CLAIMS

1. A method for generating a recombinant adenovirus comprising a nucleotide sequence encoding a heterologous gene of interest for use as a vaccine comprising the steps of:

(i) inserting the heterologous gene of interest into the adenovirus genome by recombining terminal protein complexed adenovirus genomic DNA (TPC-Ad gDNA) with a polynucleotide comprising a nucleotide sequence encoding the gene of interest and having 5' and 3' ends that are homologous to the insertion site sequence of the adenovirus genomic DNA in an *in vitro* recombination reaction,

(ii) transfecting cells growing in individual vessels with a dilution of the *in vitro* recombination reaction mixture from (i) such that a number of such individual vessels contain a single cell that is infected by a recombinant adenovirus comprising the nucleotide sequence encoding the heterologous gene of interest, and

(iii) identifying those individual vessels in which a single cell has been infected by the recombinant adenovirus comprising the nucleotide sequence encoding the heterologous gene of interest.

2. A method according to Claim 1, in which the TPC-Ad gDNA comprises serotype-matched terminal protein and adenovirus genome.

3. A method according to Claim 1 or 2, in which the gene of interest codes for a single epitope, a string of epitopes, a segment of an antigen or a complete antigen protein.

4. A method according to Claim 1, 2 or 3, in which the polynucleotide has between 5 and 50bp at its 5' end and between 5 and 50bp at its 3' end that are homologous to the insertion site sequence of the adenovirus genomic DNA, or in which the polynucleotide has between 10 and 20bp at its 5' end and between 10 and 20bp at its 3' end that are homologous to the insertion site sequence of the adenovirus genomic DNA, or in which the polynucleotide has 15bp at its 5' end and 15bp at its 3' end that are homologous to the insertion site sequence of the adenovirus genomic DNA.

5. A method according to any preceding claim, in which the insertion site sequence of the adenovirus genomic DNA is located within the E1 locus.

6. A method according to any preceding claim, in which the TPC-Ad gDNA is digested at a unique restriction site within the insertion site sequence of the adenovirus genomic DNA that is flanked at its 5' end by the long tetracycline-regulated CMV promoter that drives expression of the gene of interest and at its 3' end by the bovine growth hormone polyadenylation sequence.

7. A method according to any preceding claim, in which the *in vitro* recombination reaction comprises 40ng digested TPC-Ad gDNA and 44fmol 3' and 5' ends of synthetic DNA encoding the gene of interest.

8. A method according to any preceding claim, in which cells to be transfected are seeded in individual vessels at a density of 3.75×10^5 cells.ml^{-1} one day before transfection.

9. A method according to any preceding claim, in which cells are transfected while growing at approximately 80% confluence in individual vessels.

10. A method according to any preceding claim, in which the *in vitro* recombination reaction mixture is diluted in transfection medium and divided equally so as to transfect cells growing in 60 individual vessels.

11. A method according to any preceding claim, in which transfected cells are frozen and thawed to release cell-associated virus and presence of recombinant adenovirus is identified by quantitative PCR (qPCR) using cell lysate from each well of transfected cells and a set of primers and a probe designed to bind to the left end of the genome downstream of the adenoviral inverted terminal repeat (ITR) and upstream of the insertion site of the gene of interest in a non-coding region.

12. A method according to any preceding claim, in which the adenovirus genome is derived from a simian adenovirus.

13. A method according to Claim 12, in which the simian adenovirus is ChAdOx1 or ChAdOx2 or ChAd3 or ChAd63.

14. A method according to any of Claims 1 to 11, in which the adenovirus genome is derived from a human adenovirus.

15. A recombinant adenovirus produced by the method according to any preceding claim.