Development of Chromatin Immunoprecipitation Microarray Technology for the Identification of Regulatory Elements in the Human Genome

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Disclaimer

This dissertation is the result of my own work and includes nothing which is the outcome of work done in collaboration, except where specifically indicated in the text.

This dissertation does not exceed the word limit prescribed by the Biology Degree Committee.

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Abstract

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The recent development of methods which use chromatin immunoprecipitation in combination with genomic microarrays (ChIP-chip) have transformed the way in which the dynamics of chromatin and the regulation of gene expression are studied genome-wide. The aim of this thesis was to use both conventional and improved ChIP-chip approaches to characterize a variety of regulatory elements (promoters, enhancers, and putative insulators) across selected regions of the human genome in a number of cell types. Firstly, histone modifications which define promoter and enhancer elements have been used to map and characterise these elements in the K562 cell line. In parallel, the transcription factor CTCF and its known binding partners USF1, USF2, and mSin3a have been used to characterize putative insulator element. The findings of this work are discussed. Secondly, whilst ChIP-chip assays have been successfully used to map numerous DNA-protein interactions, there are limitations restricting their use in the study of cell populations that are rare and/or of limited availability. Therefore, this thesis describes the development of ChIP-chip assays that allow as few as 10,000 cells to be used per ChIP condition. These experiments do not necessitate the need to perform the ChIP reactions in the presence of carrier chromatin or the need to amplify the ChIP material prior to hybridization onto the microarray. The distribution of histone methyl and acetyl modifications with material derived from 10 000 cells was detected with a similar efficiency as that obtained from more conventional ChIP-chip approaches. This method has been applied in the study of a range of histone modifications across regions of the human genome using limited numbers of human monocytes and human embryonic stem cells. The results presented in this thesis demonstrate that developments in ChIP-chip technology can be used to accelerate our understanding of the general principles of gene regulation in the human genome.

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