

**[008]** To develop more effective and specific drugs, candidate targets need to be identified directly from humans and human tissues instead of transgenic mice, and the extent that these targets vary across individuals needs to be determined. Therefore, there is a long-felt need to develop a generalizable method that enables molecular studies of defined cell types in wild-type animals and humans. In addition, non-genetic technologies are needed to complement the discovery of specific characteristics of neural cell types and circuits in model systems and provide insight into potentially unique properties of the human nervous system.

## **SUMMARY**

**[009]** Various examples of the disclosure herein provide method profiling the gene expression of a nucleus, the method comprising: processing a tissue sample containing a plurality of cell types to expose nuclei; labeling at least one nucleus from the processed tissue sample by contacting the processed tissue sample with an affinity label that binds a nucleic acid transcript or protein unique to the nucleus of at least one cell type contained in the tissue sample, wherein the nucleic acid transcript or protein is encoded by a gene selected from Tables 19-24; purifying at least one labeled nucleus; and collecting nucleic acid transcript and/or protein expression data for the labeled nucleus, thereby profiling the gene expression of the nucleus.

**[009a]** In one aspect, the present disclosure refers to a method profiling the gene expression of a nucleus, the method comprising:

- (a) processing a tissue sample containing a plurality of cell types to expose nuclei;
- (b) labeling at least one nucleus from the processed tissue sample by contacting the processed tissue sample with an affinity label that binds a nucleic acid transcript or protein unique to the nucleus of at least one cell type contained in the tissue sample, wherein the nucleic acid transcript or protein is encoded by a gene selected from Tables 19-24;
- (c) purifying at least one labeled nucleus; and
- (d) collecting nucleic acid transcript and/or protein expression data for the labeled nucleus, thereby profiling the gene expression of the nucleus.

**[009b]** In another aspect, the present disclosure refers to a method of isolating granule cells, the method comprising:

- (a) processing a tissue sample containing a plurality of cell types to expose nuclei;
- (b) isolating nuclei from the processed tissue sample by contacting the processed tissue sample with at least one affinity label that binds a nucleic acid transcript or protein encoded by one of genes *Itpr*, *NeuN*, *Cdh15*, *Calb2*, *Rbfox3*, *Neurod1*, and *Reln*, wherein the nucleic acid transcript or protein is unique to the nuclei of granule cells; and

- (c) purifying at least one labeled nucleus.

**[009c]** In yet another aspect, the present disclosure refers to a method of isolating Purkinje cells, the method comprising:

- (a) processing a tissue sample containing a plurality of cell types to expose nuclei;
- (b) isolating nuclei from the processed tissue sample by contacting the processed tissue sample with an affinity label that binds a nucleic acid transcript or protein encoded by one of genes *Pcp2*, *Pvalb*, *Cabl1*, and *Itpr1*; and
- (c) purifying at least one labeled nucleus.

**[009d]** In yet another aspect, the present disclosure refers to a method of isolating Purkinje cells, the method comprising:

- (a) processing a tissue sample containing a plurality of cell types to expose nuclei;
- (b) isolating nuclei from the processed tissue sample by contacting the processed tissue sample with an affinity label that binds a nucleic acid transcript or protein encoded by one of genes *Lypd6*, *Pvalb*, *Kit*, *NeuN*, *Itpr*, and *Sorcs3*; and
- (c) purifying at least one labeled nucleus.

**[009e]** In yet another aspect, the present disclosure refers to a method of isolating astrocytes and oligodendrocytes, the method comprising:

- (a) processing a tissue sample containing a plurality of cell types to expose nuclei;
- (b) isolating nuclei from the processed tissue sample by contacting the processed tissue sample with an affinity label that binds a nucleic acid transcript or protein encoded by one of genes *Olig2*, *Pdga*, *Cspg4*, *Mag*, *Mbp*, and *Mog*; and
- (c) purifying at least one labeled nucleus.

**[009f]** In yet another aspect, the present disclosure refers to a method of isolating astrocytes, the method comprising:

- (a) processing a tissue sample containing a plurality of cell types to expose nuclei;
- (b) isolating nuclei from the processed tissue sample by contacting the processed tissue sample with an affinity label that binds a nucleic acid transcript or protein encoded by one of genes *Aldh1a1*, *Gfap*, *S110b*, and *Slc1a3*; and
- (c) purifying at least one labeled nucleus.

**[009g]** In yet another aspect, the present disclosure refers to a method of isolating basket cells, the method comprising:

- (a) processing a tissue sample containing a plurality of cell types to expose nuclei;
- (b) isolating nuclei from the processed tissue sample by contacting the processed tissue sample with at least one affinity label that binds a nucleic acid transcript or protein encoded by *Sorcs3* and *NeuN*, wherein the combination of the nucleic acid

transcript or protein encoded by Sorcs3 and is unique to the nuclei of basket cells;  
and

- (c) purifying at least one labeled nucleus.

**[009h]** In yet another aspect, the present disclosure refers to a method of isolating dopaminergic neurons, the method comprising:

- (a) processing a tissue sample containing a plurality of cell types to expose nuclei;
- (b) isolating nuclei from the processed tissue sample by contacting the processed tissue sample with at least one affinity label that binds a nucleic acid transcript or protein encoded by FoxA1, Slc6a3, TH, FoxA2, or Drd2, wherein nucleic acid transcript or protein is unique to the nuclei of dopaminergic neurons; and
- (c) purifying at least one labeled nucleus.

**[009i]** In yet another aspect, the present disclosure refers to a method of isolating brainstem motor neurons, the method comprising:

- (a) processing a tissue sample containing a plurality of cell types to expose nuclei;
- (b) isolating nuclei from the processed tissue sample by contacting the processed tissue sample with at least one affinity label that binds a nucleic acid transcript or protein encoded by VaChT or ErrB, wherein nucleic acid transcript or protein is unique to the nuclei of brainstem motor neurons; and
- (c) purifying at least one labeled nucleus.

**[009j]** In yet another aspect, the present disclosure refers to a method of profiling epigenetic expression of a nucleus, the method comprising:

- (a) processing a tissue sample containing a plurality of cell types to expose nuclei;
- (b) isolating nuclei from the processed tissue sample by contacting the processed tissue with an affinity label that binds a factor unique to at least one cell type, wherein the affinity label is selected from the group consisting of an antibody, a RNA probe, and a DNA probe;
- (c) purifying at least one labeled nucleus; and
- (d) collecting information regarding histone modifications, binding of transcription factors, and DNA modifications for the nucleus, thereby profiling the epigenetic expression.

**[009k]** In yet another aspect, the present disclosure refers to a method of profiling nuclear architecture of a nucleus, the method comprising:

- (a) processing a tissue sample containing a plurality of cell types to expose nuclei;
- (b) isolating nuclei from the processed tissue sample by contacting the processed tissue with an affinity label that binds a factor unique to at least one cell type,

wherein the affinity label is selected from the group consisting of an antibody, a RNA probe, and a DNA probe;

- (c) purifying at least one labeled nucleus; and
- (d) collecting information regarding chromosome conformation for the labeled nucleus, thereby profiling the nuclear architecture.

**[0091]** In yet another aspect, the present disclosure refers to a method of profiling nuclear architecture of a nucleus, the method comprising:

- (a) processing a tissue sample containing a plurality of cell types to expose nuclei;
- (b) isolating nuclei from the processed tissue sample by contacting the processed tissue with an affinity label that binds a factor unique to at least one cell type, wherein the affinity label is an antibody;
- (c) isolating gDNA from the nuclei;
- (d) performing oxidative bisulfite sequencing;
- (e) collecting chromosome conformation for the labeled nucleus, thereby profiling the nuclear architecture.

**[009m]** In yet another aspect, the present disclosure refers to a method of isolating a nucleus from a single cell type, the method comprising:

- (a) processing a tissue sample containing a plurality of cell types to expose nuclei;
- (b) isolating nuclei from the processed tissue sample by contacting the processed tissue sample with more than two affinity labels specific to a unique combination of nucleic acid transcripts and/or proteins for the cell type, wherein each affinity label specifically binds a nucleic acid transcript comprising a sequence selected from SEQ ID NO: 1-28,815 or the affinity label specifically binds a protein comprising an amino acid sequence selected from 28,816-38,643; and
- (c) purifying at least one labeled nucleus, thereby isolating the nucleus from the cell type.

**[0010]** In some examples, the cell type originates in cerebellar tissue, and is selected from the group consisting of: granule cells, Purkinje cells, glia, Bergmann glia, dopaminergic neurons, basket/stellate cells, astrocytes, brainstem motor neurons, oligodendrocytes, upper motor neurons, lower motor neurons, and cerebellar deep nuclei. In some examples, the protein is a transcription factor localized within the nuclei. In some examples, the cell type originates in basal ganglia tissue, and is selected from the group consisting of: striatonigral medium-sized spiny neurons (MSNs), striatopallidal MSNs, striatal cholinergic interneurons, subthalamic nucleus, dopaminergic neurons, and bed nucleus of the stria terminalis (BNST) neurons. In some

examples, the cell type originates in thalamus tissue, and is selected from the group consisting of: thalamocortical neurons, thalamostriatal neurons, and thalamic reticular nucleus neurons. In some examples, the cell type originates in cortex tissue, and is selected from the group consisting of corticostriatal neurons, entorhinal cortex layer 2/3 neurons, fast-spiking cortical interneurons, and layer 2/3 pyramidal cells from pre-frontal cortex tissue. the cell type is cholinergic projection neurons from medial habenula tissue of a pineal gland. In some examples, the cell type originates in hippocampus tissue, and is selected from the group consisting of: cornu ammonis region 1 (CA1), cornu ammonis region 2 (CA2), cornu ammonis region 3 (CA3), and dentate gyrus cells. In some examples, the cell type originates in at least one tissue selected from the group consisting of: brain tissue, brain stem tissue, and spinal cord tissue.

**[0011]** Various examples of the invention herein provide a method of isolating granule cells, the method comprising: processing a tissue sample containing a plurality of cell types to expose nuclei; isolating nuclei from the processed tissue sample by contacting the processed tissue sample with at least one affinity label that binds a nucleic acid transcript or protein encoded by one of genes *Itpr*, *NeuN*, *Cdh15*, *Calb2*, *Rbfox3*, *Neurod1*, and *Reln*, wherein the nucleic acid transcript or protein is unique to the nuclei of granule cells; and purifying at least one labeled nucleus. In some examples, the at least one labeled nucleus does not include a nucleic acid transcript encoded by *Olig2*.

**[0012]** Various examples of the disclosure herein provide a method of isolating Purkinje cells, the method comprising: processing a tissue sample containing a plurality of cell types to expose nuclei; isolating nuclei from the processed tissue sample by contacting the processed tissue sample with an affinity label that binds a nucleic acid transcript or protein encoded by one of genes *Pcp2*, *Pvalb*, *Cabl1*, and *Itpr1*; and purifying at least one labeled nucleus.

**[0013]** Various examples of the disclosure herein provide a method of isolating Purkinje cells, the method comprising: processing a tissue sample containing a plurality of cell types to expose nuclei; isolating nuclei from the processed tissue sample by contacting the processed tissue sample with an affinity label that binds a nucleic acid transcript or protein encoded by one of genes *Lypd6*, *Pvalb*, *Kit*, *NeuN*, *Itpr*, and *Sorcs3*; and purifying at least one labeled nucleus.

**[0014]** Various examples of the disclosure herein provide a method of isolating astrocytes and oligodendrocytes, the method comprising: processing a tissue sample containing a plurality of cell types to expose nuclei; isolating nuclei from the processed tissue sample by contacting the processed tissue sample with an affinity label that binds a nucleic acid transcript or protein encoded by one of genes *Olig2*, *Pdga*, *Cspg4*, *Mag*, *Mbp*, and *Mog*; and purifying at least one labeled nucleus.

**[0015]** Various examples of the disclosure herein provide a method of isolating astrocytes, the method comprising: processing a tissue sample containing a plurality of cell types to expose nuclei; isolating nuclei from the processed tissue sample by contacting the processed tissue sample with an affinity label that binds a nucleic acid transcript or protein encoded by one of genes *Aldh1a1*, *Gfap*, *S110b*, and *Slc1a3*; and purifying at least one labeled nucleus.

**[0016]** Various examples of the disclosure herein provide a method of isolating basket cells, the method comprising: processing a tissue sample containing a plurality of cell types to expose nuclei; isolating nuclei from the processed tissue sample by contacting the processed tissue sample with at least one affinity label that binds a nucleic acid transcript or protein encoded by *Sorcs3* and *NeuN*, wherein the combination of the nucleic acid transcript or protein encoded by *Sorcs3* and is unique to the nuclei of basket cells; and purifying at least one labeled nucleus.

**[0017]** Various examples of the disclosure herein provide a method of isolating dopaminergic neurons, the method comprising: processing a tissue sample containing a plurality of cell types to expose nuclei; isolating nuclei from the processed tissue sample by contacting the processed tissue sample with at least one affinity label that binds a nucleic acid transcript or protein encoded by *FoxA1*, *Slc6a3*, *TH*, *FoxA2*, or *Drd2*, wherein nucleic acid transcript or protein is unique to the nuclei of dopaminergic neurons; and purifying at least one labeled nucleus.

**[0018]** Various examples of the disclosure herein provide a method of isolating brainstem motor neurons, the method comprising: processing a tissue sample containing a plurality of cell types to expose nuclei; isolating nuclei from the processed tissue sample by contacting the processed tissue sample with at least one affinity label that binds a nucleic acid transcript or protein encoded by *VaChT* or *ErrB*, wherein nucleic acid transcript or protein is unique to the nuclei of brainstem motor neurons; and purifying at least one labeled nucleus.

**[0019]** In some examples, the affinity label is selected from the group consisting of an antibody, a RNA probe, and a DNA probe. In some examples, the method further comprises collecting nucleic acid transcript and/or protein transcript data for the labeled nucleus, thereby profiling the gene expression of the nucleus. In some examples, the method further comprises: comparing gene expression of the nucleus to gene expression in at least one nucleus of the cell type from a different tissue sample to identify variability in gene expression, thereby obtaining a drug target. Alternatively, in some examples, the method further comprises comparing gene expression of the nucleus to gene expression in at least one nucleus of a different cell type to identify variability in gene expression, thereby obtaining a drug target.

**[0020]** In some examples, the affinity label further comprising a fluorescent tag. In some examples, purifying of the nucleus is performed by fluorescence-activated cell sorting (FACS).

In some examples, the tissue sample is derived from a mammal. In some examples, the mammal is selected from the group consisting of: a mouse, a human, a rat, and another non-human primate. In some examples, the tissue sample or the different tissue sample was frozen prior to isolation of the nucleus. In some examples, the tissue sample or the different tissue sample was fresh prior to isolation of the nuclei. In some examples, the tissue sample or the different tissue sample is derived from a female. In some examples, the tissue sample or the different tissue sample is derived from a male. In some examples, the nuclei are contiguous to an endoplasmic reticulum. In some examples, the protein is a membrane protein. In some examples, the membrane protein is synthesized in the endoplasmic reticulum. In some examples, the protein is localized in the endoplasmic reticulum. In some examples, the nucleic acid transcript is localized in the nuclei. In some examples, the RNA probe specifically binds a chromosomal associated transcript (CAT) or a polyA transcript. In some examples, the DNA probe specifically binds a transporter gene. In some examples, the affinity label is more than one antibody, RNA probe, or DNA probe. In some examples, each affinity label binds a different factor. In some examples, the more than one antibody, RNA probe, or DNA probe bind to the same factor.

**[0021]** In some examples, the tissue sample is post-mortem. In some examples, the tissue sample or the different tissue sample originates from a diseased subject. In some examples, the tissue sample or the different tissue sample originates in a healthy subject. In some examples, the diseased subject is affected by at least one condition selected from the group consisting of: ataxia, Parkinson's disease, Alzheimer's disease, and amyotrophic lateral sclerosis (ALS), and Huntington's disease. In some examples, the cell type is associated with ataxia, and is selected from the group consisting of: Purkinje cells, granule cells, Bergmann glia, basket/stellate cells, astrocytes, oligodendrocytes, and cerebellar deep nuclei. In some examples, the cell type is associated with Parkinson's Disease, and is selected from the group consisting of: substantia nigra and VTA dopaminergic neurons. In some examples, the cell type is associated with Alzheimer's Disease, and is selected from: layer 2/3 entorhincortex, CA1 hippocampus, and CA2/3 hippocampus. In some examples, the cell type is associated with ALS, and is selected from the group consisting of: brain stem, cortical motor neurons, and spinal cord motor neurons. In some examples, the diseased subject is expressing a disease phenotype. In some examples, the diseased subject is not expressing a disease phenotype. In some examples, the method further comprises comparing profiling for a tissue sample originating from a diseased subject and a tissue sample from a healthy subject. In some examples, the method further comprises comparing profiling for a tissue sample originating from at least two different healthy subjects. In some examples, the method further comprises identifying variability between disease stages by

comparing profiling for a tissue sample from a diseased subject expressing a disease phenotype with profiling for a tissue sample from a diseased subject not expressing a disease phenotype.

**[0022]** In some examples, the method further comprises profiling the gene expression is performed for a plurality of nuclei from a single cell type. In some examples, the method further comprises profiling the gene expression is performed for a plurality of nuclei from different cell types. In some examples, the method further comprises identifying variability among different subjects by comparing profiling for more than one tissue sample, wherein each tissue sample is from a different subject. In some examples, the drug target is encoded by a gene selected from Tables 19-24.

**[0023]** Various examples of the disclosure herein provide a method of profiling epigenetic expression of a nucleus, the method comprising: processing a tissue sample containing a plurality of cell types to expose nuclei; isolating nuclei from the processed tissue sample by contacting the processed tissue with an affinity label that binds a factor unique to at least one cell type, wherein the affinity label is selected from the group consisting of an antibody, a RNA probe, and a DNA probe; purifying at least one labeled nucleus; and collecting information regarding histone modifications, binding of transcription factors, and DNA modifications for the nucleus, thereby profiling the epigenetic expression.

**[0024]** Various examples of the disclosure herein provide a method of profiling nuclear architecture of a nucleus, the method comprising: processing a tissue sample containing a plurality of cell types to expose nuclei; isolating nuclei from the processed tissue sample by contacting the processed tissue with an affinity label that binds a factor unique to at least one cell type, wherein the affinity label is selected from the group consisting of an antibody, a RNA probe, and a DNA probe; purifying at least one labeled nucleus; and collecting information regarding chromosome conformation for the labeled nucleus, thereby profiling the nuclear architecture.

**[0025]** Various examples of the disclosure herein provide a method of profiling nuclear architecture of a nucleus, the method comprising: processing a tissue sample containing a plurality of cell types to expose nuclei; isolating nuclei from the processed tissue sample by contacting the processed tissue with an affinity label that binds a factor unique to at least one cell type, wherein the affinity label is an antibody; isolating gDNA from the nuclei; performing oxidative bisulfite sequencing; and collecting chromosome conformation for the labeled nucleus, thereby profiling the nuclear architecture.

**[0026]** Various examples of the disclosure herein provide a method of isolating a nucleus from a single cell type, the method comprising: processing a tissue sample containing a plurality of cell types to expose nuclei; isolating nuclei from the processed tissue sample by contacting the

processed tissue sample with more than two affinity labels specific to a unique combination of nucleic acid transcripts and/or proteins for the cell type, wherein each affinity label specifically binds a nucleic acid transcript comprising a sequence selected from SEQ ID NO: 1-28,815 or the affinity label specifically binds a protein comprising an amino acid sequence selected from 28,816-38,643; and purifying at least one labeled nucleus, thereby isolating the nucleus from the cell type.