Claims:

1. A method of preparing a shuttle vector comprising a selection marker gene, a low copy bacterial replication of origin and an adenovirus genome comprising a 5' ITR, a 3' ITR, and an L5 gene, said method comprising the steps:

   a) preparing an adenovirus shuttle vector comprising ligating equal proportions the following three fragments:

      i) a vector fragment comprising a selection marker gene and a low copy bacterial replication of origin, wherein the 5' end of the vector fragment starts with a first restriction enzyme site and terminates at the 3' end of the vector fragment in a second restriction enzyme site,

      ii) a 5'-arm comprising the 5' end of the adenovirus genome including the 5' ITR, wherein the 5' end of the 5' arm starts with a second restriction enzyme site and terminates at the 3' end of the 5' arm with a third restriction enzyme site,

      iii) a 3'-arm comprising the 3' end of the adenovirus genome including the 3' ITR and the L5 gene, wherein the 5' end of the 3' arm starts with a third restriction enzyme site and terminates at the 3' end of the 3' arm with a first restriction enzyme site,

   and performing a one-step three-way ligation to join:

   the 3' end of the 3' arm (fragment iii) to the 5' end of the vector fragment (fragment i) at the first restriction enzyme site,

   the 3' end of the vector fragment (fragment i) to the 5' end of the 5' arm (fragment ii) at the second restriction enzyme site, and

   the 3' end of the 5' arm (fragment ii) to the 5' end of the 3' arm (fragment iii) at the third restriction enzyme site at least the L5 gene,

   to form a circularised shuttle vector arranged as a first restriction enzyme site followed by a vector fragment followed by a second restriction enzyme site followed by a 5' arm, followed by a third restriction enzyme site followed by a 3' arm,

   b) introducing at least one unique restriction site and/or transgene into the shuttle vector in a location between the L5 gene and a site selected from the group comprising (or consisting of) an E3 site, an E4 site or both said sites.

2. A method according to claim 1, wherein the 5' arm comprises about 2.4 to 4.7 kb of the 5' end of an adenovirus genome.

3. A method according to claim 1 or 2, wherein the 3' arm comprises about 3.3 to 4.8 kb of the 3' end of an adenovirus genome.

4. A method according to any one of claims 1 to 3, wherein the 3 way ligation is performed with the ratio of 1:1:1, for fragment i), ii) and iii).

5. A method according to any one of claims 1 to 4, wherein the one-step three-way ligation is performed for at least 50 minutes.

6. A method according to any one of claims 1 to 5, wherein the one-step three-way ligation is performed at approximately room temperature, such as 20 to 25^{o}C.

7. A method according to any one of claims 1 to 6, wherein the unique restriction site is independently selected from FseI, NotI, SbfI and SgfI, such as NotI or SbfI and SgfI or FseI and NotI and SbfI and SgfI.

8. A method according to any one of claims 1 to 7, wherein the first and second restriction enzyme sites are the same.
9. A method according to any one of claims 1 to 8, wherein the vector fragment is dephosphorylated.
10. A method according to any one of claims 1 to 9, wherein the replication of origin is p15A.
11. A method according to any one of claims 1 to 10, wherein the selection marker gene is KanR.
12. A method according to any one of claims 1 to 11, wherein the adenovirus genome is from an adenovirus capable of replication.
13. A method according to any one of claims 1 to 12, which further comprises a step c) performing homologous recombination between the shuttle vector of step a) or step b) and an adenovirus genome to form a plasmid.
14. A method according to claim 13 wherein step c) is performed at a ratio of 3.5:1.5.
15. A method according to claim 12 or 13, wherein step c) is performed in electrocompetent BJ5183 cells.
16. A method according to any one of claims 1 to 15, wherein the adenovirus genome is selected from EnAd1, OvAd1, OvAd2, Ad3, Ad5 and Ad11.
17. A method according to any one of claims 13 to 16, comprising a futher step of generating an adenovirus, for example a replication capable adenovirus or a replication deficient adenovirus vector.
18. A method according to claim 17, which comprises a further step of formulating an adenovirus as a pharmaceutical composition.
19. An adenovirus plasmid comprising:
    a) an adenovirus genome comprising an L5 gene, an E3 site and an E4 site,
    b) at least one unique restriction site in a location between the L5 gene and the E3 site, and/or at least one unique restriction site in a location between the L5 gene and the E4 site,
    c) a low copy bacterial origin of replication, and
    d) a selection marker gene.
20. An adenovirus plasmid according to claim 19, wherein the adenovirus genome is a subgroup B adenovirus genome, for example EnAd, OvAd1, OvAd2, Ad3, Ad11 and Ad5, such as EnAd.
21. An adenovirus plasmid according to claim 19 or 20, wherein the unique restriction site is independently selected from FseI, NotI, SbfI and SgfI.
22. An adenovirus plasmid according to any one of claims 19 to 21, wherein the unique restriction site is in a location between the L5 gene and the E3 site, for example wherein the original restriction site is NotI.
23. An adenovirus plasmid according to any one of claims 19 to 22, wherein there are at least two unique restriction sites in a location between the L5 gene and the E4 site, for example wherein the two original restriction sites are SbfI and SgfI.
24. An adenovirus plasmid according to any one of claims 19 to 23, wherein there are three unique restriction sites in a location between the L5 gene and the E4 site.
25. An adenovirus plasmid according to claim 24, wherein the three unique restriction sites are one each of SbfI, SgfI and FseI.

26. An adenovirus plasmid according to any one of claims 19 to 25, which further comprises at least one unique restriction site located within the E1 region.
27. An adenovirus plasmid according to any one of claims 19 to 26, which further comprises a transgene cassette, for example comprising one or more transgenes, in particular independently selected from: a therapeutic gene of interest which encodes a therapeutic protein, peptide or RNA such as an antibody or antibody domain, pro-drug converting enzyme, immunomodulator, enzyme, siRNA, transcription factor, intracellular signalling or surface membrane protein, or antigen; and a reporter gene or imaging agent, such as luciferase or eGFP.
28. A pharmaceutical composition comprising an adenovirus plasmid according to any one of claims 19 to 27, and a pharmaceutically acceptable excipient.
29. Use of an adenovirus plasmid according to any one of claims 1 to 27 or a pharmaceutical composition according to claim 28 in the manufacture of a medicament for the treatment of cancer.