



In eukaryotic cells many processes take place in the context of chromatin structure. Changes in the chromatin structure induce the recruitment of the repair proteins upon DNA damage. DNA Double-Strand breaks occur frequently in the genome during replication or are caused by DNA damaging agents and affect genome stability resulting in translocations that can lead to cancer formation. In eukaryotic cells the repair of broken DNA occurs in the context of highly structured chromatin. Thus, alterations of chromatin structure are thought to influence not only the efficiency and kinetics of repair but also the choice of the repair pathway in cells. In response to DNA damage, chromatin undergoes rapid local and global decondensation, a process that has been proposed to facilitate genome surveillance by enhancing the access of DNA damage response (DDR) proteins to sites of damage. Inaccurate repair of DNA caused by chromatin structure alterations or the absences of repair factors leads to mutations, chromosomal rearrangements and alterations of gene expression, in turn, these can lead to cancer and aging. This underlines the importance of DDR studies in the context of chromatin for the identification of novel potential therapeutic targets in cancer treatment. Our experimental system in *Drosophila* allows a unique setup to study the changes in chromatin marks during and after the repair. A powerful model for studying these aspects of chromatin structure changes has not been established in any other experimental systems until now. A better understanding of DSB repair mechanism can lead to identification of novel potential therapeutic targets in cancer treatment.

The PHCVA plasmid contains a 95 bp synthetic oligonucleotide, called 2AB, which contains the cleavage sites of six restriction endonucleases (*SmaI*, *EcoRI*, *NcoI*, *Sall*, *XhoI*, *NotI*) and the coding region of hemagglutinin epitope (HA).

In addition to the acceptor plasmid, PHCVD includes the 31 bp length synthetic oligonucleotide, called oligo 1. The oligo 1 consists of six restriction endonucleases (*SmaI*, *EcoRI*, *NcoI*, *Sall*, *XhoI*, *KpnI*) restriction sites .

The figure shows the mutated amino acid positions created by *in vitro* mutagenesis. We have designed 14 mutation primers in different regions of H2A gene. With the help of mutation primers, a single amino acid is exchanged in the histone protein. Depending on the post-translational modifications of the amino acids, the mutations could mimic or erase the possible PTMs.

According to our cloning strategy, the final version of PHCVA contains a single PTM mutant H2A in three copies. In this process the PHCVD and PHCVA help to each other. First, the given histone gene is cleaved from the PHCVD, then it has two compatible ends, so its ends could easily be ligated with the PHCVA, which is digested by the same restriction endonuclease. For this, the PHCVD contains two copies of the restriction sites in tandem orientation. The histone genes tagged with hemagglutinin epitope will be expressed as a fusion protein in the animals.

For DSB induction we plan to use the I-PpoI homing endonuclease based system. In our case the I-PpoI restriction endonuclease is fused to the hormone binding domain of estrogen receptor and tagged by hemagglutinin epitope. By adding 4OH₂ (estrogen analog) the nuclear translocation of I-PpoI-ER can be observed. Then, the I-PpoI could cleave the DNA in the nucleus.

In *Drosophila melanogaster* the histone genes (H1, H2A, H2B, H3 and H4) are located at a single genomic locus and approximately 100 copies of the histone cluster is presented in the genome. To generate a full copy of histone cluster we amplified this region by the help of polymerase chain reaction. To amplify the histone cluster, we used several strategies. First, we tried to amplify the entire locus in one part but despite all of these optimizing steps the histone cluster was not amplified in one PCR product. A new plan has been prepared considering the length of the repetitive sequence before the first copy of the cluster.

Four primer pairs were designed to produce overlapping PCR products containing all the histone genes. In that way, the histone cluster can be rebuilt from the PCR products by BamHI, SpeI, NcoI and EcoRI, considering the recognition sites of specific restriction endonucleases. After the polymerase chain reaction, the appropriate fragments were isolated from the agarose gel and ligated in pJET 1.2 cloning vector. DH5 α *E. coli* cells were used for the transformation. After the plasmid preparation, BglII endonuclease was used for the test digestion.

By using this strategy, we could amplify the four overlapping DNA fragments. (Figure 7, white stars). To make sure that there is no amino acid change or frame shift mutation in the insert sequence the DNA fragments are sequenced and proved to be error free. Our further aim is to rebuild the histone cluster with specific endonucleases and generate transgenic animals.

To reconstitute the histone PTM-containing histone cluster, we plan to delete the endogenous histones. As a following step, we reconstitute the histones by using the acceptor vector. It will allow the integration of the PTM mutant histone genes into the genome. Thus, the mutant histone proteins appear in the *Drosophila*'s nucleosomes.

This work was supported by „Kutatóegyetemi és Kiválósági EMMI támogatás” [29-39-0T147] and OTKA-PD [112115]. Furthermore this project was supported by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences.

