CLAIMS

WHAT IS CLAIMED IS:

1. A method of identifying a regulatory element that provides selective expression in a given cell type, comprising:

    a. providing cells with a mixture of vectors each comprising a candidate regulatory element operably linked to a transgene, wherein each vector further comprises a barcode;

    b. isolating RNA from a plurality of single cells expressing said transgene;

    c. identifying each of said single cells by sequencing the transcriptome of each of the single cells; and

    d. correlating the barcode in the transcriptome to a candidate regulatory element

    thereby identifying a regulatory element that provides selective expression in the cell type.

2. The method of claim 1, wherein the regulatory element selectively increases expression of the transgene in the cell type.

3. The method of claim 1, wherein the regulatory element provides selective expression of the transgene that is at least 2 fold, at least 4 fold, at least 6 fold, at least 8 fold, or at least 10 fold greater or less as compared to expression driven by another candidate regulatory and/or a control regulatory element in the same cell type.

4. The method of claim 1, wherein the regulatory element provides selective expression of the transgene that is at least 2%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% greater or less as compared to expression driven by another candidate regulatory element and/or control regulatory element in the same cell type.

5. The method of claim 1, wherein the regulatory element provides selective expression of the transgene that is about 1.5 times, 2 times, 3 times, 4 times, 5 times, 6 times, 7 times, 7.5

times, 8 times, 9 times, or 10 times greater or less as compared to expression driven by another candidate regulatory element and/or control regulatory element in the same cell type.

6. The method of claim 1, wherein the regulatory element provides selective expression of the transgene that is at least 2 fold, at least 4 fold, at least 6 fold, at least 8 fold, or at least 10 fold greater or less as compared to expression of the transgene from the same regulatory element in a different cell type.

7. The method of claim 1, wherein the regulatory element provides selective expression of the transgene that is at least 2%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% greater or less compared to expression of the transgene from the same regulatory element in a different cell type.

8. The method of claim 1, wherein the regulatory element provides selective expression of the transgene that is about 1.5 times, 2 times, 3 times, 4 times, 5 times, 6 times, 7 times, 7.5 times, 8 times, 9 times, or 10 times greater or less compared to expression of the transgene from the same regulatory element in a different cell type.

9. The method of claim 1, wherein the regulatory element provides selective expression of the transgene in one cell type over at least one other cell type.

10. The method of claim 1, wherein the regulatory element provides selective expression of the transgene in parvalbumin (PV) neurons as compared to non-PV neurons.

11. The method of claim 10, wherein the non-PV neuron is one or more of excitatory neurons, dopaminergic neurons, astrocytes, microglia, or motor neurons.

12. A method of identifying a regulatory element that provides selective expression of the transgene in a cell type, comprising:

    a. providing cells with a mixture of vectors each comprising a candidate regulatory element operably linked to a transgene, wherein each vector further comprises a barcode;

b. isolating RNA from a plurality of single cells expressing said transgene;

c. identifying each of said single cells by sequencing the transcriptome of each of the single cells;

d. correlating the barcode in the transcriptome to the candidate regulatory element; and

e. comparing expression level of the transgene provided by each candidate regulatory element to a reference expression level of the transgene;

thereby identifying the candidate regulatory element that provides selective expression of the transgene in the cell type.

13. The method of claim 12, wherein the regulatory element selectively increases or decreases expression of the transgene in the cell type.

14. The method of claim 12, wherein the reference expression level of the transgene is provided by a control regulatory element.

15. The method of claim 12, wherein the regulatory element provides selective expression of the transgene that is at least 2 fold, at least 4 fold, at least 6 fold, at least 8 fold, or at least 10 fold greater or less as compared to expression driven by another candidate regulatory element and/or control regulatory element in the same cell type.

16. The method of claim 12, wherein the regulatory element provides selective expression of the transgene that is at least 2%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% greater or less as compared to expression driven by another candidate regulatory element and/or control regulatory element in the same cell type.

17. The method of claim 12, wherein the regulatory element provides selective expression of the transgene that is about 1.5 times, 2 times, 3 times, 4 times, 5 times, 6 times, 7 times, 7.5 times, 8 times, 9 times, or 10 times greater or less as compared to expression driven by

another candidate regulatory element and/or control regulatory element in the same cell type.

18. The method of claim 12, wherein the reference expression level of the transgene is provided by a pan-cellular regulatory element.

19. The method of claim 12, wherein the pan-cellular regulatory element is selected from the group consisting of cytomegalovirus major immediate-early promoter (CMV), chicken β-actin promoter (CBA), CMV early enhancer/CBA promoter (CAG), elongation factor-1α promoter (EF1α), simian virus 40 promoter (SV40), phosphoglycerate kinase promoter (PGK), and the polyubiquitin C gene promoter (UBC).

20. The method of claim 12, wherein the regulatory element provides selective expression of the transgene that is at least 2 fold, at least 4 fold, at least 6 fold, at least 8 fold, or at least 10 fold greater or less as compared to expression driven by a pan-cellular regulatory element in the same cell type.

21. The method of claim 12, wherein the regulatory element provides selective expression of the transgene that is at least 2%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% greater or less as compared to expression driven by a pan-cellular regulatory element in the same cell type.

22. The method of claim 12, wherein the regulatory element provides selective expression of the transgene that is about 1.5 times, 2 times, 3 times, 4 times, 5 times, 6 times, 7 times, 7.5 times, 8 times, 9 times, or 10 times greater or less as compared to expression driven by a pan-cellular regulatory element in the same cell type.

23. The method of claim 12, wherein the regulatory element provides selective expression of the transgene in one cell type over at least one other cell type.

24. The method of claim 12, wherein the regulatory element results in selective expression of the transgene in PV neurons as compared to non-PV neurons.

25. The method of claim 24, wherein the non-PV neuron is one or more of excitatory neurons, dopaminergic neurons, astrocytes, microglia, or motor neurons.

26. A method of identifying a cell type that selectively expresses a transgene operably linked to a regulatory element, comprising:

    a. providing cells with a mixture of vectors each comprising a candidate regulatory element operably linked to a transgene, wherein each vector further comprises a barcode;

    b. isolating RNA from a plurality of single cells expressing said transgene;

    c. identifying each of said single cells by sequencing the transcriptome of each of the single cells;

    d. correlating the barcode in the transcriptome to the candidate regulatory element; and

    e. comparing expression level of the transgene provided by the candidate regulatory element in one cell type to expression level of the same candidate regulatory element in a different cell type

thereby identifying the cell type that selectively expresses the transgene operably linked regulatory element.

27. The method of claim 26, wherein the regulatory element selectively increases or decreases expression of the transgene in one cell type as compared to at least one other cell type.

28. The method of claim 26, wherein the regulatory element provides selective expression of the transgene in one cell type that is at least 2 fold, at least 4 fold, at least 6 fold, at least 8 fold, or at least 10 fold greater or less as compared to expression driven by the regulatory element in at least one other cell type.

29. The method of claim 26, wherein the regulatory element provides selective expression of the transgene in one cell type that is at least 2%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least

85%, at least 90%, or at least 95% greater or less as compared to expression driven by the regulatory element in at least one other cell type.

30. The method of claim 26, wherein the regulatory element provides selective expression of the transgene in one cell type that is about 1.5 times, 2 times, 3 times, 4 times, 5 times, 6 times, 7 times, 7.5 times, 8 times, 9 times, or 10 times greater or less as compared to expression driven by the regulatory element in at least one other cell type.

31. The method of claim 26, wherein the regulatory element results in selective expression of the transgene in PV neurons as compared to non-PV neurons.

32. The method of claim 31, wherein the non-PV neuron is one or more of excitatory neurons, dopaminergic neurons, astrocytes, microglia, or motor neurons.

33. The method of any one of claims 1-32, wherein the RNA is selected from the group consisting of: mRNA, long noncoding RNA, antisense transcripts, and pri-miRNAs.

34. The method of any one of claims 1-33, wherein the vector is selected from the group consisting of: a plasmid, a viral vector, or a cosmid.

35. The method of claim 34, wherein the viral vector is an adeno-associated virus (AAV) vector.

36. The method of claim 35, wherein the AAV vector is AAV1, AAV8, AAV9, scAAV1, scAAV8, or scAAV9.

37. The method of claim 36, wherein the AAV vector is AAV9.

38. The method of any one of claims 35-37, wherein the vector comprises a 5’ AAV inverted terminal repeat (ITR) sequence and a 3’ AAV ITR sequence.

39. The method of any one of claims 1-38, wherein the mixture of vectors comprises at least 10^4 candidate regulatory elements.

40. The method of any one of claims 1-39, wherein each candidate regulatory element correlates to at least one unique barcode.

41. The method of any one of claims 1-40, wherein the transgene comprises a reporter gene sequence.

42. The method of claim 41, wherein the reporter gene sequence is operably linked to a sequence encoding a nuclear binding domain.

43. The method of any one of claims 1-43, wherein the transgene comprises the barcode.

44. The method of any one of claims 42-44, wherein the reporter gene sequence comprises the barcode.

45. The method of claim 43 or 44, wherein the barcode comprises alternative codons.

46. The method of any one of claims 43-45, wherein the sequence encoding the nuclear binding domain comprises the barcode.

47. The method of any one of claims 43-46, wherein the sequence encoding the nuclear binding domain encodes a Klarsicht/ANC-1/Syne homology (KASH) domain or a Sad1p/UNC-84 (SUN) domain protein, or biologically active fragment thereof.

48. The method of any one of claims 1-47, wherein the cell type belongs to a tissue selected from the group consisting of: connective tissue, muscular tissue, nervous tissue, and epithelial tissue.