**[0012]** In the fourth subpopulation (D), at least one of genes *PAX6*, *SIX3*, and *SLC2A1* is expressed in the Day 26-34 gene profile. Desirably, more or most of these genes are expressed.

**[0013]** In the fifth subpopulation (E), at least one of genes *VSX2*, *PTH2*, *FZD5*, *RARRES2*, *DIO3*, *SOX2*, and *CYP1B1* is expressed in the Day 26-34 gene profile. Desirably, more or most of these genes are expressed.

**[0013a]** In one aspect, the present disclosure refers to a photoreceptor progenitor cell, wherein a Day 26-34 gene profile indicates that:

(A) (i) *CRX* is expressed with a Log2 normalized and cell cycle adjusted gene count of at least 5 gene count-per-million, or (ii) *C11orf96* is expressed with a Log2 normalized and cell cycle adjusted gene count of at least 5 gene count-per-million, or (iii) *NEUROD4* is expressed with a Log2 normalized and cell cycle adjusted gene count of at least 1 gene count-per-million; or

(B) (i) *STMN2* is expressed with a Log2 normalized and cell cycle adjusted gene count of at least 10 gene count-per-million, or (ii) *ONECUT2* is expressed with a Log2 normalized and cell cycle adjusted gene count of at least 10 gene count-per-million, or (iii) *ELAVL4* is expressed with a Log2 normalized and cell cycle adjusted gene count of at least 1 gene count-per-million; or

(C) (i) *CNTN2* is expressed with a Log2 normalized and cell cycle adjusted gene count of at least 5 gene count-per-million, or (ii) *POU4F2* is expressed with a Log2 normalized and cell cycle adjusted gene count of at least 5 gene count-per-million; or

(E) (i) *VSX2* is expressed with a Log2 normalized and cell cycle adjusted gene count of at least 10 gene count-per-million.

**[0013b]** In one aspect, the present disclosure refers to a method for producing a population of photoreceptor progenitors, comprising:

plating pluripotent human stem cells on a cell culture surface having a laminin matrix thereon, wherein the laminin matrix comprises (i) a first laminin which is laminin-521, wherein the laminin-521 is either an intact protein or a protein fragment; and (ii) a second laminin selected from the group consisting of laminin-323 and laminin-523, wherein the second laminin is either an intact protein or a protein fragment;

culturing the pluripotent human stem cells in a neural induction medium comprising a TGFβ inhibitor and a Wnt inhibitor for a first time period of about 120 hours to about 168 hours;

culturing the pluripotent human stem cells in a photoreceptor differentiation medium comprising a brain derived neurotrophic factor (BDNF), a ciliary neurotrophic factor (CNTF), retinoic acid, and a gamma secretase inhibitor for a second time period of about 288 hours to about 816 hours; and

identifying the population of photoreceptor progenitors by (i) preparing a Day 16-24 gene profile and selecting photoreceptor progenitors in which at least one of genes DAPL1, SIX6 SFRP2, LHX2, *PAX6*, CDH2, CLDN1, TRPM3, RELN, DCT and PMEL is expressed; and (ii) preparing a Day 26-34 gene profile and selecting photoreceptor progenitors in which:

(A) (i) *CRX* is expressed with a Log2 normalized and cell cycle adjusted gene count of at least 5 gene count-per-million, or (ii) *C11orf96* is expressed with a Log2 normalized and cell cycle adjusted gene count of at least 5 gene count-per-million, or (iii) *NEUROD4* is expressed with a Log2 normalized and cell cycle adjusted gene count of at least 1 gene count-per-million; or

(B) i) *STMN2* is expressed with a Log2 normalized and cell cycle adjusted gene count of at least 10 gene count-per-million, or (ii) *ONECUT2* is expressed with a Log2 normalized and cell cycle adjusted gene count of at least 10 gene count-per-million, or (iii) *ELAVL4* is expressed with a Log2 normalized and cell cycle adjusted gene count of at least 1 gene count-per-million; or

(C) (i) *CNTN2* is expressed with a Log2 normalized and cell cycle adjusted gene count of at least 5 gene count-per-million, or (ii) *POU4F2* is expressed with a Log2 normalized and cell cycle adjusted gene count of at least 5 gene count-per-million; or

(E) *VSX2* is expressed with a Log2 normalized and cell cycle adjusted gene count of at least 5 gene count-per-million.

**[0013c]** In one example, the present disclosure refers to a method for producing recombinant laminin-323 or recombinant laminin-523, comprising:

encoding a first expression plasmid with (A) a sequence that codes for laminin alpha-3 or alpha-5 chain, and (B) a first antibiotic resistance gene;

encoding a second expression plasmid with (A) a sequence that codes for laminin beta-2 chain, and (B) a second antibiotic resistance gene;

encoding a third expression plasmid with (A) a sequence that codes for laminin gamma-3 chain, and (B) a third antibiotic resistance gene;

transfecting the first, second, and third expression plasmids into human cells that are maintained in a basal medium containing serum;

identifying potential positive clones;

exposing the potential positive clones to a serum-free cell culture medium;

filtering the serum-free cell culture medium to obtain the recombinant laminin-323 or recombinant laminin-523;

optionally concentrating the recombinant laminin-323 or recombinant laminin-523 by centrifugation after filtering; and

optionally purifying the recombinant laminin-323 or recombinant laminin-523.

**[0014]** These and other non-limiting characteristics of the disclosure are more particularly disclosed below.

**[0095]** In the fourth subpopulation (D), at least one of genes *PAX6*, *SIX3*, and *SLC2A1* is expressed in the Day 26-34 gene profile. Combinations of any 2 or 3 of these genes are specifically contemplated. In some embodiments, the combination of expressed genes includes *PAX6* and *SIX3*.

**[0096]** In the fifth subpopulation (E), at least one of genes *VSX2*, *PTH2*, *FZD5*, *RARRES2*, *DIO3*, *SOX2*, and *CYP1B1* is expressed in the Day 26-34 gene profile. Combinations of any 2, 3, 4, 5, 6, or 7 of these genes are specifically contemplated. In some embodiments, the combination of expressed genes includes *VSX2* and *CYP1B1*.

**[0097]** In the present disclosure, reference to a gene being "expressed" means the gene expression level of that gene has a minimum Log2 normalized and cell cycle adjusted gene count value of 1 gene count-per-million (CPM). It is also noted that discussion of certain genes being expressed in these subpopulations should not be interpreted to mean that other genes are not expressed. For example, it is possible that gene *STMN2* is still expressed in subpopulation (A).

**[0098]** In addition, in these five subpopulations, the Day 16-24 gene profile usually indicates that at least one of genes *DAPL1*, *SIX6*, *SFRP2*, *LHX2*, *PAX6*, *CDH2*, *CLDN1*, *TRPM3*, *RELN*, *DCT*, or *PMEL* is expressed. Combinations of any 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11 of these genes are specifically contemplated. In more specific embodiments, at least one of genes *SIX6*, *SFRP2*, *LHX2*, *PAX6*, *DCT*, or *PMEL* is expressed, including combinations of any 2, 3, 4, 6, or 6 of these genes. In particular embodiments, *PAX6* is expressed in the Day 16-24 gene profile. The Day 16-24 gene profile can be used, if desired, to discard differentiating cells that will not end in the desired cell lineage.

**[0099]** In particular embodiments of subpopulation (A), the Day 26-34 gene profile indicates that (i) *CRX* is expressed with a Log2 normalized and cell cycle adjusted gene count of at least 5 gene CPM, and/or (ii) *C11orf96* is expressed with a Log2 normalized and cell cycle adjusted gene count of at least 5 gene CPM. In more specific embodiments, the Day 26-34 gene profile indicates that (i) *CRX* is expressed with a Log2 normalized and cell cycle adjusted gene count of at least 10 gene CPM, and/or (ii) *C11orf96* is expressed with a Log2 normalized and cell cycle adjusted gene count of at least 10 gene CPM.

**[0100]** In particular embodiments of subpopulation (B), the Day 26-34 gene profile indicates that (i) *STMN2* is expressed with a Log2 normalized and cell cycle adjusted gene count of at least 5 gene CPM, and/or (ii) *ONECUT2* is expressed with a Log2 normalized and cell cycle adjusted gene count of at least 5 gene CPM. In more specific embodiments, the Day 26-34 gene profile indicates that (i) *STMN2* is expressed with a Log2 normalized and cell cycle adjusted gene count of at least 10 gene CPM, and/or (ii) *ONECUT2* is expressed with a Log2 normalized and cell cycle adjusted gene count of at least 10 gene CPM.

**[0101]** In particular embodiments of subpopulation (C), the Day 26-34 gene profile indicates that (i) *CNTN2* is expressed with a Log2 normalized and cell cycle adjusted gene count of at least 5 gene CPM, and/or (ii) *POU4F2* is expressed with a Log2 normalized and cell cycle adjusted gene count of at least 5 gene CPM. In more specific embodiments, the Day 26-34 gene profile indicates that (i) *CNTN2* is expressed with a Log2 normalized and cell cycle adjusted gene count of at least 10 gene CPM, and/or (ii) *POU4F2* is expressed with a Log2 normalized and cell cycle adjusted gene count of at least 10 gene CPM.

**[0102]** In particular embodiments of subpopulation (D), the Day 26-34 gene profile indicates that (i) *PAX6* is expressed with a Log2 normalized and cell cycle adjusted gene count of at least 5 gene CPM, and/or (ii) *SIX3* is expressed with a Log2 normalized and cell cycle adjusted gene count of at least 5 gene CPM. In more specific embodiments, the Day 26-34 gene profile indicates that (i) *PAX6* is expressed with a Log2 normalized and cell cycle adjusted gene count of at least 10 gene CPM, and/or (ii) *SIX3* is expressed with a Log2 normalized and cell cycle adjusted gene count of at least 10 gene CPM.

**[0103]** In particular embodiments of subpopulation (E), the Day 26-34 gene profile indicates that (i) *VSX2* is expressed with a Log2 normalized and cell cycle adjusted gene count of at least 5 gene CPM, and/or (ii) *CYP1B1* is expressed with a Log2 normalized and cell cycle adjusted gene count of at least 5 gene CPM. In more specific embodiments, the Day 26-34 gene profile indicates that (i) *VSX2* is expressed with a Log2 normalized and cell cycle adjusted gene count of at least 10 gene CPM, and/or (ii) *CYP1B1* is expressed with a Log2 normalized and cell cycle adjusted gene count of at least 10 gene CPM.

**[0104]** The present disclosure is further illustrated in the following non-limiting working examples, it being understood that these examples are intended to be illustrative only and that the disclosure is not intended to be limited to the materials, conditions, process parameters and the like recited herein.

## EXAMPLES

### EXAMPLE 1

**[0105] Cell Line Development of HEK overexpressing LN-523 and LN-323**

**[0106]** A flow chart describing the steps for producing recombinant laminin-523 and laminin-323 is provided in **FIG. 1A**. Briefly, human recombinant laminin expression plasmids encoding laminin alpha-3, beta-2, and gamma-3 chains and containing three different antibiotic resistance genes were generated and sequentially transfected into HEK cells. For producing LN-523, expression plasmids encoding laminin alpha-5, beta-2, and gamma-3 chains were sequentially transfected into HEK cells. Stable clonal cell lines overexpressing the desired laminin chains were established. Third, potential positive clones were identified using qPCR and Western blot. Fourth, the cell culture

**CLAIMS:**

1. A photoreceptor progenitor cell, wherein a Day 26-34 gene profile indicates that:

(A) (i) *CRX* is expressed with a Log2 normalized and cell cycle adjusted gene count of at least 5 gene count-per-million, or (ii) *C11orf96* is expressed with a Log2 normalized and cell cycle adjusted gene count of at least 5 gene count-per-million, or (iii) *NEUROD4* is expressed with a Log2 normalized and cell cycle adjusted gene count of at least 1 gene count-per-million; or

(B) (i) *STMN2* is expressed with a Log2 normalized and cell cycle adjusted gene count of at least 10 gene count-per-million, or (ii) *ONECUT2* is expressed with a Log2 normalized and cell cycle adjusted gene count of at least 10 gene count-per-million, or (iii) *ELAVL4* is expressed with a Log2 normalized and cell cycle adjusted gene count of at least 1 gene count-per-million; or

(C) (i) *CNTN2* is expressed with a Log2 normalized and cell cycle adjusted gene count of at least 5 gene count-per-million, or (ii) *POU4F2* is expressed with a Log2 normalized and cell cycle adjusted gene count of at least 5 gene count-per-million; or

(E) (i) *VSX2* is expressed with a Log2 normalized and cell cycle adjusted gene count of at least 10 gene count-per-million.

2. The photoreceptor progenitor cell of claim 1, wherein the Day 26-34 gene profile indicates that (i) *CRX* is expressed with a Log2 normalized and cell cycle adjusted gene count of at least 5 gene count-per-million, and (ii) *C11orf96* is expressed with a Log2 normalized and cell cycle adjusted gene count of at least 5 gene count-per-million.

3. The photoreceptor progenitor cell of claim 2, wherein the Day 26-34 profile also indicates that *NEUROD4* is expressed with a Log2 normalized and cell cycle adjusted gene count of at least 1 gene count-per-million.

4. The photoreceptor progenitor cell of claim 2, wherein the Day 26-34 profile also indicates that *RCVRN, FAM57B, or SYT4* is expressed with a Log2 normalized and cell cycle adjusted gene count of at least 1 gene count-per-million.

5. The photoreceptor progenitor cell of claim 1, wherein the Day 26-34 gene profile indicates that (i) *STMN2* is expressed with a Log2 normalized and cell cycle adjusted gene count of at least 10 gene count-per-million, and (ii) *ONECUT2* is expressed with a Log2 normalized and cell cycle adjusted gene count of at least 10 gene count-per-million.

6. The photoreceptor progenitor cell of claim 5, wherein the Day 26-34 profile also indicates that *ATOH7* is expressed with a Log2 normalized and cell cycle adjusted gene count of at least 1 gene count-per-million.

7. The photoreceptor progenitor cell of claim 1, wherein the Day 26-34 gene profile indicates that (i) *CNTN2* is expressed with a Log2 normalized and cell cycle adjusted gene count of at least 5 gene count-per-million, and (ii) *POU4F2* is expressed with a Log2 normalized and cell cycle adjusted gene count of at least 5 gene count-per-million.

8. The photoreceptor progenitor cell of claim 7, wherein the Day 26-34 profile also indicates that *CXCR4* is expressed with a Log2 normalized and cell cycle adjusted gene count of at least 1 gene count-per-million.

9. The photoreceptor progenitor cell of claim 1, wherein the Day 26-34 profile indicates that (i) *VSX2* is expressed with a Log2 normalized and cell cycle adjusted gene count of at least 10 gene count-per-million, and (ii) *PTH2* is expressed with a Log2 normalized and cell cycle adjusted gene count of at least 1 gene count-per-million.

10. The photoreceptor progenitor cell of claim 1, wherein a Day 16-24 gene profile indicates that at least one of genes *DAPL1*, *SIX6*, *SFRP2*, *LHX2*, *PAX6*, *CDH2*, *CLDN1*, *TRPM3*, *RELN*, *DCT*, and *PMEL* is expressed.

11. The photoreceptor progenitor cell of claim 10, wherein the Day 16-24 gene profile indicates that *PAX6* is expressed.

12. A method for producing a population of photoreceptor progenitors, comprising:

plating pluripotent human stem cells on a cell culture surface having a laminin matrix thereon, wherein the laminin matrix comprises (i) a first laminin which is laminin-521, wherein the laminin-521 is either an intact protein or a protein fragment; and (ii) a second laminin selected from the group consisting of laminin-323 and laminin-523, wherein the second laminin is either an intact protein or a protein fragment;

culturing the pluripotent human stem cells in a neural induction medium comprising a TGFβ inhibitor and a Wnt inhibitor for a first time period of about 120 hours to about 168 hours;

culturing the pluripotent human stem cells in a photoreceptor differentiation medium comprising a brain derived neurotrophic factor (BDNF), a ciliary neurotrophic factor (CNTF), retinoic acid, and a gamma secretase inhibitor for a second time period of about 288 hours to about 816 hours; and

identifying the population of photoreceptor progenitors by (i) preparing a Day 16-24 gene profile and selecting photoreceptor progenitors in which at least one of genes DAPL1, SIX6 SFRP2, LHX2, *PAX6*, CDH2, CLDN1, TRPM3, RELN, DCT and PMEL is expressed; and (ii) preparing a Day 26-34 gene profile and selecting photoreceptor progenitors in which:

(A) (i) *CRX* is expressed with a Log2 normalized and cell cycle adjusted gene count of at least 5 gene count-per-million, or (ii) *C11orf96* is expressed with a Log2 normalized and cell cycle adjusted gene count of at least 5 gene count-per-million, or (iii) *NEUROD4* is expressed with a Log2 normalized and cell cycle adjusted gene count of at least 1 gene count-per-million; or

(B) i) *STMN2* is expressed with a Log2 normalized and cell cycle adjusted gene count of at least 10 gene count-per-million, or (ii) *ONECUT2* is expressed with a Log2 normalized and cell cycle adjusted gene count of at least 10 gene count-per-million, or (iii) *ELAVL4* is expressed with a Log2 normalized and cell cycle adjusted gene count of at least 1 gene count-per-million; or

(C) (i) *CNTN2* is expressed with a Log2 normalized and cell cycle adjusted gene count of at least 5 gene count-per-million, or (ii) *POU4F2* is expressed with a Log2 normalized and cell cycle adjusted gene count of at least 5 gene count-per-million; or

(E) *VSX2* is expressed with a Log2 normalized and cell cycle adjusted gene count of at least 5 gene count-per-million.

13. The method of claim 12, wherein the neural induction medium comprises:
from about 5 µM to about 15 µM of the TGFβ inhibitor; and
from about 5 µM to about 15 µM of the Wnt inhibitor.

14. The method of claim 12, wherein:
the TGFβ inhibitor is SB431542; or
the Wnt inhibitor is CKI-7.

15. The method of claim 12, wherein the photoreceptor differentiation medium comprises:
from about 1 ng/mL to about 20 ng/mL of the brain derived neurotrophic factor (BDNF);
from about 1 ng/mL to about 30 ng/mL of the ciliary neutrotrophic factor (CNTF);
from about 0.1 µM to about 5 µM of the retinoic acid; and
from about 5 µM to about 15 µM of the gamma secretase inhibitor.

16. The method of claim 12, wherein the gamma secretase inhibitor is DAPT.

17. The method of claim 12, wherein the weight ratio of the second laminin to the first laminin is from about 1:1 to about 4:1.