

3D-DIP-Chip: a Microarray-Based Method to Measure Genomic DNA Damage

Application Note

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Abstract

DNA damage detection by DNA ImmunoPrecipitation on microarrays (3D-DIP-ChIP) is a microarray-based method for the genome-wide analysis of DNA damage and repair characteristics. This Application Note describes a novel application of microarray technology involving the affinity capture of damaged DNA with 3D-DIP-Chip in cells treated with UV radiation and cisplatin. The method is applicable to the analysis of any genetic lesion that can be captured and separated from undamaged forms of DNA. We analyzed our data using novel associated bioinformatic software written in A Language and Environment for Statistical Computing (R).

Introduction

Exposure to DNA damage-inducing genotoxins is associated with genomic instability. The resulting DNA damage, if left unrepaired, produces diverse genetic mutations with far reaching consequences, causing many genetic aberrations that are linked to diseases including cancer. A variety of physical and chemical agents can be genotoxic, including radiations and chemicals found naturally in the environment, as well as man-made forms created by human activity across a range of industries. All newly-developed materials, therefore, are tested for their potential genotoxic effects. Recent advances in genomic technologies have enabled the genome-wide analysis of the effects of genotoxins, including damage-induced changes in the transcriptome, proteome, and metabolome. These approaches are enhancing our ability to test the safety of novel compounds¹. In addition, advances in sequencing technologies are enabling genome-wide mutation analyses and the sequencing of individual cancer genomes has revealed the presence of mutational signatures embedded within cancer cells². These signatures represent the product of the exposure of an individual to various types of genotoxin during their lifetime, and the ability of the cells of the individual to remove the damage from the genome, a process called DNA repair.



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These studies are revolutionizing the way we view the impact of natural and man-made products on the development and treatment of disease in individuals, offering great potential for personalized medicine in the future. For this reason, we have developed a sensitive DIP-chip-based method to measure the location and level of genotoxin-induced DNA damage throughout the genome³. Additionally, we have developed an R⁴ package for the visualization of this data. Combining these methods with standard ChIP-chip to measure DNA repair factor binding facilitates the elucidation of the underlying mechanisms of genotoxicity and genome stability, providing a systems view of these. We believe the method could be useful in a variety of applications in both basic and translational science.

Experimental workflow

Figure 1 shows the 3D-DIP-chip procedure. Damaged DNA or crosslinked chromatin (1A) is extracted from cells and fragmented by sonication to a fragment length appropriate to the microarray being used. DNA fragments of interest are captured by immunoprecipitation (IP) using magnetic beads and the appropriate antibody raised against either the specific type of DNA damage or chromatin binding protein of interest (1B). Following IP, damages or crosslinks are reversed, and samples are assessed by RT-PCR for quality control purposes. DNA is then amplified using either a proprietary whole genome amplification method or ligation-mediated PCR. IP samples and separate input samples are differentially labeled using the Agilent SureTag DNA labeling Kit (1C). Samples are then hybridized to the microarray using an Agilent ChIP on chip hybridization kit and the Agilent Hybridization chamber and Hybridization oven, followed by washing and drying with the Agilent ChIP on chip Wash Buffer Kit and Stabilization and drying solution (1D). The microarray is then scanned as described in the Agilent mammalian ChIP on chip protocol (http://www.genomics.agilent.com/files/Manual/G4481-90010_MammalianProtocol_10.11.pdf) using the Agilent Microarray Scanner Bundle. Information is then acquired using Feature Extraction software to produce data, which can be analyzed and plotted using the tools in the R software package Sandcastle (1E).

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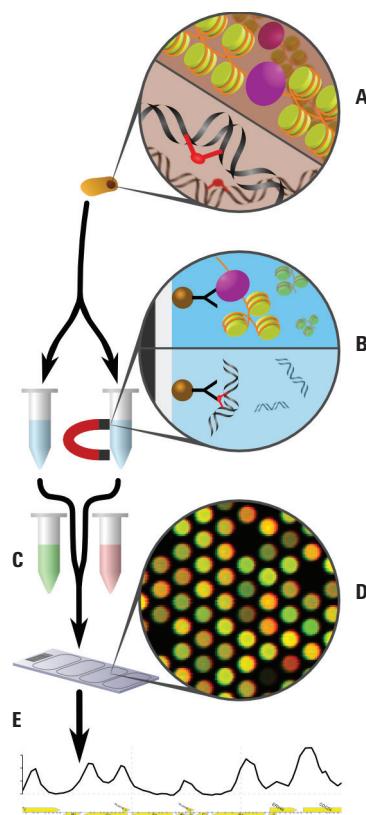


Figure 1. Representation of the 3D-DIP-Chip and ChIP-Chip procedure. A) Proteins are crosslinked to chromatin (top) or DNA damage is induced (bottom). This DNA or chromatin is extracted, sonicated, and split into two samples. B) IP is carried out on one sample to separate out the chromatin bound to the factor of interest (top) or damaged DNA (bottom). C) Both samples are purified, amplified by PCR, and differentially labeled. D) The samples are allowed to hybridize to the microarray probes and the resulting intensity values from the scanned image are converted to numerical values. E) These values may be plotted and processed as required by the investigation.

Microarray processing and data analysis

To determine the relative levels and locations of DNA damage, we examined human and yeast derived samples, which were hybridized to two-color Agilent 4 × 44 K microarrays, either a custom human design covering 10 Mbp of chromosome 17, or a yeast whole genome array G4493A, respectively. Log2 IP:IN ratio values are used for all analyses, whereby the input channel (IN) corrects for relative differences in DNA amounts, producing relative levels of DNA adducts detected at the genomic locations represented by the probes/features printed on the microarray.

Data from files created by the Agilent Feature Extraction Software⁷ were loaded into the Sandcastle package in R, described in detail in our accompanying paper. The average of two *in vivo* human cisplatin-treated datasets is shown as a Circos plot in Figure 2A, demonstrating the heterogeneity of the DNA damage distribution over the 10 Mbp genome section analyzed. A 25 Kbp section of the data is shown in more detail in 2B, showing the mean and standard error of the mean for the two datasets. Overall reproducibility is also shown as a scatter plot of one dataset against the other for the total cisplatin (2C) and oxaliplatin (2D) data.

We have measured genomic DNA damage profiles for two different forms of DNA damage in human DNA; chemically (platinum) induced lesions and UV radiation induced pyrimidine dimers (Figures 2 and 3). Genetic damage also induces epigenetic changes in the form of histone modifications^{5,6}. These are known to affect both induction of DNA damage and its removal by DNA repair. To investigate how these two parameters relate to each other in response to genotoxic exposure, we treated yeast cells with the same two classes of genotoxins as above. In addition to measuring the genetic damage, we

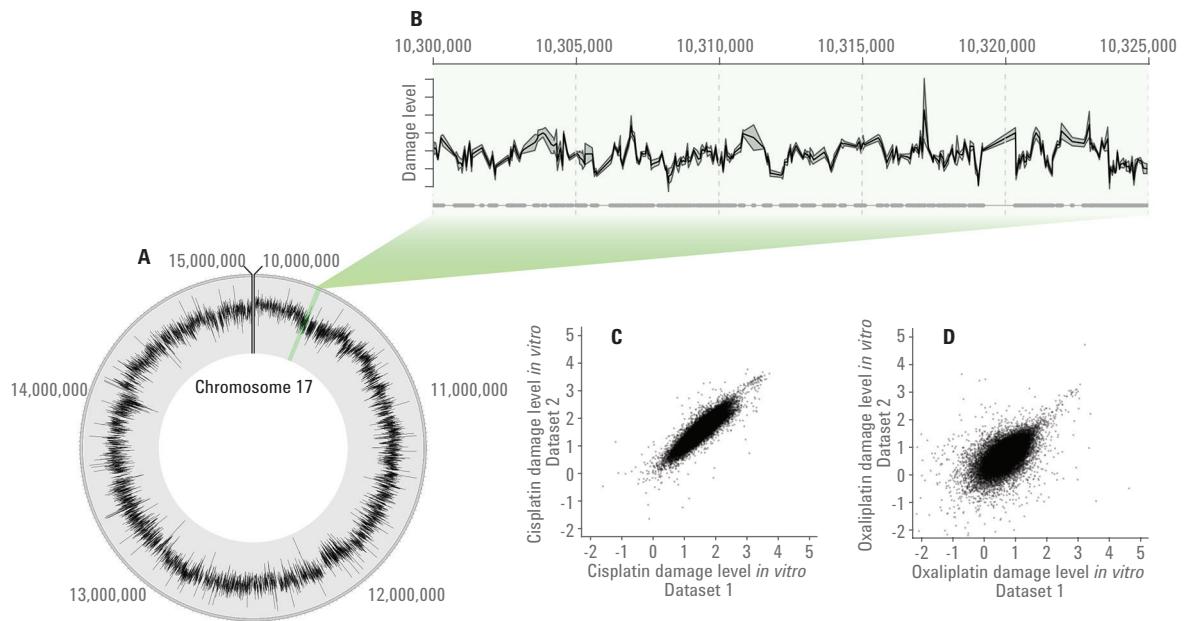


Figure 2. Human *in vivo* platinating agent damage. A) Circos plot of the whole dataset demonstrating the heterogeneity of the damage pattern. B) 25 Kbp section of data (produced in Sandcastle), showing the mean (black line) and standard error (grey shading) of two datasets and probe positions (grey dots). C and D) Scatter plots showing the relationship between cisplatin and oxaliplatin repeat datasets, respectively (plot D has been scaled to the same axis limits as plot C, resulting in a small number of probes not being shown).

employed ChIP-chip to measure DNA damage-induced changes in histone H3 acetylation (H3Ac) at lysine 9 (K9), which is known to be required for the efficient repair of UV-induced CPDs (Figure 3).

Bioinformatic analysis of genetic and epigenetic genome-wide datasets

We have developed a way of analyzing genomic DNA damage data and integrating this information with epigenetic data measuring post-translational modifications to histones. Figure 3 demonstrates how it is possible to measure the distribution of genetic damage induced in the genome by exposing yeast cells either to UV light or to treatment with the chemotherapy drug cisplatin. A scatter plot of the UV-induced versus the cisplatin-induced lesions reveals an inverse relationship (Figure 3A), which reflects the fact that

UV-induced damage is primarily induced at dipyrimidine sites in the DNA, whereas cisplatin damage is primarily induced at GG purine sites. Indeed, by analyzing and plotting the same data in relation to transcriptional start sites (Figure 3B) it is possible to visualize reciprocal patterns of cisplatin damage (solid black line) versus UV-induced damage (solid green line). Based on previous studies to measure frequency and sequence specificity of UV and cisplatin-induced DNA damage in treated DNA samples, it is possible to generate a predicted pattern of genomic DNA damage. By plotting the predicted damage spectrum for both cisplatin-induced (dashed black line) and UV-induced (dashed green line) DNA damage, it can be seen that the actual DNA damage profile generally follows the predicted pattern. Intriguingly, regions where these patterns deviate from the prediction can be observed, particularly in the promoter regions of genes upstream

of the transcriptional start site. This is particularly evident in the case of cisplatin damage, where higher levels of damage are observed in the promoter regions of genes than would be expected based on the predicted pattern.

Analysis of the spectrum of damage-induced histone acetylation reveals a positive association between the UV versus the cisplatin-induced damage (Figure 3C), which is confirmed when plotting the same data in relation to transcription start sites (Figure 3D). This indicates that the induction of histone H3 acetylation, which is known to be important for the response to and repair of DNA damage in chromatin, appears to be very similar, regardless of the individual patterns of DNA damage induced or whether a physical or chemical DNA damaging agent is used.

Conclusion

We have described a patented method for measuring genomic DNA damage on microarrays. This DIP-chip assay is available for use in human cells to measure different types of DNA damage with sensitivity and at a high resolution, representing a significant technological advance in the measurement of genetic damage at a genomic scale. This technology offers a novel way to examine genomic DNA damage in human and other cells. This, allied to the novel bioinformatic methods described, offers a functional assay capable of examining the DNA-damaging lesions directly with significant potential relevance in the fields of genotoxicity testing, translational and personalised medicine, as well as basic mechanistic laboratory studies investigating DNA damage and repair.

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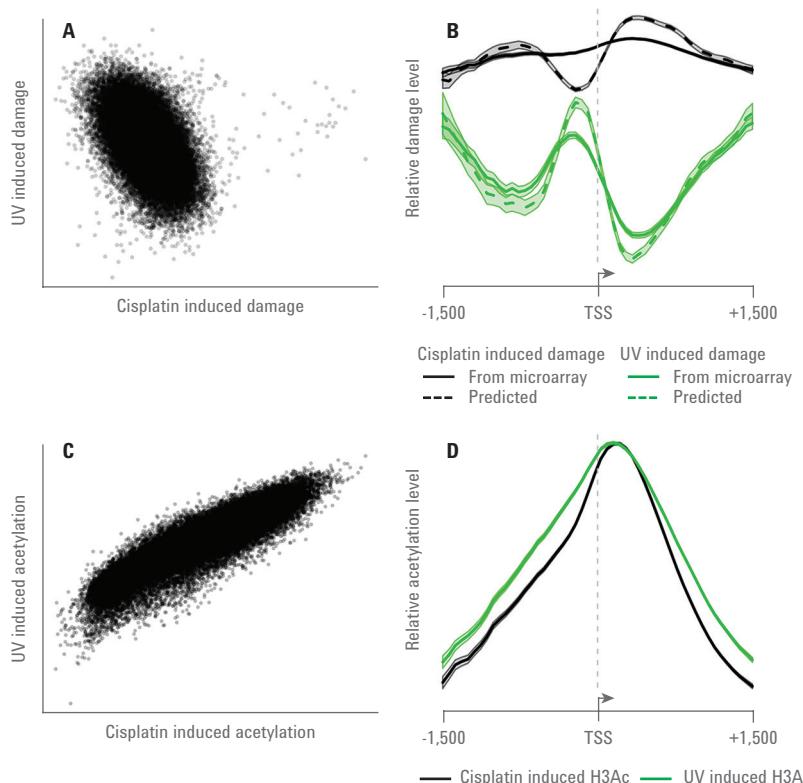


Figure 3. Examples of the types of analyses that may be undertaken with 3D-Chip and ChIP-chip. Scatter plots show an inverse association between cisplatin-induced and UV-induced DNA damage (A) but a positive association between cisplatin-induced and UV-induced histone acetylation (C). Plotting the data around transcription start sites (TSSs) shows different patterns of damage induction with the two damaging agents (B) (UV solid green line, cisplatin solid black line) along with similar predicted patterns (UV dashed green line, cisplatin dashed black line). Standard errors for all TSSs are shown as a shaded region. Histone acetylation around the same TSSs shows similar patterns with both damaging agents (D) (same coloring as B).

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