MASSEY UNIVERSITY

An investigation into the application of microfluidics to the analysis of chromosome conformation

A thesis presented in partial fulfilment of the requirements for the degree of

Master of Science

in

Molecular Bioscience

at Massey University, Albany,
New Zealand.

Steven Chun-Wei Chou

2011

Abstract

Ever since the discovery of DNA, biologists have been striving to unravel its mysteries. Many efforts have been made over the years to further our understanding of genes, what they do and how they function. Genomes exist as a 3D structure inside the nucleus and they are not randomly arranged. However, there are still many gaps in the knowledge of how the structure fills this 3D space. Using chromosome conformation capture (3C) and other methods based on proximity ligation, interactions between different sections on the chromosome can be captured. A computer simulated 3D chromosome model can then be created based on the interaction data. Currently, global interaction maps can only be created for populations of cells. The overall goal of this research is to develop a protocol that will enable the capture of chromosome interactions within a single cell. This requires the use of microfluidic chips due to the minute quantity of DNA within a single cell. Therefore the main objectives of this research are to: 1) build and test a microfluidic system (lab-on-a-chip or LOC) that will aid in the capture of inter- and intra- chromosomal interactions of a single cell; and 2) characterize the restriction and ligation of DNA that will be performed in a microfluidic system.

In order to assess the efficiency of DNA digestion within microfluidic chips, EcoRI and MspI digestion kinetics within microtubes is first characterized to establish a base line for comparison with digestion kinetics within microfluidic chips. The $K_{m,}$ V_{max} and K_{cat} for EcoRI within microtubes are 32 nM, 0.14 nM s^{-1} and 1.4 fmol s^{-1} U^{-1} respectively. The K_m, V_{max} and K_{cat} for MspI within microtubes are 125 nM, 1.46 nM s^{-1} and 29.2 fmol s^{-1} U^{-1} respectively.

On the other hand, the digestion kinetics within microfluidic chips is undetermined, because both restriction enzymes exhibit non-specific nuclease activity within microfluidic chips under the conditions tested. The exhibition of non-specific nuclease activity is unexpected and causes ligation of DNA performed in microfluidic chips to fail. The non-specific nuclease activity of EcoRI and MspI within microfluidic chips is also problematic for the overall goal of developing a protocol that will enable the capture of chromosome interactions within a single cell, because the non-specific nuclease activity would cause loss of template and random variations in results obtained.

Acknowledgements

I would like to thank Justin O'Sullivan and John Harrison for the guidance provided and patience shown throughout this project.

I would also like to thank Chris Rodley for providing most of the primer sequences used in this project.

Table of content

List of Figures and Tables

List of abbreviations

[S]	Substrate concentration
3C	Chromosome conformation capture
3D	Three dimensional
4C	3C-on-chip or open-ended 3C, or 'olfactory receptor' 3C
5C	Chromosome conformation capture carbon copy
AB	Amplification bias
ADO	Allele drop out
bp	Base pair
BSA	Bovine serum albumin
FACS	Fluorescence-activated cell sorting
GCC	Genome conformation capture
LOC	Lab-on-a-chip
MDA	Multiple displacement amplification
Microtube	1.7ml Microfuge tube
PA	Preferential amplification
PCR	Polymerase chain reaction
PDMS	Poly-dimethylsiloxane
PGD	preimplantation genetic diagnosis
qPCR	Quantitative polymerase chain reaction or real-time polymerase chain reaction
SEM	Scanning electron microscope
VI	Virtual instrument
WGA	Whole genome amplification