SUMMARY

[0006] In one example, there is provided a method for assessing the transduction efficiency and/or specificity of vectors at single cell level, said method comprising:

a) providing a plurality of different vectors,
b) transducing a heterogeneous population of cells with the plurality of different vectors;
c) partitioning the heterogeneous population of cells into a plurality of compartments, wherein each compartment comprises a single cell from the heterogeneous population of cells;
d) subjecting each partitioned cell to nucleotide sequencing;
e) detecting the presence of the any one or more of the different vectors in each partitioned cell.

[0006a] In one aspect the present disclosure refers to a method for assessing the transduction efficiency and/or specificity of vectors at single cell level, said method comprising:

a) providing a plurality of different vectors,
b) transducing a heterogeneous population of cells with the plurality of different vectors;
c) partitioning the heterogeneous population of cells into a plurality of compartments, wherein each compartment comprises a single cell from the heterogeneous population of cells;
d) subjecting each partitioned cell to nucleotide sequencing;
e) detecting the presence of the any one or more of the different vectors in each partitioned cell using sequencing results obtained in step d); and
f) classifying each partitioned cell into a specific cell type based on gene expression patterns and/or epigenetic features of said cell, as determined using sequencing results obtained in step d);

wherein the sequencing results used in steps d) and e) are obtained within a single library preparation; and

wherein the vectors are selected from the group consisting of: a viral vector, a pseudo-virus vector, a virus-like particle vector, a liposome vector, an exosome vector, a nanoparticle, and combinations thereof; wherein the vectors comprise DNA, RNA, modified RNA, modified DNA, or combinations thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

[0007] The invention will be better understood with reference to the detailed description when considered in conjunction with the non-limiting examples and the accompanying drawings.

[0008] **Figure 1** is an exemplary schematic of the present disclosure: **(A)** A first example of an Adeno-associated virus (AAV) variant wherein the Adeno-associated virus (AAV) serotype variants are individually packaged with uniquely identifiable genomes, with the genomes each containing a unique nucleotide barcode. **(B)** A second example of an Adeno-associated virus (AAV) variant wherein the genomes each containing a unique capsid-encoding nucleotide sequence is located between the flanking Adeno-associated virus (AAV) inverted terminal repeats (ITRs). **(C)** A third example of an Adeno-associated virus (AAV) variant wherein the genomes, each containing a unique capsid-encoding nucleotide sequence between the flanking AAV ITRs, are packaged in multiplex based on capsid:genotype linkage, also known as genome:phenotype linkage, known in AAV biology. **(D)** Library of AAV variants from any one of (A) to (C) consisting a multitude of capsid serotypes, each identifiable by their encapsulated and uniquely differentiable nucleotide sequences. **(E)** Library of AAV variants is transduced into a heterogenous population of cells (e.g. organs, tissues, organoids, admixtures). **(F)** In single-cell sequencing, nucleic acids (which can include RNA, DNA) within each cell are tagged by a unique cell-specific single-cell-sequencing nucleotide tag and then sequenced.

Claims

1. A method for assessing the transduction efficiency and/or specificity of vectors at single cell level, said method comprising:
    a) providing a plurality of different vectors,
    b) transducing a heterogeneous population of cells with the plurality of different vectors;
    c) partitioning the heterogeneous population of cells into a plurality of compartments, wherein each compartment comprises a single cell from the heterogeneous population of cells;
    d) subjecting each partitioned cell to nucleotide sequencing;
    e) detecting the presence of the any one or more of the different vectors in each partitioned cell using sequencing results obtained in step d); and
    f) classifying each partitioned cell into a specific cell type based on gene expression patterns and/or epigenetic features of said cell, as determined using sequencing results obtained in step d);

    wherein the sequencing results used in steps d) and e) are obtained within a single library preparation; and

    wherein the vectors are selected from the group consisting of: a viral vector, a pseudo-virus vector, a virus-like particle vector, a liposome vector, an exosome vector, a nanoparticle, and combinations thereof; wherein the vectors comprise DNA, RNA, modified RNA, modified DNA, or combinations thereof.

2. The method of claim 1, wherein the transduction efficiency of a specific vector against a specific cell type is determined by the percentage of cells of the specific cell type which have been detected positive for the presence of the specific vector.

3. The method of any of the preceding claims, wherein the transduction efficiency of a specific vector against a specific cell type is assessed by comparing the frequencies with which the presence of said specific vector is detected in the cells of said specific cell type, against the frequencies with which the presence of another vector is detected in the cells of said specific cell type.

4. The method of any of the preceding claims, wherein the transduction specificity of a specific vector against a specific cell type relative to another cell type is assessed by comparing the frequencies with which the presence of said specific vector is detected in the cells of said specific cell type, against the frequencies with which the presence of said specific vector is detected in the cells of another specific cell type.

5. The method of any of the preceding claims, wherein each of the plurality of different vectors comprises an oligonucleotide barcode sequence, wherein the barcode sequence is different between any two different vectors.

6. The method of claim 5, wherein the barcode sequence is located on an expression cassette in the vector, wherein the expression of the cassette results in the production of an RNA molecule comprising the barcode sequence, wherein the RNA molecule further comprises a polyadenylation tail.

7. The method of claim 6, wherein the barcode sequence is located on a region of the RNA molecule which allows the barcode sequence to be sequenced, optionally wherein the barcode sequence is within a distance of 98 nucleotides from the polyadenylation tail.

8. The method of any one of claims 5-7, wherein the barcode sequence is 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 nucleotides in length, preferably wherein the barcode sequence is 8 nucleotides in length.

9. The method of any one of claims 1-4, wherein each of the plurality of different vectors comprises a marker polynucleotide, wherein the marker polynucleotide is different between any two different vectors; and wherein the marker polynucleotide encodes for one or more proteins, said one or more proteins when expressed form a protein envelope which encapsulate the marker polynucleotide, so that after transfection of the vector, each marker polynucleotide is encapsulated by the one or more proteins which the marker polynucleotide encodes for.

10. The method of claim 9, wherein: (i) the marker polynucleotide is located on an expression cassette in the vector, wherein the expression of the cassette results in the production of an RNA molecule comprising the marker polynucleotide, wherein the RNA molecule further comprises a polyadenylation tail; and/or (ii) the marker polynucleotide is a viral-capsid-

encoding gene, wherein the capsid expressed by the marker polynucleotide encapsulates the marker polynucleotide, and optionally wherein the viral-capsid-encoding gene is specifically an AAV-capsid-encoding gene.

11. The method of any one of the preceding claims, wherein step e) comprises detecting the presence of one or more marker sequences specific to each different vector; wherein when each vector comprises a unique barcode sequence, said one or more marker sequences comprise the barcode sequence; wherein when each vector comprises a unique marker polynucleotide, said one or more marker sequences comprise the marker polynucleotide

12. The method of claim 11, wherein step e) comprises matching the sequence reads obtained in step d) with a reference data set;

    and optionally wherein the reference data set comprises the genomes and/or the transcriptomes of the plurality of different viral vectors, and/or the barcodes comprised in the plurality of different viral vectors, and/or the marker polynucleotides comprised in the plurality of different viral vectors.

13. The method of any one of the preceding claims, wherein the compartments are oil droplets.

14. The method of any one of the preceding claims, wherein the nucleotide sequencing is RNA sequencing.

15. The method of any one of the preceding claims, wherein the nucleotide sequencing is DNA sequencing.

16. The method of any one of the preceding claims, wherein the vectors comprise viral vectors, wherein the viral vectors are selected from the group consisting of: an adenoviral vector, an Adeno-associated virus (AAV) vector, a lentiviral vector, a coronavirus vector, an enterovirus vector, a retroviral vector, or a combination thereof.

17. The method of claim 16, wherein the viral vectors are AAV vectors, optionally wherein the viral vectors are selected from the group consisting of: AAV type 1 (AAV-1), AAV type 2 (AAV-2), AAV type 3 (AAV-3), AAV type 4 (AAV-4), AAV type 5 (AAV-5), AAV type 6 (AAV-6), AAV type 7 (AAV-7), AAV type 8 (AAV-8), AAV type 9 (AAV9), AAV type 10 (AAV10), AAV type 11 (AAV11), AAV type 12 (AAV12), AAV type 13 (AAV13),

rh10, AAVDJ, AAVAnc80, AAV-PHP.S, AAV-PHP.eB, AAV-LK03, AAV2-7m8, AAV variants thereof, and combinations thereof.

18. The method of claim 16 or 17, wherein the plurality of different viral vectors comprises viral vectors of different families, viral vectors of different genera, viral vectors of different species, viral vectors of different serotypes, viral vectors thereof carrying different mutations, or combinations thereof.

19. The method of any one of the preceding claims, wherein the heterogeneous population of cells:

(i) comprises plant cells, animal cells (e.g. mammalian or human cells), fungal cells, or combinations thereof; and/or

(ii) are cultured cells or are obtained from one or more cultured organoids, optionally wherein the one or more cultured organoids are selected from the group consisting of ocular organoid, cerebral organoid, epithelial organoid, kidney organoid, lung organoid, pancreas organoid, cardiac organoid, and hepatic organoid.