3. SUMMARY

[0005] This disclosure is based in part on the finding that including a polynucleotide sequence encoding a selection marker outside of the 5' ITR and the 3' ITR of a transposon and a transposase within the 5' ITR and the 3' ITR causes the transposase but not the selection marker to integrate into the cell. This phenomenon enables selection of the transposed cells via transiently expressed selection markers, while eliminating the need to further genetically modify a cell to remove a selection marker at a later time (see **FIGs. 1-3**). Applicant found that such a system enables cell line generation where selection markers are not required to be integrated into a cell's genome but rather exist in an episomal state, thereby allowing all the benefit of selection without the integration. This approach is particularly advantageous for cells destined to become food products, because the selection marker nucleic acid is lost relatively quickly and is therefore not intact when the cells are formulated into a food product. This approach thereby lessens the burden of creating a cell culture protocol that produces cells that are recognized as safe for consumption, because sequences encoding the selection marker are substantially and automatically eliminated during routine cell expansion and well before consumption. In one example, the system includes a transposase or polynucleotide encoding a transposase; and an exogenous nucleic acid sequence comprising: a transposon comprising: a 5' inverted terminal repeat (ITR), a first polynucleotide encoding a gene capable of modulating cell proliferation; and a 3' inverted terminal repeat (ITR); and (ii) a polynucleotide encoding an at least first selection marker located outside of the 5' ITR and 3' ITR, where upon introducing (a) and (b) into a cell the transposon is transposed into the cell's genome and the polynucleotide encoding the selection marker is episomal. In cases where the first polynucleotide comprises a sequence coding for TERT or other genes with similar functions, this method increases a cell's replicative capacity, thereby increasing population doubling potential and reducing the number of starting cells needed in order to produce cell-based meat products.

[0006] Overall, this work demonstrated the ability to generate and select for cell lines having an integrated gene of interest without integrating selection markers that may not be recognized as safe for consumption. This approach is valuable because manufacturing cells suitable for consumption, *i.e.,* those from commonly consumed animals, typically requires

one or more cell line adaptations, such as adaptation into particular culture formats (*e.g.*, suspension) and cell culture media, which require vast amounts of cells and extensive passaging and proliferation, *e.g.,* population doubling levels (PDLs) of 100 or more, and this contributes to this technology field's tight constraints on economic feasibility.

[0007] In one example, this disclosure features a transposon system for transposing and selecting of transposed cells, the system comprising: (a) a transposase; and (b) an exogenous nucleic acid sequence comprising a first polynucleotide encoding a gene of interest located between a 5' inverted terminal repeat (ITR) and a 3' inverted terminal repeat (ITR), and a second polynucleotide encoding a selection marker located outside the 5' ITR and the 3' ITR; wherein the transposase integrates the first polynucleotide while leaving the second polynucleotide episomal.

[0008] In another example, this disclosure features a vector for transposing and selecting of transposed cells comprising: (a) a first polynucleotide sequence encoding a transposon comprising: a 5' inverted terminal repeat (ITR), a first polynucleotide encoding a gene of interest; and a 3' inverted terminal repeat (ITR); and (b) a second polynucleotide encoding a selection marker located outside of the 5' ITR and 3' ITR; and wherein upon introducing the vector into a cell comprising a suitable transposase the transposon is transposed into the cell's genome leaving the second polynucleotide episomal.

[0009] In some examples, the first polynucleotide encoding the gene of interest, the second polynucleotide encoding the selection marker, or both are operably linked to a first promoter.

[0010] In some examples, the first polynucleotide and second nucleotide are covalently linked on a circular nucleic acid vector, such as a plasmid.

[0011] In some examples, the transposon comprises a third polynucleotide encoding a second gene of interest, wherein the third polynucleotide encoding a gene of interest is located between the 5' ITR and the 3' ITR.

[0012] In some examples, the third polynucleotide encoding the second gene of interest is operably coupled to the first gene of interest or a second expression control element.

[0013] In some examples, the second gene of interest is selected from: an antibiotic resistance gene, a fluorophore, or a cell surface marker.

[0014] In some examples, where upon being introduced into the cell and following transposition, the episomal polynucleotide encoding the first selection marker remains in the cell for at most 10 days, at most 11 days, at most 12 days, at most 13 days, at most 14 days, at most 15 days, at most 16 days, at most 17 days, at most 18 days, at most 19 days, at most 20 days, at most 21 days, at most 22 days, at most 23 days, at most 24 days, at most 25 days, at most 26 days, at most 27 days, at most 28 days, at most 29 days, or at most 30 days.

[0015] In some examples, where, upon being introduced into the cell and following transposition, the episomal polynucleotide encoding the first selection marker remains in the cell for at least 5 days, at least 6 days, at least 7 days, at least 8 days, at least 9 days, at least 10 days, at least 11 days, at least 12 days, at least 13 days, at least 14 days, at least 15 days, at least 16 days, at least 17 days, at least 18 days, at least 19 days, at least 20 days, at least 21 days, at least 22 days, at least 23 days, at least 24 days, at least 25 days, at least 26 days, at least 27 days, at least 28 days, at least 29 days, or at least 30 days.

[0016] In another example, this disclosure features a method for generating a cell line suitable for dietary consumption, the method comprising: (a) introducing into a population of cells an exogenous nucleic acid sequence comprising a first polynucleotide encoding a gene of interest located between a 5' inverted terminal repeat (ITR) and a 3' inverted terminal repeat (ITR), and a second polynucleotide encoding a selection marker located outside of the 5' ITR and the 3' ITR, (b) selecting a transduced cell from the population of cells using the selection marker; and (c) culturing the transduced cells under conditions sufficient to expand the transduced cells. In examples, the first and second nucleic acids are within the same nucleic acid vector, including a transposon or transposon encoding vector, and including a circular nucleic acid vector.

[0017] In some examples, the method further comprises a second selecting step.

[0018] In some examples, the second selecting step comprises using the second selection marker to further select the transduced cell.

[0019] In some examples, the second selecting step is performed 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 15 days, 16 days, 17 days, 18 days, 19 days, 20 days, 21 days, 22 days, 23 days, 24 days, 25 days, 26 days, 27 days, 28 days, 29 days, or 30 days after the first selecting step.

[0020] In some examples, the episomal polynucleotide encoding the selection marker remains in the cell following introduction for at most 10 days, at most 11 days, at most 12 days, at most 13 days, at most 14 days, at most 15 days, at most 16 days, at most 17 days, at most 18 days, at most 19 days, at most 20 days, at most 21 days, at most 22 days, at most 23 days, at most 24 days, at most 25 days, at most 26 days, at most 27 days, at most 28 days, at most 29 days, or at most 30 days.

[0021] In some examples, the cell line is derived from: livestock, poultry, or game animal species.

[0022] In some examples, the cell line comprises cells selected from: fibroblasts, mesenchymal cells, chondrocytes, myoblasts, myocytes, satellite cells, side population cells, muscle derived stem cells, myofibroblasts, mesenchymal stem cells, myogenic pericytes, or mesoangioblasts.

[0023] In another example, this disclosure features cell comprising: a genome having an integrated polynucleotide encoding a gene of interest between a 5' ITR and a 3' ITR, wherein the 5' ITR and the 3' ITR is transposable by a transpose; and an episomal polynucleotide encoding a selection marker.

[0024] In some examples, the gene of interest is selected from genes capable of modulating cell proliferation, a growth factor ligand, a growth factor receptor, a myogenic transcription factor, or a combination thereof.

[0025] In some examples, the selection marker is selected from: an essential nutrient, an antibiotic resistance gene, a fluorophore, or a cell surface marker.

[0025a] In one aspect, the disclosure features a method for generating a non-human metazoan cell line, the method comprising: (a) introducing into a population of non-human metazoan cells an exogenous nucleic acid sequence comprising a first polynucleotide encoding a gene of interest located between a 5' transposon inverted terminal repeat (ITR) and a 3' transposon inverted terminal repeat (ITR), and a second polynucleotide encoding a selection marker located outside of the 5' ITR and the 3' ITR, wherein the second polynucleotide is an episomal polynucleotide, (b) selecting a transduced cell from the population of non-human metazoan cells using the selection marker; and (c) culturing the transduced non-human metazoan cells under conditions sufficient to expand the transduced cells.

4. BRIEF DESCRIPTION OF THE DRAWINGS

[0026] These and other features, aspects, and advantages of the present invention will become better understood with regard to the following description, and accompanying drawings, where:

[0027] **FIG. 1** shows a schematic for transposition of the transposon comprising a promoter and a gene of interest into a cellular genome. ITR – inverted terminal repeats. btTERT – *Bos taurus* TERT. btCDK4^{R24C}. pA – polyA. AMP^{R} – ampicillin resistance. Ori – origin of replication. GFP-Puro –a green fluorescent protein (GFP) gene and a Puromycin resistance gene. Step 1 shows the transposition encoding the gene of interest between two

WHAT IS CLAIMED IS:

1. A method for generating a non-human metazoan cell line, the method comprising:

(a) introducing into a population of non-human metazoan cells an exogenous nucleic acid sequence comprising a first polynucleotide encoding a gene of interest located between a 5' transposon inverted terminal repeat (ITR) and a 3' transposon inverted terminal repeat (ITR), and a second polynucleotide encoding a selection marker located outside of the 5' ITR and the 3' ITR, wherein the second polynucleotide is an episomal polynucleotide,

(b) selecting a transduced cell from the population of non-human metazoan cells using the selection marker; and

(c) culturing the transduced non-human metazoan cells under conditions sufficient to expand the transduced cells.

2. The method of claim 1, further comprising a second selecting step.

3. The method of claim 2, wherein the second selecting step comprises using a second selection marker to further select the non-human metazoan transduced cell.

4. The method of any one of claims 1-3, wherein the second selecting step is performed 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 15 days, 16 days, 17 days, 18 days, 19 days, 20 days, 21 days, 22 days, 23 days, 24 days, 25 days, 26 days, 27 days, 28 days, 29 days, or 30 days after the first selecting step.

5. The method of any one of claims 1-4, wherein the second polynucleotide encoding the selection marker remains in the non-human metazoan cell following introduction for at most 10 days, at most 11 days, at most 12 days, at most 13 days, at most 14 days, at most 15 days, at most 16 days, at most 17 days, at most 18 days, at most 19 days, at most 20 days, at most 21 days, at most 22 days, at most 23 days, at most 24 days, at most 25 days, at most 26 days, at most 27 days, at most 28 days, at most 29 days, or at most 30 days.

6. The method of any one of claims 1-5, wherein the non-human metazoan cell line is derived from: livestock, poultry, or game animal species.

7. The method of any one of claims 1-6, wherein the non-human metazoan cell line comprises cells selected from: fibroblasts, mesenchymal cells, chondrocytes, myoblasts, myocytes,

satellite cells, side population cells, muscle derived stem cells, myofibroblasts, mesenchymal stem cells, myogenic pericytes, or mesoangioblasts.

8. The method of any one of claims 1-7, wherein the first polynucleotide is integrated into the genome.

9. The method of any one of claims 1-8, wherein the non-human metazoan cell line is an avian cell line or a bovine cell line.