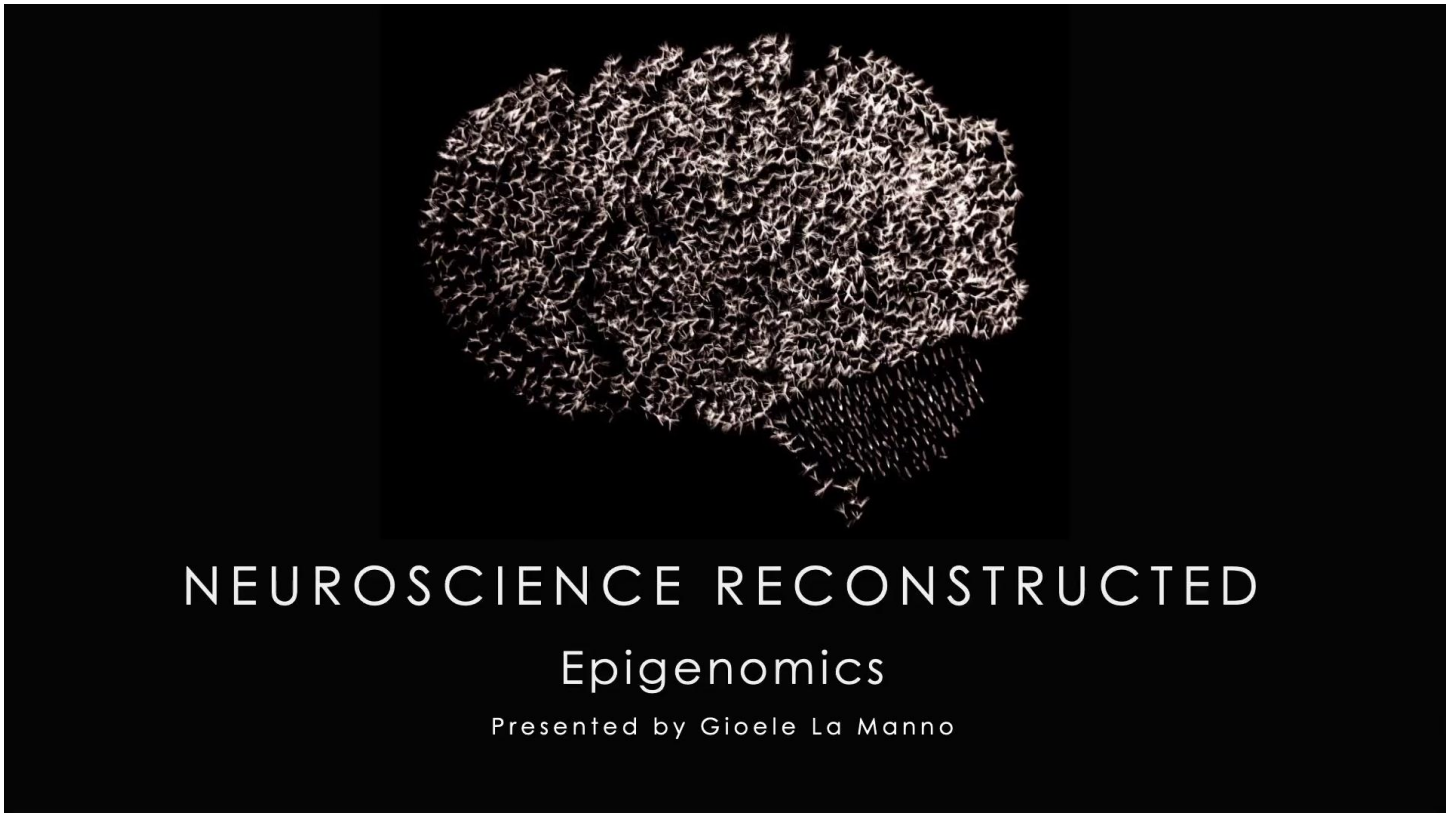
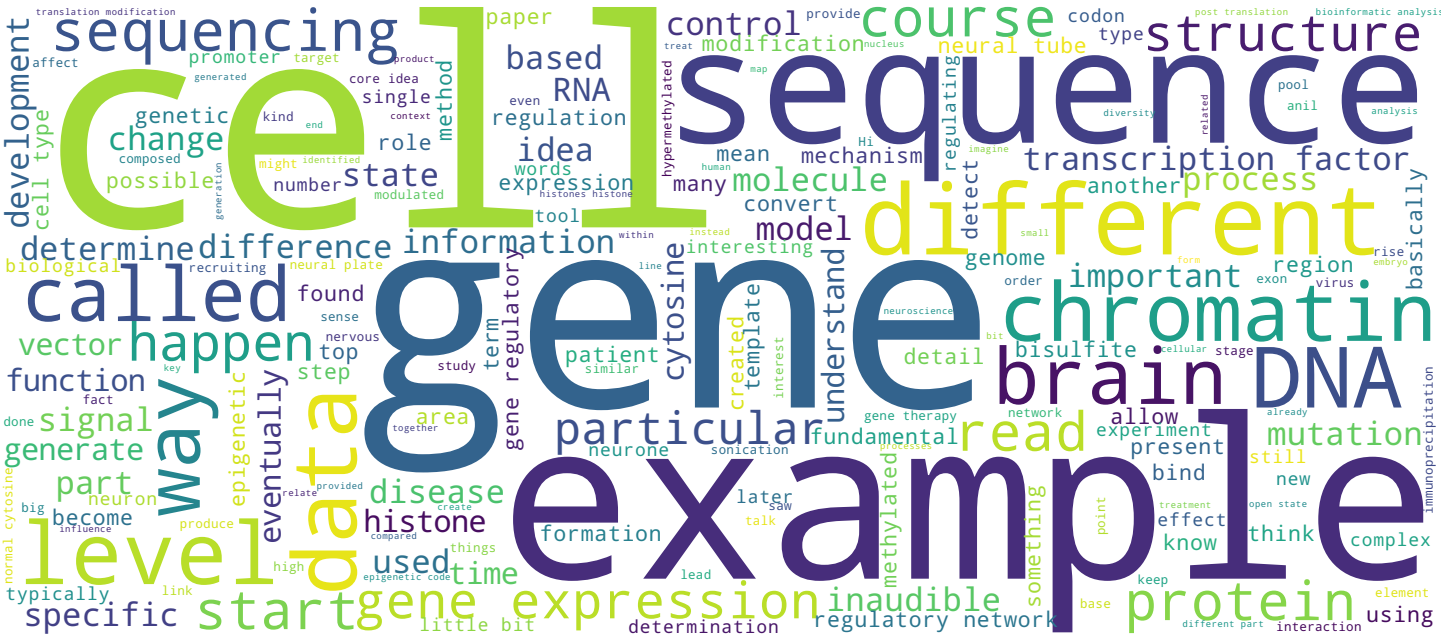
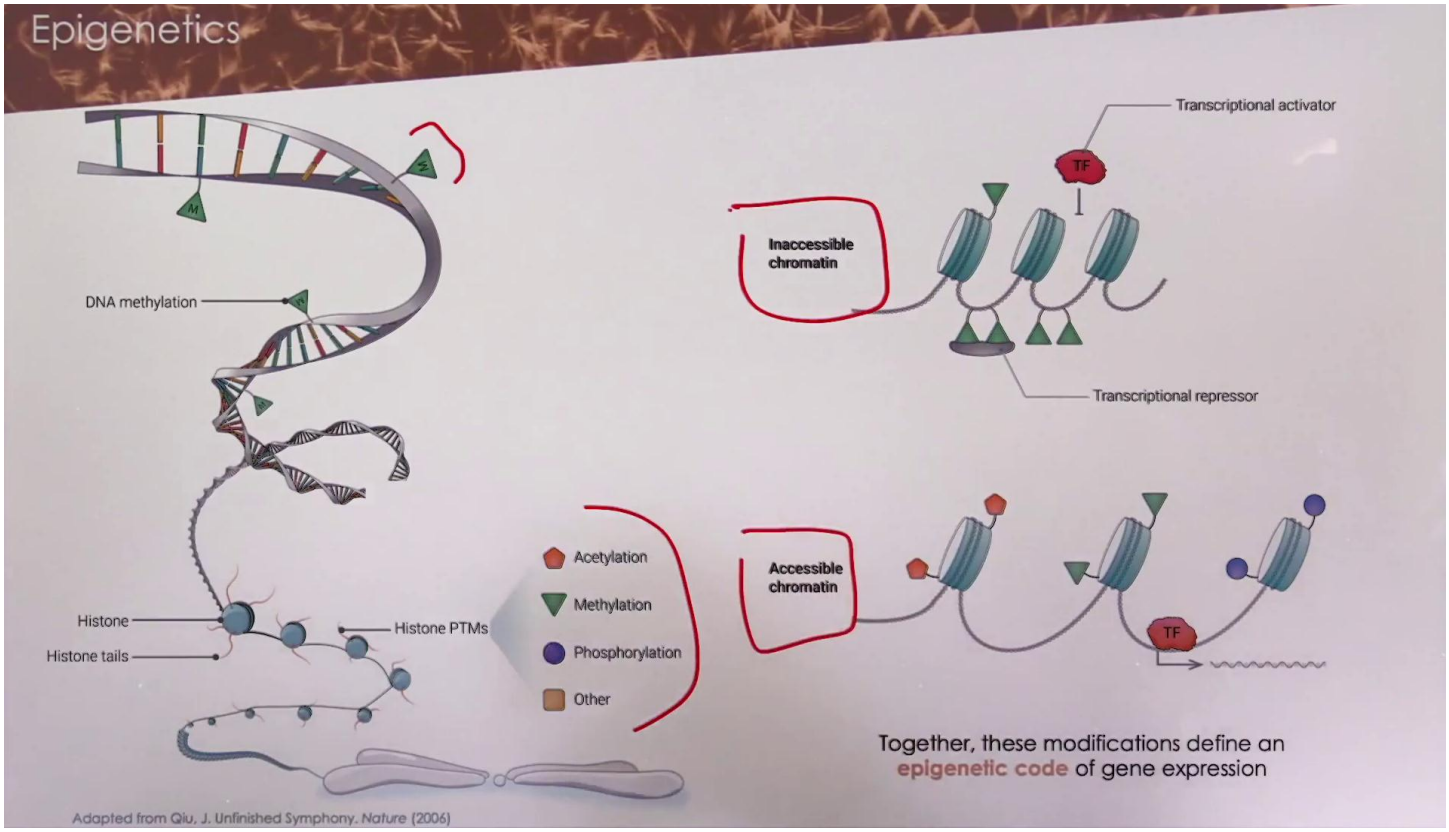




EPFL

Search MOOC

Video

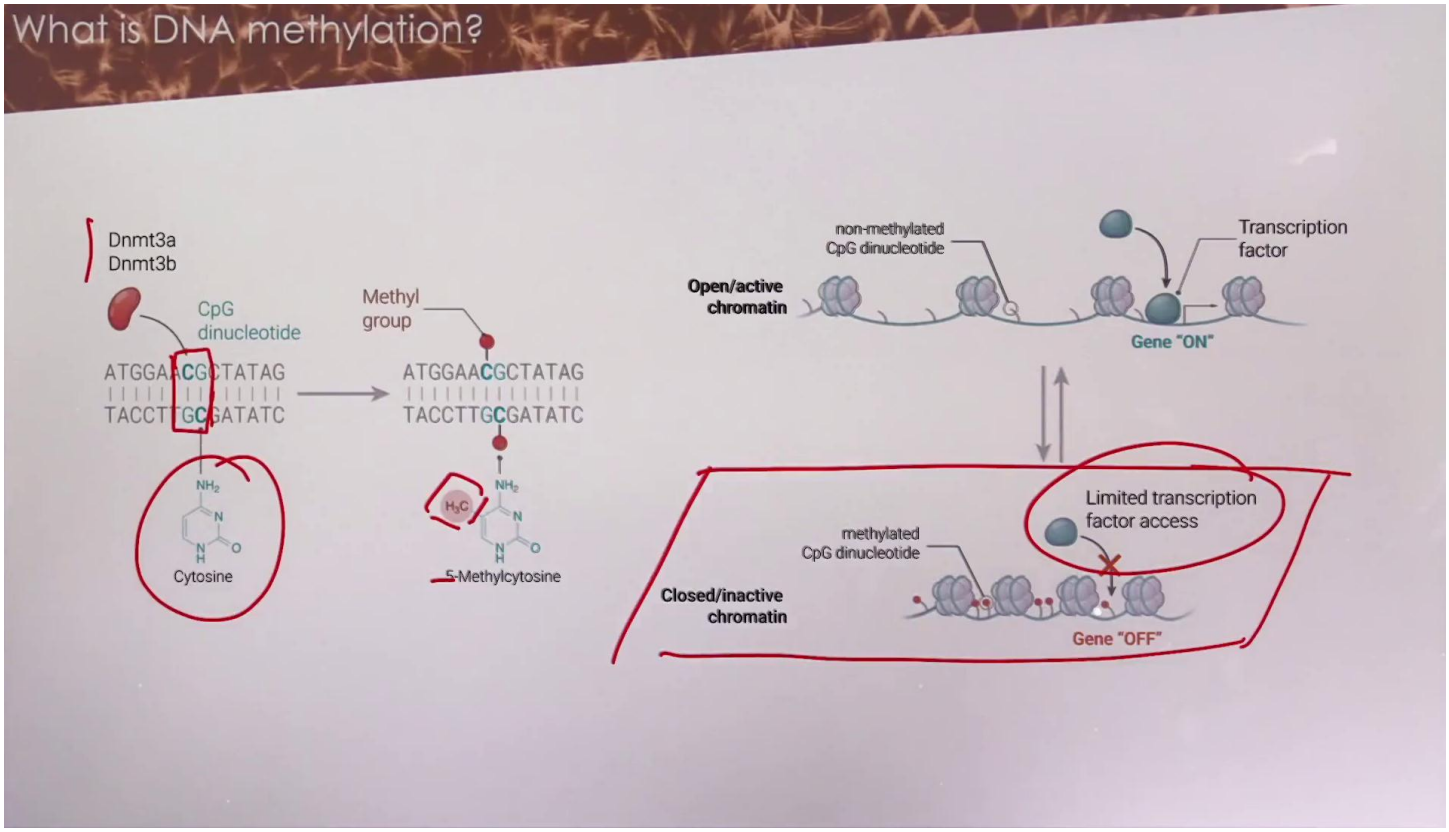


Now, we move away a bit from sequence and we think about epigenetic aspects. What I mean here with epigenetics is aspect that relate to chromatin, but which information is not held into the sequence. Therefore, as such not necessarily readable in the same way. What could be those modifications? Could be, for example, talking about things like the methylation of the DNA. The post translational modification that we can find in histone, core fundamental component of the chromatin, and for example, acetylation, methylation, phosphorylation, biotinylation, osmylation, and so on. How does those modification act on regulating how the genome gets transcribed in the [inaudible 00:01:03] and produce proteins that will have a function? The core idea, even though there might be other mechanisms that going into the details of how this happens. But generally, the way chromatin modifications, including DNA modification and cell modification, affect the regulation of genes is by making chromatin accessible or inaccessible.

Notes

Summary

0m 05s

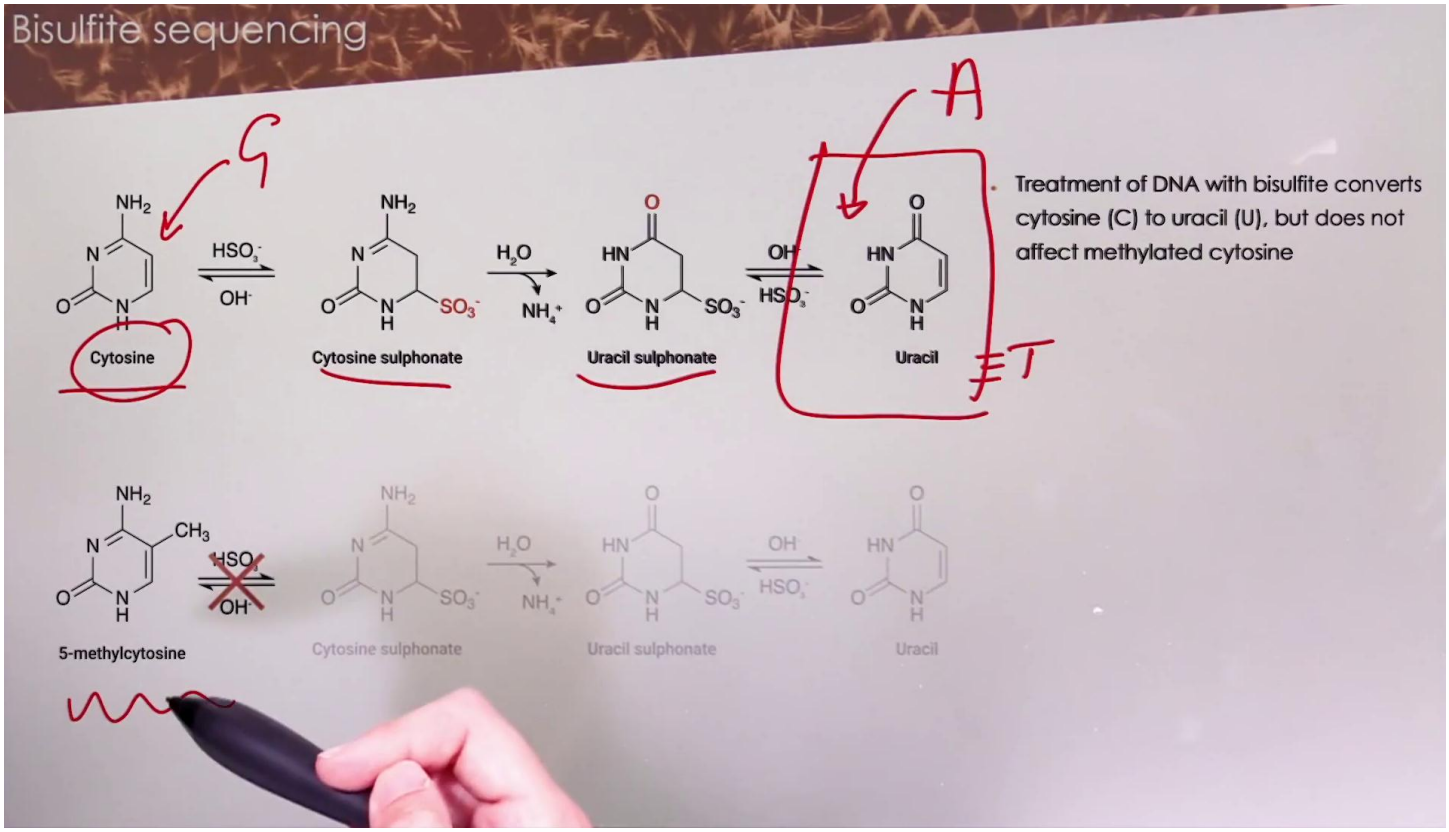


Let's go a little bit more in detail. What are the core modifications? DNA methylation, this is operated by specific enzymes, here Dnmt3 A and B. It happens at the level of CpG... Sorry, of CG dinucleotide. The base involved is cytosine, which a metal group gets added in this position, position five. When the DNA is methylated, that becomes more hydrophobic and allows also the recruiting of other regulators that produces the formation of a chromatin that is more packed, is more condensed, and therefore limits transcription factor binding. In this way, contributes to regulating the particular locus to be downregulated or repressed in terms of gene expression.

Notes

Summary

1m 37s

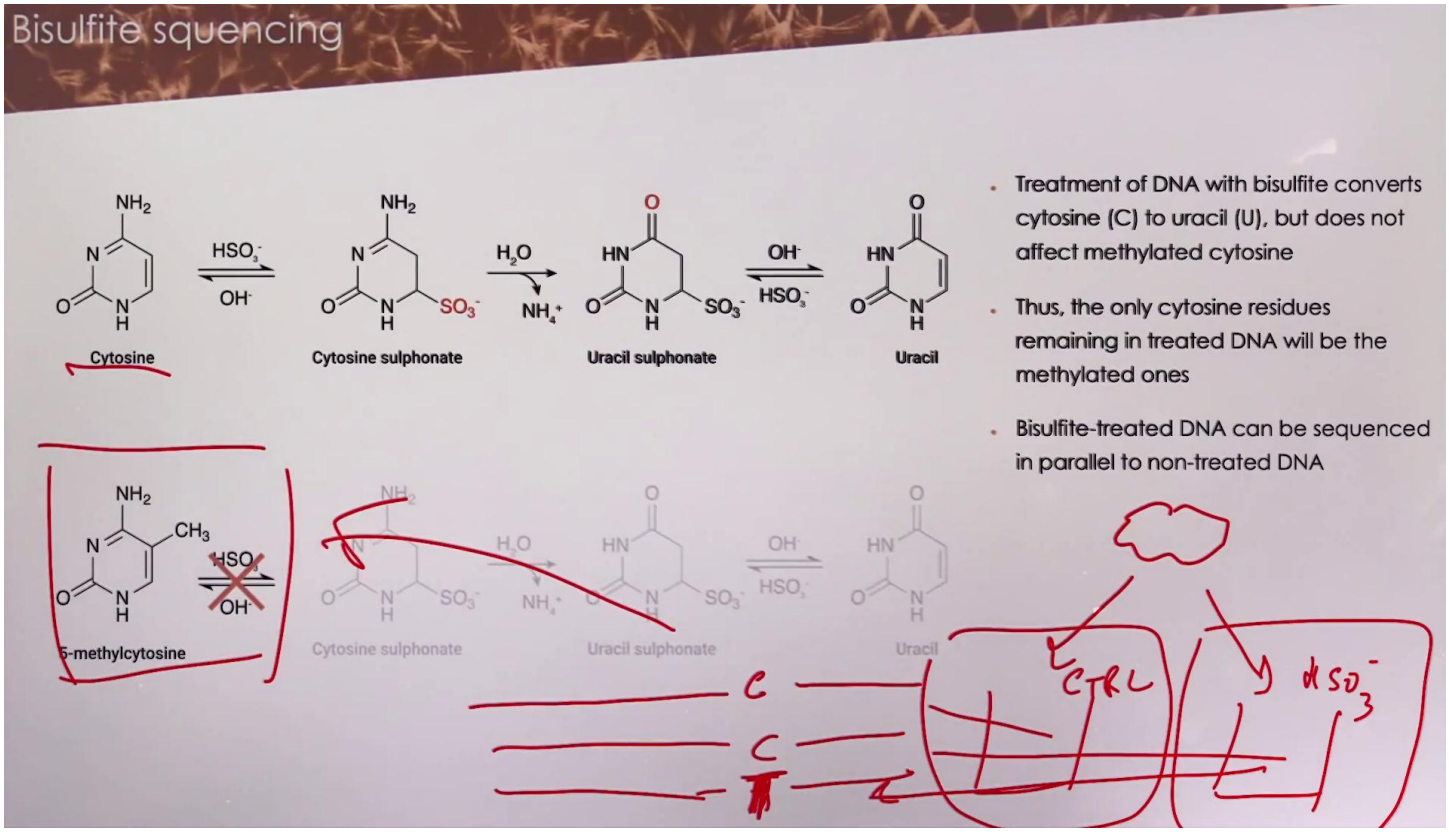


How can we detect if a particular stretch of DNA has been methylated? How can we do it particularly in a way that is similar to sequencing and have the same [inaudible 00:03:03]? We can do something that is called bisulfite sequencing. It's based on the idea that you can convert a cytosine through an organic chemistry reaction, particularly treating with bisulfite. This leads to an aromatic addition that eventually also convert in a stepwise fashion a cytosine into a sulphonate and eventually an uracil sulphonate that finally converts into an uracil. Basically, if you start from a cytosine that is not methylated, so from a normal cytosine, you get out of the treatment with bisulfite an uracil. Therefore, you converted a template that would anil with a G, with a template... Uracil, you remember, is basically equivalent for RNA to T for DNA. Therefore, you convert it to a base that will anil with A. This conversion is fundamental. It basically has created the difference between a sequence that has been created with bisulfite and a normal one. It also has created a difference... There is a difference between the fact that this process happens for cytosine, but not for methylcytosine.

Notes

Summary

2m 53s



Again, the idea is that all the cytosine residues remaining in treated DNA will be the methylated ones. One can do the following experimental design trick. I can start from the same pool of molecule, I can split it in two and now treat one of them with bisulfite, and one of them keep it as a control. Now all the sequence structures that are read as C, in other words, complementary to a G in the control and they stay C in the bisulfite reaction, then they must be methylated. Instead, if they were normal cytosine, unmethylated one, then we actually would expect instead to read in the bisulfite-treated reaction to read an A. Sorry, to read a T, that is complementary to an A. This is the basic idea. We can then at the level of the bioinformatic analysis, use the shared information of these two libraries that we sequence to determine the actual state of methylation of the sequence that we read.

Notes

Summary

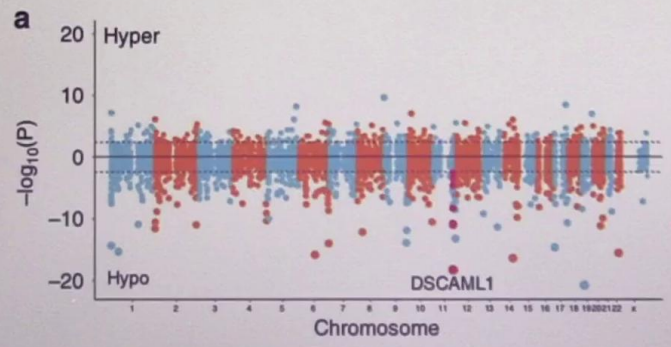
4m 33s

Methylome profiling – example

ARTICLE

https://doi.org/10.1038/s41467-019-10101-7 OPEN

Epigenetic dysregulation of enhancers in neurons is associated with Alzheimer's disease pathology and cognitive symptoms



- More than 1000 regions were differentially methylated in neurons showing more advanced pathology
- Most perturbed regulatory regions were hypomethylated

Li P et al. Epigenetic dysregulation of enhancers in neurons is associated with Alzheimer's disease pathology and cognitive symptoms. Nat Commun. 2019 May 21;10(1):2246. doi: 10.1038/s41467-019-10101-7

An example of how this can be used is provided by this paper that is detecting an association of Alzheimer's disease pathology, cognitive symptom, and methylation state of the chromatin. In this paper, they identified more than 1,000 regions that are differentially methylated in neuronal samples for more advanced pathology as compared to controls. They draw the conclusion, actually, that the difference is there in such a direction. Actually, the most perturbed regulatory regions were hypermethylated. This, of course, I guess is interesting and it is in line with the idea that if a regimen is hypermethylated, it's more susceptible to changes of its transcriptional activation state.

Notes

Summary

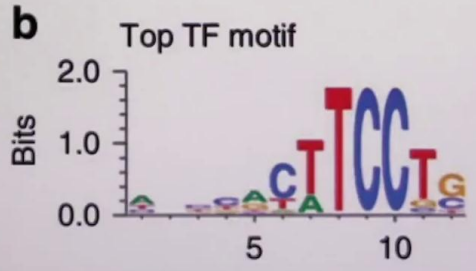
6m 02s

Methylome profiling – example

ARTICLE

https://doi.org/10.1038/s41467-019-10101-7 OPEN

Epigenetic dysregulation of enhancers in neurons is associated with Alzheimer's disease pathology and cognitive symptoms



- Binding site of the ETS transcription factor family were strongly hypomethylated
- ETS play a role in cell differentiation, cell cycle and apoptosis

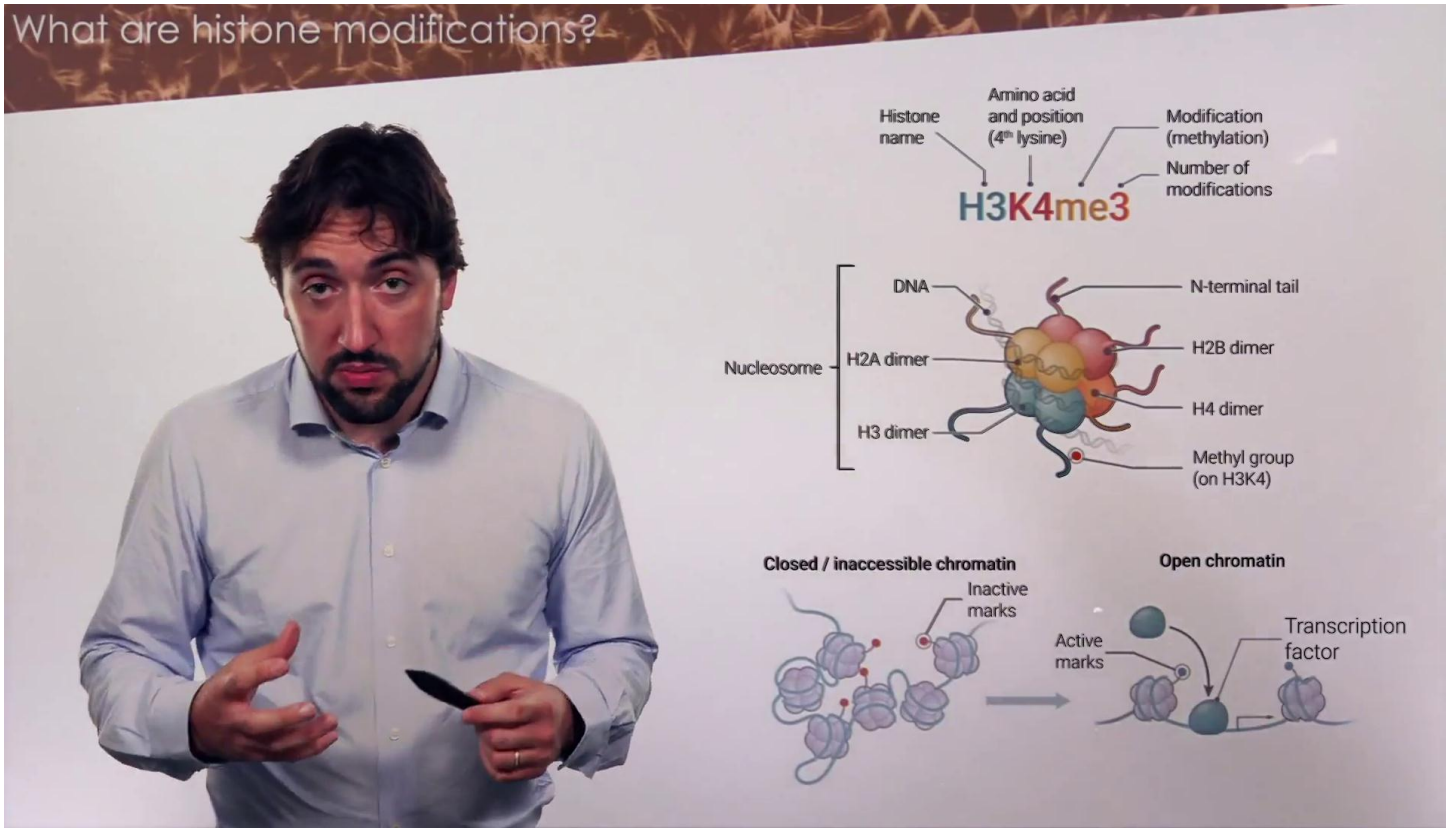
Li P et al. Epigenetic dysregulation of enhancers in neurons is associated with Alzheimer's disease pathology and cognitive symptoms. Nat Commun. 2019 May 21;10(1):2246. doi: 10.1038/s41467-019-10101-7

The analysis of similar data set can further involve the determination of specific transcription factor family binding sites that are found in proximity of hypomethylated or hypermethylated regions, and therefore the determination of also the role of the downstream genes to this particular transcription factor, and so then allowing to link the more functional aspect related to the activation and gene regulatory network to the epigenetic information.

Notes

Summary

6m 52s

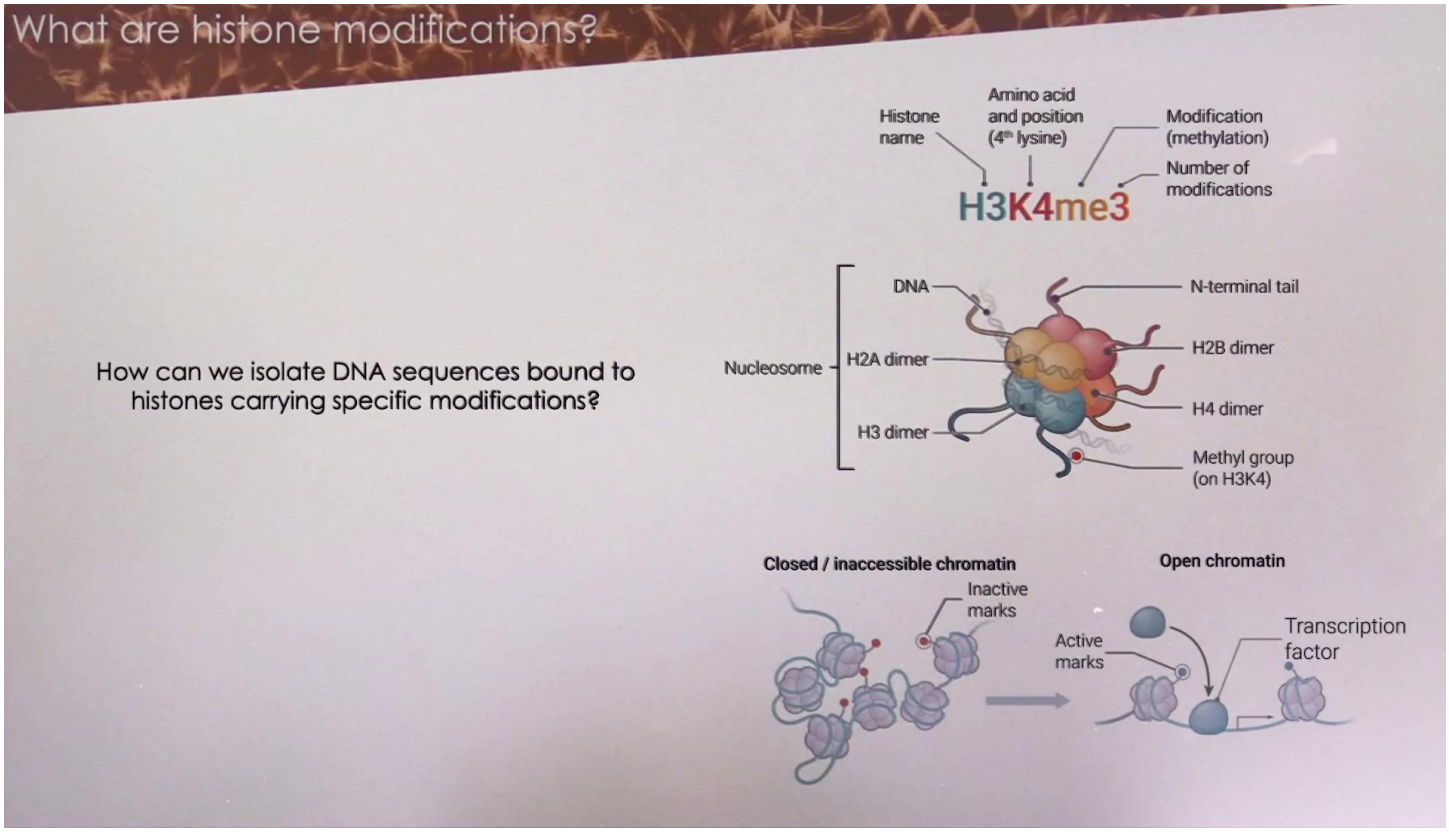


Another important aspect of the epigenetic code, in other words, of the information that is not present at the level of the sequence but is still part of the chromatin, can be modulated and also changed at the influence gene expression, exist at the level of modification, post translation modification of histones. Histones are core proteins that compose nucleosomes.

Notes

Summary

7m 30s

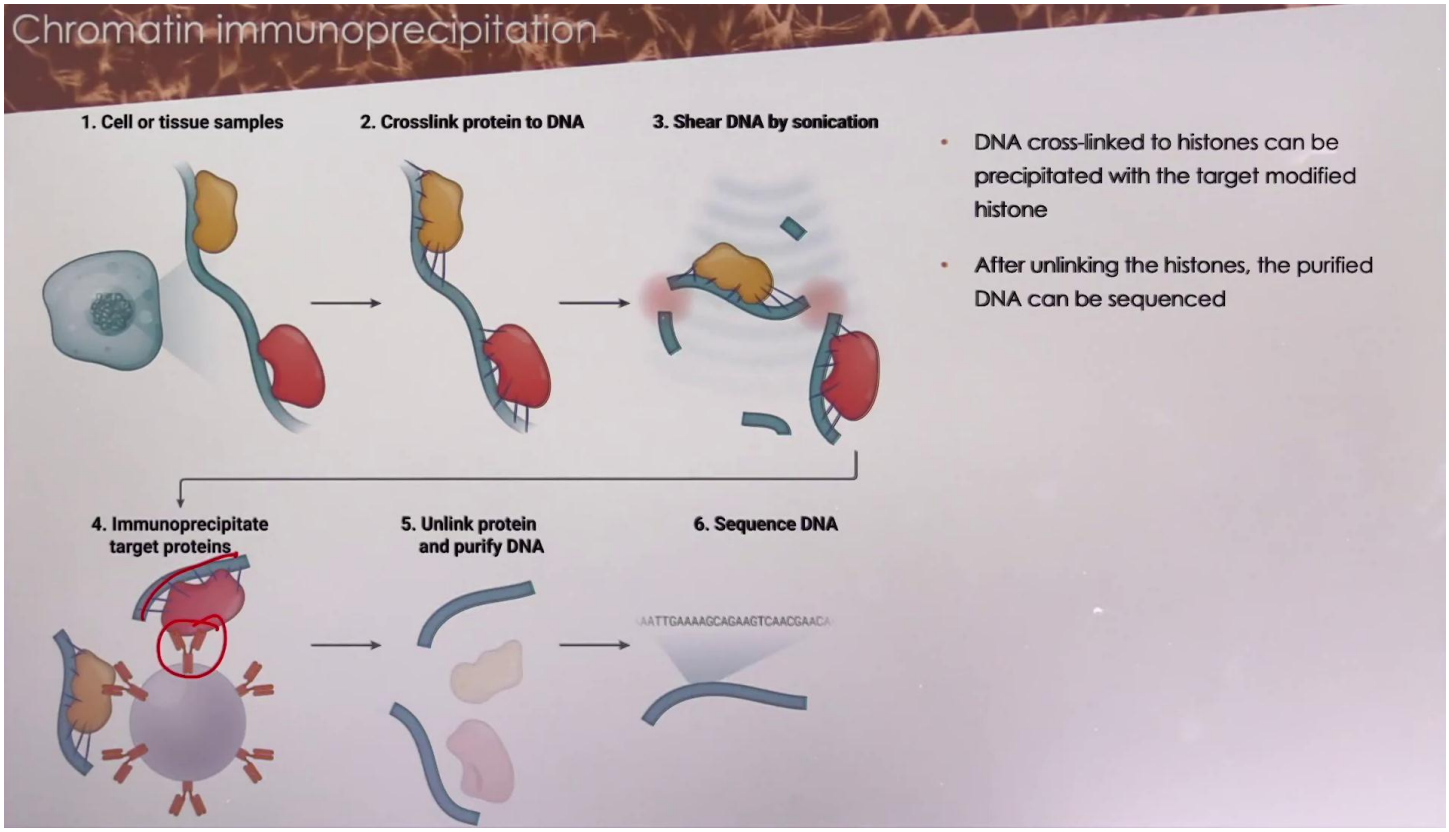


You can see here a typical DNA strand wraps around the nucleosome that is composed of an octamer of four... Actually dimers of the four fundamental histones, histone 2A, 2B, H3, and H4. Histones also have less structured long N-terminal tail that is typically where modifications occur. There are different post-translation modification that can happen in histones and change their structural or electrostatic properties or affect the recruiting of other proteins and chromatin remodelers, and therefore have an effect on changing the state of a chromatin from a closed to an open state or vice versa, from an open state to a closed state. In these different states, the chromatin can, of course, be differently accessible from transcription factors. In this way, gene expression can be modulated. How can we detect these changes that happens now at the level of the histone itself, not of the DNA? How can sequencing help us to tell us whether the histones that are present in a particular genomic locus are modified or not? The core idea that is used in immunoprecipitation.

Notes

Summary

7m 59s

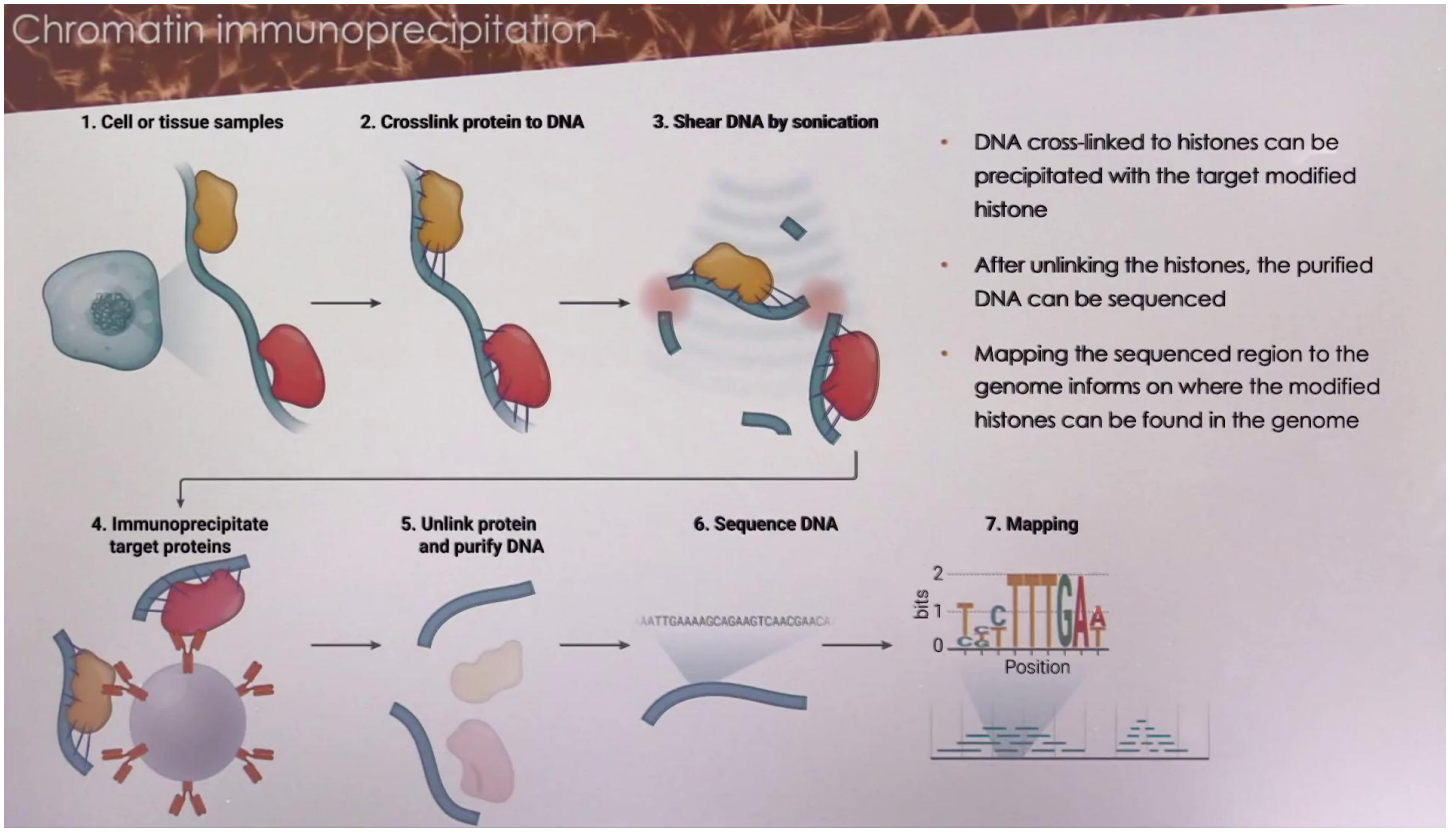


How can we use this idea of immunoprecipitation for chromatin and how can we associate it with sequencing? We start from chromatin, the nuclear material in its native state. You have the histones, they're bound to their DNA. In this state, you can proceed with the crosslinking step. You can use, for example, formaldehyde or other more mild crosslinker. This will determine the formation of covalent bonds between the distance and the DNA. Then the chromatin can be shared by sonication. This will fragment DNA. The proteins will remain associated with the specific strand. Now one can exploit specific antibodies that have been generated to recognize specifically the epitope and terminus tail of different histones with a specific modification and not to bind... Been selected not to bind other naive, non-modified version of this thing. This way, one can immunoprecipitate, as you see here, specific modified histone of interest, the crosslinking can be reverted, DNA can be released, and from these sequences, a sequencing library can be prepared. The sequences that are read and found in this library can be, for example, compared to just a control where instead of immunoprecipitating the target, we just use the [inaudible 00:11:21], where we just do not precipitate.

Notes

Summary

9m 34s

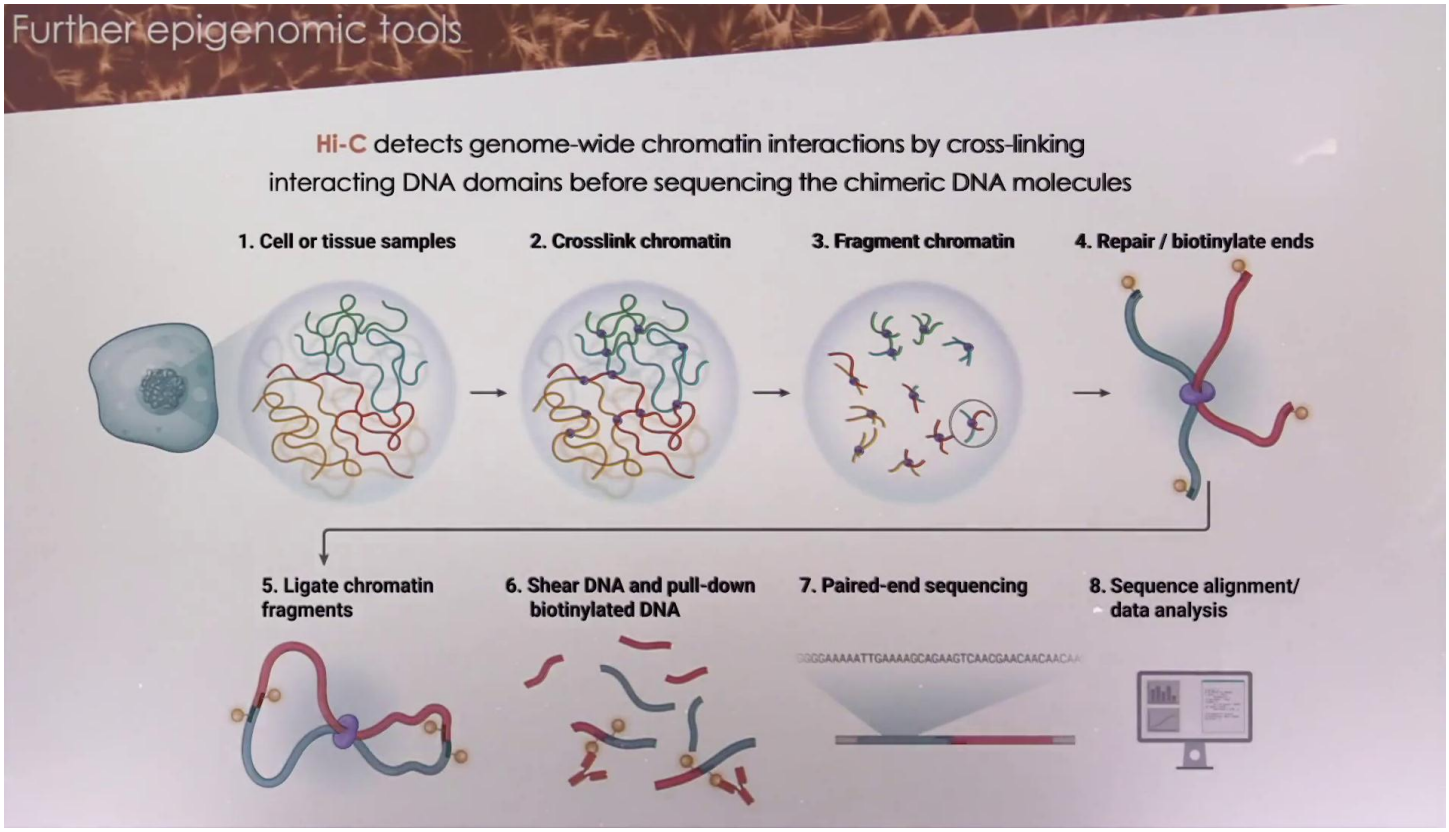


We, for example, provide... We just use the entire pool of molecules after sonication as here. Then we're going to go, of course, we can bioinformatic analysis. We're going to map the sequences and we're going to see these particular regions corresponding to specific loci that were hyper or hypo modified, can be highlighted by mapping the rates due to the reference genome.

Notes

Summary

11m 25s



Another method that is based on sequencing that gives information about the state of the chromatin, now not about modification, but about the structure is Hi-C. Hi-C is based on the idea of using crosslinking to capturing long distance interaction that could be intra or interchromosoma. In other words, we can detect different parts of the chromatin coming together. This can have an important relevance not only to understand the [inaudible 00:12:25] organization of the chromatin in the nucleus, but also to detect situations where distal sequences, the [inaudible 00:12:33] are contacting the promoter of other genes in not immediate proximity.

Notes

Summary

11m 56s