Claims

1. A population of engineered cells, wherein the engineered cells comprise: at least one exogenous GDNF gene or functional fragment thereof.

2. The population of cells of claim 1, wherein the engineered cells are human DA neuronal cells.

3. The population of cells of claim 1 or claim 2, wherein the engineered cells are engineered to be hemizygous null for SNCA.

4. The population of cells of any one claims 1 to 3, wherein the exogenous GDNF gene or functional fragment thereof is within or near a safe harbor locus.

5. The population of cells of claim 4, wherein the safe harbor locus is selected from chr11: 116192498-116194401; chr4: 180058397-180059507; chr1: 214186905-214187961; chr1: 91164906-91165855; chr1: 88180241-88181223; chr1: 72546731-72548018; chr1: 199684394-199685410; chr1: 104647871-104648861; chr10: 109456554-109457498; chr10: 84613037-84614143; chr10: 128540929-128541943; chr10: 128693340-128695044; chr10: 36778899-36780002; chr10: 36725218-36726233; chr11: 121721801-121724194; chr11: 42524286-42525334; chr11: 81647375-81648903; chr11: 114774331-114775325; chr11: 128021283-128022329; chr11: 116224105-116225916; chr11: 20309899-20311995; chr12: 92649604-92650584; chr12: 94763797-94764936; chr13: 103882813-103883879; chr13: 52967785-52968920; chr13: 52904228-52906942; chr13: 59317916-59319213; chr13: 70309932-70311460; chr13: 76473185-76474229; chr15: 96947520-96948565; chr16: 66127013-66128412; chr17: 56715590-56716621; chr18: 61088416-61089493; chr18: 40576326-40578671; chr18: 27749740-27750778; chr18: 67199846-67201011; chr2: 133886374-133887591; chr2: 163347709-163348850; chr2: 147082542-147083491; chr2: 180125287-180126295; chr2: 121974721-121975718; chr2: 122660609-122661835; chr2: 75920596-75921844; chr2: 103401408-103402401; chr2: 133843863-133844997; chr2: 180123973-180124967; chr2: 160582472-160583418; chr20: 55667282-55669174; chr22: 49103355-49104388; chr3: 67113422-67114525; chr3: 74614218-74615242; chr3: 5426026-5426982; chr3: 117399281-117400399; chr3: 28915343-28917419; chr3: 117439560-117440548; chr3: 117237013-117239704; chr3: 137162914-137164165; chr3: 106609403-106610465; chr3: 67109328-67110455; chr3: 16048355-16049420; chr3: 117397641-117399034; chr3: 104908816-104909868; chr3: 104596179-104597355; chr4: 180057145-

180058232; chr4: 27684257-27685441; chr4: 116925637-116926672; chr4: 166165437-166166501; chr4: 156168620-156169693; chr4: 18689105-18690087; chr4: 85370123-85371213; chr4: 64537927-64540013; chr4: 154982074-154983202; chr4: 130563237-130564339; chr5: 71937993-71940439; chr5: 113806007-113808129; chr5: 18393819-18394795; chr5: 18465687-18466781; chr5: 123874455-123875520; chr5: 123875580-123876580; chr5: 71932489-71933717; chr5: 34474174-34475918; chr5: 18113286-18115121; chr5: 103808080-103809225; chr5: 144598204-144599350; chr5: 108521605-108522526; chr5: 113692453-113694380; chr5: 101678253-101679223; chr5: 87944237-87945265; chr5: 101389859-101390987; chr5: 113678518-113679693; chr5: 88089287-88091255; chr5: 121797421-121798482; chr5: 87949701-87952291; chr5: 103894512-103895553; chr5: 63401153-63402160; chr6: 85922657-85923735; chr6: 104472033-104473578; chr6: 104525429-104526360; chr6: 47097062-47097998; chr6: 87968250-87969278; chr6: 16964556-16965560; chr6: 137415153-137416177; chr6: 91081186-91082896; chr6: 99746005-99747236; chr6: 99822427-99823526; chr6: 140439792-140441984; chr6: 137313918-137314874; chr6: 48765024-48766063; chr6: 90890535-90891762; chr7: 152925055-152926297; chr7: 12111174-12112347; chr7: 42493270-42494306; chr7: 31192574-31193687; chr7: 22000950-22002150; chr7: 96785947-96787328; chr8: 141715497-141716580; chr8: 26189940-26191013; chr8: 131803831-131805003; chr8: 106856111-106857217; chr8: 75975412-75977242; chr8: 115076228-115077322; chr8: 131815871-131817817; chr8: 137492454-137493716; chr9: 85258346-85259505; chr9: 17904998-17907161; chr9: 78439098-78440151; chr9: 16132791-16134459; chr9: 29513212-29515120; chr9: 75265233-75266237; chr9: 7542343-7543943; chr9: 118169738-118170714; chr9: 71517517-71519983; chr9: 7401713-7402906; chr9: 26325995-26327156; chr9: 1453442-1454897; chr9: 105156511-105157922; chr9: 12425914-12426947; chrX: 68894085-68895495; chrX: 20530099-20531285; chrX: 40996550-40997770; chrX: 20527023-20528155; chrX: 94058277-94059471; or chrX: 138127607-138128791.

6. A population of engineered cells, wherein the engineered cells comprise: at least one exogenous GBA gene or functional fragment thereof.

7. The population of cells of claim 6, wherein the engineered cells are human DA neuronal cells.

8. Method for preparing engineered pluripotent cells, the method comprising:

- providing a population of cells comprising pluripotent cells;

- introducing into a proportion of the pluripotent cells: i) at least one nucleic acid comprising an exogenous polynucleotide sequence to be integrated at a selected endogenous locus to encode at least one GDNF gene;

ii) at least one sequence-specific reagent that specifically targets the selected endogenous locus,

wherein the exogenous polynucleotide sequence is inserted by targeted gene integration into the endogenous locus.

9. The method according to claim 8, wherein the sequence specific reagent is a nuclease.

10. The method according to claim 8 or claim 9, wherein the targeted gene integration is operated by homologous recombination or NHEJ into the pluripotent cells.

11. The method according to any one of claims 8 to 10, wherein the exogenous polynucleotide sequence is integrated under transcriptional control of an endogenous promoter present at the locus.

12. The method according to any one of claims 8 to 11, wherein the engineered pluripotent cells are differentiated to DA neuronal cells to form engineered DA neuronal cells and the engineered DA neuronal cells are transplanted into a human and wherein the transplanted engineered DA neuronal cells do not initially express GDNF.

13. Method for preparing engineered pluripotent cells, the method comprising:

- providing a population of cells comprising pluripotent cells;

- introducing into a proportion of the pluripotent cells:

i) at least one nucleic acid comprising an exogenous polynucleotide sequence to be integrated at a selected endogenous locus to encode at least one GBA gene and at least one hemizygous null SCNA gene;

ii) at least one sequence-specific reagent that specifically targets the selected endogenous locus,

wherein the exogenous polynucleotide sequence is inserted by targeted gene integration into the endogenous locus.

14. The method according to claim 13, wherein the engineered pluripotent cells are differentiated to DA neuronal cells to form engineered DA neuronal cells and wherein the engineered DA neuronal cells are transplanted into a human.

15. The method according to claim 14, wherein the transplanted engineered DA neuronal cells express GBA and SCNA upon transplantation and do not exhibit α-synuclein accumulation.