while the *pol* gene products are involved in the synthesis polynucleotides and recombination. The *env* gene codes for the envelope components of the retrovirus particle. 5' and 3' LTR regions include promoters and assist in the integration of the viral genome into the chromosomal DNA of the host cell. The psi signal refers to the retroviral packaging signal that controls the efficient packaging of the RNA into the viral particle.

[0006] Because of their ability to form proviruses, retroviruses are useful to modify the genome of a target or host cell and various modifications have been made to retroviruses for use in gene therapy. Gene therapy using retroviral vectors is generally performed by adding a heterologous polynucleotide to the viral genome which encodes or produces a polypeptide or transcript of interest, packaging the recombinant genome into a viral particle and infecting a target host cell. The target cell will then incorporate the exogenous gene as being a part of a provirus.

[0007] Most retroviral vectors have been rendered "defective" to avoid uncontrolled spread and production of virions. However, little is reported about the development of replication competent retroviral vector systems.

SUMMARY

[0008] The disclosure ~~provides~~describes cell lines and viral particle producing cells useful for producing recombinant replication competent retroviral vectors for gene therapy.

[0008a] In one aspect, the present invention refers to a retrovirus producing HT1080 cell line that produces a replication competent retrovirus particle, said HT1080 cell line stably expresses a recombinant retroviral genome comprising a *gag* gene, *pol* gene, *env* gene, a heterologous polynucleotide, and retroviral *psi* (Ψ) factor for the assembly of the recombinant retroviral genome, wherein the HT1080 cell line been adapted to being grown and continuously passaged in serum free media and in suspension.

[0008b] In another aspect, the present invention refers to a method of producing the retrovirus producing HT1080 cell line as disclosed herein comprising:

transforming a 293 cell line with a plasmid encoding a retroviral vector comprising from 5′ to 3′:

a CMV-R-U5 fusion of the immediate early promoter from human cytomegalovirus to an MLV R-U5 region;

a PBS, primer binding site for reverse transcriptase;

a 5′ splice site;

ψ packaging signal;

a gag coding sequence for MLV group specific antigen;

a pol coding sequence for MLV polymerase polyprotein;

a 3′ splice site;

a 4070A env coding sequence for envelope protein of MLV strain 4070A;

an internal ribosome entry site (IRES) from encephalomyocarditis virus or a nucleic acid regulatory domain;

a modified cytosine deaminase coding sequence;

a polypurine tract; and

a U3-R-U5 MLV long terminal repeat;

culturing the 293 cell to produce viral particles;

isolating the viral particles;

infecting an HT1080 cell line with the viral particles thereby affording a retrovirus producing HT1080 cell line that produces replication competent retrovirus particles, wherein the retrovirus producing HT1080 cell line is grown in serum free media and in suspension.

[0008c] In yet another aspect, the present invention refers to a method for producing a composition for gene therapy comprising culturing the cell line as disclosed herein to produce viral particles and substantially purifying the viral particles.

[0009] The disclosure ~~provides~~describes retrovirus producing cell line for the production of a replication competent retrovirus particle, the cell line comprising a fibrosarcoma, an osteosarcoma or a thymoma cell line, said cell line stably expressing a recombinant retroviral genome comprising a *gag* gene, *pol* gene, *env* gene, a heterologous polynucleotide, and retroviral *psi* (Ψ) factor for the assembly of the recombinant retroviral genome. In one ~~embodiment~~example, the replication competent retrovirus particle is stably expressed. In another ~~embodiment~~example, the half life is greater than 7 days at 2-8°C. In yet another ~~embodiment~~example, viral particles produced from the cell line show no loss of infectivity after 12 months of storage of the cell line at 65 C. In yet another ~~embodiment~~example, the vector produced is approximately 100% stable for 3 months or longer and the

same vector produced from a cell line transiently transfected with the same replication competent retrovirus loses at least five-fold activity at 2 to 8 weeks under the same storage conditions, compared to initial titers. In yet another ~~embodiment~~example the replication competent retrovirus comprises: a retroviral GAG protein; a retroviral POL protein; a retroviral envelope; a retroviral polynucleotide comprising Long-Terminal Repeat (LTR) sequences at the 3' end of the retroviral polynucleotide sequence, a promoter sequence at the 5' end of the retroviral polynucleotide, said promoter being suitable for expression in a mammalian cell, a gag nucleic acid domain, a pol nucleic acid domain and an env nucleic acid domain; a cassette comprising an internal ribosome entry site (IRES) or regulatory nucleic acid domain operably linked to a heterologous polynucleotide, wherein the cassette is positioned 5' to the 3' LTR and 3' to the env nucleic acid domain encoding the retroviral envelope; and cis-acting sequences necessary for reverse transcription, packaging and integration in a target cell,

[0010] wherein the RCR maintains higher replication competency after 6 passages compared to a pACE vector. In one ~~embodiment~~example, the retroviral polynucleotide sequence is derived from murine leukemia virus (MLV), Moloney murine leukemia virus (MoMLV), or Gibbon ape leukemia virus (GALV), murine mammary tumor virus (MuMTV), Rous Sarcoma Virus (RSV), Gibbon ape leukemia virus (GALV), baboon endogenous virus (BEV), and the feline virus RD114. In a further ~~embodiment~~example, the MLV is an amphotropic MLV. In yet another ~~embodiment~~example, retrovirus is a gammaretrovirus. In one ~~embodiment~~example, the promoter sequence is associated with a growth regulatory gene. In yet another ~~embodiment~~example, the nucleic acid regulatory domain comprises a pol II promoter. In yet another ~~embodiment~~example, the promoter sequence comprises a tissue-specific promoter sequence such as an androgen response element. In one ~~embodiment~~example, the androgen response element is derived from a probasin promoter.

[0011] The retroviral vector produced by the producer cell line comprise various domain. For example, the promoter comprises a CMV promoter having a sequence as set forth in SEQ ID NO:19, 20 or 22 from nucleotide 1 to about nucleotide 582 and may include modification to one or more nucleic acid bases and which is capable

of directing and initiating transcription; a CMV-R-U5 domain polynucleotide comprises a sequence as set forth in SEQ ID NO:19, 20 or 22 from about nucleotide 1 to about nucleotide 1202 or sequences that are at least 95% identical to a sequence as set forth in SEQ ID NO:19, 20 or 22, wherein the polynucleotide promotes transcription of a nucleic acid molecule operably linked thereto; the gag nucleic acid domain comprises a sequence from about nucleotide number 1203 to about nucleotide 2819 of SEQ ID NO: 19 or 22 or a sequence having at least 95%, 98%, 99% or 99.8% identity thereto; the pol domain comprises a sequence from about nucleotide number 2820 to about nucleotide 6358 of SEQ ID NO:19 or 22 or a sequence having at least 95%, 98%, 99% or 99.9% identity thereto; the env domain comprises a sequence from about nucleotide number 6359 to about nucleotide 8323 of SEQ ID NO:19 or 22 or a sequence having at least 95%, 98%, 99% or 99.8% identity thereto; the IRES comprises a sequence from about nucleotide number 8327 to about nucleotide 8876 of SEQ ID NO:19 or 22 or a sequence having at least 95%, 98%, or 99% identity thereto; and the heterologous nucleic acid comprises a polynucleotide having a sequence as set forth in SEQ ID NO:3, 5, 11, 13, 15 or 17.

[0012] The disclosure also ~~provides~~describes a cell free preparation comprising viral particles obtained from the retrovirus producing cell line described herein. In some ~~embodiments~~examples a pharmaceutical preparation is prepared from the isolated viral particles.

[0013] The disclosure also ~~provides~~describes a method of producing a vector producing cell line described herein comprising transforming a 293 cell line with a plasmid encoding a retroviral vector comprising from 5′ to 3′: a CMV-R-U5 fusion of the immediate early promoter from human cytomegalovirus to an MLV R-U5 region; a PBS, primer binding site for reverse transcriptase; a 5′ splice site; ψ packaging signal; a gag coding sequence for MLV group specific antigen; a pol coding sequence for MLV polymerase polyprotein; a 3′ splice site; a 4070A env coding sequence for envelope protein of MLV strain 4070A; an internal ribosome entry site (IRES) from encephalomyocarditis virus or a nucleic acid regulatory domain; a modified cytosine deaminase coding sequence; a polypurine tract; and a U3-R-U5 MLV long terminal repeat; culturing the 293 cell to produce viral particles; isolating the viral

particles; infecting an HT1080 cell line with the viral particles thereby producing the viral particle producing cell line. The disclosure also ~~provides~~describes a cell line generated by the foregoing method.

[0014] The disclosure also ~~provides~~describes a method for producing a composition for gene therapy comprising culturing the cell line described herein to produce viral particles and substantially purifying the viral particles.

[0015] The disclosure also ~~provides~~describes a cell bank comprising the cell line of the disclosure. In some ~~embodiments~~examples, the cell line of the disclosure is grown in suspension. In some ~~embodiment~~example, the cell line of the disclosure is grown in serum free medium. In some ~~embodiment~~example, the cell line is grown in suspension in serum free medium.

[0016] An electronic copy of a sequence listing is submitted herewith and is incorporated herein in its entirety.

[0017] The details of one or more ~~embodiments~~examples of the disclosure are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the disclosure will be apparent from the description and drawings, and from the claims.

DESCRIPTION OF DRAWINGS

[0018] **Figure 1** shows a general process of producing a producer cell line and bank of the disclosure.

[0019] **Figure 2** shows a graph of cell killing data showing that modified vectors are more effective compared to the original wildtype CD. The graph also shows that the modified backbone (T5.0007) is more effective at killing than the backbone of pACE-CD. Also shown is a table cataloguing the various vector constructs and their names.

[0020] **Figure 3A-F** shows (a) a schematic of a recombinant retroviral vector of the disclosure; (b and c) a plasmid map of a polynucleotide of the disclosure (CMV Promotor:1 - 582; R:583 - 650; U5:651 - 1202; Primer binding site (PBS):728 - 776; 5' slicing site:788 - 789; gag:1203 - 2819; pol:2820 - 6358; 3'splicing site:3314 - 3315; 4070A env: 6359 - 8323; EMCV IRES:8327 - 8876; yCD2:8877 - 9353; Poly purine tract (PPT):9386 - 9404; U3:9405 -