

CLAIMS

1. A method for analyzing chromatin interaction, comprising:
 - (a) contacting a sample with a first probe and a second probe simultaneously or sequentially in either order, wherein:
 - the sample comprises a first chromatin region and a second chromatin region in proximity to each other, wherein the proximity is associated with a chromatin interaction event between the first and second chromatin regions in a cell,
 - the first probe hybridizes to a first nucleic acid strand in the first chromatin region and the second probe hybridizes to a second nucleic acid strand in the second chromatin region, and
 - the first and second probes are bridged by one another or by one or more bridging probes;
 - and
 - (b) connecting the ends of the first and/or second probes or the ends of the one or more bridging probes to form a circular probe, wherein the circular probe comprises a sequence of the first nucleic acid strand or complement thereof and/or a sequence of the second nucleic acid strand or complement thereof,wherein an amplification product of the circular probe is detected, thereby analyzing the chromatin interaction event.
2. The method of claim 1, wherein the first and second chromatin regions are in the same molecule, e.g., the same chromosome in the cell.
3. The method of claim 2, wherein the first and second chromatin regions do not overlap in nucleic acid sequence and are not directly linked by a common phosphodiester bond in the cell.
4. The method of claim 3, wherein the genomic distance between the first and second chromatin regions are at least 0.5 kb, 1 kb, 2 kb, 5 kb, 10 kb, 15 kb, 20 kb, 25 kb, 30 kb, 35 kb, 40 kb, 45 kb, 50 kb, 100 kb, 150 kb, or 200 kb, or in any range in between, or more than 200 kb.
5. The method of claim 1, wherein the first and second chromatin regions are in different molecules, e.g., different chromosomes in the cell.
6. The method of any of claims 1-5, wherein the first and/or second chromatin regions comprise chromosomal nucleic acid, e.g., chromosomal DNA in heterochromatin or euchromatin.

7. The method of any of claims 1-6, wherein the first and/or second chromatin regions are in a nucleosome region or a nucleosome-free region, e.g., in a cellular compartment such as a nucleus or mitochondrion.

8. The method of any of claims 1-7, wherein the first and/or second chromatin regions are in a 30 nm fiber, an interphase chromosome, a metaphase chromosome, a telomere, or a centromere.

9. The method of any of claims 1-8, wherein the first and/or second chromatin regions comprise a regulatory element.

10. The method of any of claims 1-9, wherein the first and/or second chromatin regions comprise a promoter or element thereof (e.g., a core promoter element or a proximal promoter element) and/or a long-range regulatory element (e.g., an enhancer, a silencer, an insulator, or a locus control region (LCR)).

11. The method of claim 10, wherein the first and second probes hybridize to a promoter sequence and an enhancer sequence, respectively, or vice versa.

12. The method of any of claims 1-11, wherein the chromatin interaction event is mediated by one or more chromatin associated factors.

13. The method of claim 12, wherein the one or more chromatin associated factors comprise a polynucleotide (e.g., a DNA or RNA), a polypeptide (e.g., a protein or peptide), a carbohydrate, a lipid, a small molecule, and/or a conjugate, derivative, metabolite, or analogue thereof.

14. The method of claim 12 or 13, wherein the one or more chromatin associated factors comprise a transcription factor, an activator, a repressor, a chromatin-remodeler, a polymerase, a replication factor, a DNA repair factor, a histone (e.g., histones comprising post-translational modifications), a histone-modifying enzyme, a DNA-modifying enzyme (e.g., DNA methylases), and/or a cofactor or complex thereof.

15. The method of any of claims 1-14, wherein the chromatin interaction event occurs prior to or during the contacting step.

16. The method of any of claims 1-15, wherein the chromatin interaction event holds the first and second chromatin regions together in a chromatin conformation.

17. The method of claim 16, further comprising preserving or capturing the chromatin conformation.

18. The method of any of claims 1-17, further comprising treating the sample with a cross-linking agent prior to the contacting step.

19. The method of claim 18, wherein nucleic acid in the first and/or second chromatin regions is cross-linked to one another and/or to one or more chromatin associated factors by the cross-linking agent.

20. The method of claim 18 or 19, wherein the cross-linking agent is selected from the group consisting of formaldehyde, UV radiation, glutaraldehyde, bis(imido esters), bis(succinimidyl esters), diisocyanates, diacid chlorides, 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide, mitomycin C, nitrogen mustard, melphalan, 1,3-butadiene diepoxide, cis diaminedichloroplatinum (II), cyclophosphamide, disuccinimidylglutarate, and dithiobis-succinimidyl propionate.

21. The method of any of claims 1-20, wherein the first nucleic acid strand is a sense strand or antisense strand, and/or wherein the second nucleic acid strand is a sense strand or antisense strand.

22. The method of any of claims 1-21, wherein the first and/or second probes are chromatin accessing probes, optionally wherein the complementary strand to the first nucleic acid strand in the first chromatin region and/or the complementary strand to the second nucleic acid strand in the second chromatin region is not hybridized to a probe, e.g., a chromatin accessing probe.

23. The method of any of claims 1-21, wherein the complementary strand to the first nucleic acid strand in the first chromatin region is hybridized to a first chromatin accessing probe, and/or wherein the complementary strand to the second nucleic acid strand in the second chromatin region is hybridized to a second chromatin accessing probe.

24. The method of claim 22 or 23, wherein the chromatin accessing probe (e.g., the first and/or second chromatin accessing probes) comprises one or more natural nucleic acids and/or one or more synthetic nucleic acid analogues.

25. The method of claim 24, wherein the one or more synthetic nucleic acid analogues are xeno nucleic acids (XNAs), optionally selected from the group consisting of 1,5-anhydrohexitol nucleic acid (HNA), cyclohexene nucleic acid (CeNA), threose nucleic acid (TNA), glycol nucleic acid (GNA), locked nucleic acid (LNA), peptide nucleic acid (PNA), and fluoro arabino nucleic acid (FANA), and combinations thereof.

26. The method of any of claims 22-25, wherein the chromatin accessing probe (e.g., the first and/or second chromatin accessing probes) comprises peptide nucleic acid (PNA).

27. The method of claim 26, wherein the first and/or second chromatin accessing probes are PNA probes, and the method further comprises a step of contacting the sample with the first and second chromatin accessing probes simultaneously or sequentially in either order.

28. The method of any of claims 23-27, wherein the sample is contacted with the first and/or second chromatin accessing probes prior to contacting the sample with the first and/or second probes.

29. The method of any of claims 23-28, wherein the melting temperature (T_m) of the hybridization between the first or second nucleic acid strand and the corresponding complementary strand is lower than the T_m of the hybridization between the first or second chromatin accessing probe and the corresponding complementary strand, e.g., by about 5°C, about 10°C, about 15°C, about 20°C, about 25°C, about 30°C, about 35°C, about 40°C, about 45°C, about 50°C, or by more than 50°C.

30. The method of any of claims 22-29, wherein the sample is contacted with the chromatin accessing probe (e.g., the first and/or second chromatin accessing probes) under conditions that allow probe access to single-stranded sequences in the first and/or second chromatin regions.

31. The method of claim 30, wherein the sample is contacted with the chromatin accessing probe at a probe concentration of at least 100 nM, 200 nM, 500 nM, 1 μ M, 2 μ M, or more than 2 μ M.

32. The method of claim 30 or 31, wherein the sample is contacted with the chromatin accessing probe at a temperature of at least 40°C, 45°C, 50°C, or more than 50°C.

33. The method of any of claims 30-32, wherein the sample is incubated with the chromatin accessing probe for at least 30 minutes, 1 hours, 2 hours, 5 hours, or more than 5 hours.

34. The method of any of claims 30-33, further comprising removing molecules of the chromatin accessing probe that are not specifically hybridized, e.g., using one or more washes such as a stringency wash.

35. The method of any of claims 30-34, further comprising removing molecules of the first and/or second probes and/or the one or more bridging probes that are not specifically hybridized, e.g., using one or more washes such as a stringency wash.

36. The method of any of claims 1-35, wherein the first and/or second probes comprise a 3' overhang and a 5' overhang flanking a sequence hybridized to the first and second nucleic acid strands, respectively.

37. The method of claim 36, wherein the 3' overhang of the first probe is at least partially complementary to the 5' overhang of the second probe, and/or the 5' overhang of the first probe is at least partially complementary to the 3' overhang of the second probe.

38. The method of claim 37, further comprising extending the 3' end of the first probe (e.g., using primer extension and/or ligation) using the second probe as a template, and connecting (e.g., using ligation) the extended 3' end of the first probe to the optionally extended 5' end of the first probe, thereby circularizing the first probe to form the circular probe.

39. The method of claim 37 or 38, further comprising extending the 3' end of the second probe (e.g., using primer extension and/or ligation) using the first probe as a template, and connecting (e.g., using ligation) the extended 3' end of the second probe to the optionally extended 5' end of the second probe, thereby circularizing the second probe to form the circular probe.

40. The method of claim 36, wherein the 3' overhang of the first probe and the 5' overhang of the second probe are at least partially complementary to a first bridging probe sequence, and the 5' overhang of the first probe and the 3' overhang of the second probe are at least partially complementary to a second bridging probe sequence, optionally wherein the first and second bridging probe sequences are in the same bridging probe or in separate bridging probes.

41. The method of claim 40, further comprising extending the 3' end of the first probe (e.g., using primer extension and/or ligation) using the bridging probe(s) as a template, and connecting (e.g., using ligation) the extended 3' end of the first probe to the optionally extended 5' end of the second probe.

42. The method of claim 41, further comprising extending the 3' end of the second probe (e.g., using primer extension and/or ligation) using the bridging probe(s) as a template, and connecting (e.g., using ligation) the extended 3' end of the second probe to the optionally extended 5' end of the first probe, thereby connecting the first and second probes to form the circular probe.

43. The method of any of claims 40-42, wherein the first and/or second bridging probe sequences are in a probe (e.g., a chromatin accessing probe) hybridized to the complementary strand to the first nucleic acid strand in the first chromatin region and/or a probe (e.g., a chromatin accessing probe) hybridized to the complementary strand to the second nucleic acid strand in the second chromatin region.

44. The method of claim 36, wherein the 3' overhang of the first probe and the 5' overhang of the second probe are at least partially complementary to a first bridging probe, and the 5' overhang of the first probe and the 3' overhang of the second probe are at least partially complementary to a second bridging probe.

45. The method of claim 44, further comprising extending the 3' end of the first bridging probe (e.g., using primer extension and/or ligation) using the first probe as a template, and connecting (e.g., using ligation) the extended 3' end of the first bridging probe to the optionally extended 5' end of the second bridging probe.

46. The method of claim 45, further comprising extending the 3' end of the second bridging probe (e.g., using primer extension and/or ligation) using the second probe as a template, and connecting (e.g., using ligation) the extended 3' end of the second bridging probe to the optionally extended 5' end of the first bridging probe, thereby connecting the first and second bridging probes to form the circular probe.

47. The method of any of claims 1-46, wherein the circular probe is formed *in situ* in the sample.

48. The method of any of claims 1-47, wherein the first probe, the second probe, the bridging probe, the circular probe, and/or the chromatin accessing probe comprises a barcode sequence.

49. The method of claim 48, wherein the circular probe comprises one or more barcode sequences that correspond to a nucleic acid sequence of interest, e.g., in the first chromatin region and/or in the second chromatin region.

50. The method of claim 49, wherein the amplification product comprises multiple copies of the one or more barcode sequences or a complement thereof, the sequence of the first nucleic acid strand or complement thereof, and the sequence of the second nucleic acid strand or complement thereof.

51. The method of any of claims 1-50, wherein the amplification product is formed *in situ* in the sample.

52. The method of any of claims 1-51, wherein the amplification product is formed using rolling circle amplification (RCA) of the circular probe, optionally wherein the RCA is a linear RCA, a branched RCA, a dendritic RCA, or any combination thereof, and optionally wherein the amplification product is formed using a Phi29 polymerase, wherein the amplification is performed at a temperature between about 20°C and about 60°C, optionally between about 30°C and about 40°C.

53. The method of any of claims 1-52, wherein the detecting of the amplification product comprises labeling the amplification product with a fluorophore, an isotope, a mass tag, or a combination thereof.

54. The method of any of claims 1-53, wherein the detecting of the amplification product comprises directly or indirectly hybridizing one or more probes to the amplification product, optionally wherein a fluorescently labeled probe is directly hybridized to the amplification product, or optionally wherein a fluorescently labeled probe is directly hybridized to an intermediate probe which is hybridized to the amplification product.

55. The method of any of claims 1-54, wherein the amplification product is detected *in situ* in the sample.

56. The method of any of claims 1-55, wherein the detecting of the amplification product comprises imaging the sample, e.g., using fluorescent microscopy.

57. The method of any of claims 1-56, wherein the detecting of the amplification product comprises determining a sequence of the amplification product, e.g., by sequencing all or a portion of the amplification product and/or *in situ* hybridization (e.g., sequential fluorescence *in situ* hybridization) to the amplification product.

58. The method of claim 57, wherein the sequencing comprises sequencing by hybridization, sequencing by ligation, sequencing by synthesis, and/or sequencing by binding.

59. The method of any of claims 1-58, wherein the sample is a tissue sample, e.g., a non-homogenized tissue sample, such as a tissue slice optionally between about 1 μm and about 50 μm in thickness, e.g., between about 5 μm and about 35 μm in thickness.

60. The method of any of claims 1-59, wherein the sample is a processed or cleared sample.

61. The method of any of claims 1-60, wherein the sample is fixed or not fixed, e.g., a formalin-fixed, paraffin-embedded (FFPE) sample, a frozen tissue sample, or a fresh tissue sample.

62. The method of any of claims 1-61, wherein the sample is on a substrate such as a slide.

63. The method of any of claims 1-62, wherein the sample is embedded in a matrix, e.g., a hydrogel functionalized for attaching biomolecules and/or products thereof from the sample.

64. The method of any of claims 1-63, wherein the sample is analyzed to detect a plurality of chromatin interaction events, e.g., in parallel or sequentially.

65. The method of claim 64, wherein a first circular probe is associated with a first chromatin interaction event and a second circular probe is associated with a second chromatin interaction event, and the first and second circular probe each comprises a barcode sequence or complement thereof that corresponds to the first and/or second probe or the chromatin interaction event.

66. The method of any of claims 1-65, wherein the chromatin interaction event involves one or more chromatin regions other than the first and second chromatin regions, and the circular probe further comprises a sequence or complement thereof of a nucleic acid strand in the one or more other chromatin regions.

67. A method for analyzing chromatin interaction, comprising:

(a) contacting a sample with a first chromatin accessing probe and a second chromatin accessing probe simultaneously or sequentially in either order, wherein:

the sample comprises a first chromatin region and a second chromatin region in proximity to each other, wherein the proximity is associated with a chromatin interaction event between the first and second chromatin regions in a cell, and

the first chromatin accessing probe hybridizes to the complementary strand of a first nucleic acid strand in the first chromatin region and the second chromatin accessing probe hybridizes to the complementary strand of a second nucleic acid strand in the second chromatin region,

thereby allowing access to the first and second nucleic acid strands for further probes (e.g., by providing single-stranded binding sites in the first and second nucleic acid strands for further probes);

(b) contacting the sample with a first detection probe and a second detection probe simultaneously or sequentially in either order, wherein:

the first detection probe hybridizes to the first nucleic acid strand and the second detection probe hybridizes to the second nucleic acid strand;

(c) contacting the sample with a first bridging probe that bridges the 3' end of the first detection probe and the 5' end of the second detection probe, and a second bridging probe that bridges the 3' end of the second detection probe and the 5' end of the first detection probe;

(d) circularizing the first and second bridging probes using the first and second detection probes as templates to form a circular probe, wherein the circular probe comprises a sequence of the first nucleic acid strand and a sequence of the second nucleic acid strand;

(e) generating a rolling circle amplification (RCA) product of the circular probe *in situ* in the sample; and

(f) detecting the RCA product, thereby analyzing the chromatin interaction event.

68. The method of claim 67, wherein the chromatin interaction event holds the first and second chromatin regions together in a chromatin conformation, and the method further comprises preserving or capturing the chromatin conformation with a cross-linking agent prior to (a).

69. The method of claim 67 or 68, wherein the first and second chromatin accessing probes are peptide nucleic acid (PNA) probes.

70. The method of any of claims 67-69, wherein the first and second detection probes do not displace the first and second chromatin accessing probes from the first and second chromatin regions, respectively.

71. A kit, comprising a first probe capable of hybridizing to a first nucleic acid strand in a first chromatin region, and a second probe capable of hybridizing to a second nucleic acid strand in a second chromatin region, wherein:

the first chromatin region and the second chromatin region are known to engage or suspected of engaging in a chromatin interaction event in a cell,

upon hybridization to the first and second nucleic acid strands in a sample in which a chromatin conformation associated with the chromatin interaction event is preserved or captured, the first and second probes are bridged by one another or by one or more bridging probes, and

upon connection of the ends of the first and/or second probes or the ends of the one or more bridging probes, a circular probe is formed and the circular probe comprises a sequence of the first nucleic acid strand or complement thereof and/or a sequence of the second nucleic acid strand or complement thereof.

72. The kit of claim 71, further comprising the one or more bridging probes.

73. The kit of claim 71 or 72 for analyzing a plurality of chromatin interaction events, comprising multiple sets of the first probe, the second probe, and optionally the one or more bridging probes, wherein each set is for analyzing a different chromatin interaction event.

74. A kit, comprising:

(i) a first chromatin accessing probe and a second chromatin accessing probe, wherein:

the first chromatin accessing probe is capable of hybridizing to the complementary strand of a first nucleic acid strand in a first chromatin region,

the second chromatin accessing probe is capable of hybridizing to the complementary strand of a second nucleic acid strand in a second chromatin region, and

the first chromatin region and the second chromatin region are known to engage or suspected of engaging in a chromatin interaction event in a cell;

(ii) a first detection probe and a second detection probe, wherein:

the first detection probe is capable of hybridizing to the first nucleic acid strand and the second detection probe is capable of hybridizing to the second nucleic acid strand; and

(iii) a first bridging probe capable of bridging the 3' end of the first detection probe and the 5' end of the second detection probe, and a second bridging probe capable of bridging the 3' end of the second detection probe and the 5' end of the first detection probe,

upon hybridization to the first and second nucleic acid strands in a sample in which a chromatin conformation associated with the chromatin interaction event is preserved or captured, the first and second detection probes are bridged by the first and second bridging probes, and

upon connection of the ends of the first and second bridging probes using the first and second detection probes as templates, a circular probe is formed and the circular probe comprises a sequence of the first nucleic acid strand and a sequence of the second nucleic acid strand.

75. The kit of claim 74, wherein the first and second chromatin accessing probes are peptide nucleic acid (PNA) probes.

76. The kit of claim 74 or 75 for analyzing a plurality of chromatin interaction events, comprising multiple sets of the first and second chromatin accessing probes, the first and second detection probes, and/or the first and second bridging probes, wherein each set is for analyzing a different chromatin interaction event.

77. The kit of any of claims 71-76, further comprising an enzyme and optionally comprising a polymerase (e.g., a Phi29 polymerase) and/or a ligase.