzyme which is conferring resistance to a host cell harbouring such nucleic acid sequence coding for an enzyme.

13. The combination according to any one of claims 9 to 12, wherein the first part of a genome of an adenovirus of the first nucleic acid molecule is an adenoviral terminal repeat.

14. The combination according to any one of claims 9 to 13, wherein the first part of a genome of an adenovirus of the first nucleic acid molecule contains the adenoviral promoter pIX, preferably the adenoviral promoter pIX is a pIX promoter from human adenovirus 19a.

15. The combination according to any one of claims 1 to 14, wherein the adenovirus genome of the second nucleic acid molecule is a human adenovirus genome, whereby in case of the combination according to claim 1 the adenovirus genome of the second nucleic acid molecule is preferably a human adenovirus type 5 genome or a human adenoviral type 19a genome and in case of the combination according to claim 9 the adenovirus genome of the second nucleic acid molecule is preferably a human adenovirus genome which is different from human adenovirus type 5 genome, more preferably the virus genome of the second nucleic acid molecule is a human adenoviral type 19a genome.

16. The combination according to any one of claims 1 to 15, wherein the bacterial nucleotide sequence for single copy replication of the second nucleic acid molecule comprises a replication origin for single copy maintenance in prokaryotic host cells.

17. The combination according to any one of claims 1 to 16, wherein the nucleotide sequence providing for a selection marker of the second nucleic acid molecule is a nucleic acid sequence coding for an enzyme which is conferring resistance to a host cell harbouring such nucleic acid sequence coding to an enzyme.

18. The combination according to any one of claims 1 to 17, wherein the second part of a genome of an adenovirus of the second nucleic acid molecule comprises an adenoviral right inverted terminal repeat and the first part of a genome of an adenovirus of the first nucleic acid molecule and the third nucleic acid molecule comprises an adenoviral left inverted terminal repeat.

19. A method for the generation of a nucleic acid molecule coding for a virus comprising the following steps

a) providing a third nucleic acid molecule as defined in any one of claims 1 to 8;

b) providing a second nucleic acid molecule as defined in any one of claims 1 to 8; or

c) providing a combination of a third nucleic acid molecule and a second nucleic acid molecule according to any one of claims 1 to 8 and 15 to 18;

d) allowing the third and the second nucleic acid molecule to react so that a site-specific recombination occurs, wherein the site-specific recombination is mediated by a site-specific recombinase and the site-specific recombination forms a recombination product comprising a copy, preferably single copy of the genome of a or the virus, whereby the genome is a complemented complete genome and the complemented complete genome is complemented by the site-specific recombination;

e) selecting the recombination product; and

f) optionally cleaving the recombination product with the first and second restriction enzyme.

20. A method for the generation of a nucleic acid molecule coding for a virus comprising the following steps

a) providing a combination of a first nucleic acid molecule and a second nucleic acid molecule according to any one of claims 9 to 18;

b) allowing the first and the second nucleic acid molecule to react so that a site-specific recombination occurs, wherein the site-specific recombination is mediated by a site-specific recombinase and the site-specific recombination forms a recombination product comprising a copy, preferably single copy of the genome of a or the virus, whereby the genome is a complemented complete genome and the complemented complete genome is complemented by the site-specific recombination;

c) selecting the recombination product; and

d) optionally cleaving the recombination product with the first and second restriction enzyme.

21. The method according to claim 19, wherein the third and the second nucleic acid molecule are reacted in a prokaryotic host cell, preferably in *E. coli*.

22. The method according to claim 20, wherein the first and the second nucleic acid molecule are reacted in a prokaryotic host cell, preferably in *E. coli*.

23. A method for generating a library of nucleotide sequences, wherein said library comprises a plurality of individual nucleotide sequences, wherein said library is represented by a plurality of viral genomes and each viral genome contains a single one of the individual nucleotide sequences, comprising the steps of the method as defined in any of claims 19 and 21, wherein the individual nucleotide sequence is part of the transcription unit of the third nucleic acid molecule.

24. A method for generating a library of nucleotide sequences, wherein said library comprises a plurality of individual nucleotide sequences, wherein said library is represented by a plurality of viral genomes and each viral genome contains a single one of the individual nucleotide sequences, comprising the steps of the method as defined in any of claims 20 and 22, wherein the individual nucleotide sequence is part of the transcription unit of the first nucleic acid molecule.

25. A kit comprising optionally a package insert, and, in (a) suitable container(s), at least a combination of the third nucleic acid molecule and the second nucleic acid molecule according to any one of claims 1 to 8 and 15 to 18.

26. A kit comprising optionally a package insert, and, in (a) suitable container(s), at least a combination of the first nucleic acid molecule and the second nucleic acid molecule according to any one of claims 9 to 18.

27. The kit according to any one of claims 25 and 26, wherein the nucleic acid molecule(s) is/are contained in a ready- to-use form and/or wherein the kit contains a permissive cell line providing a recombinase and instructions for use.