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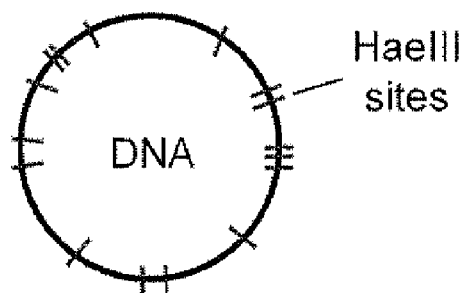
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(54) **Title:** CHROMATIN REMODELING ACTIVITY ASSAYS AND USES THEREOF



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Full
digestion
of DNA

Figure 1

(57) **Abstract:** Provided herein are chromatin remodeling activity assays and methods for identifying a test compound that is a modulator of chromatin remodeling activity, methods for identifying a candidate compound for treating a subject with a chromatin remodeling-related condition (e.g., a cancer, a neurodevelopmental disorder, an autoimmune disease, or a cardiovascular disease) in a subject, and methods for identifying a candidate compound for maintaining or inducing pluripotency in a mammalian cell. Also provided are kits that may be used, e.g., to perform these methods.

CHROMATIN REMODELING ACTIVITY ASSAYS AND USES THEREOF

TECHNICAL FIELD

This invention relates generally to the fields of cell biology and drug screening.

BACKGROUND

5 A mammalian genome exists in the nucleus of a cell as a protein-double stranded DNA (dsDNA) complex called chromatin. The basic repeating unit of chromatin about 147 base pairs of dsDNA wrapped around a core of histone proteins. The packaging of a mammalian genome into chromatin serves to compact and organize the genome, and also
10 regulates the accessibility of the dsDNA, e.g., to proteins that allow for transcription of genes in the dsDNA. A large number of chromatin remodeling proteins can alter or modify chromatin (either the dsDNA or histone(s) present in nucleosomes), and make the dsDNA more accessible or less accessible to factors that allow for the transcription of genes in the dsDNA. Exemplary chromatin remodeling proteins are chromatin remodeling ATPases,
15 which directly alter the structure of chromatin by breaking histone-dsDNA contacts. Additional examples of chromatin remodeling proteins are histone acetyltransferases.

Dysregulation of different chromatin remodeling proteins has been implicated in the development of different types of disorders including cancers (Dawson and Kouzarides, *Cell* 150:12-27, 2012, Bartholomew *Ann. Rev. Biochem.* 83:671-696, 2014, and Helming et
20 al., *Cancer Cell* 26:309-317, 2014), neurodevelopmental disorders (Ronan et al., *Nat. Rev. Genet.* 14:347-359, 2013, and Tyssowski et al., *Neuroscience* 264:4-16, 2014), autoimmune disorders (Seelig et al., *Arthritis Rheum.* 38:1389-99, 1995, and Lu et al., *J. Biol. Chem.* 283:13825-33, 2008), and cardiovascular disorders (Bevilacqua et al., *Cardiovasc. Pathol.* 23:85-91, 2014, and Nührenberg et al., *Cell Tissue Res.* 356:585-600, 2014). The activity
25 of chromatin remodeling proteins have also been shown to play a role in the maintenance and induction of pluripotency in a mammalian cell (Hu and Wade, *Cell Stem Cell* 10:497-503, 2012, and Chen and Dent, *Nat. Rev. Genet.* 15:93-106, 2014).

SUMMARY

The present disclosure is based, at least in part, on the discovery that polymerase
30 chain reaction (PCR)-based assays can be used rapidly and sensitively to detect chromatin remodeling activity in an assay that includes, in part, the use of at least one chromatin

remodeling protein, at least one restriction endonuclease, and a molecular complex comprising a dsDNA molecule and at least one nucleosome, where the dsDNA molecule includes at least one restriction endonuclease recognition site recognized by one of the at least one restriction endonuclease(s). In view of this discovery, provided herein are

5 methods of identifying a test compound that is a modulator of chromatin remodeling activity, methods for identifying a candidate compound for treating a subject with a chromatin remodeling-related condition (e.g., a cancer, a neurodevelopmental disorder, an autoimmune disease, or a cardiovascular disease) in a subject, and methods for identifying a candidate compound for maintaining or inducing pluripotency in a mammalian cell. Also

10 provided are kits that may be used, e.g., to perform these methods.

Provided herein are methods for identifying a test compound that is a modulator of chromatin remodeling activity that include: (a) combining in a reaction mixture: a molecular complex including a double stranded deoxyribonucleic acid (dsDNA) molecule and at least one nucleosome, where the dsDNA molecule comprises at least one restriction

15 endonuclease recognition site, a test compound, and at least one chromatin remodeling protein, under conditions sufficient to allow for activity of the at least one remodeling protein; (b) contacting the dsDNA molecule with at least one restriction endonuclease that is capable of cleaving the dsDNA molecule at one or more of the at least one restriction endonuclease recognition site(s) under conditions sufficient to allow for activity of the at

20 least one remodeling protein and the at least one restriction endonuclease; (c) determining the level of cleavage of the dsDNA molecule by the at least one restriction endonuclease using a polymerase chain reaction assay; (d) comparing the determined level of cleavage in step (c) to a control total level of cleavage; and (e) identifying a test compound that causes a modulation in the determined level of cleavage as compared to the control level of cleavage

25 as a modulator of chromatin remodeling activity. In some embodiments of the methods, (e) includes identifying a test compound that causes a decrease in the determined level of cleavage as compared to the control level of cleavage as an inhibitor of chromatin remodeling activity. In some embodiments of the methods, step (e) includes identifying a test compound that causes an increase in the determined level of cleavage as compared to

30 the control level of cleavage as an activator of chromatin remodeling activity. In some embodiments of the methods, the dsDNA molecule comprises a sequence of a gene.

Also provided are methods for identifying a candidate compound for treating a cancer in a subject that include: (a) combining in a reaction mixture: a molecular complex

comprising a double stranded deoxyribonucleic acid (dsDNA) molecule and at least one nucleosome, where the dsDNA molecule includes at least one restriction endonuclease recognition site, a test compound, and at least one chromatin remodeling protein, under conditions sufficient to allow for activity of the at least one remodeling protein; (b) 5 contacting the dsDNA molecule with at least one restriction endonuclease that is capable of cleaving the dsDNA molecule at one or more of the at least one restriction endonuclease recognition site(s) under conditions sufficient to allow for activity of the at least one remodeling protein and the at least one restriction endonuclease; (c) determining the level of cleavage of the dsDNA molecule by the at least one restriction endonuclease using a 10 polymerase chain reaction assay; (d) comparing the determined level of cleavage in step (c) to a control total level of cleavage; and (e) identifying a test compound that causes a modulation in the determined level of cleavage as compared to the control level of cleavage as a candidate compound for treating a cancer. In some embodiments of the methods, the dsDNA molecule includes a sequence of a cancer-associated gene, and the at least one 15 restriction endonuclease recognition site is/are located within the sequence of the cancer-associated gene. In some embodiments of the methods, the cancer-associated gene is an oncogene. In some embodiments of the methods, the oncogene is Wnt, β -catenin, and myc. In some embodiments of the methods, step (e) includes identifying a test compound that causes an increase in the determined level of cleavage as compared to the control level of 20 cleavage as a candidate compound for treating cancer. In some embodiments of the methods, the cancer-associated gene is a tumor suppressor gene. In some embodiments of the methods, the tumor suppressor gene is WEE1, Rb, p21, p16, SFRP1/2, TIMP3, TGF- β , or SNAIL. In some embodiments of the methods, step (e) includes identifying a test compound that causes a decrease in the determined level of cleavage as compared to the 25 control level of cleavage as a candidate compound for treating cancer. Some embodiments of the methods further include: (f) contacting a cancer cell with the candidate compound identified in step (e); (g) determining the level of proliferation of the cancer cell in step (f); (h) comparing the determined level of proliferation to a control level of proliferation; and (i) further identifying a candidate compound that has a decreased proliferation as compared to 30 the control level of proliferation as a candidate compound for treating a cancer in a subject.

Also provided are methods for identifying a candidate compound for treating a subject with a chromatin remodeling-related condition that include: (a) combining in a reaction mixture: a molecular complex including a double stranded deoxyribonucleic acid

(dsDNA) molecule and at least one nucleosome, wherein the dsDNA molecule comprises at least one restriction endonuclease recognition site, a test compound, and at least one chromatin remodeling protein, under conditions sufficient to allow for activity of the at least one remodeling protein; (b) contacting the dsDNA molecule with at least one restriction endonuclease that is capable of cleaving the dsDNA molecule at one or more of the at least one restriction endonuclease recognition site(s) under conditions sufficient to allow for activity of the at least one remodeling protein and the at least one restriction endonuclease; (c) determining the level of cleavage of the dsDNA molecule by the at least one restriction endonuclease using a polymerase chain reaction assay; (d) comparing the determined level of cleavage in step (c) to a control total level of cleavage; and (e) identifying a test compound that causes a modulation in the determined level of cleavage as compared to the control level of cleavage as a candidate compound for treating a subject with a chromatin remodeling-related condition. In some embodiments of the methods, the chromatin remodeling-related condition is a neurodevelopmental disorder, an autoimmune disease, or a cardiovascular disease. In some embodiments of the methods, step (e) includes identifying a test compound that causes an increase in the determined level of cleavage as compared to the control level of cleavage as a candidate compound for treating a subject with a neurodevelopmental disorder, an autoimmune disease, or a cardiovascular disease. In some embodiments of the methods, the dsDNA molecule includes a sequence of a gene related to the pathogenesis of the neurodevelopmental disorder, the autoimmune disease, or the cardiovascular disease. In some embodiments of the methods, the neurodevelopmental disorder is autism-spectrum disorders, epilepsy, Alpha-thalassemia X-linked mental retardation, Cockayne syndrome, Coffin-Siris syndrome, Nicolaides-Baraitser syndrome, Floating-Harbo syndrome, Schimke immunoosseous dysplasia (SIOD), Williams syndrome, or CHARGE syndrome. In some embodiments of the methods, the at least one chromatin remodeling protein is selected from the group of: MECP2, ATRX, H2AFY, SMC1A, MACROD2, KDM5C, MBD1, ARID1B, SMARCC2, JMJD1C, CHD8, CHD1, CHD2, CHD3, CHD4, CHD5, CHD6, CHD7, CSB, BRG1, BRM, and HARP.

Also provided are methods for identifying a candidate compound for maintaining or inducing pluripotency in a mammalian cell that include: (a) combining in a reaction mixture: a molecular complex including a double stranded deoxyribonucleic acid (dsDNA) molecule and at least one nucleosome, wherein the dsDNA molecule includes at least one restriction endonuclease recognition site, a test compound, and at least one chromatin

remodeling protein, under conditions sufficient to allow for activity of the at least one remodeling protein; (b) contacting the dsDNA molecule with at least one restriction endonuclease that is capable of cleaving the dsDNA molecule at one or more of the at least one restriction endonuclease recognition site(s) under conditions sufficient to allow for activity of the at least one remodeling protein and the at least one restriction endonuclease; (c) determining the level of cleavage of the dsDNA molecule by the at least one restriction endonuclease using a polymerase chain reaction assay; (d) comparing the determined level of cleavage in step (c) to a control total level of cleavage; and (e) identifying a test compound that causes a modulation in the determined level of cleavage as compared to the control level of cleavage as a candidate compound for maintaining or inducing pluripotency in a mammalian cell. In some embodiments, step (e) includes identifying a test compound that causes a decrease in the determined level of cleavage as compared to the control level of cleavage as a candidate compound for maintaining or inducing pluripotency in a mammalian cell. In some embodiments of the methods, the at least one chromatin remodeling protein is selected from the group of: NuRD, Mi-2, SWI, SNF, ISWI, CHD1, CHD2, CHD3, CHD4, CHD5, CHD6, CHD7, CHD8, CHD9, and INO80. Some embodiments of the methods further include: (f) contacting a differentiated cell with the candidate compound identified in step (e); (g) determining the level of one or more stem cell marker genes in the cell in step (f); (h) comparing the determined level of the one or more stem cell marker genes to a control level(s) of the one or more stem cell marker genes; and (i) further identifying a candidate compound that has an increased level of the one or more stem cell marker genes proliferation as compared to the control level(s) of the one or more stem cell marker genes as a candidate compound for maintaining or inducing pluripotency in a mammalian cell.

In some embodiments of any of the methods described herein, the dsDNA molecule only includes a single restriction endonuclease recognition site. In some embodiments of any of the methods described herein, the dsDNA molecule includes at least two restriction endonuclease recognition sites. In some embodiments of any of the methods described herein, the dsDNA molecule comprises at least three restriction endonuclease recognition sites. In some embodiments of any of the methods described herein, the dsDNA molecule includes at least four restriction endonuclease recognition sites.

In some embodiments of any of the methods described herein, each of the at least two restriction endonuclease recognition sites is spaced about 100 base pairs to about

12,500 base pairs away from another of the restriction endonuclease recognition site(s) in the dsDNA molecule. In some embodiments of any of the methods described herein, step (b) includes contacting the dsDNA molecule with at least two different restriction endonucleases, where each of the different restriction endonucleases is capable of cleaving the dsDNA molecule at one of the restriction endonuclease recognition site(s).

In some embodiments of any of the methods described herein, the restriction endonuclease recognition site(s) include about 4 base pairs to about 10 base pairs. In some embodiments of any of the methods described herein, the restriction endonuclease recognition site(s) include about 4 base pairs to about 8 base pairs.

In some embodiments of any of the methods described herein, the restriction endonuclease(s) is selected from the group of: HaeIII, AatI, AatII, AccI, AccIII, Acc65I, AclI, AcsI, AclI, AflI, AflII, AflIII, AgeI, AhaII, AhaIII, AluI, AlwI, Alw44I, AlwNI, AocI, AosI, ApaI, ApaLI, ApoI, ApyI, AscI, AseI, AsnI, AspI, Asp700, Asp718, AspEI, AspHI, AsuII, AvaI, AvaII, AviII, AvrII, Ball, BamHI, BanI, BanII, BbrPI, BbsI, BbvI, BcgI, BclI, BfaI, BfrI, BglI, BglII, BinI, BmyI, BpmI, BpuAI, Bpu1102I, BsaI, BsaAI, BsaBI, BsaHI, BsaJI, BseAI, BsePI, BsgI, BsiEI, BsiWI, BsiYI, BslI, BsmI, BsmAI, Bsp1286I, Bsp1407I, BspDI, BspEI, BspHI, BspLU11I, BspMI, BsrI, BsrFI, BssGI, BssHII, Bst1107I, BstBI, BstEII, BstNI, BstUI, BstXI, BstYI, Bsu36I, CelI, CfoI, CfrI, Cfr10I, ClaI, DdeI, DpnI, DpnII, DraI, DraII, DraIII, DrdI, DsaI, EaeI, EagI, Eam1105I, EarI, Ecl136II, EclXI, Eco47III, Eco57I, EcoNI, EcoO109I, EcoRI, EcoRII, EcoRV, EspI, Esp3I, FnuDII, Fnu4HI, FokI, FseI, FspI, GsuI, HaeII, HgaI, HgiAI, HhaI, HincII, HindII, HindIII, HinfI, HinfPI, HpaI, HpaII, HphI, ItaI, KasI, KpnI, KspI, MaeI, MaeII, MaeIII, MamI, MboI, MboII, MfeI, MluI, MluNI, MnlI, MroI, MscI, MseI, MspI, MstI, MstII, MunI, MvaI, MvnI, NaeI, NarI, NciI, NcoI, NdeI, NdeII, NgoMI, NheI, NlaIII, NlaIV, NotI, NruI, NsiI, NspBII, NspI, NspII, NspV, PacI, PaeR7I, PflMI, PinAI, PleI, PmaCI, PmeI, PmlI, PpuMI, Psp1406I, PstI, PvuI, PvuII, RcaI, RmaI, RsaI, RsrII, SacI, SacII, SalI, Saul, Sau3AI, Sau96I, ScaI, ScrFI, SexAI, SfaNI, SfcI, SfiI, SfuI, SgrAI, SmaI, SnaBI, SnaI, SpeI, SphI, SrfI, Sse8387I, SspI, SspBI, SstI, SstII, StuI, StyI, SwaI, TaqI, TfiI, ThaI, Tru9I, Tth111I, Van9II, XbaI, XcmI, XhoI, XhoII, XmaI, XmaIII, XmaCI, and XmnI.

In some embodiments of any of the methods described herein, the dsDNA molecule includes a plasmid. In some embodiments of any of the methods described herein, the plasmid has a size of about 500 base pairs to about 25,000 base pairs, or about 1000 base

pairs to about 20,000 base pairs. In some embodiments of any of the methods described herein, the at least one chromatin remodeling proteins is selected from the group of:

BRG1/SMARCA4, BRM/SMARCA2, SNF2H/ISWI/SMARCA5, SNF2L/SMARCA1, INO80, EP400, SRCAP, RAD54, RAD54B, ATRX, CHD1, CHD2, CHD3/Mi-2alpha,
 5 CHD4/Mi-2beta, CHD5, CHD6, CHD7, CHD8, CHD9, CSB/ERCC6,
 ETL1/SMARCA1/HEL1, HELLS/SMARCA6, HARP/SMARCA1, ZRANB3/AH2,
 MOT1, SHRP, HLTF/SMARCA3, MECP2, H2AFY, SMC1A, MACROD2, KDM5C,
 MBD1, ARID1B, SMARCC2, JMJD1C, NuRD, Mi-2, SWI, SNF, SWI1, ADR6, SWI3,
 SNF6, and BAF200/250a,b/ARID1a,b, BAF180/Polybromo, BAF170, BAF155,
 10 SNF5/BAF47/INI1, BAF60a-c, BAF57, BAF53a,b, BAF47, BAF45a-d, BRD7,9, Actin,
 MBD2/3, RbAP46/48, HDAC1/2, MTA1-3, P66alpha/beta, LSD1, BPTF, ACF1,
 CHAC15/17, WSTF, EPC1, ING3, TIP60, GAS41, DMAP1, MRG15, MRGBP, TRRAP,
 BDF6, and RVB1/2. In some embodiments of any of the methods described herein, the at
 least one chromatin remodeling protein includes ACF1 or CHD5.

15 In some embodiments of any of the methods described herein, the reaction mixture includes only one chromatin remodeling protein. In some embodiments of any of the methods described herein, the reaction mixture includes at least two chromatin remodeling proteins.

In some embodiments of any of the methods described herein, the polymerase chain
 20 reaction assay is a real-time polymerase chain reaction assay. In some embodiments of any of the methods described herein, the real-time quantitative polymerase chain reaction assay includes the use of at least one pair of real-time quantitative polymerase chain reaction primers, where a first member of the at least one pair specifically hybridizes to a sequence that is 5' of one of the at least one restriction endonuclease recognition site on a first strand
 25 of the dsDNA molecule and the second member of the at least one pair specifically hybridizes to a sequence that is 5' of the one of the at least one restriction endonuclease recognition site on a second strand of the dsDNA molecule.

Some embodiments of any of the methods described herein further include between steps (b) and (c) inactivating one or both of the at least one restriction endonuclease and the
 30 at least one chromatin remodeling protein. In some embodiments of any of the methods described herein, the inactivating includes one or both of heating the reaction mixture to a temperature of about 50 °C to about 98 °C or adding to the reaction mixture an amount of a magnesium chelator sufficient to inhibit the activity of the restriction endonuclease.

In some embodiments of any of the methods described herein, the reaction mixture comprises an amount of magnesium ion sufficient to activate the at least one restriction endonuclease. In some embodiments of any of the methods described herein, the reaction mixture in step (a) includes an amount of adenine triphosphate (ATP) sufficient to activate
 5 the at least one chromatin remodeling protein.

In some embodiments of any of the methods described herein, the reaction mixture is a liquid. In some embodiments of any of the methods described herein, the liquid has a volume of between about 5 μ L and about 50 μ L. In some embodiments of any of the methods described herein, the method is a high throughput method.

Also provided herein are kits comprising, consisting, or consisting essentially of: (i)
 10 at least one chromatin remodeling protein; and (ii) a molecular complex including a double stranded deoxyribonucleic acid (dsDNA) molecule and at least one nucleosome, wherein the dsDNA molecule includes at least one restriction endonuclease recognition site. In some embodiments of the kits, the at least one chromatin remodeling protein is selected
 15 from the group of: BRG1/SMARCA4, BRM/SMARCA2, SNF2H/ISWI/SMARCA5, SNF2L/SMARCA1, INO80, EP400, SRCAP, RAD54, RAD54B, ATRX, CHD1, CHD2, CHD3/Mi-2alpha, CHD4/Mi-2beta, CHD5, CHD6, CHD7, CHD8, CHD9, CSB/ERCC6, ETL1/SMARCA1/HEL1, HELLS/SMARCA6, HARP/SMARCA1, ZRANB3/AH2, MOT1, SHRP, HLTF/SMARCA3, MECP2, H2AFY, SMC1A, MACROD2, KDM5C,
 20 MBD1, ARID1B, SMARCC2, JMJD1C, NuRD, Mi-2, SWI, SNF, SWI1, ADR6, SWI3, SNF6, and BAF200/250a,b/ARID1a,b, BAF180/Polybromo, BAF170, BAF155, SNF5/BAF47/INI1, BAF60a-c, BAF57, BAF53a,b, BAF47, BAF45a-d, BRD7,9, Actin, MBD2/3, RbAP46/48, HDAC1/2, MTA1-3, P66alpha/beta, LSD1, BPTF, ACF1, CHRA15/17, WSTF, EPC1, ING3, TIP60, GAS41, DMAP1, MRG15, MRGBP, TRRAP,
 25 BDF6, and RVB1/2. In some embodiments of the kits, the at least one chromatin remodeling protein includes ACF1 or CHD5. In some embodiments of the kits, the kit includes only one chromatin remodeling protein. In some embodiments of the kits, the kit includes at least two chromatin remodeling proteins.

In some embodiments of the kits, the dsDNA molecule includes a sequence of a
 30 gene, wherein the at least one restriction endonuclease recognition site is within the sequence of the gene. In some embodiments of the kits, the gene is a cancer-associated gene or a gene related to the pathogenesis of a neurodevelopmental disorder, an autoimmune disease, or a cardiovascular disease. In some embodiments of the kits, the

cancer-associated gene is an oncogene. In some embodiments of the kits, the cancer-associated gene is a tumor suppressor gene.

In some embodiments of the kits, the dsDNA molecule only includes a single restriction endonuclease recognition site. In some embodiments of the kits, the dsDNA molecule includes at least two restriction endonuclease recognition sites. In some
5 embodiments of the kits, the dsDNA molecule includes at least three restriction endonuclease recognition sites. In some embodiments of the kits, the dsDNA molecule includes at least four restriction endonuclease recognition sites. In some embodiments of the kits, each of the at least two restriction endonuclease recognition sites is spaced about
10 100 base pairs to about 12,500 base pairs away from another of the restriction endonuclease recognition site(s) in the dsDNA molecule. In some embodiments of the kits, the dsDNA molecule includes at least two different restriction endonuclease recognition sites. In some embodiments of the kits, the restriction endonuclease recognition site(s) include(s) about 4 base pairs to about 10 base pairs, or about 4 base pairs to about 8 base pairs.

15 Some embodiments of the kits further include one or both of a heat-stable DNA polymerase or a polynucleotide mix. In some embodiments of the kits, the heat-stable DNA polymerase is a Taq DNA polymerase. Some embodiments of the kits further include at least one restriction endonuclease that is capable of cleaving the dsDNA molecule at one of the at least one restriction endonuclease recognition site(s). Some embodiments of the kits
20 further include: at least one pair of polymerase chain reaction primers, where a first member of the at least one pair specifically hybridizes to a sequence that is 5' of one of the at least one restriction endonuclease recognition site(s) on a first strand of the dsDNA molecule and the second member of the at least one pair specifically hybridizes to a sequence that is 5' of the one of the at least one restriction endonuclease recognition site(s) on a second strand of
25 the dsDNA molecule. In some embodiments of the kits, the at least one pair of polymerase chain reaction primers is at least one pair of real-time polymerase chain reaction primers. Some embodiments of the kits further include a buffered solution including one or both of a sufficient concentration of magnesium ion to activate a restriction endonuclease and a sufficient concentration of adenosine triphosphate to activate the at least one chromatin
30 remodeling protein.

As used herein, the word "a" before a noun represents one or more of the particular noun. For example, the phrase "a chromatin remodeling protein" represents "one or more chromatin remodeling proteins."

The term “nucleosome” means a complex of at least a core of eukaryotic (e.g., mammalian, yeast, insect, or plant) mammalian histone proteins (e.g., two H2A proteins, two H2B proteins, two H3 proteins, and two H4 proteins) with about 147 base pairs of a dsDNA molecule wrapped around the core of mammalian histone proteins. Structural
5 features of nucleosomes are well known in the art. A nucleosome can include mammalian histone proteins, e.g., human histone proteins, mouse histone proteins, rat histone proteins, or pig histone proteins. Additional exemplary features of nucleosomes are described herein.

The phrase “molecular complex of a double stranded DNA molecule and at least one nucleosome” means a double stranded DNA molecule that includes at least one nucleosome.

10 The term “molecular complex” or “complex” means the association of two or more molecules into a tertiary structure via non-covalent bonds. An example of a molecular complex of a dsDNA molecule and at least one nucleosome.

The term “restriction endonuclease” means an enzyme capable of cleaving a specific phosphodiester bond in the backbone of both strands of a dsDNA molecule, wherein the
15 specific phosphodiester bond in the backbone of both strands of the dsDNA molecule is located within or proximal (e.g., between 1 nucleotide to about 25 nucleotides, between 1 nucleotide to about 20 nucleotides, between 1 nucleotide to about 15 nucleotides, between 1 nucleotide to about 10 nucleotides, between 1 nucleotide to about 5 nucleotides, between 1 nucleotide to about 4 nucleotides, or between 1 nucleotide to about 3 nucleotides) to a
20 palindromic sequence specifically recognized by the restriction endonuclease (also called a restriction endonuclease recognition site). Features of restriction endonucleases and their corresponding restriction endonuclease recognition sites are well known in the art. Non-limiting examples of restriction endonucleases and their corresponding restriction endonuclease recognition sites are described herein.

25 The term “restriction endonuclease recognition site” means a palindromic nucleotide sequence within a dsDNA molecule that is specifically recognized by a restriction endonuclease and that indicates the specific phosphodiester bond in the backbone of both strands of the dsDNA molecule to be cleaved by the restriction endonuclease. The specific phosphodiester bond in the backbone of both strands of the dsDNA to be cleaved by a
30 restriction endonuclease can be located within or proximal (e.g., between 1 nucleotide to about 25 nucleotides, between 1 nucleotide to about 20 nucleotides, between 1 nucleotide to about 15 nucleotides, between 1 nucleotide to about 10 nucleotides, between 1 nucleotide to about 5 nucleotides, between 1 nucleotide to about 4 nucleotides, or between 1 nucleotide to

about 3 nucleotides) to the restriction endonuclease recognition site. Non-limiting examples of restriction endonuclease recognition sites are described herein.

The term “chromatin remodeling” means modification of the structure of one or both of a dsDNA molecule and a histone (e.g., a histone within a core of histones in a nucleosome) in a molecular complex (e.g., chromatin) that results in an increase in the accessibility of the dsDNA, e.g., to proteins that initiate and/or mediate transcription of a gene in the dsDNA molecule. For example, the modification can be a post-translational modification to one or more histone proteins present in a nucleosome by an enzyme (e.g., a histone acetyltransferase and a histone kinase). In other examples, the modification can be either the physical movement, removal, or restructuring of a nucleosome by an enzyme (e.g., a chromatin remodeling ATPase).

The term “chromatin remodeling protein” means a protein that modifies, alone or as part of a protein complex, the structure of one or both of a dsDNA molecule and a histone (e.g., a histone present in a core of histone proteins present in a nucleosome) in a molecular complex (e.g., chromatin) that results in an increase in the accessibility of the dsDNA, e.g., to proteins that initiate and/or mediate transcription of a gene in the dsDNA molecule. Non-limiting examples of chromatin remodeling proteins include histone acetyltransferases, histone kinases, and chromatin remodeling ATPases. Specific non-limiting examples of histone acetyltransferases, histone kinases, and chromatin remodeling ATPases are described herein. Additional examples and features of chromatin remodeling proteins are known in the art.

The phrase “sequence of a gene” means all or part of a contiguous nucleic acid sequence found at a mammalian gene locus. For example, the nucleic acid sequence found at a mammalian gene locus can include, e.g., a promoter, an enhancer, and a transcribed sequence (e.g., a sequence encoding one or more exons and/or one or more introns). The sequences of any of the exemplary genes described herein can be found by searching the National Center for Biotechnology Information (NCBI) website.

The term “subject” means any mammal, e.g., such as a human, a monkey, a mouse, a rat, a rabbit, or a goat. A subject can be, e.g., a subject diagnosed or identified as having a chromatin-remodeling-related condition (e.g., a cancer, a neurodevelopmental disorder, an autoimmune disease, or a cardiovascular disease). Additional examples of subjects are described herein.

The phrase “consisting essentially of” means the specified materials and those that do not materially affect the basic characteristics of any of the kits described herein.

Unless otherwise defined, all technical terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs.

5 Methods and materials are described herein for use in the present invention; other, suitable methods and materials known in the art can also be used. The materials, methods, and examples are illustrative only and not intended to be limiting. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will
10 control.

Other features and advantages of the invention will be apparent from the following detailed description and figures, and from the claims.

DESCRIPTION OF DRAWINGS

15 Figure 1 is a schematic showing a circular dsDNA molecule including HaeIII restriction endonuclease recognition sites.

Figure 2 is a schematic showing the binding of a pair of real-time PCR primers to both strands of a dsDNA molecule in order to achieve amplification of a region of the dsDNA molecule that includes a HaeIII restriction endonuclease recognition site. In such
20 an assay, the performance of real-time PCR will result in amplification of a dsDNA molecule that has not been cleaved by HaeIII, while the performance of real-time PCR will not result in amplification of a dsDNA molecule that has been cleaved by HaeIII.

Figure 3 is a flow chart showing an exemplary assay for detecting chromatin remodeling activity.

25 Figure 4 is a graph showing the inverse of the quantitated real-time PCR signal measured in a chromatin remodeling assay that included a molecular complex of a dsDNA molecule and at least one nucleosome, where the dsDNA molecule includes multiple HaeIII restriction endonuclease recognition site (“Chromatin”) or the dsDNA molecule (“DNA”), in the presence or absence of HaeIII (light bars and dark bars, respectively). Quantitative
30 real-time PCR was used to amplify one of the HaeIII restriction endonuclease recognition sites in the dsDNA molecule.

Figure 5 is a graph showing the inverse of the quantitated real-time PCR signal measured in a chromatin remodeling assay that included (i) a molecular complex of a

dsDNA molecule and at least one nucleosome, where the dsDNA molecule includes multiple HaeIII restriction endonuclease recognition sites; (ii) HaeIII, (iii) no chromatin remodeling protein, ACF, or CHD5; (iv) adenosine triphosphate (ATP) or adenylyl-imidodiphosphate (AMP-PNP); and (v) magnesium ion. Quantitative real-time PCR was
5 used to amplify one of the HaeIII restriction endonuclease recognition sites in the dsDNA molecule.

DETAILED DESCRIPTION

Dysregulation of chromatin remodeling proteins has been implicated in the
10 pathogenesis of different chromatin remodeling-related conditions (e.g., cancers, neurodevelopmental disorders, autoimmune diseases, and cardiovascular diseases) and increased chromatin remodeling activity has been implicated for a role in maintaining or inducing pluripotency in a mammalian cell.

Provided herein are methods, e.g., high throughput and sensitive methods, for
15 identifying a test compound that is a modulator of chromatin remodeling activity, for identifying a candidate compound for treating or reducing the risk of developing a chromatin remodeling-related condition (e.g., any of the chromatin remodeling-related conditions described herein) in a subject, and for identifying a candidate compound for maintaining or inducing pluripotency in a mammalian cell. Also provided are kits for
20 performing any of the methods described herein.

The methods can be performed on a large scale to screen a large number of test compounds for their effect on chromatin remodeling activity and/or to determine whether the test compound is a candidate compound for treating a chromatin remodeling-related condition in a subject or a candidate compound for maintaining or inducing pluripotency in
25 a mammalian cell. The methods described herein can be performed using a reaction mixture (e.g., a liquid reaction mixture) having a volume of between about 3 μ L to about 100 μ L, between about 3 μ L to about 95 μ L, between about 3 μ L to about 90 μ L, between about 3 μ L to about 85 μ L, between about 3 μ L to about 80 μ L, between about 3 μ L to about 75 μ L, between about 3 μ L to about 70 μ L, between about 3 μ L to about 65 μ L,
30 between about 3 μ L to about 60 μ L, between about 3 μ L to about 55 μ L, between about 3 μ L to about 50 μ L, between about 3 μ L to about 45 μ L, between about 3 μ L to about 40 μ L, between about 3 μ L to about 35 μ L, between about 3 μ L to about 30 μ L, between about 3 μ L to about 25 μ L, between about 3 μ L to about 20 μ L, between about 3 μ L to about 15 μ L,

between about 3 μ L to about 10 μ L, between about 3 μ L to about 5 μ L, between about 5 μ L to about 100 μ L, between about 5 μ L to about 95 μ L, between about 5 μ L to about 90 μ L, between about 5 μ L to about 85 μ L, between about 5 μ L to about 80 μ L, between about 5 μ L to about 75 μ L, between about 5 μ L to about 70 μ L, between about 5 μ L to about 65 μ L, between about 5 μ L to about 60 μ L, between about 5 μ L to about 55 μ L, between about 5 μ L to about 50 μ L, between about 5 μ L to about 45 μ L, between about 5 μ L to about 40 μ L, between about 5 μ L to about 35 μ L, between about 5 μ L to about 30 μ L, between about 5 μ L to about 25 μ L, between about 5 μ L to about 20 μ L, between about 5 μ L to about 15 μ L, between about 5 μ L to about 10 μ L, between about 10 μ L to about 100 μ L, between about 10 μ L to about 95 μ L, between about 10 μ L to about 90 μ L, between about 10 μ L to about 85 μ L, between about 10 μ L to about 80 μ L, between about 10 μ L to about 75 μ L, between about 10 μ L to about 70 μ L, between about 10 μ L to about 65 μ L, between about 10 μ L to about 60 μ L, between about 10 μ L to about 55 μ L, between about 10 μ L to about 50 μ L, between about 10 μ L to about 45 μ L, between about 10 μ L to about 40 μ L, between about 10 μ L to about 35 μ L, between about 10 μ L to about 30 μ L, between about 10 μ L to about 25 μ L, between about 10 μ L to about 20 μ L, or between about 10 μ L to about 15 μ L.

Exemplary aspects of the methods and kits provided herein are described below. As one of skill in the art would appreciate, the various aspects of the methods and kits described below can be used in any combination.

Chromatin Remodeling Proteins

Chromatin remodeling proteins modify, alone or as part of a protein complex, the structure of one or both of a dsDNA molecule and a histone (e.g., a histone within a core of histones present in a nucleosome) in a molecular complex (e.g., chromatin) that results in an increase in the accessibility of the dsDNA, e.g., to proteins that initiate and/or mediate transcription of a gene in the dsDNA molecule. Examples of chromatin remodeling proteins include histone acetyltransferases, histone methyltransferases, ubiquitin ligases, histone deacetylases, histone kinases, histone phosphatases, histone demethylases, and histone deubiquitinating enzymes, and chromatin remodeling ATPases.

Non-limiting examples of chromatin remodeling proteins that are human histone acetyltransferases are listed in Table 1.

Table 1. Exemplary Human Histone Acetyltransferases

Name (Synonym)	Protein NCBI Access. No.	Nucleic Acid NCBI Acc. No.
GCN5	AAC50641.1	U57316.1
PCAF (KAT2B)	NP_003875.3	NM_003884.4
Tip60 (KAT5)	NP_006379.2	NM_006388.3
MOZ (KAT6A)	NP_001092883.1	NM_001099413.1
MORF (KAT6B)	NP_036462.2	NM_012330.3
HBO1 (KAT7)	NP_001186087.1	NM_001199158.1
p300	NP_001420.2	NM_001429.3
SRC-1	NP_003734.3	NM_003743.4
ACTR (RAC3, AIB1, TRAM-1)	NP_858045.1	NM_181659.2
CLOCK	AAB83969.1	AF011568.1

Non-limiting examples of chromatin remodeling proteins that are human histone
5 kinases are listed in Table 2.

Table 2. Exemplary Human Histone Kinases

Name (Synonym)	Protein NCBI Access. No.	Nucleic Acid NCBI Acc. No.
Aurora-B	NP_004208.2	NM_004217.3
Haspin	NP_114171.2	NM_031965.2
Dlk/ZIP	NP_001339.1	NM_001348.2
MSK1	NP_004746.2	NM_004755.2
MSK2	NP_001287731.1	NM_001300802.1
IKK- α	NP_001269.3	NM_001278.3
JNK	NP_620637.1	NM_139049.2
PKA	NP_002723.2	NM_002732.3
Akt	NP_001014431.1	NM_001014431.1
Cot	NP_005195.2	NM_005204.3
PIM1	NP_001230115.1	NM_001243186.1
CDK8	NP_001251.1	NM_001260.1
JAK2	NP_004963.1	NM_004972.3
PKC β	NP_997700.1	NM_212535.2

Chk1	NP_001107594.1	NM_001114122.2
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Non-limiting examples of human chromatin remodeling proteins that are human chromatin remodeling ATPases are listed in Table 3. The chromatin remodeling ATPases listed in Table 3 do not require a protein binding partner for activity. Some of the chromatin remodeling ATPases listed in Table 3 can, e.g., have increased activity when contacted with a protein binding partner (e.g., any one of the exemplary protein binding partners listed in Table 3 or listed in Table 4). These protein binding partners are another class of chromatin remodeling proteins. Any of the methods described herein that include a chromatin remodeling ATPase in the reaction mixture can further include an amount of ATP sufficient to activate the chromatin remodeling ATPase in the reaction mixture. Any of the kits described herein that include a chromatin remodeling ATPase can further include a buffer that can include an amount of ATP sufficient to activate the chromatin remodeling ATPase.

Table 3. Exemplary Human Chromatin Remodeling ATPases

Name (Synonyms) Protein NCBI Acc. No. Nucleic Acid NCBI Acc. No.

BRG1 (SMARCA4)	NP_001122321	NM_001128849
BRM (SMARCA2)	NP_003061	NM_003070
SNF2H (ISWI, SMARCA5)	NP_003592	NM_003601
SNF2L (SMARCA1)	NP_003060	NM_003069
INO80	NP_060023	NM_017553
EP400	NP_056224	NM_015409
SRCAP	NP_006653	NM_006662
RAD54B	NP_036547	NM_012415
ATRX	NP_000480	NM_000489
CHD1	NP_001261	NM_001270
CHD2	NP_001262	NM_001271
CHD3 (Mi-2alpha)	NP_001005273	NM_001005273
CHD4 (Mi-2beta)	NP_001264	NM_001273
CHD5	NP_056372	NM_015557
CHD6	NP_115597	NM_032221

CHD7	NP_060250	NM_017780
CHD8	NP_001164100	NM_001170629
CHD9	NP_079410	NM_025134
CSB (ERCC6)	NP_000115	NM_000124
ETL1 (SMARCA1, HEL1)	NP_001121901	NM_001128429
HELLS (SMARCA6)	NP_060533	NM_018063
HARP (SMARCA1)	NP_054859	NM_014140
ZRANB3 (AH2)	NP_115519	NM_032143
MOT1	NP_003963	NM_003972
SHPRH	NP_001036148	NM_001042683
HLTF (SMARCA3)	NP_003062	NM_003071

Table 4. Exemplary Human Protein Binding Partners of Chromatin Remodeling ATPases

Name (Synonym)	Protein NCBI Access. No.	Nucleic Acid NCBI Acc. No.
BAF250a (ARID1a)	NP_006006	NM_006015
BAF250b (ARID1b)	NP_059989	NM_017519
BAF180 (Polybromo)	NP_060783	NM_018313
BAF170 (SMARCC2)	NP_003066	NM_003075
BAF155 (SMARCC1)	NP_003065	NM_003074
SNF5 (BAF47, INI1)	NP_003064	NM_003073
BAF60a (SMARCD1)	NP_003067	NM_003076
BAF60b (SMARCD2)	NP_001091896	NM_001098426
BAF60c (SMARCD3)	NP_003069	NM_003078
BAF57 (SMARCE1)	NP_003070	NM_003079
BAF53a (ACTL6A)	NP_004292	NM_004301
BAF53b (ACTL6B)	NP_057272	NM_016188
BAF47	NP_003064	NM_003073
BAF45a	NP_060758	NM_018288
BAF45b	NP_004638	NM_004647
BAF45c	NP_036206	NM_012074

BAF45d	NP_006259	NM_006268
BRD7	NP_001167455	NM_001173984
BRD9	NP_076413	NM_023924
Actin (ACTB)	NP_001092	NM_001101
MBD2	NP_003918	NM_003927
MBD3	NP_001268382	NM_001281453
RbAP46 (RBBP7)	NP_001185648	NM_001198719
RbAP48 (RBBP4)	NP_005601	NM_005610
HDAC1	NP_004955	NM_004964
HDAC2	NP_001518	NM_001527
MTA1	NP_004680	NM_004689
MTA2	NP_004730	NM_004739
MTA3	NP_001269685	NM_001282756
P66-alpha (GATAD2A)	NP_001287875	NM_001300946
P66-beta (GATAD2B)	NP_065750	NM_020699
LSD1	NP_001009999	NM_001009999
BPTF	NP_872579	NM_182641
ACF1 (BAZ1A)	NP_038476	NM_013448
CHRA15	NP_059140	NM_017444
CHRA17	NP_059139	NM_017443
WSTF (BAZ1B)	NP_115784	NM_032408
EPC1	NP_079485	NM_025209
ING3	NP_061944	NM_019071
TIP60 (KAT5)	NP_874369	NM_182710
GAS41	NP_006521	NM_006530
DMAP1	NP_061973	NM_019100
MRG15	NP_006782	NM_006791
MRGBP	NP_060740	NM_018270
TRRAP	NP_001231509	NM_001244580
RUVBL1	NP_003698	NM_003707
RUVBL2	NP_006657	NM_006666

Examples of chromatin remodeling proteins that are histone methyltransferases, histone ubiquitin ligases, histone deacetylases, histone phosphatases, histone demethylases, and histone deubiquitinating enzymes are known in the art.

Exemplary chromatin remodeling proteins implicated for a role in the development
5 of a neurodevelopmental disorder are listed in Table 5.

Table 5. Exemplary Human Chromatin Remodeling Proteins Implicated for a Role in Neurodevelopmental Disorders

Name (Synonym)	Protein NCBI Access. No.	cDNA NCBI Access. No.
MECP2	NP_004983	NM_004992
ATRX	NP_000480	NM_000489
H2AFY	NP_613075	NM_138609
SMC1A	NP_006297	NM_006306
MACROD2	NP_542407	NM_080676
KDM5C	NP_004178	NM_004187
MBD1	NP_056671	NM_015846
BAF250B (ARID1B)	NP_059989	NM_017519
BAF170 (SMARCC2)	NP_003066	NM_003075
JMJD1C	NP_116165	NM_032776
CHD1	NP_001261	NM_001270
CHD2	NP_001262	NM_001271
CHD5	NP_056372	NM_015557
CHD6	NP_115597	NM_032221
CHD7	NP_060250	NM_017780
CHD8	NP_001164100	NM_001170629
CSB (ERCC6)	NP_000115	NM_000124
BRG1 (SMARCA4)	NP_001122321	NM_001128849
BRM (SMARCA2)	NP_003061	NM_003070
HARP (SMARCA1)	NP_054859	NM_014140

10

Any of methods or kits described herein can include the use of or include at least one chromatin remodeling protein selected from the group of mammalian (e.g., human) BRG1/SMARCA4, BRM/SMARCA2, SNF2H/ISWI/SMARCA5, SNF2L/SMARCA1,

INO80, EP400, SRCAP, RAD54, RAD54B, ATRX, CHD1, CHD2, CHD3/Mi-2alpha, CHD4/Mi-2beta, CHD5, CHD6, CHD7, CHD8, CHD9, CSB/ERCC6, ETL1/SMARCA1/HEL1, HELLS/SMARCA6, HARP/SMARCA1, ZRANB3/AH2, MOT1, SHRPH, HLTF/SMARCA3, MECP2, H2AFY, SMC1A, MACROD2, KDM5C, MBD1, ARID1B, SMARCC2, JMJD1C, CHD8, NuRD, Mi-2, SWI, SNF, SWI1, ADR6, SWI3, SNF6, and BAF200/250a,b/ARID1a,b, BAF180/Polybromo, BAF170, BAF155, SNF5/BAF47/INI1, BAF60a-c, BAF57, BAF53a,b, BAF47, BAF45a-d, BRD7,9, Actin, MBD2/3, RbAP46/48, HDAC1/2, MTA1-3, P66-alpha/beta, LSD1, BPTF, ACF1, CHRA15/17, WSTF, EPC1, ING3, TIP60, GAS41, DMAP1, MRG15, MRGBP, TRRAP, and RUVBL1/2. In any of the methods or kits described herein, the at least one chromatin remodeling protein includes mammalian (e.g., human) ACF1 or CHD5.

In any of the methods described herein, the reaction mixture can include only one chromatin remodeling protein (e.g., any of the chromatin remodeling proteins described herein). In any of the methods described herein, the reaction mixture can include at least two (e.g., three, four, five, six, or seven) chromatin remodeling proteins (e.g., any of the chromatin remodeling proteins described herein). In some embodiments, the reaction mixture can include a SWI/SNF complex (e.g., a complex comprising one or more of SWI1, SWI2, SNF2, SWI3, SWI5, and SWI6) and optionally, further includes BRG1 and/or BRM ATPase. In some embodiments, the reaction mixture can include a SWI/SNF complex and optionally further includes a NURD complex (e.g., a complex comprising CHD3, CHD4, or CHD5).

Any of the kits described herein can include only one chromatin remodeling protein (e.g., any of the chromatin remodeling proteins described herein). Additional examples of any of the kits described herein can include at least two (e.g., three, four, five, six, or seven) chromatin remodeling proteins (e.g., any of the chromatin remodeling proteins described herein).

Any of the methods described herein can include the use of any combination of one or more of the chromatin remodeling proteins described herein (without any limitation) and any of the kits described herein can include any combination of one or more of the chromatin remodeling proteins described herein (without any limitation). Any of the methods described herein can include the use of a combination of a chromatin remodeling protein described herein and a chromatin remodeling protein known in the art. Additional examples of chromatin remodeling proteins are known in the art. For example, although

specific examples of human chromatin remodeling proteins are described in this section, other mammalian homologues of the human chromatin remodeling proteins can be identified by performing sequence searching using, e.g., the BLAST® software on the National Center for Biotechnology Information (NCBI) website.

5

Nucleosomes

A nucleosome is a complex of at least a core of eukaryotic (e.g., mammalian) histone proteins (e.g., two H2A proteins, two H2B proteins, two H3 proteins, and two H4 proteins) with about 147 base pairs of a dsDNA molecule wrapped around the core of eukaryotic (e.g., mammalian) histone proteins.

10

In some examples of any of the methods described herein, a nucleosome includes, in part, a core of mammalian histone proteins (e.g., an octamer of two H2A proteins, two H2B proteins, two H3 proteins, and two H4 proteins). The amino acid and cDNA sequences for human H2A, H2B, H3, and H4 are listed in Table 6 below.

15

In other examples of any of the methods described herein, a nucleosome includes, in part, a core of mammalian (e.g., human) histone proteins (e.g., an octamer of two H2A proteins, two H2B proteins, two H3 proteins, and two H4 proteins) and at least one mammalian (e.g., human) H1 protein. The amino acid and cDNA sequences for human H1 are also listed in Table 6 below.

20

Table 6. Amino Acid and cDNA Sequences for Human Histones

Human Histone	Protein NCBI Access. No.	Nucleic Acid NCBI Accession No.
H1	NP_005309	NM_005318
H2A	NP_003507	NM_003516
H2B	NP_066406	NM_021062
H3	NP_003522	NM_003531
H4	NP_003529	NM_003538

25

Human H1, H2A, H2B, H3, and H4 proteins can be purchased from a variety of commercial vendors including, e.g., New England BioLabs (Ipswich, MA). A core of human histone proteins (e.g., two H2A proteins, two H2B proteins, two H3 proteins, and two H4 proteins) can be purchased from Genway Biotech Inc. (San Diego, CA) and EpiCypher (Research Triangle Park, NC).

Restriction Endonucleases and Restriction Endonuclease Recognition Sites

The methods described herein include the use of at least one (e.g., two, three, four, five, six, seven, or eight) restriction endonuclease (e.g., any of the restriction endonucleases described herein) and the kits described herein optionally include at least one (e.g., two, three, four, five, six, seven, or eight) restriction endonuclease (e.g., any of the restriction endonucleases described herein). Non-limiting examples of restriction endonucleases that can be used in any of the methods or can be included in any of the kits described herein include: HaeIII, AatI, AatII, AccI, AccIII, Acc65I, AciI, AcsI, AcyI, AflI, AflII, AflIII, AgeI, AhaII, AhaIII, AluI, AlwI, Alw44I, AlwNI, AocI, AosI, ApaI, ApaLI, ApoI, ApyI, AscI, AseI, AsnI, AspI, Asp700, Asp718, AspEI, AspHI, AsuII, Aval, AvaII, AviII, AvrII, Ball, BamHI, BanI, BanII, BbrPI, BbsI, BbvI, BcgI, BclI, BfaI, BfrI, BglI, BglII, BinI, BmyI, BpmI, BpuAI, Bpu1102I, BsaI, BsaAI, BsaBI, BsaHI, BsaJI, BseAI, BsePI, BsgI, BsiEI, BsiWI, BsiYI, BslI, BsmI, BsmAI, Bsp1286I, Bsp1407I, BspDI, BspEI, BspHI, BspLU11I, BspMI, BsrI, BsrFI, BssGI, BssHII, Bst1107I, BstBI, BstEII, BstNI, BstUI, BstXI, BstYI, Bsu36I, CelII, CfoI, CfrI, Cfr10I, ClaI, DdeI, DpnI, DpnII, DraI, DraII, DraIII, DrdI, DsaI, EaeI, EagI, Eam1105I, EarI, Ecl136II, EclXI, Eco47III, Eco57I, EcoNI, EcoO109I, EcoRI, EcoRII, EcoRV, EspI, Esp3I, FnuDII, Fnu4HI, FokI, FseI, FspI, GsuI, HaeII, HgaI, HgiAI, HhaI, HincII, HindII, HindIII, HinfI, HinPI, HpaI, HpaII, HphI, ItaI, KasI, KpnI, KspI, MaeI, MaeII, MaeIII, MamI, MboI, MboII, MfeI, MluI, MluNI, MnlI, MroI, MscI, MseI, MspI, MstI, MstII, MunI, MvaI, MvnI, NaeI, NarI, NciI, NcoI, NdeI, NdeII, NgoMI, NheI, NlaIII, NlaIV, NotI, NruI, NsiI, NspBII, NspI, NspII, NspV, PacI, PaeR7I, PflMI, PinAI, PleI, PmaCI, PmeI, PmlI, PpuMI, Psp1406I, PstI, PvuI, PvuII, RcaI, RmaI, RsaI, RsrII, SacI, SacII, SalI, SauI, Sau3AI, Sau96I, ScaI, ScrFI, SexAI, SfaNI, SfcI, SfiI, SfuI, SgrAI, SmaI, SnaBI, SnaI, SpeI, SphI, SrfI, Sse8387I, SspI, SspBI, SstI, SstII, StuI, StyI, SwaI, TaqI, TfiI, Thal, Tru9I, Tth111I, Van91I, XbaI, XcmI, XhoI, XhoII, XmaI, XmaIII, XmaCI, and XmnI. Restriction endonucleases are commercially available from a variety of different commercial vendors (e.g., New England BioLabs).

A list of the restriction endonuclease recognition sites that correspond to each of the exemplary restriction endonucleases listed above is shown below.

30

AatI	AGG/CCT
AatII	GACGT/C
AccI	GT/MKAC
AccIII	T/CCGGA
35 Acc65I	G/GTACC

	AciI	C/CGC and G/CGG
	AcsI	R/AATTY
	AcyI	GR/CGYC
	AflI	G/GWCC
5	AflII	C/TTAAG
	AflIII	A/CRYGT
	AgeI	A/CCGGT
	AhaII	GR/CGYC
	AhaIII	TTT/AAA
10	AluI	AG/CT
	AlwI	GGATCNNNN/ and /NNNNNGATCC (SEQ ID NO: 1)
	Alw44I	G/TGCAC
	AlwNI	CAGNNN/CTG
	AocI	CC/TNAGG
15	AosI	TGC/GCA
	ApaI	GGGCC/C
	ApaLI	G/TGCAC
	ApoI	R/AATTY
	ApyI	/CCWGG
20	AscI	GG/CGCGCC
	AseI	AT/TAAT
	AsnI	AT/TAAT
	AspI	GACN/NNGTC
	Asp700	GAANN/NNTTC (SEQ ID NO: 2)
25	Asp718	G/GTACC
	AspEI	GACNNN/NNGTC (SEQ ID NO: 3)
	AspHI	GWGCW/C
	AsuII	TT/CGAA
	AvaI	C/YCGRG
30	AvaII	G/GWCC
	AviII	TGC/GCA
	AvrII	C/CTAGG
	BalI	TGG/CCA
	BamHI	G/GATCC
35	BanI	G/GYRCC
	BanII	GRGCY/C
	BbrPI	CAC/GTG
	BbsI	GAAGACNN/ and /NNNNNNGTCTTC (SEQ ID NO: 4)
	BbvI	GCAGCNNNNNNNN/ (SEQ ID NO: 5) and
40		/NNNNNNNNNNNGCTGC (SEQ ID NO: 6)
	BcgI	/NNNNNNNNNNCGANNNNNNNTGCNNNNNNNNNNNN/ (SEQ ID
		NO: 7) and
		/NNNNNNNNNNCGANNNNNNNTCGNNNNNNNNNNNN/ (SEQ ID
		NO: 8)
45	BclI	T/GATCA
	BfaI	C/TAG
	BfrI	C/TTAAG
	BglI	GCCNNNN/NGGC (SEQ ID NO: 9)
	BglII	A/GATCT
50	BinI	C/CTAGG

	BmyI	GDGCH/C
	BpmI	CTGGAGNNNNNNNNNNNNNNNN/ (SEQ ID NO: 10) and /NNNNNNNNNNNNNNNCTCCAG (SEQ ID NO: 11)
	BpuAI	GAAGACNN/ and /NNNNNNGTCTTC (SEQ ID NO: 12)
5	Bpu1102I	GC/TNAGC
	BsaI	GGTCTCN/ and /NNNNNGAGACC (SEQ ID NO: 13)
	BsaAI	YAC/GTR
	BsaBI	GATNN/NNATC (SEQ ID NO: 14)
	BsaHI	GR/CGYC
10	BsaJI	C/CNNGG
	BseAI	T/CCGGA
	BsePI	G/CGCGC
	BsgI	GTGCAGNNNNNNNNNNNNNNNN/ (SEQ ID NO: 15) and /NNNNNNNNNNNNNNNCTGCAC (SEQ ID NO: 16)
15	BsiEI	CGRY/CG
	BsiWI	C/GTACG
	BsiYI	CCNNNNN/NNGG (SEQ ID NO: 17)
	BslI	CCNNNNN/NNGG (SEQ ID NO: 18)
	BsmI	GAATGCN/ and /NGCATTC
20	BsmAI	GTCTCN/ and /NNNNNGAGAC (SEQ ID NO: 19)
	Bsp1286I	GDGCH/C
	Bsp1407I	T/GTACA
	BspDI	AT/CGAT
	BspEI	T/CCGGA
25	BspHI	T/CATGA
	BspLU11I	A/CATGT
	BspMI	ACCTGCNNNN/ (SEQ ID NO: 20) and /NNNNNNNNGCAGGT (SEQ ID NO: 21)
	BsrI	ACTGGN/ and /NCCAGT
30	BsrFI	R/CCGGY
	BssGI	CCANNNNN/NTGG (SEQ ID NO: 22)
	BssHII	G/CGCGC
	Bst1107I	GTA/TAC
	BstBI	TT/CGAA
35	BstEII	G/GTNACC
	BstNI	CC/WGG
	BstUI	CG/CG
	BstXI	CCANNNNN/NTGG (SEQ ID NO: 23)
	BstYI	R/GATCY
40	Bsu36I	CC/TNAGG
	CelII	GC/TNAGC
	CfoI	GCG/C
	CfrI	Y/GGCCR
	Cfr10I	R/CCGGY
45	ClaI	AT/CGAT
	DdeI	C/TNAG
	DpnI	GA/TC (only if G methylated)
	DpnII	/GATC
	DraI	TTT/AAA
50	DraII	RG/GNCCY

	DraIII	CACNNN/GTG
	DrdI	GACNNNN/NNGTC (SEQ ID NO: 24)
	DsaI	C/CRYGG
	EaeI	Y/GGCCR
5	EagI	C/GGCCG
	Eam1105I	GACNNN/NNGTC (SEQ ID NO: 25)
	EarI	CTCTTCN/ and /NNNNGAAGAG (SEQ ID NO: 26)
	Ecl136II	GAG/CTC
	EclXI	C/GGCCG
10	Eco47III	AGC/GCT
	Eco57I	CTGAAGNNNNNNNNNNNNNNNN/ (SEQ ID NO: 27) and /NNNNNNNNNNNNNNCTTCAG (SEQ ID NO: 28)
	EcoNI	CCTNN/NNNAGG (SEQ ID NO: 29)
	EcoO109I	RG/GNCCY
15	EcoRI	G/AATTC
	EcoRII	/CCWGG
	EcoRV	GAT/ATC
	EspI	GC/TNAGC
	Esp3I	CGTCTCN/ and /NNNNNGAGACG (SEQ ID NO: 30)
20	FnuDII	CG/CG
	Fnu4HI	GC/NGC
	FokI	GGATGNNNNNNNN/ (SEQ ID NO: 31) and /NNNNNNNNNNNNNCATCC (SEQ ID NO: 32)
	FseI	GGCCGG/CC
25	FspI	TGC/GCA
	GsuI	CTGGAGNNNNNNNNNNNNNNNN/ (SEQ ID NO: 33) and /NNNNNNNNNNNNNNCTCCAG (SEQ ID NO: 34)
	HaeII	RGCGC/Y
	HaeIII	GG/CC
30	HgaI	GACGCNNNNN/ (SEQ ID NO: 35) and /NNNNNNNNNNNGCGTC (SEQ ID NO: 36)
	HgiAI	GWGCW/C
	HhaI	GCG/C
	HincII	GTY/RAC
35	HindII	GTY/RAC
	HindIII	A/AGCTT
	HinfI	G/ANTC
	HinPI	G/CGC
	HpaI	GTT/AAC
40	HpaII	C/CGG
	HphI	GGTGANNNNNNNN/ (SEQ ID NO: 37) and /NNNNNNNTCACC (SEQ ID NO: 38)
	ItaI	GC/NGC
	KasI	G/GCGCC
45	KpnI	GGTAC/C
	KspI	CCGC/GG
	MaeI	C/TAG
	MaeII	A/CGT
	MaeIII	/GTNAC
50	MamI	GATNN/NNATC

	MboI	/GATC
	MboII	GAAGANNNNNNNN/ (SEQ ID NO: 39) and /NNNNNNNTCTTC (SEQ ID NO: 40)
	MfeI	C/AATTG
5	MluI	A/CGCGT
	MluNI	TGG/CCA
	MnlI	CCTCNNNNNNN/ (SEQ ID NO: 41) and /NNNNNNGAGG (SEQ ID NO: 42)
	MroI	T/CCGGA
10	MscI	TGG/CCA
	MseI	T/TAA
	MspI	C/CGG
	MstI	TGC/GCA
	MstII	CC/TNAGG
15	MunI	C/AATTG
	MvaI	CC/WGG
	MvnI	CG/CG
	NaeI	GCC/GGC
	NarI	GG/CGCC
20	NciI	CC/SGG
	NcoI	C/CATGG
	NdeI	CA/TATG
	NdeII	/GATC
	NgoMI	G/CCGGC
25	NheI	G/CTAGC
	NlaIII	CATG/
	NlaIV	GGN/NCC
	NotI	GC/GGCCGC
	NruI	TCG/CGA
30	NsiI	ATGCA/T
	NspBII	CMG/CKG
	NspI	RCATG/Y
	NspII	GDGCH/C
	NspV	TT/CGAA
35	PacI	TTAAT/TAA
	Paer7I	C/TCGAG
	PflMI	CCANNNN/NTGG (SEQ ID NO: 43)
	PinAI	A/CCGGT
	PleI	GAGTCNNNN/ and /NNNNNGACTC (SEQ ID NO: 44)
40	PmaCI	CAC/GTG
	PmeI	GTTT/AAAC
	PmlI	CAC/GTG
	PpuMI	RG/GWCCY
	Psp1406I	AA/CGTT
45	PstI	CTGCA/G
	PvuI	CGAT/CG
	PvuII	CAG/CTG
	RcaI	T/CATGA
	RmaI	C/TAG
50	RsaI	GT/AC

	RsrII	CG/GWCCG
	SacI	GAGCT/C
	SacII	CCGC/GG
	SalI	G/TCGAC
5	SauI	CC/TNAGG
	Sau3AI	/GATC
	Sau96I	G/GNCC
	ScaI	AGT/ACT
	ScrFI	CC/NGG
10	SexAI	A/CCWGGT
	SfaNI	GCATCNNNNN/ (SEQ ID NO: 45) and /NNNNNNNNNGATGC (SEQ ID NO: 46)
	SfcI	C/TRYAG
	SfiI	GGCCNNNN/NGGCC (SEQ ID NO: 47)
15	SfuI	TT/CGAA
	SgrAI	CR/CCGGYG
	SmaI	CCC/GGG
	SnaBI	TAC/GTA
	SnoI	G/TGCAC
20	SpeI	A/CTAGT
	SphI	GCATG/C
	SrfI	GCCC/GGGC
	Sse8387I	CCTGCA/GG
	SspI	AAT/ATT
25	SspBI	T/GTACA
	SstI	GAGCT/C
	SstII	CCGC/GG
	StuI	AGG/CCT
	StyI	C/CWWGG
30	SwaI	ATTT/AAAT
	TaqI	T/CGA
	TfiI	G/AWTC
	ThaI	CG/CG
	Tru9I	T/TAA
35	Tth111I	GACN/NNGTC
	Van91I	CCANNNN/NTGG (SEQ ID NO: 48)
	XbaI	T/CTAGA
	XcmI	CCANNNNN/NNNNTGG (SEQ ID NO: 49)
	XhoI	C/TCGAG
40	XhoII	R/GATCY
	XmaI	C/CCGGG
	XmaIII	C/GGCCG
	XmaCI	C/CCGGG
	XmnI	GAANN/NNTTC (SEQ ID NO: 50)

45

In some examples of any of the methods described herein, the dsDNA molecule is contacted with at least two (e.g., two, three, four, five, six, seven, or eight) different restriction endonucleases (e.g., any of the restriction endonucleases described herein),

where each of the different restriction endonucleases is capable of cleaving the dsDNA molecule at one of the restriction endonuclease recognition site(s). In some examples of any of the methods and kits described herein, the restriction endonuclease recognition site(s) include about 4 base pairs to about 15 base pairs (e.g., about 4 base pairs to about 14
5 base pairs, about 4 base pairs to about 13 base pairs, about 4 base pairs to about 12 base pairs, about 4 base pairs to about 11 base pairs, about 4 base pairs to about 10 base pairs, about 4 base pairs to about 9 base pairs, about 4 base pairs to about 8 base pairs, about 4 base pairs to about 7 base pairs, about 4 base pairs to about 6 base pairs, about 5 base pairs to about 15 base pairs, about 5 base pairs to about 14 base pairs, about 5 base pairs to about
10 13 base pairs, about 5 base pairs to about 12 base pairs, about 5 base pairs to about 11 base pairs, about 5 base pairs to about 10 base pairs, about 5 base pairs to about 9 base pairs, about 5 base pairs to about 8 base pairs, about 6 base pairs to about 15 base pairs, about 6 base pairs to about 14 base pairs, about 6 base pairs to about 13 base pairs, about 6 base pairs to about 12 base pairs, about 6 base pairs to about 11 base pairs, about 6 base pairs to
15 about 10 base pairs, about 6 base pairs to about 9 base pairs, about 7 base pairs to about 15 base pairs, about 7 base pairs to about 14 base pairs, about 7 base pairs to about 13 base pairs, about 7 base pairs to about 12 base pairs, about 7 base pairs to about 11 base pairs, about 7 base pairs to about 10 base pairs, about 8 base pairs to about 15 base pairs, about 8 base pairs to about 14 base pairs, about 8 base pairs to about 13 base pairs, about 8 base
20 pairs to about 12 base pairs, about 8 base pairs to about 11 base pairs, about 9 base pairs to about 15 base pairs, about 9 base pairs to about 14 base pairs, about 9 base pairs to about 13 base pairs, about 9 base pairs to about 12 base pairs, or about 10 base pairs to about 15 base pairs).

Any of the reaction mixtures described herein can include an amount of magnesium
25 ion sufficient to activate the at least one restriction endonuclease present in the reaction mixture. Any of the kits described herein can include a buffer that includes an amount of magnesium ion sufficient to activate the at least one restriction endonuclease provided in the kit.

30 **Double Stranded DNA Molecules**

The dsDNA molecule used in any of the methods or included in any of the kits described herein include at least one (e.g., two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen or twenty,

or two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, ten or more, eleven or more, twelve or more, thirteen or more, fourteen or more, or fifteen or more) restriction endonuclease recognition site. The dsDNA molecule in any of the methods or kits described herein can be a linear dsDNA molecule (e.g., a human artificial chromosome, a yeast artificial chromosome, or a viral vector) or can be a plasmid (e.g., a bacterial plasmid, a yeast plasmid, or a cosmid). A dsDNA molecule that is a plasmid can have a size of about 500 base pairs to about 35,000 base pairs, about 500 base pairs to about 30,000 base pairs, about 500 base pairs to about 25,000 base pairs, about 500 base pairs to about 20,000 base pairs, about 500 base pairs to about 15,000 base pairs, about 500 base pairs to about 10,000 base pairs, between about 500 base pairs to about 8,000 base pairs, about 500 base pairs to about 5,000 base pairs, about 500 base pairs to about 2,500 base pairs, about 1,000 base pairs to about 35,000 base pairs, about 1,000 base pairs to about 30,000 base pairs, about 1,000 base pairs to about 25,000 base pairs, about 1,000 base pairs to about 20,000 base pairs, about 1,000 base pairs to about 15,000 base pairs, about 1,000 base pairs to about 10,000 base pairs, about 1,000 base pairs to about 8,000 base pairs, about 1,000 base pairs to about 5,000 base pairs, about 1,000 base pairs to about 2,500 base pairs, about 2,000 base pairs to about 35,000 base pairs, about 2,000 base pairs to about 30,000 base pairs, about 2,000 base pairs to about 25,000 base pairs, about 2,000 base pairs to about 20,000 base pairs, about 2,000 base pairs to about 15,000 base pairs, about 2,000 base pairs to about 10,000 base pairs, about 2,000 base pairs to about 8,000 base pairs, about 2,000 base pairs to about 5,000 base pairs, about 2,500 base pairs to about 35,000 base pairs, about 2,500 base pairs to about 30,000 base pairs, about 2,500 base pairs to about 25,000 base pairs, about 2,500 base pairs to about 20,000 base pairs, about 2,500 base pairs to about 15,000 base pairs, about 2,500 base pairs to about 10,000 base pairs, about 2,500 base pairs to about 8,000 base pairs, about 2,500 base pairs to about 5,000 base pairs, about 5,000 base pairs to about 35,000 base pairs, about 5,000 base pairs to about 30,000 base pairs, about 5,000 base pairs to about 25,000 base pairs, about 5,000 base pairs to about 20,000 base pairs, about 5,000 base pairs to about 15,000 base pairs, about 5,000 base pairs to about 10,000 base pairs, or about 5,000 base pairs to about 8,000 base pairs. A dsDNA molecule that is a plasmid can further include one or both of a selection marker (e.g., an antibiotic resistance gene) and an origin of replication. Plasmids can be purchased from a number of commercial vendors. Methods for introducing at least one restriction endonuclease recognition site into a dsDNA molecule (e.g., a

plasmid) are well known in the art. A dsDNA molecule that is a plasmid can have, e.g., one of the at least one restriction endonuclease recognition sites within a selection marker (e.g., an antibiotic resistance gene, such as an ampicillin resistance gene).

A dsDNA molecule for use in any of the methods described herein need not include
5 a specific sequence (i.e., other than the at least one restriction endonuclease recognition site). Any dsDNA molecule including at least one restriction endonuclease recognition site can be tested in an initial set of validation experiments (e.g., experiments similar to those described in Example 2) to determine whether the dsDNA molecule can form a molecular complex that includes at least one nucleosome and provide for sensitive and accurate
10 detection of chromatin remodeling activity in any of the methods described herein. Examples of dsDNA molecules that are plasmids that can be used in any of the methods or included in any of the kits described herein include: pBluescript.

In some examples, the dsDNA molecule in any of the methods or kits described herein includes a single restriction endonuclease recognition site (e.g., any of the restriction
15 endonuclease recognition sites described herein). In other examples, the dsDNA molecule in any of the methods or kits described herein includes at least two (e.g., three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, or fifteen) restriction endonuclease recognition sites. In examples where the dsDNA molecule includes at least two restriction endonuclease sites, each of the at least two restriction endonuclease
20 recognition sites is spaced about 100 base pairs to about 12,500 base pairs (e.g., about 100 base pairs to about 10,000 base pairs, about 100 base pairs to about 7,500 base pairs, about 100 base pairs to about 5,000 base pairs, about 100 base pairs to about 2,500 base pairs, about 100 base pairs to about 1,000 base pairs, about 100 base pairs to about 500 base pairs, about 500 base pairs to about 12,500 base pairs, about 500 base pairs to about 10,000 base
25 pairs, about 500 base pairs to about 7,500 base pairs, about 500 base pairs to about 5,000 base pairs, about 500 base pairs to about 2,500 base pairs, about 500 base pairs to about 1,000 base pairs, about 1,000 base pairs to about 12,500 base pairs, about 1,000 base pairs to about 10,000 base pairs, about 1,000 base pairs to about 7,500 base pairs, about 1,000 base pairs to about 5,000 base pairs, about 1,000 base pairs to about 2,500 base pairs, about
30 2,500 base pairs to about 12,500 base pairs, about 2,500 base pairs to about 10,000 base pairs, about 2,500 base pairs to about 7,500 base pairs, between about 2,500 base pairs to about 5,000 base pairs, about 5,000 base pairs to about 12,500 base pairs, about 5,000 base pairs to about 10,000 base pairs, about 5,000 base pairs to about 7,500 base pairs, or about

10,000 base pairs to about 12,500 base pairs) away from another of the restriction endonuclease recognition site(s) in the dsDNA molecule. As is well-known in the art, the positioning of each of the at least two restriction endonuclease recognition sequences within the dsDNA molecule should allow for the amplification of each of the at least two
5 restriction endonuclease recognition sequences using a PCR assay (e.g., any of the PCR assays described herein).

In any of the methods or kits described herein, the dsDNA molecule can include a sequence of a gene, e.g., a cancer-associate gene (e.g., an oncogene or tumor suppressor gene) or a sequence of a gene related to the pathogenesis of a chromatin remodeling-related
10 condition (e.g., a neurodevelopmental disorder, an autoimmune disease, or a cardiovascular disease), and the at least one restriction endonuclease recognition site is/are located within the sequence of the gene. A sequence of a gene can include about 100 base pairs to about 100,000 base pairs, about 100 base pairs to about 90,000 base pairs, about 100 base pairs to about 80,000 base pairs, about 100 base pairs to about 70,000 base pairs, about 100 base
15 pairs to about 60,000 base pairs, about 100 base pairs to about 50,000 base pairs, about 100 base pairs to about 40,000 base pairs, about 100 base pairs to about 30,000 base pairs, about 100 base pairs to about 20,000 base pairs, about 100 base pairs to about 10,000 base pairs, about 100 base pairs to about 8,000 base pairs, about 100 base pairs to about 6,000 base pairs, about 100 base pairs to about 4,000 base pairs, about 100 base pairs to about 2,000
20 base pairs, about 100 base pairs to about 1,000 base pairs, about 100 base pairs to about 500 base pairs, about 500 base pairs to about 100,000 base pairs, about 500 base pairs to about 90,000 base pairs, about 500 base pairs to about 80,000 base pairs, about 500 base pairs to about 70,000 base pairs, about 500 base pairs to about 60,000 base pairs, about 500 base pairs to about 50,000 base pairs, about 500 base pairs to about 40,000 base pairs, about 500
25 base pairs to about 30,000 base pairs, about 500 base pairs to about 20,000 base pairs, about 500 base pairs to about 10,000 base pairs, about 500 base pairs to about 8,000 base pairs, about 500 base pairs to about 6,000 base pairs, about 500 base pairs to about 4,000 base pairs, about 500 base pairs to about 2,000 base pairs, about 500 base pairs to about 1,000 base pairs, about 1000 base pairs to about 100,000 base pairs, about 1,000 base pairs to
30 about 80,000 base pairs, about 1,000 base pairs to about 60,000 base pairs, about 1,000 base pairs to about 40,000 base pairs, about 1,000 base pairs to about 20,000 base pairs, about 1,000 base pairs to about 10,000 base pairs, about 1,000 base pairs to about 8,000 base pairs, about 1,000 base pairs to about 6,000 base pairs, about 1,000 base pairs to about

4,000 base pairs, about 1,000 base pairs to about 2,000 base pairs, about 2,000 base pairs to about 100,000 base pairs, about 2,000 base pairs to about 80,000 base pairs, about 2,000 base pairs to about 60,000 base pairs, about 2,000 base pairs to about 40,000 base pairs, about 2,000 base pairs to about 20,000 base pairs, about 2,000 base pairs to about 10,000 base pairs, about 2,000 base pairs to about 8,000 base pairs, about 2,000 base pairs to about 6,000 base pairs, about 2,000 base pairs to about 4,000 base pairs, about 5,000 base pairs to about 100,000 base pairs, about 5,000 base pairs to about 80,000 base pairs, about 5,000 base pairs to about 60,000 base pairs, about 5,000 base pairs to about 40,000 base pairs, about 5,000 base pairs to about 20,000 base pairs, or about 5,000 base pairs to about 10,000 base pairs. Non-limiting examples of an oncogene are human Wnt, human Beta-catenin, and human myc. Non-limiting examples of a tumor suppressor are human WEE1, Retinoblastoma (Rb), p21, p16, SFRP1/2, TIMP3, TGFbeta, and human SNAIL.

A sequence of a gene (e.g., any of the exemplary genes described herein) included in any of the dsDNA molecules described herein can include all or a partial sequence of one or more of any of the following gene locus elements: an enhancer, a promoter, a ribosome binding site, a start codon, a transcribed sequence (e.g., a coding sequence), one or more exons, one or more introns, and a 3' untranslated region. For example, a sequence of a gene can include all or a partial sequence of an enhancer and/or all or partial sequence of a promoter and/or the start codon and/or all or a partial sequence of the first intron. It is understood that dsDNA molecules can be any of the above described sequences of a gene inserted into, e.g., any of the plasmids described herein or known in the art.

Formation of a Molecular Complex of a dsDNA Molecule and a Nucleosome

Methods for forming a molecular complex of a dsDNA molecule (e.g., any of the dsDNA molecules described herein) and at least one nucleosome (e.g., any of the nucleosomes described herein) are known in the art. See, e.g., the methods described in Carruthers et al., *Methods Enzymol.* 304:19-35, 1999, Lusser et al., *Nature Methods* 1:19-26, 2004, and Hizume et al., *Archives Histol. Cytol.* 65:405-413, 2003. An example of such a method is the salt-dialysis method, which involves mixing (e.g., human) histones (e.g., a combination of H2A, H2B, H3, and H4 (e.g., human) proteins) with the dsDNA molecule at a high salt concentration and performing dialysis to reduce the salt concentration and to form a molecular complex of the dsDNA molecule and at least one nucleosome. The histone proteins used to form the molecular complexes can be purchased from a vendor

(e.g., New England BioLabs, Ipswich, MA, or Worthington Biochem. Corp., Lakewood, NJ).

Another example of such a method is to first purify histones (e.g., any of the histones described herein, such as human histones) or histone octamers (e.g., human histone octamers) (e.g., two H2A proteins, two H2B proteins, two H3 proteins, and two H4 proteins) from a mammalian (e.g., human) cell (see, e.g., the methods described in Johns, *Biochem. J.* 92:55-59, 1962, and Simon and Felsenfeld, *Nucl. Acids. Res.* 6:689-696, 1979) and contacting the purified histones or histone octamers with the dsDNA molecule (e.g., any of the dsDNA molecules described herein).

The formation of a molecular complex of a dsDNA molecule and at least one nucleosome can include a step of incubating a dsDNA molecule with the histones (e.g., human histones) or histone octamers (e.g., human histone octamers) at a temperature of between about 1 °C and about 40 °C (e.g., between about 2 °C and about 37 °C, between about 3 °C to about 37 °C, between about 4 °C to about 37 °C, between about 10 °C to about 37 °C, between about 15 °C and about 37 °C, between about 20 °C and about 37 °C, between about 25 °C and about 37 °C, between about 30 °C and about 37 °C, between 1 °C and about 30 °C, between about 2 °C and about 30 °C, between about 3 °C and about 30 °C, between about 4 °C and about 30 °C, between about 10 °C to about 30 °C, between about 15 °C and about 30 °C, between about 20 °C and about 30 °C, between about 25 °C and about 30 °C, between about 1 °C and about 25 °C, between about 2 °C and about 25 °C, between about 3 °C and about 25 °C, between about 4 °C and about 25 °C, between about 10 °C and about 25 °C, between about 15 °C and about 25 °C, between about 20 °C and about 25 °C, between about 1 °C and about 20 °C, between about 2 °C and about 20 °C, between about 3 °C and about 20 °C, between about 4 °C and about 20 °C, between about 10 °C and about 20 °C, between about 15 °C and about 20 °C, between about 1 °C and about 15 °C, between about 2 °C and about 15 °C, between about 3 °C and about 15 °C, between about 4 °C and about 15 °C, between about 10 °C and about 15 °C, between about 1 °C and about 10 °C, between about 2 °C and about 10 °C, between about 3 °C and about 10 °C, or between about 4 °C and about 10 °C) for between about 1 minute and about 5 hours, between about 1 minute and about 4 hours, between about 1 minute and about 3 hours, between about 1 minute and about 2.5 hours, between about 1 minute and about 2.0 hours, between about 1 minute and about 1.5 hours, between about 10 minutes and about

1.5 hours, between about 15 minutes and about 1.5 hours, or between about 15 minutes and about 1 hour).

PCR Assay

5 Examples of a PCR assay include a PCR assay, a real-time PCR assay, a quantitative PCR assay, a real-time quantitative PCR assay, and a multi-plex PCR assay. Additional examples of PCR assays are known in the art. Methods and reagents for performing any of the PCR assays described herein are also well known in the art. See, e.g., PCR Technology: Current Innovations, Third Edition, Nolan et al. (Eds.), CRC Press, Boca Raton, FL, 2013; 10 Quantitative Real-Time PCR: Methods and Protocols, Biassoni et al. (Eds.), Humana Press, 2014. In some examples, the real-time quantitative PCR assay uses TaqMan® dual-labeled probes and molecular beacon probes (see, e.g., Tyagi et al., *Nature Biotech.* 14(3):303-308, 1996) for detection.

 In any of the methods described herein, the PCR assay is used to amplify a nucleic 15 acid sequence within the dsDNA molecule that includes a restriction endonuclease recognition site. As is well known in the art, a pair of PCR primers (e.g., a pair of real-time PCR primers or a pair of real-time quantitative polymerase chain reaction primers) can be designed to amplify the nucleic acid sequence within the dsDNA molecule that includes the restriction endonuclease recognition site. In dsDNA molecules that include two or more 20 restriction endonuclease sites, a pair of primers can be designed for each restriction endonuclease recognition site.

Chromatin Remodeling-Related Conditions

 A dysregulation (e.g., an aberrant increase or an aberrant decrease) in chromatin 25 remodeling has been implicated for a role in the pathogenesis of different conditions (chromatin remodeling-related conditions). Non-limiting examples of chromatin remodeling-related conditions include cancer, neurodevelopmental disorders, autoimmune diseases, or cardiovascular diseases.

 A loss in the SWI/SNF chromatin remodeling complex can lead to increased 30 expression of the oncogene WNT and increased or decreased (depending on tumor type) expression of the oncogene myc (Jordan et al., *Mol. Cell Biol.* 33:3011-3025, 2013, Mora-Blanco et al., *Oncogene* 33:933-938, 2014, Cheng et al., *Nat. Genet.* 22:102-105, 1999, and Park et al., *Mol. Cell Biol.* 22:1307-1316, 2002). The SWI/SNF chromatin remodeling

complex has also been shown to bind and inactivate the tumor suppressor proteins p53 and RB, and to promote cell growth (Lee et al., *J. Biol. Chem.* 277:22330-7, 2002, Naidu et al., *Oncogene* 28:2492-501, 2009, Alessio et al., *Oncogene* 29:5452-63, 2010, Trouche et al., *Proc. Natl. Acad. Sci. U.S.A.* 94:11268-73, 1997, and Strobeck et al., *Proc. Natl. Acad. Sci. U.S.A.* 97:7748-53, 2000). Non-limiting examples of cancers that are chromatin remodeling-related conditions include: acute lymphoblastic leukemia, acute myeloid leukemia, adrenocortical carcinoma, AIDS-related cancers, Kaposi's sarcoma, lymphoma, anal cancer, appendix cancer, astrocytomas, teratoid/rhabdoid tumor, basal cell carcinoma, bile duct cancer, bladder cancer, bone cancer, brain stem glioma, brain tumor, breast cancer, bronchial tumor, Burkitt's lymphoma, carcinoid tumor, cardiac tumors, cervical cancer, chronic lymphocytic leukemia, chronic myelogenous leukemia, chronic myeloproliferative neoplasms, colon cancer, colorectal cancer, craniopharyngioma, endometrial cancer, ependymoma, esophageal cancer, Ewing's sarcoma, eye cancer, fallopian tube cancer, gallbladder cancer, gastric cancer, glioma, hairy cell leukemia, head and neck cancer, liver cancer, Hodgkin's lymphoma, hypopharyngeal cancer, kidney cancer, laryngeal cancer, leukemia, lip and oral cavity cancer, lung cancer, melanoma, mesothelioma, mouth cancer, nasopharyngeal cancer, neuroblastoma, non-small cell lung cancer, oropharyngeal cancer, osteosarcoma, ovarian cancer, pancreatic cancer, parathyroid cancer, penile cancer, pheochromocytoma, pituitary tumor, prostate cancer, rectal cancer, rhabdomyosarcoma, salivary gland cancer, sarcoma, Sezary's syndrome, skin cancer, small intestine cancer, gastric cancer, testicular cancer, throat cancer, thymoma and thymic carcinoma, thyroid cancer, urethral cancer, uterine cancer, vaginal cancer, vulvar cancer, and Wilms' tumor.

An aberrant activity (e.g., an aberrant decrease or an aberrant increase) in chromatin remodeling has been implicated for a role in a number of different neurodevelopmental and autoimmune disorders (e.g., autism-spectrum disorders, epilepsy, or Alpha-thalassemia X-linked mental retardation, Cockayne, Coffin-Siris, Nicolaides-Baraitser, Floating-Harbor, SIOD, Williams, or CHARGE syndromes, and dermatomyositis). For example, aberrant activity of chromatin remodeling proteins MECP2, ATRX, H2AFY, SMC1A, MACROD2, KDM5C, MBD1, ARID1B, SMARCC2, JMJD1C, CHD2, CHD3, CHD4, CHD5, CHD7, and CHD8 have been implicated for a role in the development of neurodevelopmental and autoimmune disorders (see, e.g., LaSalle, *OA Autism* 1:14, 2013).

Aberrant activity (e.g., an aberrant increase or an aberrant decrease) in chromatin remodeling has also been implicated for a role in autoimmune disorders and cardiovascular disorders (e.g., CHARGE syndrome and Williams syndrome).

It will be appreciated that in conditions in which an aberrant increase in chromatin remodeling results in the pathogenesis of a chromatin remodeling-related condition, it will be desired to decrease or inhibit such aberrant remodeling activity. It will also be appreciated that in conditions in which an aberrant decrease in chromatin remodeling results in the pathogenesis of a chromatin remodeling-related condition, it will be desired to increase or activate remodeling activity or decrease an opposing remodeling activity. For example, in cancer it may be desired to, e.g., decrease a related, compensatory remodeling activity.

Methods for Identifying a Test Compound that is a Modulator of Chromatin Remodeling Activity

Provided herein are methods (e.g., high throughput methods) for identifying a test compound that is a modulator of chromatin remodeling activity. These methods include: (a) combining in a reaction mixture a molecular complex comprising a dsDNA molecule (e.g., any of the dsDNA molecules described herein) and at least one nucleosome (e.g., any of the nucleosomes described herein), wherein the dsDNA molecule includes at least one (e.g., two, three, four, five, six, seven, eight, nine, or ten) restriction endonuclease recognition site, a test compound, and at least one (e.g., two, three, four, five, or six) chromatin remodeling protein (e.g., any of the chromatin remodeling proteins described herein), under conditions sufficient to allow for activity of the at least one chromatin remodeling protein; (b) contacting the dsDNA molecule with at least one (e.g., two, three, four, or five) restriction endonuclease (e.g., any of the restriction endonucleases described herein) that is capable of cleaving the dsDNA molecule at one or more of the at least one restriction endonuclease recognition site(s) under conditions sufficient to allow for activity of the at least one chromatin remodeling protein and the at least one restriction endonuclease; (c) determining the level of cleavage of the dsDNA molecule by the at least one restriction endonuclease using a PCR assay; (d) comparing the determined level of cleavage in step (c) to a control level of cleavage; and (e) identifying a test compound that causes a modulation in the determined level of cleavage as compared to the control level of cleavage as a modulator of chromatin remodeling activity.

A test compound can be a nucleic acid, a protein, a lipid, a carbohydrate, or mixture thereof, or an inorganic or organic molecule having a molecular weight of between 5 g/mole and 5000 g/mole (e.g., between 5 g/mole and 4000 g/mole, between 5 g/mole and 3000 g/mole, between 5 g/mole and 2000 g/mole, between 5 g/mole and 1000 g/mole, between 5 g/mole and 500 g/mole, between 5 g/mole and 250 g/mole). Libraries of test compounds
5 can be purchased from commercial vendors.

In some examples of these methods, step (e) includes identifying a test compound that causes a decrease (e.g., a significant or detectable decrease) in the determined level of cleavage as compared to the control level of cleavage as an inhibitor of chromatin
10 remodeling activity. In other examples of these methods, step (e) includes identifying a test compound that causes an increase (e.g., a significant or detectable increase) in the determined level of cleavage as compared to the control level of cleavage as an activator of chromatin remodeling activity.

As can be appreciated by those skilled in the art, steps (a) and (b) can be performed
15 at substantially the same time (e.g., within 10 minutes, within 9 minutes, within 8 minutes, within 7 minutes, within 6 minutes, within 5 minutes, within 4 minutes, within 3 minutes, within 2 minutes, or within 1 minute of each other) or can be reordered such that the different components of the reaction mixture are added in a different order. It is generally required that a molecular complex of the dsDNA molecule (which includes at least one
20 restriction endonuclease recognition site) and the at least one nucleosome, the test compound, the at least one chromatin remodeling protein, and the at least one restriction endonuclease that is capable of cleaving the dsDNA molecule at one or more of the at least one restriction endonuclease recognition site(s) are contacted under conditions sufficient to allow for activity of the at least one remodeling protein and the at least one restriction
25 endonuclease.

In some examples, the contacting step is performed for a specific period of time, e.g., between about 1 minute and about 5.0 hours, between about 1 minute and about 4.5 hours, between about 1 minute and about 4.0 hours, between about 1 minute and about 3.5 hours, between about 1 minute and about 3.0 hours, between about 1 minute and about 2.5
30 hours, between about 1 minute and about 2.0 hours, between about 1 minute and about 1.5 hours, between about 1 minute and about 1.0 hours, between about 1 minute and about 50 minutes, between about 1 minute and about 40 minutes, between about 1 minute and about 30 minutes, between about 1 minute and about 25 minutes, between about 1 minute and

about 20 minutes, between about 1 minute and about 15 minutes, between about 1 minute and about 10 minutes, between about 1 minute and about 5 minutes, between about 5 minutes and about 5 hours, between about 5 minutes and about 4.5 hours, between about 5 minutes and about 4.0 hours, between about 5 minutes and about 3.5 hours, between about 5 minutes and about 3.0 hours, between about 5 minutes and about 2.5 hours, between about 5 minutes and about 2.0 hours, between about 5 minutes and about 1.5 hours, between about 5 minutes and about 1.0 hour, between about 5 minutes and about 50 minutes, between about 5 minutes and about 40 minutes, between about 5 minutes and about 30 minutes, between about 5 minutes and about 25 minutes, between about 5 minutes and about 20 minutes, between about 5 minutes and about 15 minutes, between about 5 minutes and about 10 minutes, between about 10 minutes and about 5.0 hours, between about 10 minutes and about 4.5 hours, between about 10 minutes and about 4.0 hours, between about 10 minutes and about 3.5 hours, between about 10 minutes and about 3.0 hours, between about 10 minutes and about 2.5 hours, between about 10 minutes and about 2.0 hours, between about 10 minutes and about 1.5 hours, between about 10 minutes and about 1.0 hours, between about 10 minutes and about 50 minutes, between about 10 minutes and about 40 minutes, between about 10 minutes and about 30 minutes, between about 10 minutes and about 25 minutes, between about 10 minutes and about 20 minutes, between about 10 minutes and about 15 minutes, between about 15 minutes and about 5.0 hours, between about 15 minutes and about 4.5 hours, between about 15 minutes and about 4.0 hours, between about 15 minutes and about 3.5 hours, between about 15 minutes and about 3.0 hours, between about 15 minutes and about 2.5 hours, between about 15 minutes and about 2.0 hours, between about 15 minutes and about 1.5 hours, between about 15 minutes and about 1.0 hour, between about 15 minutes and about 50 minutes, between about 15 minutes and about 45 minutes, between about 15 minutes and about 40 minutes, between about 15 minutes and about 35 minutes, between about 15 minutes and about 30 minutes, between about 15 minutes and about 25 minutes, between about 15 minutes and about 20 minutes, between about 20 minutes and about 5.0 hours, between about 20 minutes and about 4.5 hours, between about 20 minutes and about 4.0 hours, between about 20 minutes and about 3.5 hours, between about 20 minutes and about 3.0 hours, between about 20 minutes and about 2.5 hours, between about 20 minutes and about 2.0 hours, between about 20 minutes and about 1.5 hours, between about 20 minutes and about 1.0 hours, between about 20 minutes and about 50 minutes, between about 20 minutes and about 40 minutes, between about 20

minutes and about 30 minutes, between about 20 minutes and about 25 minutes, between about 30 minutes and about 5.0 hours, between about 30 minutes and about 4.5 hours, between about 30 minutes and about 4.0 hours, between about 30 minutes and about 3.5 hours, between about 30 minutes and about 3.0 hours, between about 30 minutes and about 2.5 hours, between about 30 minutes and about 2.0 hours, between about 30 minutes and about 1.5 hours, between about 30 minutes and about 1.0 hour, between about 1.0 hour and about 5.0 hours, between about 1.0 hour and about 4.5 hours, between about 1.0 hour and about 4.0 hours, between about 1.0 hour and about 3.5 hours, between about 1.0 hour and about 3.0 hours, between about 1.0 hour and about 2.5 hours, between about 1.0 hour and about 2.0 hours, between about 1.0 hour and about 1.5 hour, between about 1.5 hour and about 5.0 hour, between about 1.5 hour and about 4.5 hours, between about 1.5 hour and about 4.0 hour, between about 1.5 hour and about 3.5 hour, between about 1.5 hour and about 3.0 hour, between about 1.5 hour and about 2.5 hours, between about 2.0 hours and about 5.0 hours, between about 2.0 hours and about 4.5 hours, between about 2.0 hours and about 4.0 hours, between about 2.0 hours and about 3.5 hours, between about 2.0 hours and about 3.0 hours, between about 2.5 hours and about 5.0 hours, between about 2.5 hours and about 4.5 hours, between about 2.5 hours and about 4.0 hours, between about 2.5 hours and about 3.5 hours, between about 3.0 hours and about 5.0 hours, between about 3.0 hours and about 4.5 hours, between about 3.0 hours and about 4.0 hours, between about 3.5 hours and about 5.0 hours, between about 3.5 hours and about 4.5 hours, or between about 4.0 hours and about 5.0 hours. The contacting step can be performed at a temperature of between about 10 °C and about 40 °C (e.g., between about 15 °C and about 37 °C, between about 20 °C and about 37 °C, between about 25 °C and about 37 °C, between about 25 °C and about 35 °C, between about 28 °C and about 32 °C, or between about 30 °C and about 37 °C).

Some embodiments of these methods further include, between steps (b) and (c), a step of inactivating one or both of the at least one restriction endonuclease and the at least one chromatin remodeling protein. In some examples, the inactivating includes one or both of heating the reaction mixture to a temperature of about 50 °C to about 98 °C (e.g., between about 55 °C and about 98 °C, between about 55 °C and about 95 °C, between about 55 °C and about 90 °C, between about 55 °C and about 85 °C, between about 55 °C and about 80 °C, between about 55 °C and about 75 °C, between about 55 °C and about 70 °C, between about 55 °C and about 65 °C, between about 60 °C and about 98 °C, between about 60 °C and about 95 °C, between about 60 °C and about 90 °C, between about 60 °C and

about 85 °C, between about 60 °C and about 80 °C, between about 60 °C and about 75 °C, between about 60 °C and about 70 °C, between about 60 °C and about 65 °C, between about 65 °C and about 98 °C, between about 65 °C and about 95 °C, between about 65 °C and about 90 °C, between about 65 °C and about 85 °C, between about 65 °C and about 80 °C, 5 between about 65 °C and about 75 °C, between about 65 °C and about 70 °C, between about 70 °C and about 98 °C, between about 70 °C and about 95 °C, between about 70 °C and about 90 °C, between about 70 °C and about 85 °C, between about 70 °C and about 80 °C, between about 70 °C and about 75 °C, between about 75 °C and about 98 °C, between about 75 °C and about 95 °C, between about 75 °C and about 90 °C, between about 75 °C and 10 about 85 °C, between about 75 °C and about 80 °C, between about 80 °C and about 98 °C, between about 80 °C and about 95 °C, between about 80 °C and about 90 °C, between about 80 °C and about 85 °C, between about 85 °C and about 98 °C, between about 85 °C and about 95 °C, between about 85 °C and about 90 °C, between about 90 °C and about 98 °C, between about 90 °C and about 95 °C, or between about 95 °C and about 98 °C) (e.g., for a 15 time period of between about 1 minute to about 20 minutes, between about 2 minutes and about 15 minutes, between about 2 minutes and about 10 minutes, or between about 2 minutes and about 5 minutes) and adding to the reaction mixture an amount of a magnesium chelator (e.g., ethylene glycol tetraacetic acid or ethylenediaminetetraacetic acid) sufficient to inhibit the activity of the at least one restriction endonuclease.

20 The control level of cleavage can be the amount of cleavage that occurs in a reaction mixture after the contacting step (b), except that the reaction mixture lacks the test compound. The control level can be a threshold level of cleavage that corresponds to the amount of cleavage that occurs in a reaction mixture after the contacting step (b), except that the reaction mixture lacks the test compound.

25 The reaction mixture can be a solid composition or can be a liquid. The volume of the reaction mixture can be any of the exemplary reaction mixture volumes described herein.

In any of the methods, the PCR assay can be a PCR assay, a real-time PCR assay, a quantitative PCR assay, a real-time quantitative PCR assay, or a multi-plex PCR assay. 30 For example, a real-time PCR assay can, e.g., include the use of at least one pair of real-time quantitative PCR primers, where a first member of the at least one pair specifically hybridizes to a sequence that is 5' of one of the at least one restriction endonuclease

recognition site on a first strand of the dsDNA molecule and the second member of the at least one pair specifically hybridizes to a sequence that is 5' of the one of the at least one restriction endonuclease recognition site on a second strand of the dsDNA molecule.

5 Methods for Identifying a Candidate Compound for Treating a Chromatin Remodeling-Related Condition in a Subject

Provided herein are methods (e.g., high throughput methods) for identifying a candidate compound for treating a subject with a chromatin remodeling-related condition (e.g., any of the cancers, neurodevelopmental disorders, autoimmune diseases, or cardiovascular disorders described herein). These methods include: (a) combining in a reaction mixture a molecular complex comprising a dsDNA molecule (e.g., any of the dsDNA molecules described herein) and at least one nucleosome (e.g., any of the nucleosomes described herein), wherein the dsDNA molecule includes at least one (e.g., two, three, four, five, six, seven, eight, nine, or ten) restriction endonuclease recognition site, a test compound, and at least one (e.g., two, three, four, five, or six) chromatin remodeling protein (e.g., any of the chromatin remodeling proteins described herein), under conditions sufficient to allow for activity of the at least one chromatin remodeling protein; (b) contacting the dsDNA molecule with at least one (e.g., two, three, four, or five) restriction endonuclease (e.g., any of the restriction endonucleases described herein) that is capable of cleaving the dsDNA molecule at one or more of the at least one restriction endonuclease recognition site(s) under conditions sufficient to allow for activity of the at least one chromatin remodeling protein and the at least one restriction endonuclease; (c) determining the level of cleavage of the dsDNA molecule by the at least one restriction endonuclease using a PCR assay; (d) comparing the determined level of cleavage in step (c) to a control level of cleavage; and (e) identifying a test compound that causes a modulation in the determined level of cleavage as compared to the control level of cleavage as a candidate compound for treating a subject with a chromatin remodeling-related condition.

A test compound can be a nucleic acid, a protein, a lipid, a carbohydrate, or mixture thereof, or an inorganic or organic molecule having a molecular weight of between 5 g/mole and 5000 g/mole (e.g., between 5 g/mole and 4000 g/mole, between 5 g/mole and 3000 g/mole, between 5 g/mole and 2000 g/mole, between 5 g/mole and 1000 g/mole, between 5 g/mole and 500 g/mole, between 5 g/mole and 250 g/mole). Libraries of test compounds can be purchased from commercial vendors.

As can be appreciated by those skilled in the art, steps (a) and (b) can be performed at substantially the same time (e.g., within 10 minutes, within 9 minutes, within 8 minutes, within 7 minutes, within 6 minutes, within 5 minutes, within 4 minutes, within 3 minutes, within 2 minutes, or within 1 minute of each other) or can be reordered such that the
5 different components of the reaction mixture are added in a different order. It is generally required that a molecular complex of the dsDNA molecule (which includes at least one restriction endonuclease recognition site) and the at least one nucleosome, the test compound, the at least one chromatin remodeling protein, and the at least one restriction endonuclease that is capable of cleaving the dsDNA molecule at one or more of the at least
10 one restriction endonuclease recognition site(s) are contacted under conditions sufficient to allow for activity of the at least one remodeling protein and the at least one restriction endonuclease.

In some examples, the contacting step is performed for a specific period of time, e.g., between about 1 minute and about 5.0 hours, between about 1 minute and about 4.5
15 hours, between about 1 minute and about 4.0 hours, between about 1 minute and about 3.5 hours, between about 1 minute and about 3.0 hours, between about 1 minute and about 2.5 hours, between about 1 minute and about 2.0 hours, between about 1 minute and about 1.5 hours, between about 1 minute and about 1.0 hours, between about 1 minute and about 50 minutes, between about 1 minute and about 40 minutes, between about 1 minute and about
20 30 minutes, between about 1 minute and about 25 minutes, between about 1 minute and about 20 minutes, between about 1 minute and about 15 minutes, between about 1 minute and about 10 minutes, between about 1 minute and about 5 minutes, between about 5 minutes and about 5 hours, between about 5 minutes and about 4.5 hours, between about 5 minutes and about 4.0 hours, between about 5 minutes and about 3.5 hours, between about 5
25 minutes and about 3.0 hours, between about 5 minutes and about 2.5 hours, between about 5 minutes and about 2.0 hours, between about 5 minutes and about 1.5 hours, between about 5 minutes and about 1.0 hour, between about 5 minutes and about 50 minutes, between about 5 minutes and about 40 minutes, between about 5 minutes and about 30 minutes, between about 5 minutes and about 25 minutes, between about 5 minutes and about 20 minutes,
30 between about 5 minutes and about 15 minutes, between about 5 minutes and about 10 minutes, between about 10 minutes and about 5.0 hours, between about 10 minutes and about 4.5 hours, between about 10 minutes and about 4.0 hours, between about 10 minutes and about 3.5 hours, between about 10 minutes and about 3.0 hours, between about 10

minutes and about 2.5 hours, between about 10 minutes and about 2.0 hours, between about 10 minutes and about 1.5 hours, between about 10 minutes and about 1.0 hours, between about 10 minutes and about 50 minutes, between about 10 minutes and about 40 minutes, between about 10 minutes and about 30 minutes, between about 10 minutes and about 25 minutes, between about 10 minutes and about 20 minutes, between about 10 minutes and about 15 minutes, between about 15 minutes and about 5.0 hours, between about 15 minutes and about 4.5 hours, between about 15 minutes and about 4.0 hours, between about 15 minutes and about 3.5 hours, between about 15 minutes and about 3.0 hours, between about 15 minutes and about 2.5 hours, between about 15 minutes and about 2.0 hours, between about 15 minutes and about 1.5 hours, between about 15 minutes and about 1.0 hour, between about 15 minutes and about 50 minutes, between about 15 minutes and about 45 minutes, between about 15 minutes and about 40 minutes, between about 15 minutes and about 35 minutes, between about 15 minutes and about 30 minutes, between about 15 minutes and about 25 minutes, between about 15 minutes and about 20 minutes, between about 20 minutes and about 5.0 hours, between about 20 minutes and about 4.5 hours, between about 20 minutes and about 4.0 hours, between about 20 minutes and about 3.5 hours, between about 20 minutes and about 3.0 hours, between about 20 minutes and about 2.5 hours, between about 20 minutes and about 2.0 hours, between about 20 minutes and about 1.5 hours, between about 20 minutes and about 1.0 hours, between about 20 minutes and about 50 minutes, between about 20 minutes and about 40 minutes, between about 20 minutes and about 30 minutes, between about 20 minutes and about 25 minutes, between about 30 minutes and about 5.0 hours, between about 30 minutes and about 4.5 hours, between about 30 minutes and about 4.0 hours, between about 30 minutes and about 3.5 hours, between about 30 minutes and about 3.0 hours, between about 30 minutes and about 2.5 hours, between about 30 minutes and about 2.0 hours, between about 30 minutes and about 1.5 hours, between about 30 minutes and about 1.0 hour, between about 1.0 hour and about 5.0 hours, between about 1.0 hour and about 4.5 hours, between about 1.0 hour and about 4.0 hours, between about 1.0 hour and about 3.5 hours, between about 1.0 hour and about 3.0 hours, between about 1.0 hour and about 2.5 hours, between about 1.0 hour and about 2.0 hours, between about 1.0 hour and about 1.5 hour, between about 1.5 hour and about 5.0 hour, between about 1.5 hour and about 4.5 hours, between about 1.5 hour and about 4.0 hour, between about 1.5 hour and about 3.5 hour, between about 1.5 hour and about 3.0 hour, between about 1.5 hour and about 2.5 hours, between about 2.0 hours and

about 5.0 hours, between about 2.0 hours and about 4.5 hours, between about 2.0 hours and about 4.0 hours, between about 2.0 hours and about 3.5 hours, between about 2.0 hours and about 3.0 hours, between about 2.5 hours and about 5.0 hours, between about 2.5 hours and about 4.5 hours, between about 2.5 hours and about 4.0 hours, between about 2.5 hours and about 3.5 hours, between about 3.0 hours and about 5.0 hours, between about 3.0 hours and about 4.5 hours, between about 3.0 hours and about 4.0 hours, between about 3.5 hours and about 5.0 hours, between about 3.5 hours and about 4.5 hours, or between about 4.0 hours and about 5.0 hours. The contacting step can be performed at a temperature of between about 10 °C and about 40 °C (e.g., between about 15 °C and about 37 °C, between about 20 °C and about 37 °C, between about 25 °C and about 37 °C, between about 25 °C and about 35 °C, between about 28 °C and about 32 °C, or between about 30 °C and about 37 °C).

Some embodiments of these methods further include, between steps (b) and (c), a step of inactivating one or both of the at least one restriction endonuclease and the at least one chromatin remodeling protein. In some examples, the inactivating includes one or both of heating the reaction mixture to a temperature of about 50 °C to about 98 °C (e.g., between about 55 °C and about 98 °C, between about 55 °C and about 95 °C, between about 55 °C and about 90 °C, between about 55 °C and about 85 °C, between about 55 °C and about 80 °C, between about 55 °C and about 75 °C, between about 55 °C and about 70 °C, between about 55 °C and about 65 °C, between about 60 °C and about 98 °C, between about 60 °C and about 95 °C, between about 60 °C and about 90 °C, between about 60 °C and about 85 °C, between about 60 °C and about 80 °C, between about 60 °C and about 75 °C, between about 60 °C and about 70 °C, between about 60 °C and about 65 °C, between about 65 °C and about 98 °C, between about 65 °C and about 95 °C, between about 65 °C and about 90 °C, between about 65 °C and about 85 °C, between about 65 °C and about 80 °C, between about 65 °C and about 75 °C, between about 65 °C and about 70 °C, between about 70 °C and about 98 °C, between about 70 °C and about 95 °C, between about 70 °C and about 90 °C, between about 70 °C and about 85 °C, between about 70 °C and about 80 °C, between about 70 °C and about 75 °C, between about 75 °C and about 98 °C, between about 75 °C and about 95 °C, between about 75 °C and about 90 °C, between about 75 °C and about 85 °C, between about 75 °C and about 80 °C, between about 80 °C and about 98 °C, between about 80 °C and about 95 °C, between about 80 °C and about 90 °C, between about 80 °C and about 85 °C, between about 85 °C and about 98 °C, between about 85 °C and

about 95 °C, between about 85 °C and about 90 °C, between about 90 °C and about 98 °C, between about 90 °C and about 95 °C, or between about 95 °C and about 98 °C) (e.g., for a time period of between about 1 minute to about 20 minutes, between about 2 minutes and about 15 minutes, between about 2 minutes and about 10 minutes, or between about 2
5 minutes and about 5 minutes) and adding to the reaction mixture an amount of a magnesium chelator (e.g., ethylene glycol tetraacetic acid or ethylenediaminetetraacetic acid) sufficient to inhibit the activity of the at least one restriction endonuclease.

The control level of cleavage can be the amount of cleavage that occurs in a reaction mixture after the contacting step (b), except that the reaction mixture lacks the test
10 compound. The control level can be a threshold level of cleavage that corresponds to the amount of cleavage that occurs in a reaction mixture after the contacting step (b), except that the reaction mixture lacks the test compound.

The reaction mixture can be a solid composition or can be a liquid. The volume of the reaction mixture can be any of the exemplary reaction mixture volumes described
15 herein.

In any of the methods, the PCR assay can be a PCR assay, a real-time PCR assay, a quantitative PCR assay, a real-time quantitative PCR assay, or a multi-plex PCR assay. For example, a real-time PCR assay can, e.g., include the use of at least one pair of real-time quantitative PCR primers, where a first member of the at least one pair specifically
20 hybridizes to a sequence that is 5' of one of the at least one restriction endonuclease recognition site on a first strand of the dsDNA molecule and the second member of the at least one pair specifically hybridizes to a sequence that is 5' of the one of the at least one restriction endonuclease recognition site on a second strand of the dsDNA molecule.

25 ***Cancers***

In some embodiments of these methods, the chromatin remodeling-related condition is a cancer (e.g., any of the cancers described herein). In such methods, the dsDNA molecule can include a sequence of a cancer-associated gene, and the at least one restriction endonuclease recognition site is/are located within the sequence of the cancer-associated
30 gene.

In such examples, the cancer-associated gene can be an oncogene (e.g., Wnt or myc) and optionally, step (c) can include identifying a test compound that causes an increase in

the determined level of cleavage as compared to the control level of cleavage as a candidate compound for treating cancer.

In other examples, the cancer-associated gene can be a tumor suppressor gene (e.g., WEE1, Rb, p21, p16, SFRP1/2, TIMP3, TGF- β , and SNAIL) and optionally, step (c) can
5 include identifying a test compound that causes a decrease in the determined level of cleavage as compared to the control level of cleavage as a candidate compound for treating cancer.

Some examples of these methods further include: (f) contacting a cancer cell with the candidate compound identified in step (e); (g) determining the level of proliferation of
10 the cancer cell in step (f); (h) comparing the determined level of proliferation to a control level of proliferation; and (i) further identifying a candidate compound that has a decreased proliferation as compared to the control level of proliferation as a candidate compound for treating a cancer in a subject. In such methods, the control level of proliferation is the level of proliferation of the cancer cell in the absence of the candidate compound, e.g., when
15 cultured under otherwise similar conditions.

Neurodevelopmental Disorders, Autoimmune Diseases, or a Cardiovascular Disease

In some embodiments of these methods, the chromatin remodeling-related disease is a neurodevelopmental disorder (e.g., any of the neurodevelopmental disorders described
20 herein), an autoimmune disease (e.g., any of the autoimmune diseases described herein), or a cardiovascular disease (e.g., any of the cardiovascular diseases described herein). In such examples, step (e) can include, e.g., identifying a test compound that causes an increase in the determined level of cleavage as compared to the control level of cleavage as a candidate compound for treating a subject with a neurodevelopmental disorder, an autoimmune
25 disease, or a cardiovascular disease. In some examples, step (e) can include, e.g., identifying a test compound that causes a decrease in the determined level of cleavage as compared to the control level of cleavage as a candidate compound for treating a subject with a neurodevelopmental disorder, an autoimmune disorder, or a cardiovascular disease. In such methods, the dsDNA molecule can include, e.g., a sequence of a gene related to the
30 pathogenesis of the neurodevelopmental disorder, the autoimmune disease, or the cardiovascular disease. In these methods, the at least one chromatin remodeling protein can be, e.g., selected from the group of: MECP2, ATRX, H2AFY, SMC1A, MACROD2,

KDM5C, MBD1, ARID1B, SMARCC2, JMJD1C, CHD8, CHD1, CHD2, CHD3, CHD4, CHD5, CHD6, CHD7, CSB, BRG1, BRM, and HARP.

Some embodiments of these methods further include a step of administering the candidate compound identified in step (e) to an animal model of the neurodevelopmental disorder, an animal model of autoimmune disease, or an animal model of cardiovascular disease, determining whether the administration of the candidate compound reduces the number and/or the frequency and/or severity of one or more symptoms of the neurodevelopmental disorder, the autoimmune disease, or the cardiovascular disease in the animal model, and further identifying a candidate compound identified in step (e) that reduces the number or the frequency and/or severity of the one or more symptoms of the neurodevelopmental disorder, the autoimmune disease, or the cardiovascular disease as being a candidate compound for treating a subject with the neurodevelopmental disorder, the autoimmune disease, or the cardiovascular disease.

Methods for Identifying a Candidate Compound for Maintaining or Inducing Pluripotency in a Mammalian Cell

Provided herein are methods (e.g., high throughput methods) for identifying a candidate compound for maintaining or inducing pluripotency in a mammalian cell (e.g., a stem cell, a pluripotent cell, or a differentiated cell) (e.g., a human cell, a mouse cell, a monkey cell, a rat cell, or a pig cell). These methods include: (a) combining in a reaction mixture a molecular complex comprising a dsDNA molecule (e.g., any of the dsDNA molecules described herein) and at least one nucleosome (e.g., any of the nucleosomes described herein), wherein the dsDNA molecule includes at least one (e.g., two, three, four, five, six, seven, eight, nine, or ten) restriction endonuclease recognition site, a test compound, and at least one (e.g., two, three, four, five, or six) chromatin remodeling protein (e.g., any of the chromatin remodeling proteins described herein), under conditions sufficient to allow for activity of the at least one chromatin remodeling protein; (b) contacting the dsDNA molecule with at least one (e.g., two, three, four, or five) restriction endonuclease (e.g., any of the restriction endonucleases described herein) that is capable of cleaving the dsDNA molecule at one or more of the at least one restriction endonuclease recognition site(s) under conditions sufficient to allow for activity of the at least one chromatin remodeling protein and the at least one restriction endonuclease; (c) determining the level of cleavage of the dsDNA molecule by the at least one restriction endonuclease

using a PCR assay; (d) comparing the determined level of cleavage in step (c) to a control level of cleavage; and (e) identifying a test compound that causes a modulation in the determined level of cleavage as compared to the control level of cleavage as a candidate compound for maintaining or inducing pluripotency in a mammalian cell.

5 A test compound can be a nucleic acid, a protein, a lipid, a carbohydrate, or mixture thereof, or an inorganic or organic molecule having a molecular weight of between 5 g/mole and 5000 g/mole (e.g., between 5 g/mole and 4000 g/mole, between 5 g/mole and 3000 g/mole, between 5 g/mole and 2000 g/mole, between 5 g/mole and 1000 g/mole, between 5 g/mole and 500 g/mole, between 5 g/mole and 250 g/mole). Libraries of test compounds
10 can be purchased from commercial vendors.

 In some examples of these methods, step (e) includes identifying a test compound that causes a decrease (e.g., a significant or detectable decrease) in the determined level of cleavage as compared to the control level of cleavage as a candidate compound for maintaining or inducing pluripotency in a mammalian cell. In some examples of these
15 methods, step (e) includes identifying a test compound that causes an increase (e.g., a significant or detectable increase) in the determined level of cleavage as compared to the control level of cleavage as a candidate compound for maintaining or inducing pluripotency in a mammalian cell. In some embodiments of these methods, the at least one chromatin remodeling protein is selected from the group of: SWI/SNF, ISWI, CHD1-9, or INO80.

20 As can be appreciated by those skilled in the art, steps (a) and (b) can be performed at substantially the same time (e.g., within 10 minutes, within 9 minutes, within 8 minutes, within 7 minutes, within 6 minutes, within 5 minutes, within 4 minutes, within 3 minutes, within 2 minutes, or within 1 minute of each other) or can be reordered such that the different components of the reaction mixture are added in a different order. It is generally
25 required that a molecular complex of the dsDNA molecule (which includes at least one restriction endonuclease recognition site) and the at least one nucleosome, the test compound, the at least one chromatin remodeling protein, and the at least one restriction endonuclease that is capable of cleaving the dsDNA molecule at one or more of the at least one restriction endonuclease recognition site(s) are contacted under conditions sufficient to
30 allow for activity of the at least one remodeling protein and the at least one restriction endonuclease.

 In some examples, the contacting step is performed for a specific period of time, e.g., between about 1 minute and about 5.0 hours, between about 1 minute and about 4.5

hours, between about 1 minute and about 4.0 hours, between about 1 minute and about 3.5 hours, between about 1 minute and about 3.0 hours, between about 1 minute and about 2.5 hours, between about 1 minute and about 2.0 hours, between about 1 minute and about 1.5 hours, between about 1 minute and about 1.0 hours, between about 1 minute and about 50 minutes, between about 1 minute and about 40 minutes, between about 1 minute and about 30 minutes, between about 1 minute and about 25 minutes, between about 1 minute and about 20 minutes, between about 1 minute and about 15 minutes, between about 1 minute and about 10 minutes, between about 1 minute and about 5 minutes, between about 5 minutes and about 5 hours, between about 5 minutes and about 4.5 hours, between about 5 minutes and about 4.0 hours, between about 5 minutes and about 3.5 hours, between about 5 minutes and about 3.0 hours, between about 5 minutes and about 2.5 hours, between about 5 minutes and about 2.0 hours, between about 5 minutes and about 1.5 hours, between about 5 minutes and about 1.0 hour, between about 5 minutes and about 50 minutes, between about 5 minutes and about 40 minutes, between about 5 minutes and about 30 minutes, between about 5 minutes and about 25 minutes, between about 5 minutes and about 20 minutes, between about 5 minutes and about 15 minutes, between about 5 minutes and about 10 minutes, between about 10 minutes and about 5.0 hours, between about 10 minutes and about 4.5 hours, between about 10 minutes and about 4.0 hours, between about 10 minutes and about 3.5 hours, between about 10 minutes and about 3.0 hours, between about 10 minutes and about 2.5 hours, between about 10 minutes and about 2.0 hours, between about 10 minutes and about 1.5 hours, between about 10 minutes and about 1.0 hours, between about 10 minutes and about 50 minutes, between about 10 minutes and about 40 minutes, between about 10 minutes and about 30 minutes, between about 10 minutes and about 25 minutes, between about 10 minutes and about 20 minutes, between about 10 minutes and about 15 minutes, between about 15 minutes and about 5.0 hours, between about 15 minutes and about 4.5 hours, between about 15 minutes and about 4.0 hours, between about 15 minutes and about 3.5 hours, between about 15 minutes and about 3.0 hours, between about 15 minutes and about 2.5 hours, between about 15 minutes and about 2.0 hours, between about 15 minutes and about 1.5 hours, between about 15 minutes and about 1.0 hour, between about 15 minutes and about 50 minutes, between about 15 minutes and about 45 minutes, between about 15 minutes and about 40 minutes, between about 15 minutes and about 35 minutes, between about 15 minutes and about 30 minutes, between about 15 minutes and about 25 minutes, between about 15 minutes and about 20 minutes, between

about 20 minutes and about 5.0 hours, between about 20 minutes and about 4.5 hours, between about 20 minutes and about 4.0 hours, between about 20 minutes and about 3.5 hours, between about 20 minutes and about 3.0 hours, between about 20 minutes and about 2.5 hours, between about 20 minutes and about 2.0 hours, between about 20 minutes and about 1.5 hours, between about 20 minutes and about 1.0 hours, between about 20 minutes and about 50 minutes, between about 20 minutes and about 40 minutes, between about 20 minutes and about 30 minutes, between about 20 minutes and about 25 minutes, between about 30 minutes and about 5.0 hours, between about 30 minutes and about 4.5 hours, between about 30 minutes and about 4.0 hours, between about 30 minutes and about 3.5 hours, between about 30 minutes and about 3.0 hours, between about 30 minutes and about 2.5 hours, between about 30 minutes and about 2.0 hours, between about 30 minutes and about 1.5 hours, between about 30 minutes and about 1.0 hour, between about 1.0 hour and about 5.0 hours, between about 1.0 hour and about 4.5 hours, between about 1.0 hour and about 4.0 hours, between about 1.0 hour and about 3.5 hours, between about 1.0 hour and about 3.0 hours, between about 1.0 hour and about 2.5 hours, between about 1.0 hour and about 2.0 hours, between about 1.0 hour and about 1.5 hour, between about 1.5 hour and about 5.0 hour, between about 1.5 hour and about 4.5 hours, between about 1.5 hour and about 4.0 hour, between about 1.5 hour and about 3.5 hour, between about 1.5 hour and about 3.0 hour, between about 1.5 hour and about 2.5 hours, between about 2.0 hours and about 5.0 hours, between about 2.0 hours and about 4.5 hours, between about 2.0 hours and about 4.0 hours, between about 2.0 hours and about 3.5 hours, between about 2.0 hours and about 3.0 hours, between about 2.5 hours and about 5.0 hours, between about 2.5 hours and about 4.5 hours, between about 2.5 hours and about 4.0 hours, between about 2.5 hours and about 3.5 hours, between about 3.0 hours and about 5.0 hours, between about 3.0 hours and about 4.5 hours, between about 3.0 hours and about 4.0 hours, between about 3.5 hours and about 5.0 hours, between about 3.5 hours and about 4.5 hours, or between about 4.0 hours and about 5.0 hours. The contacting step can be performed at a temperature of between about 10 °C and about 40 °C (e.g., between about 15 °C and about 37 °C, between about 20 °C and about 37 °C, between about 25 °C and about 37 °C, between about 25 °C and about 35 °C, between about 28 °C and about 32 °C, or between about 30 °C and about 37 °C).

Some embodiments of these methods further include, between steps (b) and (c), a step of inactivating one or both of the at least one restriction endonuclease and the at least one chromatin remodeling protein. In some examples, the inactivating includes one or both

of heating the reaction mixture to a temperature of about 50 °C to about 98 °C (e.g., between about 55 °C and about 98 °C, between about 55 °C and about 95 °C, between about 55 °C and about 90 °C, between about 55 °C and about 85 °C, between about 55 °C and about 80 °C, between about 55 °C and about 75 °C, between about 55 °C and about 70 °C, between about 55 °C and about 65 °C, between about 60 °C and about 98 °C, between about 60 °C and about 95 °C, between about 60 °C and about 90 °C, between about 60 °C and about 85 °C, between about 60 °C and about 80 °C, between about 60 °C and about 75 °C, between about 60 °C and about 70 °C, between about 60 °C and about 65 °C, between about 65 °C and about 98 °C, between about 65 °C and about 95 °C, between about 65 °C and about 90 °C, between about 65 °C and about 85 °C, between about 65 °C and about 80 °C, between about 65 °C and about 75 °C, between about 65 °C and about 70 °C, between about 70 °C and about 98 °C, between about 70 °C and about 95 °C, between about 70 °C and about 90 °C, between about 70 °C and about 85 °C, between about 70 °C and about 80 °C, between about 70 °C and about 75 °C, between about 75 °C and about 98 °C, between about 75 °C and about 95 °C, between about 75 °C and about 90 °C, between about 75 °C and about 85 °C, between about 75 °C and about 80 °C, between about 80 °C and about 98 °C, between about 80 °C and about 95 °C, between about 80 °C and about 90 °C, between about 80 °C and about 85 °C, between about 85 °C and about 98 °C, between about 85 °C and about 95 °C, between about 85 °C and about 90 °C, between about 90 °C and about 98 °C, between about 90 °C and about 95 °C, or between about 95 °C and about 98 °C) (e.g., for a time period of between about 1 minute to about 20 minutes, between about 2 minutes and about 15 minutes, between about 2 minutes and about 10 minutes, or between about 2 minutes and about 5 minutes) and adding to the reaction mixture an amount of a magnesium chelator (e.g., ethylene glycol tetraacetic acid or ethylenediaminetetraacetic acid) sufficient to inhibit the activity of the at least one restriction endonuclease.

The control level of cleavage can be the amount of cleavage that occurs in a reaction mixture after the contacting step (b), except that the reaction mixture lacks the test compound. The control level can be a threshold level of cleavage that corresponds to the amount of cleavage that occurs in a reaction mixture after the contacting step (b), except that the reaction mixture lacks the test compound.

The reaction mixture can be a solid composition or can be a liquid. The volume of the reaction mixture can be any of the exemplary reaction mixture volumes described herein.

In any of the methods, the PCR assay can be a PCR assay, a real-time PCR assay, a quantitative PCR assay, a real-time quantitative PCR assay, or a multi-plex PCR assay. For example, a real-time PCR assay can, e.g., include the use of at least one pair of real-time quantitative PCR primers, where a first member of the at least one pair specifically hybridizes to a sequence that is 5' of one of the at least one restriction endonuclease recognition site on a first strand of the dsDNA molecule and the second member of the at least one pair specifically hybridizes to a sequence that is 5' of the one of the at least one restriction endonuclease recognition site on a second strand of the dsDNA molecule.

Some embodiments of these methods further include: (f) contacting a differentiated cell with the candidate compound identified in step (e); (g) determining the level of one or more stem cell marker genes (e.g., 5T4, ABCG2, Activin RIB/ALK-4, Activin RIIB, Alkaline Phosphatase, E-Cadherin, Cbx2, CD9, CD30/TNFRSF8, CD117/c-kit, CDX2, CHD1, Cripto, DNMT3B, DPPA2, DPPA4, DPPA5/ESG1, EpCAM, TROP1, ERR beta, NR3B2, ESGP, F-box protein 15, FGF-4, FGF-5, FoxD3, GBX2, GCNF, GDF-3, Gi24, Integrin alpha 6, Integrin alpha 6 beta 1, integrin alpha 6 beta 4, integrin beta 1, KLF4, KFL5, L1TD1, Lefty, Lefty-1, Lefty-A, LIN-28A, LIN-28B, LIN-41, c-Maf, c-Myc, Nanog, Oct-3/4, Oct-4A, Podocalyxin, Rex-1/ZFP42, Smad2, Smad2/3, SOX2, SSEA-1, SSEA-3, STAT3, Stella/Dppa3, SUZ12, TBX2, TBX3, TERT, TEX19, TEX19.1, THAP11, TRA-1-60, TRA-1-81, TROP-2, UTF1, and ZIC3) in the cell in step (f); (h) comparing the determined level of the one or more stem cell marker genes to a control level(s) of the one or more stem cell marker genes; and (i) further identifying a candidate compound that has an increased level of the one or more stem cell marker genes proliferation as compared to the control level(s) of the one or more stem cell marker genes as a candidate compound for maintaining or inducing pluripotency in a mammalian cell. In some examples, the control level(s) of the one or more stem cell marker genes is the level of the one or more stem cell marker genes in a differentiated cell that is not contacted with the candidate compound identified in step (e).

Kits

Also provided herein are kits that comprise, consist, or consist essentially of: (i) at least one (e.g., two, three, four, or five) chromatin remodeling protein (e.g., any of the chromatin remodeling proteins described herein); and (ii) a molecular complex comprising a double stranded deoxyribonucleic acid (dsDNA) molecule (e.g., any of the dsDNA molecules described herein) and at least one nucleosome (e.g., any of the nucleosomes described herein), wherein the dsDNA molecule comprises at least one (e.g., two, three, four, five, six, or seven) restriction endonuclease recognition site (e.g., any of the restriction endonuclease recognition sites described herein). Also provided are kits that comprise, consist, or consist essentially of: (i) at least one (e.g., two, three, four, or five) chromatin remodeling protein (e.g., any of the chromatin remodeling proteins described herein); (ii) a double stranded deoxyribonucleic acid (dsDNA) molecule (e.g., any of the dsDNA molecules described herein), wherein the dsDNA molecule comprises at least one (e.g., two, three, four, five, six, or seven) restriction endonuclease recognition site (e.g., any of the restriction endonuclease recognition sites described herein), and (iii) at least one histone (e.g., one or more of any of the histones described herein) or histone octamers (e.g., any of the histone octamers described herein).

In some examples of the kits, the at least one chromatin remodeling protein includes ACF1 and/or CHD5. In some examples, the kit includes only one chromatin remodeling protein. In other examples, the kit includes at least two (e.g., two, three, four, five, six, seven, eight, nine, or ten) chromatin remodeling proteins.

In some examples, the dsDNA molecule can include a sequence of a gene (e.g., a cancer-associated gene or a gene related to the pathogenesis of a neurodevelopmental disorder, an autoimmune disease, or a cardiovascular disease), where the at least one restriction endonuclease recognition site is within the sequence of the gene. Additional exemplary features of the dsDNA molecule included in the kits are described herein.

Some embodiments of the kit further include one or both of a heat-stable DNA polymerase (e.g., a Taq DNA polymerase) and a polynucleotide mix. Some embodiments of the kit further include at least one restriction endonuclease that is capable of cleaving the dsDNA molecule at one of the at least one restriction endonuclease recognition site(s).

Some examples of the kit further include at least one pair of polymerase chain reaction primers (e.g., a pair of real-time quantitative PCR primers), where a first member of the at least one pair specifically hybridizes to a sequence that is 5' of one of the at least

one restriction endonuclease recognition site(s) on a first strand of the dsDNA molecule and the second member of the at least one pair specifically hybridizes to a sequence that is 5' of the one of the at least one restriction endonuclease recognition site(s) on a second strand of the dsDNA molecule. Some examples of the kit further include a labeled probe to detect
5 the amplified product of the PCR assay (e.g., a TaqMan® probe).

Some embodiments of the kits further include a buffered solution including one or both of a sufficient concentration of magnesium ion to activate a restriction endonuclease and a sufficient concentration of adenosine triphosphate to activate the at least one chromatin remodeling protein. Some embodiments of the kits further include second
10 solution that includes a sufficient concentration of a magnesium chelator to inactivate the at least one restriction endonuclease.

Some embodiments of the kits further include instructions for using the kit to perform any of the methods described herein.

15 **EXAMPLES**

Several general protocols are described below, which may be used in any of the methods described herein and do not limit the scope of the invention described in the claims.

20 **Example 1. Design of Chromatin Remodeling Activity Assay**

An exemplary high-throughput assay for sensitively quantitating chromatin remodeling activity was designed and is described in this Example. A plasmid DNA (e.g., pBluescript, ~2.9 kb) was incubated with human H2A, H2B, H3, and H4 histones in order to form a molecular complex of the plasmid DNA and at least one nucleosome (e.g., see
25 Carruthers et al., *Methods Enzymol.* 304:19-35, 1999). The plasmid DNA contained multiple HaeIII restriction endonuclease recognition sites (similar to that in Figure 1).

If the sequence of one of the HaeIII restriction endonuclease recognition sites is protected from cleavage by the presence of a nucleosome when the molecular complex is contacted with HaeIII, HaeIII is unable to mediate cleavage of the phosphodiester bond in
30 the backbone of both strands of the dsDNA molecule within the HaeIII restriction endonuclease recognition site. In this situation, quantitative PCR primers can be used to amplify the sequence of the HaeIII restriction endonuclease recognition site in the dsDNA molecule (Figure 2, top schematic).

If the sequence of one of the HaeIII restriction endonuclease recognition sites is not protected from cleavage by the presence of a nucleosome when the molecular complex is contacted with HaeIII, HaeIII is able to mediate cleavage of the phosphodiester bond in the backbone of both strands of the dsDNA molecule within the HaeIII restriction endonuclease recognition site. In this situation, quantitative PCR primers will not amplify the sequence of the HaeIII restriction endonuclease recognition site in the dsDNA molecule, as it has been cleaved (Figure 2, bottom schematic).

In the exemplary assays described in these Examples, HaeIII was added to the molecular complex of the plasmid DNA and at least one nucleosome. The mixture of the (i) molecular complex of the plasmid DNA and the at least one nucleosome, and (ii) HaeIII was then incubated in the presence or absence of at least one chromatin remodeling protein, and quantitative PCR was performed using primers designed to amplify one of the HaeIII restriction endonuclease recognition sites (Figure 3). Assays including the at least one chromatin remodeling protein (with chromatin remodeling activity) will have a low quantitative PCR signal, while assays not including the at least one chromatin remodeling protein (with no chromatin remodeling activity) will have a high quantitative PCR signal.

In these assays, the reaction mixtures must be incubated under conditions sufficient to allow for HaeIII activity and the activity of the at least one chromatin remodeling protein. For example, the reaction mixture can include an amount of magnesium ion sufficient to allow for HaeIII restriction endonuclease activity. If the chromatin remodeling protein is a chromatin remodeling ATPase, then the reaction mixture can include an amount of ATP sufficient to allow for activity of the chromatin remodeling ATPase.

Example 2. Validation of Chromatin Remodeling Activity Assay

A set of experiments was performed to validate the chromatin remodeling activity assay described in Example 1.

Materials and Methods

In these experiments, molecular complexes of a plasmid DNA and at least one nucleosome were generated by incubating the plasmid DNA with histone cores (e.g., histone octamers including two H2A, two H2B, two H3, and two H4 proteins) and performing the salt-dialysis method as described in Carruthers et al., *Methods Enzymol.* 304:19-35, 1999.

An assay mixture was placed into each well of a multi-well plate. The assay mixture includes an aliquot of the molecular complex of the plasmid DNA and at least one nucleosome, and ACF or CDH5 (a chromatin remodeling protein), in the presence or absence of ATP. A buffered salt solution containing magnesium ion was also included in the reaction mixture. The HaeIII restriction endonuclease was added to each assay mixture, and each assay mixture (10 μ L) was incubated for 90 minutes at 30 °C. The contents of the assay mixture used is listed below.

Assay Mixture (10 μ L total volume)

Buffer (final concentration of 20 mM Tris-acetate, 50 mM potassium acetate, 10 mM magnesium acetate, and 1 mM dithiothreitol)
 100 ng molecular complex of the plasmid DNA and at least one nucleosome;
 100 ng ACF or CDH5 (chromatin remodeling protein)
 3 mM adenosine triphosphate (ATP) or adenylyl-imidodiphosphate (AMP-PNP) (the later as a negative control)
 0.2 μ L HaeIII

After the 90-minute incubation at 30 °C, each assay mixture was heated to 80 °C for 5 minutes to inactivate the restriction endonuclease and ACF or CDH5. After heat-inactivation, 1 μ L of each assay mixture was combined with 99 μ L of water. One μ L of the diluted reaction mixture was used for each 10 μ L real-time PCR reaction. The real-time PCR reaction was performed using one pair of primers to amplify the sequence of a single HaeIII restriction endonuclease recognition site present in an ampicillin resistance gene in the plasmid DNA.

Results

A first set of assays was performed without any added chromatin remodeling factor, and using either plasmid DNA or the molecular complex of the plasmid DNA and at least one nucleosome in the presence or absence of HaeIII. The data show that high levels of qPCR signal is observed in these assays, in the presence or absence of HaeIII, when the assays include the molecular complex of the plasmid DNA and at least one nucleosome, indicating that the nucleosome(s) prevent the ability of HaeIII to cleave the plasmid DNA at one of the HaeIII restriction endonuclease recognition sites (Figure 4). In contrast, in assays

including the plasmid DNA and HaeIII, there are low levels of qPCR signal, indicating that in the absence of the nucleosome(s), HaeIII is able to cleave the plasmid DNA at the same HaeIII restriction endonuclease recognition site (Figure 4).

5 An additional set of experiments was performed to test whether the chromatin remodeling activity assays can be used to sensitively detect the activity of the chromatin remodeling proteins ACF and CHD5. The data show that there are high levels of qPCR signal when no chromatin remodeling protein is included in the assays, and that there is a low level of qPCR signal when chromatin remodeling proteins ACF and CHD5 are included in the assay (and that this low level of qPCR signal is dependent on the presence of ATP, 10 which is consistent with the ATPase activity of ACF and CHD5) (Figure 5).

These data demonstrate that the chromatin remodeling activity assays described herein can be used to sensitively detect chromatin remodeling activity in a high throughput manner.

OTHER EMBODIMENTS

15 It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

WHAT IS CLAIMED IS:

1. A method for identifying a test compound that is a modulator of chromatin remodeling activity, the method comprising:

(a) combining in a reaction mixture:

a molecular complex comprising a double stranded deoxyribonucleic acid (dsDNA) molecule and at least one nucleosome, wherein the dsDNA molecule comprises at least one restriction endonuclease recognition site,

a test compound, and

at least one chromatin remodeling protein,

under conditions sufficient to allow for activity of the at least one remodeling protein;

(b) contacting the dsDNA molecule with at least one restriction endonuclease that is capable of cleaving the dsDNA molecule at one or more of the at least one restriction endonuclease recognition site(s) under conditions sufficient to allow for activity of the at least one remodeling protein and the at least one restriction endonuclease;

(c) determining the level of cleavage of the dsDNA molecule by the at least one restriction endonuclease using a polymerase chain reaction assay;

(d) comparing the determined level of cleavage in step (c) to a control total level of cleavage; and

(e) identifying a test compound that causes a modulation in the determined level of cleavage as compared to the control level of cleavage as a modulator of chromatin remodeling activity.

2. The method of claim 1, wherein (e) comprises identifying a test compound that causes a decrease in the determined level of cleavage as compared to the control level of cleavage as an inhibitor of chromatin remodeling activity.

3. The method of claim 1, wherein (e) comprises identifying a test compound that causes an increase in the determined level of cleavage as compared to the control level of cleavage as an activator of chromatin remodeling activity.

4. The method of any one of claims 1-3, wherein the dsDNA molecule comprises a sequence of a gene.

5. A method for identifying a candidate compound for treating a cancer in a subject, the method comprising:

(a) combining in a reaction mixture:

a molecular complex comprising a double stranded deoxyribonucleic acid (dsDNA) molecule and at least one nucleosome, wherein the dsDNA molecule comprises at least one restriction endonuclease recognition site,

a test compound, and

at least one chromatin remodeling protein,

under conditions sufficient to allow for activity of the at least one remodeling protein;

(b) contacting the dsDNA molecule with at least one restriction endonuclease that is capable of cleaving the dsDNA molecule at one or more of the at least one restriction endonuclease recognition site(s) under conditions sufficient to allow for activity of the at least one remodeling protein and the at least one restriction endonuclease;

(c) determining the level of cleavage of the dsDNA molecule by the at least one restriction endonuclease using a polymerase chain reaction assay;

(d) comparing the determined level of cleavage in step (c) to a control total level of cleavage; and

(e) identifying a test compound that causes a modulation in the determined level of cleavage as compared to the control level of cleavage as a candidate compound for treating a cancer.

6. The method of claim 5, wherein the dsDNA molecule comprises a sequence of a cancer-associated gene, and the at least one restriction endonuclease recognition site is/are located within the sequence of the cancer-associated gene

7. The method of claim 6, wherein the cancer-associated gene is an oncogene.

8. The method of claim 6, wherein the oncogene is Wnt, β -catenin, and myc.

9. The method of claim 6 or 7, wherein (e) comprising identifying a test compound that causes an increase in the determined level of cleavage as compared to the control level of cleavage as a candidate compound for treating cancer.

10. The method of claim 6, wherein the cancer-associated gene is a tumor suppressor gene.

11. The method of claim 10, wherein the tumor suppressor gene is WEE1, Rb, p21, p16, SFRP1/2, TIMP3, TGF- β , and SNAIL.

12. The method of claim 11, wherein (e) comprises identifying a test compound that causes a decrease in the determined level of cleavage as compared to the control level of cleavage as a candidate compound for treating cancer.

13. The method of any one of claims 5-12, further comprising:

(f) contacting a cancer cell with the candidate compound identified in step (e);

(g) determining the level of proliferation of the cancer cell in step (f);

(h) comparing the determined level of proliferation to a control level of proliferation; and

(i) further identifying a candidate compound that has a decreased proliferation as compared to the control level of proliferation as a candidate compound for treating a cancer in a subject.

14. A method for identifying a candidate compound for treating a subject with a chromatin remodeling-related condition, the method comprising:

(a) combining in a reaction mixture:

a molecular complex comprising a double stranded deoxyribonucleic acid (dsDNA) molecule and at least one nucleosome, wherein the dsDNA molecule comprises at least one restriction endonuclease recognition site,

a test compound, and

at least one chromatin remodeling protein,

under conditions sufficient to allow for activity of the at least one remodeling protein;

(b) contacting the dsDNA molecule with at least one restriction endonuclease that is capable of cleaving the dsDNA molecule at one or more of the at least one restriction endonuclease recognition site(s) under conditions sufficient to allow for activity of the at least one remodeling protein and the at least one restriction endonuclease;

- (c) determining the level of cleavage of the dsDNA molecule by the at least one restriction endonuclease using a polymerase chain reaction assay;
- (d) comparing the determined level of cleavage in step (c) to a control total level of cleavage; and
- (e) identifying a test compound that causes a modulation in the determined level of cleavage as compared to the control level of cleavage as a candidate compound for treating a subject with a chromatin remodeling-related condition.

15. The method of claim 14, wherein the chromatin remodeling-related condition is a neurodevelopmental disorder, an autoimmune disease, or a cardiovascular disease.

16. The method of claim 15 wherein step (e) comprises identifying a test compound that causes an increase in the determined level of cleavage as compared to the control level of cleavage as a candidate compound for treating a subject with a neurodevelopmental disorder, an autoimmune disease, or a cardiovascular disease.

17. The method of claim 15 or 16, wherein the dsDNA molecule comprises a sequence of a gene related to the pathogenesis of the neurodevelopmental disorder, the autoimmune disease, or the cardiovascular disease.

18. The method of any one of claims 15-17, wherein the neurodevelopmental disorder is autism-spectrum disorders, epilepsy, Alpha-thalassemia X-linked mental retardation, Cockayne syndrome, Coffin-Siris syndrome, Nicolaides-Baraitser syndrome, Floating-Harbo syndrome, Schimke immunoosseous dysplasia (SIOD), Williams syndrome, or CHARGE syndrome.

19. The method of any one of claims 15-18, wherein the at least one chromatin remodeling protein is selected from the group consisting of: MECP2, ATRX, H2AFY, SMC1A, MACROD2, KDM5C, MBD1, ARID1B, SMARCC2, JMJD1C, CHD8, CHD1, CHD2, CHD3, CHD4, CHD5, CHD6, CHD7, CSB, BRG1, BRM, and HARP.

20. A method for identifying a candidate compound for maintaining or inducing pluripotency in a mammalian cell, the method comprising:

- (a) combining in a reaction mixture:
 - a molecular complex comprising a double stranded deoxyribonucleic acid (dsDNA) molecule and at least one nucleosome, wherein the dsDNA molecule comprises at least one restriction endonuclease recognition site,
 - a test compound, and
 - at least one chromatin remodeling protein,under conditions sufficient to allow for activity of the at least one remodeling protein;
- (b) contacting the dsDNA molecule with at least one restriction endonuclease that is capable of cleaving the dsDNA molecule at one or more of the at least one restriction endonuclease recognition site(s) under conditions sufficient to allow for activity of the at least one remodeling protein and the at least one restriction endonuclease;
- (c) determining the level of cleavage of the dsDNA molecule by the at least one restriction endonuclease using a polymerase chain reaction assay;
- (d) comparing the determined level of cleavage in step (c) to a control total level of cleavage; and
- (e) identifying a test compound that causes a modulation in the determined level of cleavage as compared to the control level of cleavage as a candidate compound for maintaining or inducing pluripotency in a mammalian cell.

21. The method of claim 1, wherein step (e) comprises identifying a test compound that causes a decrease in the determined level of cleavage as compared to the control level of cleavage as a candidate compound for maintaining or inducing pluripotency in a mammalian cell.

22. The method of claim 20 or 21, wherein the at least one chromatin remodeling protein is selected from the group consisting of: NuRD, Mi-2, SWI, SNF, ISWI, CHD1, CHD2, CHD3, CHD4, CHD5, CHD6, CHD7, CHD8, CHD9, and INO80.

23. The method of any one of claims 20-22, further comprising:
- (f) contacting a differentiated cell with the candidate compound identified in step (e);
 - (g) determining the level of one or more stem cell marker genes in the cell in step

(f);

(h) comparing the determined level of the one or more stem cell marker genes to a control level(s) of the one or more stem cell marker genes; and

(i) further identifying a candidate compound that has an increased level of the one or more stem cell marker genes proliferation as compared to the control level(s) of the one or more stem cell marker genes as a candidate compound for maintaining or inducing pluripotency in a mammalian cell.

24. The method of any one of claims 1-23, wherein the dsDNA molecule only comprises a single restriction endonuclease recognition site.

25. The method of any one of claims 1-23, wherein dsDNA molecule comprises at least two restriction endonuclease recognition sites.

26. The method of claim 25, wherein the dsDNA molecule comprises at least three restriction endonuclease recognition sites.

27. The method of claim 26, wherein the dsDNA molecule comprises at least four restriction endonuclease recognition sites.

28. The method of claim 25, wherein each of the at least two restriction endonuclease recognition sites is spaced about 100 base pairs to about 12,500 base pairs away from another of the restriction endonuclease recognition site(s) in the dsDNA molecule.

29. The method of claim 25, wherein step (b) comprises contacting the dsDNA molecule with at least two different restriction endonucleases, wherein each of the different restriction endonucleases is capable of cleaving the dsDNA molecule at one of the restriction endonuclease recognition site(s).

30. The method of any one of claims 1-29, wherein the restriction endonuclease recognition site(s) comprise about 4 base pairs to about 10 base pairs.

31. The method of claim 30, wherein the restriction endonuclease recognition site(s) comprise about 4 base pairs to about 8 base pairs.

32. The method of any one of claims 1-31, wherein the restriction endonuclease(s) is selected from the group consisting of: HaeIII, AatI, AatII, AccI, AccII, Acc65I, AciI, AcsI, AcyI, AflI, AflII, AflIII, AgeI, AhaII, AhaIII, AluI, AlwI, Alw44I, AlwNI, AocI, AoiI, ApaI, ApaLI, ApoI, ApyI, AscI, AseI, AsnI, AspI, Asp700, Asp718, AspEI, AspHI, AsuII, Aval, AvaII, AviII, AvrII, Ball, BamHI, BanI, BanII, BbrPI, BbsI, BbvI, BcgI, BclI, BfaI, BfrI, BglI, BglII, BinI, BmyI, BpmI, BpuAI, BpuI 102I, BsaI, BsaAI, BsaBI, BsaHI, BsaJI, BseAI, BsePI, BsgI, BsiEI, BsiWI, BsiYI, BslI, BsmI, BsmAI, Bsp1286I, Bsp1407I, BspDI, BspEI, BspHI, BspLU11I, BspMI, BsrI, BsrFI, BssGI, BssHII, Bst1107I, BstBI, BstEII, BstNI, BstUI, BstXI, BstYI, Bsu36I, CelII, CfoI, CfrI, Cfr10I, ClaI, DdeI, DpnI, DpnII, DraI, DraII, DraIII, DrdI, DsaI, EaeI, EagI, Eam1105I, EarI, Ecl136II, EclXI, Eco47III, Eco57I, EcoNI, EcoO109I, EcoRI, EcoRII, EcoRV, EspI, Esp3I, FnuDII, Fnu4HI, FokI, FseI, FspI, GsuI, HaeII, HgaI, HgiAI, HhaI, HincII, HindII, HindIII, HinfI, HinPI, HpaI, HpaII, HphI, ItaI, KasI, KpnI, KspI, MaeI, MaeII, MaeIII, MamI, MboI, MboII, MfeI, MluI, MluNI, MnlI, MroI, MscI, MseI, MspI, MstI, MstII, MunI, MvaI, MvnI, NaeI, NarI, NciI, NcoI, NdeI, NdeII, NgoMI, NheI, NlaIII, NlaIV, NotI, NruI, NsiI, NspBII, NspI, NspII, NspV, PacI, Paer7I, PflMI, PinAI, PleI, PmaCI, PmeI, PmlI, PpuMI, Psp1406I, PstI, PvuI, PvuII, RcaI, RmaI, RsaI, RsrII, SacI, SacII, SalI, SauI, Sau3AI, Sau96I, Scal, ScrFI, SexAI, SfaNI, SfcI, SfiI, SfuI, SgrAI, SmaI, SnaBI, SnoI, SpeI, SphI, SrfI, Sse8387I, SspI, SspBI, SstI, SstII, StuI, StyI, SwaI, TaqI, TfiI, Thal, Tru9I, Tth111I, Van9II, XbaI, XcmI, XhoI, XhoII, XmaI, XmaIII, XmaCI, and XmnI.

33. The method of any one of claims 1-32, wherein the dsDNA molecule comprises a plasmid.

34. The method of claim 33, wherein the plasmid has a size of about 500 base pairs to about 25,000 base pairs.

35. The method of claim 34, wherein the plasmid has a size of about 1000 base pairs to about 20,000 base pairs.

36. The method of any one of claims 1-18, 20, 21, and 23-35, wherein the at least one chromatin remodeling proteins is selected from the group consisting of: BRG1/SMARCA4, BRM/SMARCA2, SNF2H/ISWI/SMARCA5, SNF2L/SMARCA1, INO80, EP400, SRCAP, RAD54, RAD54B, ATRX, CHD1, CHD2, CHD3/Mi-2alpha, CHD4/Mi-2beta, CHD5, CHD6, CHD7, CHD8, CHD9, CSB/ERCC6, ETL1/SMARCA1/HEL1, HELLS/SMARCA6, HARP/SMARCA1, ZRANB3/AH2, MOT1, SHRP, HLTF/SMARCA3, MECP2, H2AFY, SMC1A, MACROD2, KDM5C, MBD1, ARID1B, SMARCC2, JMJD1C, NuRD, Mi-2, SWI, SNF, SWI1, ADR6, SWI3, SNF6, and BAF200/250a,b/ARID1a,b, BAF180/Polybromo, BAF170, BAF155, SNF5/BAF47/INI1, BAF60a-c, BAF57, BAF53a,b, BAF47, BAF45a-d, BRD7,9, Actin, MBD2/3, RbAP46/48, HDAC1/2, MTA1-3, P66alpha/beta, LSD1, BPTF, ACF1, CHAC15/17, WSTF, EPC1, ING3, TIP60, GAS41, DMAP1, MRG15, MRGBP, TRRAP, BDF6, and RVB1/2.

37. The method of claim 36, wherein the at least one chromatin remodeling protein comprises ACF1 or CHD5.

38. The method of any one of claims 1-37, wherein the reaction mixture comprises only one chromatin remodeling protein.

39. The method of any one of claims 1-38, wherein the reaction mixture comprises at least two chromatin remodeling proteins.

40. The method of any one of claims 1-39, wherein the polymerase chain reaction assay is a real-time polymerase chain reaction assay.

41. The method of any one of claims 40, wherein the real-time quantitative polymerase chain reaction assay includes the use of at least one pair of real-time quantitative polymerase chain reaction primers, where a first member of the at least one pair specifically hybridizes to a sequence that is 5' of one of the at least one restriction endonuclease recognition site on a first strand of the dsDNA molecule and the second member of the at least one pair specifically hybridizes to a sequence that is 5' of the one of

the at least one restriction endonuclease recognition site on a second strand of the dsDNA molecule.

42. The method of any one of claims 1-41, further comprising between steps (b) and (c) inactivating one or both of the at least one restriction endonuclease and the at least one chromatin remodeling protein.

43. The method of claim 42, wherein the inactivating includes one or both of heating the reaction mixture to a temperature of about 50 °C to about 98 °C or adding to the reaction mixture an amount of a magnesium chelator sufficient to inhibit the activity of the restriction endonuclease.

44. The method of any one of claims 1-43, wherein the reaction mixture comprises an amount of magnesium ion sufficient to activate the at least one restriction endonuclease.

45. The method of any one of claims 1-44, wherein the reaction mixture in step (a) comprises an amount of adenine triphosphate (ATP) sufficient to activate the at least one chromatin remodeling protein.

46. The method of any one of claims 1-45, wherein the reaction mixture is a liquid.

47. The method of claim 46, wherein the liquid has a volume of between about 5 µL and about 50 µL.

48. The method of any one of claims 1-47, wherein the method is a high throughput method.

49. A kit consisting essentially of:

- (i) at least one chromatin remodeling protein; and
- (ii) a molecular complex comprising a double stranded deoxyribonucleic acid (dsDNA) molecule and at least one nucleosome, wherein the dsDNA molecule comprises at least one restriction endonuclease recognition site.

50. The kit of claim 49, wherein the at least one chromatin remodeling protein is selected from the group consisting of: BRG1/SMARCA4, BRM/SMARCA2, SNF2H/ISWI/SMARCA5, SNF2L/SMARCA1, INO80, EP400, SRCAP, RAD54, RAD54B, ATRX, CHD1, CHD2, CHD3/Mi-2alpha, CHD4/Mi-2beta, CHD5, CHD6, CHD7, CHD8, CHD9, CSB/ERCC6, ETL1/SMARCA1/HEL1, HELLS/SMARCA6, HARP/SMARCA1, ZRANB3/AH2, MOT1, SHRPH, HLT/SMARCA3, MECP2, H2AFY, SMC1A, MACROD2, KDM5C, MBD1, ARID1B, SMARCC2, JMJD1C, NuRD, Mi-2, SWI, SNF, SWI1, ADR6, SWI3, SNF6, and BAF200/250a,b/ARID1a,b, BAF180/Polybromo, BAF170, BAF155, SNF5/BAF47/INI1, BAF60a-c, BAF57, BAF53a,b, BAF47, BAF45a-d, BRD7,9, Actin, MBD2/3, RbAP46/48, HDAC1/2, MTA1-3, P66alpha/beta, LSD1, BPTF, ACF1, CHRAC15/17, WSTF, EPC1, ING3, TIP60, GAS41, DMAP1, MRG15, MRGBP, TRRAP, BDF6, and RVB1/2.

51. The kit of claim 50, wherein the at least one chromatin remodeling protein comprises ACF1 or CHD5.

52. The kit of any one of claims 49-51, wherein the kit comprises only one chromatin remodeling protein.

53. The kit of any one of claims 49-51, wherein the kit comprises at least two chromatin remodeling proteins.

54. The kit of any one of claims 49-53, wherein the dsDNA molecule comprises a sequence of a gene, wherein the at least one restriction endonuclease recognition site is within the sequence of the gene.

55. The kit of claim 54, wherein the gene is a cancer-associated gene or a gene related to the pathogenesis of a neurodevelopmental disorder, an autoimmune disease, or a cardiovascular disease.

56. The kit of claim 55, wherein the cancer-associated gene is an oncogene.

57. The kit of claim 55, wherein the cancer-associated gene is a tumor suppressor gene.

58. The kit of any one of claims 49-57, wherein the dsDNA molecule only comprises a single restriction endonuclease recognition site.

59. The kit of any one of claims 49-57, wherein dsDNA molecule comprises at least two restriction endonuclease recognition sites.

60. The kit of claim 59, wherein the dsDNA molecule comprises at least three restriction endonuclease recognition sites.

61. The kit of claim 60, wherein the dsDNA molecule comprises at least four restriction endonuclease recognition sites.

62. The kit of claim 59, wherein each of the at least two restriction endonuclease recognition sites is spaced about 100 base pairs to about 12,500 base pairs away from another of the restriction endonuclease recognition site(s) in the dsDNA molecule.

63. The kit of any one of claims 49-57 and 59-62, wherein the dsDNA molecule comprises at least two different restriction endonuclease recognition sites.

64. The kit of any one of claims 49-63, wherein the restriction endonuclease recognition site(s) comprise(s) about 4 base pairs to about 10 base pairs.

65. The kit of claim 64, wherein the restriction endonuclease recognition site(s) comprise(s) about 4 base pairs to about 8 base pairs.

66. The kit of any one of claims 49-65, wherein the kit further comprises one or both of a heat-stable DNA polymerase or a polynucleotide mix.

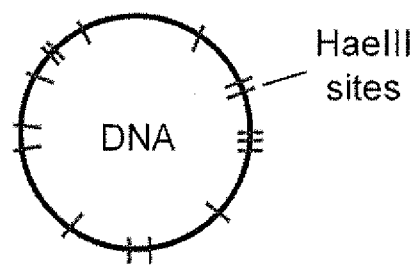
67. The kit of any one of claims 66, wherein the heat-stable DNA polymerase is a Taq DNA polymerase.

68. The kit of any one of claims 49-67, wherein the kit further comprises at least one restriction endonuclease that is capable of cleaving the dsDNA molecule at one of the at least one restriction endonuclease recognition site(s).

69. The kit of any one of claims 49-68, further comprising: at least one pair of polymerase chain reaction primers, where a first member of the at least one pair specifically hybridizes to a sequence that is 5' of one of the at least one restriction endonuclease recognition site(s) on a first strand of the dsDNA molecule and the second member of the at least one pair specifically hybridizes to a sequence that is 5' of the one of the at least one restriction endonuclease recognition site(s) on a second strand of the dsDNA molecule.

70. The kit of claim 69, wherein the at least one pair of polymerase chain reaction primers is at least one pair of real-time polymerase chain reaction primers.

71. The kit of any one of claims 49-70, wherein the kit further comprises a buffered solution comprising one or both of a sufficient concentration of magnesium ion to activate a restriction endonuclease and a sufficient concentration of adenosine triphosphate to activate the at least one chromatin remodeling protein.



+ HaeIII

↓
Full
digestion
of DNA

Figure 1

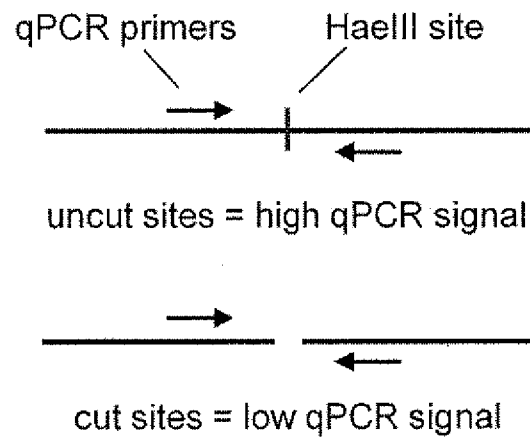
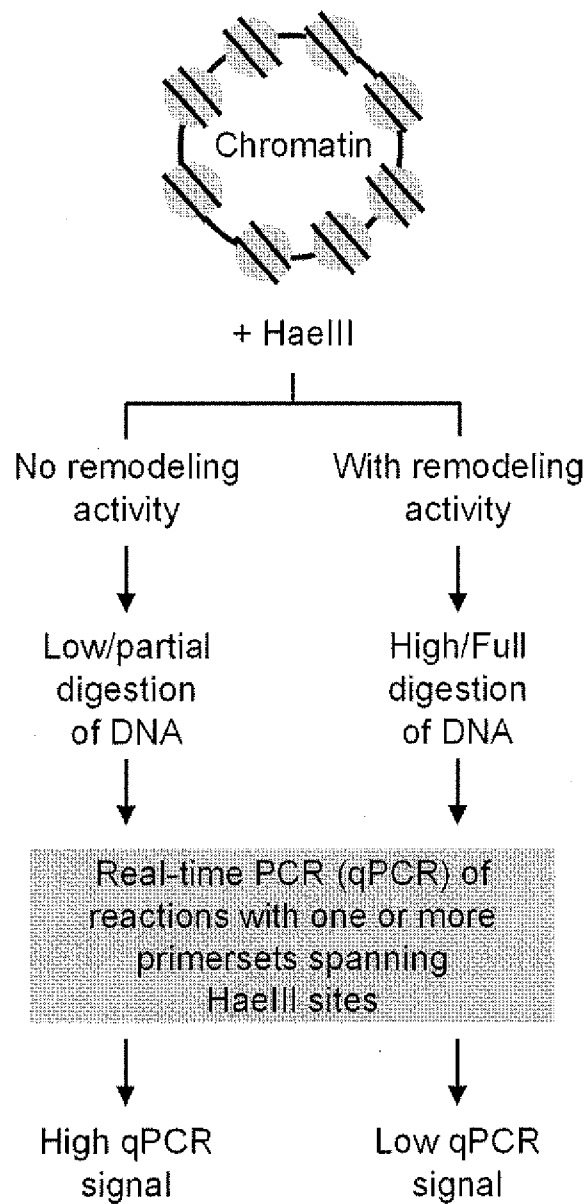


Figure 2

**Figure 3**

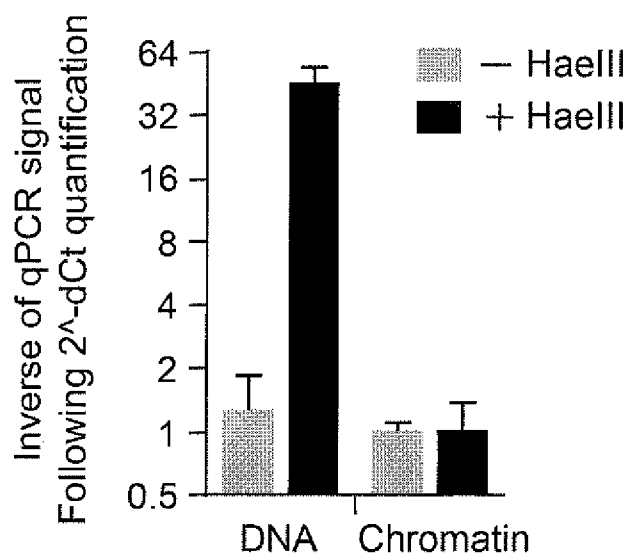


Figure 4

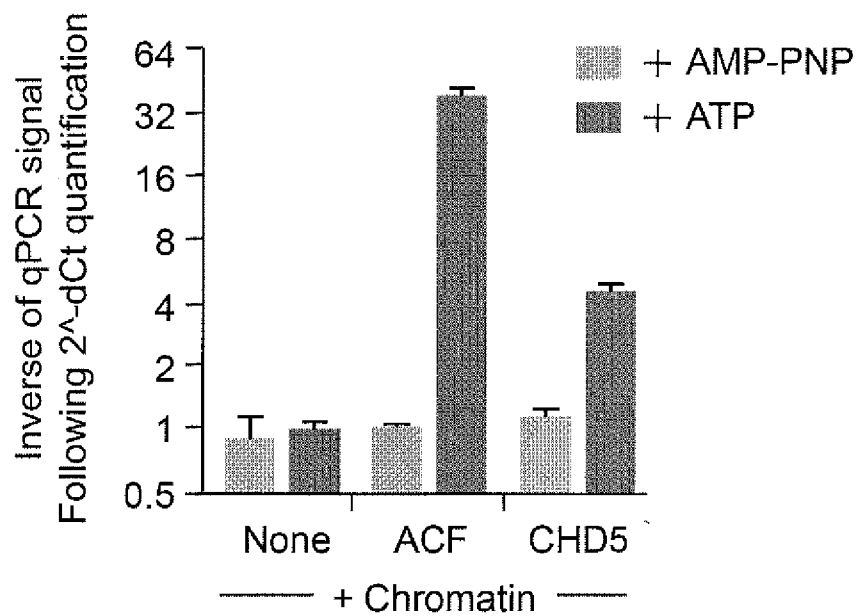


Figure 5

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 16/25609

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C12N 15/11, C12Q 1/68 (2016.01)

CPC - C12N2830/46, C12N15/09, C12N15/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC(8): C12N 15/11, C12Q 1/68 (2016.01)

CPC: C12N2830/46, C12N15/09, C12N15/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC: 506/16, 435/6.14, 435/6.1Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatBase, Google Patents, Google Scholar, Google Web, search terms: chromatin remodeling, modulator, nucleosome, test compound, dsDNA molecule, level of cleavage, cancer-associated gene, WEE1, Rb, p21, pl6, SFRP1/2, TIMP3, TGF-p, SNAIL, neurodevelopmental disorder, autoimmune disease, NuRD, Mi-2, SWI, SNF, ISWI, CHDI, CHD2

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 5,972,608 A (PETERSON et al.) 26 October 1999 (26.10.1999) abstract col 3, ln 11-13, ln 22-42, ln 44-59, col 4, ln 32-34, col 6, ln 6-19, col 8, ln 26-36, ln 53-59, col 9, ln 4-8, ln 15-18, col 10, ln 35-52	49-53 ----- 1-12, 14-17, 20-22
Y	US 2011/0237447 A1 (WEINZIERL) 29 September 2011 (29.09.2011) claim 7, para [0002], [0011], [0013], [0019], [0029], [0047], [0050], [0051], [0113], [0116], [0291], [0293], [0361], [0365]	1-12, 14-17, 20-22
Y	US 2006/0030626 A1 (KAMMER et al.) 9 February 2006 (09.02.2006) abstract, para [0096], [0102], [0103]	15-17
Y	Quan et al. The Chromatin Remodeling Factor CHD5 Is a Transcriptional Repressor of WEE1. PLoS ONE (23 September 2014) vol 9 no 9, pp 1-8, e108066. doi:10.1371/journal.pone.0108066, abstract	10-12

☐ Further documents are listed in the continuation of Box C.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

8 June 2016 (08.06.2016)

Date of mailing of the international search report

12 JUL 2016

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents

P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-8300

Authorized officer:

Lee W. Young

PCT Helpdesk: 571-272-4300

PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 16/25609

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
 - a. ☒ forming part of the international application as filed:
 - ☒ in the form of an Annex C/ST.25 text file.
 - ☐ on paper or in the form of an image file.
 - b. ☐ furnished together with the international application under PCT Rule 13ter.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
 - c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
 - ☐ in the form of an Annex C/ST.25 text file (Rule 13ter.1(a)).
 - ☐ on paper or in the form of an image file (Rule 13ter.1(b) and Administrative Instructions, Section 713).
2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 16/25609

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 13, 18-19, 23-48, 54-71
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.