

We claim:

1. A method for producing a modified cell with an increased number of copies of a repeat sequence at a target genomic locus, comprising:
 - (a) providing a population of cells comprising a repeat expansion sequence comprising a plurality of copies of the repeat sequence at the target genomic locus;
 - (b) introducing into the population of cells a nuclease agent or a nucleic acid encoding the nuclease agent,
wherein the nuclease agent is a zinc finger nuclease (ZFN), a Transcription Activator-Like Effector Nuclease (TALEN), or a Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-associated (Cas) protein and a guide RNA, and
wherein the nuclease agent makes a double-strand break or a single-strand break at a nuclease target site within about 25 nucleotides of the 5' end or the 3' end of the repeat expansion sequence to produce a population of modified cells; and
 - (c) quantifying the number of copies of the repeat sequence in the population of modified cells and selecting a modified cell in which the number of copies of the repeat sequence has been increased.
2. The method of claim 1, wherein an exogenous repair template is not introduced into the population of cells.
3. The method of claim 1 or 2, wherein the nuclease target site is within about 20 nucleotides of the 5' end or the 3' end of the repeat expansion sequence or overlaps with the 5' end or the 3' end of the repeat expansion sequence.
4. The method of any one of claims 1-3, wherein the nuclease target site is within about 15 nucleotides of the 5' end or the 3' end of the repeat expansion sequence or overlaps with the 5' end or the 3' end of the repeat expansion sequence.
5. The method of any one of claims 1-4, wherein the nuclease target site overlaps with the 5' end or the 3' end of the repeat expansion sequence.

6. The method of claim 1 or 2, wherein the nuclease target site is within about 25 nucleotides of the 5' end of the repeat expansion sequence.
7. The method of claim 1 or 2, wherein the nuclease target site is within about 25 nucleotides of the 3' end of the repeat expansion sequence.
8. The method of any one of claims 1-4, 6, and 7, wherein the nuclease target site is outside of the repeat expansion sequence.
9. The method of any one of claims 1-8, wherein the site at which the nuclease agent makes the double-strand break or the single-strand break is within about 25 nucleotides of the 5' end or the 3' end of the repeat expansion sequence.
10. The method of any one of claims 1-9, wherein the site at which the nuclease agent makes the double-strand break or the single-strand break is within about 15 nucleotides of the 5' end or the 3' end of the repeat expansion sequence.
11. The method of any one of claims 1-9, wherein the site at which the nuclease agent makes the double-strand break or the single-strand break is within about 25 nucleotides of the 5' end of the repeat expansion sequence.
12. The method of any one of claims 1-9, wherein the site at which the nuclease agent makes the double-strand break or the single-strand break is within about 25 nucleotides of the 3' end of the repeat expansion sequence.
13. The method of any one of claims 1-12, wherein the site at which the nuclease agent makes the double-strand break or the single-strand break is outside of the repeat expansion sequence.
14. The method of any one of claims 1-13, wherein the nuclease target site is retained after repair of the double-strand break or the single strand-break by the cells.

15. The method of any one of claims 1-14, wherein repair of the double-strand break or the single strand-break does not result in insertions or deletions outside of the repeat expansion sequence.

16. The method of any one of claims 1-9 and 13-15, wherein the nuclease agent makes the double-strand break within about 25 nucleotides of the 5' end or the 3' end of the repeat expansion sequence.

17. The method of any one of claims 1-9 and 13-15, wherein the nuclease agent is a nickase that makes the single-strand break within about 25 nucleotides of the 5' end or the 3' end of the repeat expansion sequence.

18. The method of any one of claims 1, 2, 8, and 13-15, wherein a first nuclease agent or a nucleic acid encoding the first nuclease agent and a second nuclease agent or a nucleic acid encoding the second nuclease agent are introduced into the population of cells, wherein the first nuclease agent makes a double-strand break or a single-strand break at a first nuclease target site within about 25 nucleotides of the 5' end of the repeat expansion sequence, and the second nuclease agent makes a double-strand break or a single-strand break at a second nuclease target site within about 25 nucleotides of the 3' end of the repeat expansion sequence.

19. The method of any one of claims 1, 2, 8, 13-15, and 18, wherein the first nuclease agent makes the double-strand break at the first nuclease target site within about 25 nucleotides of the 5' end of the repeat expansion sequence, and the second nuclease agent makes the double-strand break at the second nuclease target site within about 25 nucleotides of the 3' end of the repeat expansion sequence.

20. The method of any one of claims 1, 2, 8, 13-15, and 18, wherein the first nuclease agent makes the single-strand break at the first nuclease target site within about 25 nucleotides of the 5' end of the repeat expansion sequence, and the second nuclease agent makes

the single-strand break at the second nuclease target site within about 25 nucleotides of the 3' end of the repeat expansion sequence.

21. The method of any one of claims 1-20, wherein the nuclease agent is the Cas protein and the guide RNA.

22. The method of any one of claims 1-21, wherein the Cas protein is a Cas9 protein.

23. The method of any one of claims 1-22, wherein the repeat expansion sequence is a heterologous repeat expansion sequence.

24. The method of any one of claims 1-23, wherein the repeat expansion sequence comprises at least about 10, at least about 20, at least about 30, at least about 40, at least about 50, at least about 60, at least about 70, at least about 80, or at least about 90 copies of the repeat sequence.

25. The method of any one of claims 1-24, wherein the copies of the repeat sequence are contiguous in the repeat expansion sequence.

26. The method of any one of claims 1-25, wherein the repeat sequence is a trinucleotide repeat, a tetranucleotide repeat, a pentanucleotide repeat, a hexanucleotide repeat, or a dodecanucleotide repeat.

27. The method of any one of claims 1-26, wherein the repeat sequence comprises any one of SEQ ID NOS: 1-12.

28. The method of any one of claims 1-27, wherein:

(i) the repeat sequence comprises SEQ ID NO: 2, and the target genomic locus is an *HTT*, *AR*, *ATN1*, *ATXN1*, *ATXN2*, *ATXN3*, *CACNA1A*, *ATXN7*, *PPP2R2B*, or *TBP* locus;

- (ii) the repeat sequence comprises SEQ ID NO: 3, and the target genomic locus is an *FMR1* locus;
- (iii) the repeat sequence comprises SEQ ID NO: 4, and the target genomic locus is a *DMPK*, *JPH3*, *ATXN8*, or *TCF4* locus;
- (iv) the repeat sequence comprises SEQ ID NO: 5, and the target genomic locus is an *FXN* locus;
- (v) the repeat sequence comprises SEQ ID NO: 6, and the target genomic locus is an *AFF2* locus;
- (vi) the repeat sequence comprises SEQ ID NO: 7, and the target genomic locus is a *PABPN1* locus;
- (vii) the repeat sequence comprises SEQ ID NO: 8, and the target genomic locus is a *CNBP* locus;
- (viii) the repeat sequence comprises SEQ ID NO: 9, and the target genomic locus is an *ATXN10* locus;
- (ix) the repeat sequence comprises SEQ ID NO: 10, and the target genomic locus is a *TK2* or *BEAN1* locus;
- (x) the repeat sequence comprises SEQ ID NO: 11, and the target genomic locus is a *NOP56* locus;
- (xi) the repeat sequence comprises SEQ ID NO: 1, and the target genomic locus is a *C9ORF72* locus; or
- (xii) the repeat sequence comprises SEQ ID NO: 12, and the target genomic locus is a *CSTB* locus.

29. The method of any one of claims 1-28, wherein the repeat sequence comprises SEQ ID NO: 1, and the target genomic locus is the *C9ORF72* locus.

30. The method of any one of claims 1-29, wherein the nuclease agent is the Cas protein and the guide RNA, wherein the Cas protein is a Cas9 protein, and the nuclease target site comprises SEQ ID NO: 28 or 33.

31. The method of any one of claims 1-30, wherein the cells are non-human animal cells.
32. The method of any one of claims 1-31, wherein the cells are non-human animal embryonic stem cells, embryonic stem-cell-derived motor neurons, brain cells, cortical cells, neuronal cells, muscle cells, heart cells, or germ cells.
33. The method of any one of claims 1-31, wherein the cells are non-human animal one-cell stage embryos.
34. The method of any one of claims 1-33, wherein the cells are rodent cells.
35. The method of any one of claims 1-34, wherein the cells are mouse cells or rat cells.
36. The method of any one of claims 1-35, wherein the cells are mouse cells.
37. The method of any one of claims 1-31, wherein the cells are mouse embryonic stem cells or mouse one-cell stage embryos.
38. The method of any one of claims 1-30, wherein the cells are human induced pluripotent stem cells.
39. The method of any one of claims 1-38, wherein the cells are *in vitro*.
40. The method of any one of claims 1-37, wherein the cells are *in vivo*.
41. The method of any one of claims 1-40, wherein the method comprises multiple rounds of steps (a)-(c), wherein the population of cells in step (a) in each round after the first round is a clonal population of cells expanded from the modified cell selected in step (c) of the previous round.

42. The method of claim 41, wherein the method comprises at least 3 rounds or at least 4 rounds.

43. The method of claim 41 or 42, wherein:

(I) the method is performed a first time with a first nuclease agent that makes a double-strand break or a single-strand break at a first nuclease target site within about 25 nucleotides of the 5' end of the repeat expansion sequence to produce a first modified cell, and the method is performed a second time on the first modified cell with a second nuclease agent that makes a double-strand break or a single-strand break at a second nuclease target site within about 25 nucleotides of the 3' end of the repeat expansion sequence to produce a second modified cell;

(II) the method is performed a first time with a first nuclease agent that makes a double-strand break or a single-strand break at a first nuclease target site within about 25 nucleotides of the 3' end of the repeat expansion sequence to produce a first modified cell, and the method is performed a second time on the first modified cell with a second nuclease agent that makes a double-strand break or a single-strand break at a second nuclease target site within about 25 nucleotides of the 5' end of the repeat expansion sequence to produce a second modified cell;

(III) the method is performed a first time with a first nuclease agent that makes a double-strand break or a single-strand break at a first nuclease target site within about 25 nucleotides of the 5' end of the repeat expansion sequence to produce a first modified cell, and the method is performed a second time on the first modified cell with a second nuclease agent that makes a double-strand break or a single-strand break at a second nuclease target site within about 25 nucleotides of the 5' end of the repeat expansion sequence to produce a second modified cell;

(IV) the method is performed a first time with a first nuclease agent that makes a double-strand break or a single-strand break at a first nuclease target site within about 25 nucleotides of the 3' end of the repeat expansion sequence to produce a first modified cell, and the method is performed a second time on the first modified cell with a second nuclease agent that makes a double-strand break or a single-strand break at a second nuclease target site within

about 25 nucleotides of the 3' end of the repeat expansion sequence to produce a second modified cell;

(V) the method is performed a first time with a first nuclease agent that makes a double-strand break at a first nuclease target site within about 25 nucleotides of the 5' end of the repeat expansion sequence to produce a first modified cell, and the method is performed a second time on the first modified cell with a second nuclease agent that makes a double-strand break at a second nuclease target site within about 25 nucleotides of the 3' end of the repeat expansion sequence to produce a second modified cell;

(VI) the method is performed a first time with a first nuclease agent that makes a double-strand break at a first nuclease target site within about 25 nucleotides of the 3' end of the repeat expansion sequence to produce a first modified cell, and the method is performed a second time on the first modified cell with a second nuclease agent that makes a double-strand break at a second nuclease target site within about 25 nucleotides of the 5' end of the repeat expansion sequence to produce a second modified cell;

(VII) the method is performed a first time with a first nuclease agent that makes a double-strand break at a first nuclease target site within about 25 nucleotides of the 5' end of the repeat expansion sequence to produce a first modified cell, and the method is performed a second time on the first modified cell with a second nuclease agent that makes a double-strand break at a second nuclease target site within about 25 nucleotides of the 5' end of the repeat expansion sequence to produce a second modified cell;

(VIII) the method is performed a first time with a first nuclease agent that makes a double-strand break at a first nuclease target site within about 25 nucleotides of the 3' end of the repeat expansion sequence to produce a first modified cell, and the method is performed a second time on the first modified cell with a second nuclease agent that makes a double-strand break at a second nuclease target site within about 25 nucleotides of the 3' end of the repeat expansion sequence to produce a second modified cell;

(IX) the method is performed a first time with a first nuclease agent that makes a single-strand break at a first nuclease target site within about 25 nucleotides of the 5' end of the repeat expansion sequence to produce a first modified cell, and the method is performed a second time on the first modified cell with a second nuclease agent that makes a single-strand break at a

second nuclease target site within about 25 nucleotides of the 3' end of the repeat expansion sequence to produce a second modified cell;

(X) the method is performed a first time with a first nuclease agent that makes a single-strand break at a first nuclease target site within about 25 nucleotides of the 3' end of the repeat expansion sequence to produce a first modified cell, and the method is performed a second time on the first modified cell with a second nuclease agent that makes a single-strand break at a second nuclease target site within about 25 nucleotides of the 5' end of the repeat expansion sequence to produce a second modified cell;

(XI) the method is performed a first time with a first nuclease agent that makes a single-strand break at a first nuclease target site within about 25 nucleotides of the 5' end of the repeat expansion sequence to produce a first modified cell, and the method is performed a second time on the first modified cell with a second nuclease agent that makes a single-strand break at a second nuclease target site within about 25 nucleotides of the 5' end of the repeat expansion sequence to produce a second modified cell; or

(XII) the method is performed a first time with a first nuclease agent that makes a single-strand break at a first nuclease target site within about 25 nucleotides of the 3' end of the repeat expansion sequence to produce a first modified cell, and the method is performed a second time on the first modified cell with a second nuclease agent that makes a single-strand break at a second nuclease target site within about 25 nucleotides of the 3' end of the repeat expansion sequence to produce a second modified cell.

44. The method of claim 43, wherein the first nuclease target site in option (III), (IV), (VII), (VIII), (XI), or (XII) is the same as the second nuclease target site.

45. The method of any one of claims 1-4 and 6-44, wherein the nuclease target site is outside of the repeat expansion sequence, wherein the nuclease target site is within about 15 nucleotides of the 5' end or the 3' end of the repeat expansion sequence, and wherein the nuclease agent is the Cas protein and the guide RNA, wherein the Cas protein is a Cas9 protein.

46. The method of claim 45, wherein the nuclease agent is a nickase that makes the single-strand break within about 15 nucleotides of the 5' end or the 3' end of the

repeat expansion sequence, wherein the nuclease target site is retained after repair of the single strand-break by the cells, and wherein repair of the single strand-break does not result in insertions or deletions outside of the repeat expansion sequence.

47. The method of any one of claims 1-27 and 30-46, wherein the target genomic locus is an *HTT*, *AR*, *ATN1*, *ATXN1*, *ATXN2*, *ATXN3*, *CACNA1A*, *ATXN7*, *PPP2R2B*, *TBP*, *FMR1*, *DMPK*, *JPH3*, *ATXN8*, *TCF4*, *FXN*, *AFF2*, *PABPN1*, *CNBP*, *ATXN10*, *TK2*, *BEAN1*, *NOP56*, *C9ORF72*, or *CSTB* locus.