

Claims

1. A method for assessing genomic integration of a transgene sequence, the method comprising:

(a) separating a high molecular weight fraction of deoxyribonucleic acid (DNA) of greater than or greater than about 10 kilobases (kb) from DNA isolated from one or more cells, said one or more cells comprising, or suspected of comprising, at least one engineered cell comprising a transgene sequence encoding a recombinant protein; and

(b) from the high molecular weight fraction, determining the presence, absence or amount of the transgene sequence integrated into the genome of the one or more cells.

2. The method of claim 1, wherein, prior to the separating in (a), isolating deoxyribonucleic acid (DNA) from the one or more cells.

3. The method of claim 1 or claim 2, wherein the determining the presence, absence or amount of the transgene sequence in (b) comprises determining the mass, weight or copy number of the transgene sequence per diploid genome or per cell in the one or more cells, optionally wherein the copy number is an average or mean copy number per diploid genome or per cell among the population of cells.

4. The method of any of claims 1-3, wherein the one or more cells comprises a population of cells in which a plurality of cells of the population comprise the transgene sequence encoding the recombinant protein.

5. The method of any of claims 1-4, wherein, prior to the separating in (a), a polynucleotide comprising the transgene sequence encoding the recombinant protein has been introduced into the at least one engineered cell of the one or more cells.

6. The method of claim 5, wherein the at least one engineered cell has not been incubated at a temperature greater than 25°C, optionally at or about 37°C $\pm$ 2°C, for more than 96 hours, more than 72 hours, or more than 48 hours following the introduction of the polynucleotide comprising the transgene sequence.

7. The method of any of claims 1-6, wherein the one or more cells has been cryopreserved prior to the separating of the high molecular weight fraction of DNA in (a).

8. The method of any of claims 1-7, wherein:
the one or more cells is a cell line;
the one or more cells is a primary cell obtained from a sample from a subject; and/or
the one or more cells is an immune cell.

9. A method for assessing a transgene sequence in a biological sample from a subject, the method comprising:

(a) separating a high molecular weight fraction of deoxyribonucleic acid (DNA) of greater than or greater than about 10 kilobases (kb) from DNA isolated from one or more cells present in a biological sample from a subject, wherein the biological sample comprises, or is suspected of comprising, at least one engineered cell comprising a transgene sequence encoding a recombinant protein; and

(b) from the high molecular weight fraction, determining the presence, absence or amount of transgene sequence in all or a portion of the biological sample.

10. The method of claim 9, wherein the determining the presence, absence or amount of transgene sequence in (b) comprises determining the mass, weight or copy number of the transgene sequence in all or a portion of the biological sample.

11. The method of claim 9 or claim 10, wherein the biological sample is obtained from a subject that had been administered a composition comprising the at least one engineered cell comprising the transgene sequence.

12. The method of any of claims 9-11, wherein:
the biological sample is a tissue sample or bodily fluid sample;
the biological sample is a tissue sample and the tissue is a tumor;
the biological sample is a tumor biopsy; and/or

the biological sample is a bodily fluid sample and the bodily fluid sample is a blood or serum sample.

13. The method of any of claims 9-12, wherein, prior to the separating in (a):
the method further comprises isolating the DNA from one or more cells present in the biological sample; and/or

a polynucleotide comprising the transgene sequence encoding the recombinant protein has been introduced into the at least one engineered cell of the one or more cells.

14. The method of any of claims 9-13, wherein the one or more cells in the biological sample comprises an immune cell.

15. The method of claim 8 or claim 14, wherein the immune cell is a T cell, optionally a CD3+, CD4+ and/or CD8+ T cell, or an NK cell.

16. The method of any of claims 1-15, wherein the separating is carried out by pulse field gel electrophoresis or size exclusion chromatography.

17. A method for assessing genomic integration of a transgene sequence, the method comprising:

(a) separating, by pulse field gel electrophoresis, a high molecular weight fraction of deoxyribonucleic acid (DNA) of greater than or greater than about 10 kilobases (kb) from DNA isolated from a population of cells, said population of cells comprising a plurality of engineered cells that each comprise, or are suspected of comprising, a transgene sequence encoding a recombinant protein; and

(b) from the high molecular weight fraction, determining the average or mean copy number per diploid genome or per cell of the transgene sequence integrated into the genome of the plurality of engineered cells of the population of cells.

18. The method of claim 17, wherein, prior to the separating in (a), a polynucleotide comprising the transgene sequence encoding the recombinant protein has been introduced into at least one of the plurality of engineered cells of the population of cells.

19. The method of claim 18, wherein the population of cells has not been incubated at a temperature greater than 25°C, optionally at or about 37°C $\pm$ 2°C, for more than 96 hours, more than 72 hours, or more than 48 hours following the introduction of the polynucleotide comprising the transgene sequence into the at least one engineered cell.

20. The method of any of claims 17-19, wherein the population of cells has been cryopreserved prior to the separating of the high molecular weight fraction of DNA in (a).

21. The method of any of claims 1-20, wherein the high molecular weight fraction is of greater than or greater than about 15 kilobases (kb), greater than or greater than about 17.5 kilobases (kb), or greater than or greater than about 20 kilobases (kb).

22. The method of any of claims 1-21, the transgene sequence comprises a regulatory element operably linked to a nucleic acid sequence encoding the recombinant protein.

23. The method of any of claims 1-22, wherein the determining the presence, absence or amount of the transgene sequence is carried out by polymerase chain reaction (PCR).

24. The method of claim 23, wherein the PCR is carried out using one or more primers that is complementary to or is capable of specifically amplifying at least a portion of the transgene sequence.

25. The method of claim 24, wherein the one or more primers is complementary to or is capable of specifically amplifying sequences of the regulatory element.

26. The method of any of claims 1-25, wherein:
the determining the amount of the transgene sequence comprises assessing the mass, weight or copy number of the transgene sequence per mass or weight of DNA isolated from the one or more cells, optionally per microgram of DNA isolated from the one or more cells; or
the determining the amount of the transgene sequence comprises assessing the mass, weight or copy number of the transgene sequence per the one or more cells, optionally per CD3+, CD4+ and/or CD8+ cell, and/or per cell expressing the recombinant protein.

27. The method of any of claims 9-16 and 21-25, wherein:

the determining the presence, absence or amount of the transgene sequence comprises assessing the mass, weight or copy number of the transgene sequence per diploid genome or per cell in the biological sample, optionally wherein the copy number is an average or mean copy number per diploid genome or per cell among the one or more cells in the biological sample;

the determining the amount of the transgene sequence comprises assessing the mass, weight or copy number of the transgene sequence per volume of the biological sample, optionally per microliter or per milliliter of the biological sample; or

the determining the amount of the transgene sequence comprises assessing the mass, weight or copy number of the transgene sequence per body weight or body surface area of the subject.

28. The method of any of claims 1-27, wherein determining the amount of the transgene sequence comprises assessing the mass, weight or copy number of the transgene sequence in the high molecular weight fraction and normalizing the mass, weight or copy number to the mass, weight or copy number of a reference gene in the high molecular weight fraction or to a standard curve.

29. The method of claim 28, wherein the copy number of a reference gene in the isolated DNA is carried out by PCR using one or more primers that is complementary to or is capable of specifically amplifying at least a portion of the reference gene.

30. The method of any of claims 1-29, wherein:

the transgene sequence does not encode a complete viral gag protein; and/or

the transgene sequence does not comprise a complete HIV genome, a replication competent viral genome, and/or accessory genes, which accessory genes are optionally Nef, Vpu, Vif, Vpr, and/or Vpx.

31. The method of any of claims 5-8, 13-16, and 18-30, wherein:

the introduction of the polynucleotide is carried out by transduction with a viral vector comprising the polynucleotide; or

the introduction of the polynucleotide is carried out by a physical delivery method, optionally by electroporation.

32. The method of claim 31, wherein the viral vector is a retroviral vector, a gammaretroviral vector, a lentiviral vector, or an AAV Vector, optionally selected from among AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7 or AAV8 vector.

33. The method of any of claims 1-32, wherein the recombinant protein is a recombinant receptor.

34. The method of claim 33, wherein:
the recombinant receptor specifically binds to an antigen associated with a disease or condition or an antigen that is expressed in cells of the environment of a lesion associated with a disease or condition;

the recombinant receptor is a recombinant T cell receptor (TCR) or a functional non-T cell receptor; and/or

the recombinant receptor is a chimeric antigen receptor (CAR).

35. The method of claim 34, wherein:
the disease or condition is a cancer; and/or
the antigen is selected from $\alpha v\beta 6$ integrin ($\alpha v\beta 6$ integrin), B cell maturation antigen (BCMA), B7-H3, B7-H6, carbonic anhydrase 9 (CA9, also known as CAIX or G250), a cancer-testis antigen, cancer/testis antigen 1B (CTAG, also known as NY-ESO-1 and LAGE-2), carcinoembryonic antigen (CEA), a cyclin, cyclin A2, C-C Motif Chemokine Ligand 1 (CCL-1), CD19, CD20, CD22, CD23, CD24, CD30, CD33, CD38, CD44, CD44v6, CD44v7/8, CD123, CD133, CD138, CD171, chondroitin sulfate proteoglycan 4 (CSPG4), epidermal growth factor protein (EGFR), type III epidermal growth factor receptor mutation (EGFR vIII), epithelial glycoprotein 2 (EPG-2), epithelial glycoprotein 40 (EPG-40), ephrinB2, ephrin receptor A2 (EPHa2), estrogen receptor, Fc receptor like 5 (FCRL5; also known as Fc receptor homolog 5 or FCRH5), fetal acetylcholine receptor (fetal AchR), a folate binding protein (FBP), folate receptor alpha, ganglioside GD2, O-acetylated GD2 (OGD2), ganglioside GD3, glycoprotein 100 (gp100), glypican-3 (GPC3), G protein-coupled receptor class C group 5 member D (GPRC5D), Her2/neu (receptor tyrosine kinase erb-B2), Her3 (erb-B3), Her4 (erb-B4), erbB dimers, Human high molecular weight-melanoma-associated antigen (HMW-MAA), hepatitis B surface antigen, Human leukocyte antigen A1 (HLA-A1), Human leukocyte antigen A2 (HLA-A2), IL-22 receptor alpha (IL-22R α), IL-13 receptor alpha 2 (IL-13R α 2), kinase insert domain

receptor (kdr), kappa light chain, L1 cell adhesion molecule (L1-CAM), CE7 epitope of L1-CAM, Leucine Rich Repeat Containing 8 Family Member A (LRRC8A), Lewis Y, Melanoma-associated antigen (MAGE)-A1, MAGE-A3, MAGE-A6, MAGE-A10, mesothelin (MSLN), c-Met, murine cytomegalovirus (CMV), mucin 1 (MUC1), MUC16, natural killer group 2 member D (NKG2D) ligands, melan A (MART-1), neural cell adhesion molecule (NCAM), oncofetal antigen, Preferentially expressed antigen of melanoma (PRAME), progesterone receptor, a prostate specific antigen, prostate stem cell antigen (PSCA), prostate specific membrane antigen (PSMA), Receptor Tyrosine Kinase Like Orphan Receptor 1 (ROR1), survivin, Trophoblast glycoprotein (TPBG also known as 5T4), tumor-associated glycoprotein 72 (TAG72), Tyrosinase related protein 1 (TRP1, also known as TYRP1 or gp75), Tyrosinase related protein 2 (TRP2, also known as dopachrome tautomerase, dopachrome delta-isomerase or DCT), vascular endothelial growth factor receptor (VEGFR), vascular endothelial growth factor receptor 2 (VEGFR2), Wilms Tumor 1 (WT-1), a pathogen-specific or pathogen-expressed antigen, or an antigen associated with a universal tag, and/or biotinylated molecules, and/or molecules expressed by HIV, HCV, HBV or other pathogens.

36. The method of claim 34, wherein:

the CAR comprises an extracellular antigen-recognition domain that specifically binds to the antigen and an intracellular signaling domain comprising an ITAM;

the intracellular signaling domain comprising an ITAM comprises an intracellular domain of a CD3-zeta (CD3 ζ) chain, optionally a human CD3-zeta chain;

the intracellular signaling domain further comprises a costimulatory signaling region; and/or

the costimulatory signaling region comprises a signaling domain of CD28 or 4-1BB, optionally human CD28 or human 4-1BB.

37. A method for assessing a residual non-integrated transgene sequence, the method comprising:

(1) performing the method of any of claims 1-8 and 16-36, to determine the presence, absence or amount of the transgene sequence in the high molecular weight fraction of DNA, thereby assessing genomic integration of a transgene sequence;

(2) determining the presence, absence or amount of the transgene sequence in the isolated DNA without separating the high molecular weight fraction; and

(3) comparing the amount determined in (1) to the amount determined in (2), thereby determining the amount of a residual non-integrated recombinant sequence.

38. The method of claim 37, wherein the determining the presence, absence or amount of the transgene sequence comprises determining the mass, weight or copy number of the transgene sequence per diploid genome or per cell in the one or more cells, optionally wherein the copy number is an average or mean copy number per diploid genome or per cell among the population of cells.

39. The method of claim 37 or claim 38, wherein the one or more cell comprises a population of cells in which a plurality of cells of the population comprises, or are suspected of comprising, the transgene sequence encoding the recombinant protein.

40. The method of claim 38 or claim 39, wherein comparing the amount comprises: subtracting the copy number determined in (1) from the copy number determined in (2);
or
determining the ratio of the copy number determined in (1) to the copy number determined in (2).

41. The method of any of claims 37-40, wherein the determining the presence, absence or amount in (2) is carried out by polymerase chain reaction (PCR).

42. The method of any of claims 23-36 and 41, wherein the PCR is quantitative polymerase chain reaction (qPCR), digital PCR or droplet digital PCR.

43. The method of claim 41 or claim 42, wherein the PCR is carried out using one or more primers that is complementary to or is capable of specifically amplifying at least a portion of the transgene sequence.

44. The method of any of claims 37-43 wherein determining the presence, absence or amount in (2) comprises assessing the mass, weight or copy number of the transgene sequence in the isolated DNA without separating the high molecular weight fraction and normalizing the

mass, weight or copy number to the mass, weight or copy number of a reference gene in the isolated DNA without separating the high molecular weight fraction or to a standard curve.

45. The method of any of claims 28-36 and 44, wherein:
the reference gene is a housekeeping gene;
the reference gene is a gene encoding albumin (*ALB*); and/or.
the reference gene is a gene encoding ribonuclease P protein subunit p30 (*RPP30*).

46. The method of claim 44 or claim 45, wherein the determining the mass, weight or copy number of a reference gene in the isolated DNA is carried out by PCR using one or more primers that is complementary to or is capable of specifically amplifying at least a portion of the reference gene.

47. The method of any of claims 37-46, wherein the determining the presence, absence or amount in (1) and the determining the presence, absence or amount in (2) is carried out by polymerase chain reaction (PCR) using the same primer or the same sets of primers.

48. The method of any of claims 47-47, wherein the residual non-integrated recombinant sequence comprises one or more of vector plasmids, linear complementary DNA (cDNA), autointegrants or long terminal repeat (LTR) circles.