

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 formed and detected, thereby analyzing the chromatin interaction event.
2. The method of claim 1, 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.
3. The method of claim 1 or 2, wherein the chromatin interaction event is mediated by one or more chromatin associated factors selected from the group consisting of a transcription factor, an activator, a repressor, a chromatin-remodeler, a polymerase, a replication factor, a DNA repair factor, a histone, a histone-modifying enzyme, a DNA-modifying enzyme, and a cofactor or complex thereof.
4. The method of any of claims 1-3, wherein the chromatin interaction event holds the first and second chromatin regions together in a chromatin conformation, and the method comprises preserving or capturing the chromatin conformation.
5. The method of any of claims 1-4, further comprising treating the sample with a cross-linking agent prior to the contacting step, 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.

6. The method of claim 5, 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.

7. The method of any of claims 1-6, 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.

8. The method of any of claims 1-7, 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.

9. The method of any of claims 1-8, 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.

10. The method of claim 8 or 9, wherein the first and/or second chromatin accessing probe comprises one or more natural nucleic acids and/or one or more synthetic nucleic acid analogues.

11. The method of any of claims 8-10, wherein the chromatin accessing probe comprises peptide nucleic acid (PNA).

12. The method of claim 11, 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.

13. The method of any of claims 9-12, 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.

14. The method of any of claims 9-13, 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.

15. The method of any of claims 8-14, 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;

the sample is contacted with the first and/or second chromatin accessing probes at a temperature of at least 40°C, 45°C, 50°C, or more than 50°C; and/or

the sample is incubated with the first and/or second chromatin accessing probes for at least 30 minutes, 1 hours, 2 hours, 5 hours, or more than 5 hours.

16. The method of any of claims 1-15, 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.

17. The method of claim 16, 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, and wherein the method further comprises (i) extending the 3' end of the first probe using the second probe as a template, and connecting 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, and/or (ii) extending the 3' end of the second probe using the first probe as a template, and connecting 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.

18. The method of claim 16, 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.

19. The method of claim 18, further comprising extending the 3' end of the first probe using the bridging probe(s) as a template, and connecting the extended 3' end of the first probe to the optionally extended 5' end of the second probe and

(ii) extending the 3' end of the second probe using the bridging probe(s) as a template, and connecting 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.

20. The method of any of claim 18 or 19, wherein the first and/or second bridging probe sequences are in a probe hybridized to the complementary strand to the first nucleic acid

strand in the first chromatin region and/or a probe hybridized to the complementary strand to the second nucleic acid strand in the second chromatin region.

21. The method of claim 16, 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 and the method further comprises:

(i) extending the 3' end of the first bridging probe using the first probe as a template and connecting the extended 3' end of the first bridging probe to the optionally extended 5' end of the second bridging probe, and

(ii) extending the 3' end of the second bridging probe using the second probe as a template, and connecting 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.

22. The method of any of claims 1-21, wherein the circular probe is formed *in situ* in the sample.

23. The method of any of claims 1-22, wherein the first probe, the second probe, the one or more bridging probes, and/or the circular probe comprise on or more barcode sequences corresponding to a nucleic acid sequence of interest in the first chromatin region and/or in the second chromatin region.

24. The method of any of claims 1-23, wherein the amplification product is formed *in situ* in the sample.

25. The method of any of claims 1-24, wherein the amplification product is formed using rolling circle amplification (RCA) of the circular probe.

26. The method of any of claims 1-24, wherein a fluorescently labeled probe is directly hybridized to the amplification product, or wherein a fluorescently labeled probe is directly hybridized to an intermediate probe which is hybridized to the amplification product.

27. The method of any of claims 1-26, wherein the amplification product is detected *in situ* in the sample.

28. The method of any of claims 1-27, wherein the detecting of the amplification product comprises imaging the sample, using fluorescent microscopy.

29. The method of any of claims 1-28, wherein the sample is a tissue sample, e.g., a non-homogenized tissue sample.

30. 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;

(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.

31. The method of claim 30, wherein the first and second chromatin accessing probes are peptide nucleic acid (PNA) probes.