

CLAIMS

WHAT IS CLAIMED IS:

1. A method of analyzing a genomic region of interest, said method comprising:
 - a) contacting genomic DNA comprising said genomic region of interest with a Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR)-associated endonuclease and an outer pair of guide RNAs (gRNAs), thereby generating a first excised fragment comprising said genomic region of interest;
 - b) contacting said first excised fragment with a CRISPR-associated endonuclease and an inner pair of gRNAs, thereby generating a second excised fragment comprising said genomic region of interest; and
 - c) analyzing said genomic region of interest contained within said second excised fragment.
2. The method of claim 1, wherein said CRISPR-associated endonuclease and said outer pair of gRNAs of a) associate with and block the 5' and 3' ends of said first excised fragment.
3. The method of claim 2, further comprising, prior to b), contacting the product of a) with one or more exonucleases, such that background genomic DNA is digested and said first excised fragment is not digested.
4. The method of any one of the preceding claims, wherein said one or more exonucleases are selected from the group consisting of: exonuclease I, exonuclease II, exonuclease III, exonuclease IV, exonuclease V, exonuclease VI, exonuclease VII, exonuclease VIII, and any combination thereof.
5. The method of any one of the preceding claims, wherein said outer pair of gRNAs comprises a first outer gRNA and a second outer gRNA.
6. The method of claim 5, wherein said first outer gRNA comprises a nucleotide sequence that is substantially complementary to a first nucleotide sequence present in said genomic DNA, and said second outer gRNA comprises a nucleotide sequence that is substantially complementary to a second nucleotide sequence present in said genomic DNA.
7. The method of claim 6, wherein said first nucleotide sequence and said second nucleotide sequence are different.
8. The method of claim 7, wherein said first nucleotide sequence and said second nucleotide sequence flank said genomic region of interest.
9. The method of claim 8, wherein said first nucleotide sequence, said second nucleotide sequence, or both, are present in said genomic DNA up to about 100 kilobases in length from said genomic region of interest.

10. The method of any one of the preceding claims, wherein said inner pair of gRNAs comprises a first inner gRNA and a second inner gRNA.
11. The method of claim 10, wherein said first inner gRNA comprises a nucleotide sequence that is substantially complementary to a third nucleotide sequence present in said genomic DNA, and said second inner gRNA comprises a nucleotide sequence that is substantially complementary to a fourth nucleotide sequence present in said genomic DNA.
12. The method of claim 11, wherein said third nucleotide sequence and said fourth nucleotide sequence are different.
13. The method of claim 12, wherein said third nucleotide sequence and said fourth nucleotide sequence flank said genomic region of interest.
14. The method of any one of claims 6-9 or 11-13, wherein said third nucleotide sequence and said fourth nucleotide sequence are present on said genomic DNA at a base length closer to said genomic region of interest than said first nucleotide sequence and said second nucleotide sequence.
15. The method of any one of the preceding claims, wherein said second excised fragment is smaller in base length than said first excised fragment.
16. The method of claim 1, wherein said analyzing comprises sequencing said genomic region of interest contained within said second excised fragment.
17. The method of any one of the preceding claims, wherein said genomic DNA is provided at an amount of about 10 µg or greater.
18. The method of any one of the preceding claims, wherein said analyzing comprises genotyping said genomic region of interest contained within said second excised fragment.
19. The method of any one of the preceding claims, wherein said analyzing comprises performing structural analysis on said genomic region of interest contained within said second excised fragment.
20. The method of any one of the preceding claims, further comprising, prior to b), isolating said first excised fragment.
21. The method of any one of the preceding claims, further comprising, prior to c), isolating said second excised fragment.
22. The method of any one of the preceding claims, wherein said method does not involve DNA amplification.
23. The method of any one of the preceding claims, further comprising, prior to c), attaching one or more adapters to the 5' end, the 3' end, or both, of said second excised fragment.

24. The method of any one of the preceding claims, wherein said CRISPR-associated endonuclease is a Class 1 CRISPR-associated endonuclease or a Class 2 CRISPR-associated endonuclease.
25. The method of claim 24, wherein said Class 1 CRISPR-associated endonuclease is selected from the group consisting of: Cas3, Cas5, Cas8a, Cas8b, Cas8c, Cas10d, Cse1, Cse2, Csy1, Csy2, Csy3, GSU0054, Cas10, Csm2, Cmr5, Csx11, Csx10, and Csf1.
26. The method of claim 24, wherein said Class 2 CRISPR-associated endonuclease is selected from the group consisting of: Cas9, Cas12a, Csn2, Cas4, Cas12b, Cas12c, Cas13a, Cas13b, Cas13c, and Cas13d.
27. The method of any one of the preceding claims, wherein said CRISPR-associated endonuclease comprises an amino acid sequence having at least 80% sequence identity to a wild-type CRISPR-associated endonuclease.
28. The method of any one of the preceding claims, wherein said CRISPR-associated endonuclease is Cas9 or a variant thereof.
29. The method of claim 28, wherein said Cas9 is a *Streptococcus pyogenes* Cas9 (spCas9).
30. The method of claim 28 or 29, wherein said Cas9 variant comprises one or more point mutations, relative to a wild-type *Streptococcus pyogenes* Cas9 (spCas9), selected from the group consisting of: R780A, K810A, K848A, K855A, H982A, K1003A, R1060A, D1135E, N497A, R661A, Q695A, Q926A, L169A, Y450A, M495A, M694A, and M698A.
31. The method of any one of the preceding claims, wherein said genomic DNA is not fragmented, digested, or sheared prior to a).
32. The method of any one of the preceding claims, wherein said genomic DNA is not subjected to restriction enzyme digestion prior to a).
33. The method of any one of the preceding claims, wherein said genomic region of interest is a complex genomic region.
34. The method of claim 33, wherein said complex genomic region comprises a gene of interest and one or more pseudogenes thereof.
35. The method of claim 34, wherein said one or more pseudogenes comprise a nucleotide sequence having at least 75% sequence identity to said gene of interest.
36. The method of any one of claim 33, wherein said complex genomic region comprises one or more repetitive regions, one or more duplications, one or more insertions, one or more inversions, one or more tandem repeats, one or more retrotransposons, or any combination thereof.

37. The method of any one of the preceding claims, wherein said genomic region of interest is a highly polymorphic gene locus.
38. The method of any one of the preceding claims, wherein said first excised fragment is at least about 0.06 kilobases in length.
39. The method of any one of the preceding claims, wherein said first excised fragment is up to about 200 kilobases in length.
40. The method of any one of the preceding claims, wherein said second excised fragment is at least about 0.02 kilobases in length.
41. The method of any one of the preceding claims, wherein said second excised fragment is up to about 199.98 kilobases in length.
42. The method of any one of the preceding claims, wherein said sequencing comprises long-read sequencing.
43. The method of claim 42, wherein said long-read sequencing comprises single-molecule real-time sequencing or nanopore sequencing.
44. The method of any one of the preceding claims, wherein said method does not involve any one of polymerase chain reaction (PCR) or isothermal amplification.
45. The method of claim 44, wherein said method does not involve any one of multiple displacement amplification (MDA), strand displacement amplification (SDA), nucleic acid sequence based amplification (NASBA), loop-mediated isothermal amplification, rolling circle amplification (RCA), ligase chain reaction (LCR), helicase dependent amplification, or ramification amplification method.
46. The method of any one of the preceding claims, wherein said genomic DNA is provided or obtained in a biological sample.
47. The method of claim 46, wherein said biological sample comprises a body fluid or a solid tissue sample; optionally, wherein the body fluid is selected from the group consisting of: blood, urine, saliva, bone marrow, spinal fluid, sputum, ascites, lymphatic fluid, pleural fluid, amniotic fluid, semen, vaginal fluid, sweat, stool, glandular secretions, ocular fluids, and breast milk; optionally, wherein the blood is selected from the group consisting of: whole blood, plasma, and serum.
48. The method of claim 47, wherein said biological sample is a diagnostic sample.
49. The method of any one of the preceding claims, wherein said genomic region of interest is a genetic locus comprising CYP2D6, CYP2D7, and CYP2D8.
50. The method of claim 49, wherein said analyzing comprises identifying one or more genetic variations in CYP2D6.

51. The method of claim 50, further comprising, identifying a subject as having a reduction, a loss of, or an increase in CYP2D6 function based on said genetic variation.
52. The method of claim 51, further comprising, recommending a treatment or an alternative treatment to said subject based on said identifying.
53. The method of claim 51, wherein, when said subject is identified as having a reduction in, a loss of, or an increase in CYP2D6 function, recommending an alternative treatment to said subject.
54. The method of claim 51, further comprising, recommending a dosage of a therapeutic to said subject based on said identifying.
55. The method of claim 51, wherein, when said subject is identified as having a reduction in, a loss of, or an increase in CYP2D6 function, altering a dosage of a therapeutic.
56. The method of any one of the preceding claims, wherein said outer pair of gRNAs, said inner pair of gRNAs, or both, comprise gRNAs selected from any one of SEQ ID NOS: 1-418.