

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.



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

Abstract	ii
Acknowledgement	iii
Table of content	iv
List of Figures and Tables	vii
List of abbreviations	x
1. Introduction	p.1
1.1. Methods of analyzing chromosome interactions	p.1
1.2. Background information on restriction enzymes used	p.8
1.3. Isolating single cells	p.10
1.4. Whole genome amplification	p.12
1.5. Microfluidic chips	p.16
1.6. Mapping chromosomal interactions within a single cell	p.22
2. Equipment	p.24
2.1. Microfluidic chips	p.24
2.2. Syringes	p.29
2.3. Syringe control	p.29
2.4. Protocols for testing lab-on-a-chip	p.30
2.4.1. Testing for leakages	p.30
2.4.2. Characterization of microfluidic chip	p.31
2.4.2.1. Phenol red indicator test	p.31
2.4.2.2. Blue Dextran test	p.31
2.4.2.3. Using a gas bubble to stop expansion of blue Dextran	p.32
3. Materials and methods	p.34
3.1. Enzymes and buffers	p.34

3.2. Primers	p.35
3.3. Polymerase chain reaction (PCR)	p.36
3.4. Restriction enzyme digestion in microtubes	p.39
3.5. Restriction enzyme digestion in microreactor chips	p.39
3.6. EcoRI digestion kinetics within microtubes	p.40
3.7. MspI digestion kinetics within microtubes	p.40
3.8. EcoRI digestion time course in micromixer chips	p.41
3.9. EcoRI digestion kinetics in micromixer chips	p.42
3.10. MspI digestion kinetics within micromixer chips	p.42
3.11. Determination of non-specific activity	p.43
3.12. Restriction enzyme digestion in microtubes with glass beads	p.44
3.13. Ligation of EcoRI or MspI digested template by T4 DNA ligase	p.45
4. Results	p.46
4.1. Preliminary comparison between MspI digestion in microfluidic chip and MspI digestion in microtube.	p.46
4.2. Preliminary comparison between EcoRI digestion in microfluidic chip and EcoRI digestion in microtube.	p.48
4.3. EcoRI kinetics within microtubes	p.50
4.4. EcoRI kinetics within microfluidic chips	p.52
4.5. MspI enzyme kinetics within microtubes	p.53
4.6. MspI kinetics within microfluidic chips	p.55
4.7. EcoRI and MspI exhibit non-specific nuclease activity within microfluidic chips	p.56
4.8. Ligation in lab-on-a-chip	p.60

5. Discussion	p.64
5.1. Restriction enzyme digestion	p.65
5.1.1. EcoRI kinetics within microtubes	p.66
5.1.2. MspI kinetics within microtubes	p.67
5.1.3. Non-specific nuclease activity within microfluidic chips	p.67
5.1.4. Implications of non-specific activity of EcoRI and MspI within microfluidic chips	p.72
5.2. Ligation by T4 DNA ligase	p.72
5.3. Single cell isolation	p.73
6. Conclusion and future work	p.75
Appendices	p.77
Appendix A. Block diagram of VI for activation of pumps.	p.77
Appendix B. Core block diagram of VI to change pump settings.	p.78
References	p.79

List of Figures and Tables

Figure 1	Schematic of the chromosome conformation capture (3C) methodology.	p.2
Figure 2	Comparison of various 4C methodologies.	p.3
Figure 3	Genome conformation capture (GCC) methodology.	p.4
Figure 4	The Duan modified Hi-C method.	p.5
Figure 5	Structure of EcoRI and EcoRV branches of Type II restriction enzymes.	p.10
Figure 6	Scanning electron microscope (SEM) image of PDMS chip.	p.11
Figure 7	Mechanism of chimera formation of phage polymerase phi 29.	p.16
Figure 8	Microfluidic chips used in this project.	p.17
Figure 9	Disposable microfluidic device.	p.18
Figure 10	Photograph of a single cell isolation and genome amplification chip that can run up to 9 samples in parallel.	p.19
Figure 11	Schematics of a cross chip connected to electrospray orifice.	p.20
Figure 12	A schematic of a simple one channel microfluidic chip.	p.21
Figure 13	An example of a more complex microfluidic chip design.	p.21
Figure 14	Diagram illustrating the modification of the Teflon capillary ends.	p.24
Figure 15	Schematics of chip holders.	p.25

Figure 16	Assembly of chip holder with dimensions for top and base padding.	p.26
Figure 17	Schematic of reaction-mixing-reaction chip series (R-M-R series).	p.27
Figure 18	Schematic of two-chip setup.	p.27
Figure 19	Schematic of single chip setup.	p.28
Table 1	Syringes used in this study.	p.29
Table 2	Volume taken up by blue Dextran through R-M-R chip series	p.32
Table 3	Table of primers used in this study.	p.35
Table 4	Reagents for end point PCR reaction.	p.36
Table 5	Reagents for qPCR reaction.	p.36
Figure 20	MspI digestion is more efficient in microfluidic chip than in microtubes.	p.47
Figure 21	EcoRI digestion is more efficient in a microfluidic chip than in microtubes.	p.49
Figure 22	EcoRI template concentration and initial rate of digestion was used to estimate EcoRI kinetics in a microtube.	p.51
Figure 23	The parameters for assaying the initial rate of EcoRI digestion were not found.	p.52
Table 6	The initial rate of EcoRI digestion was not measured for digestion within single micromixer chip	p.53
Figure 24	Substrate concentration and initial rate of digestion were used to estimate MspI kinetics in microtube.	p.54

Table 7	The initial rate of MspI digestion was not measured for digestion within single micromixer chip	p.55
Figure 25	EcoRI exhibited non-specific nuclease activity when acting within the micromixer chip.	p.57
Figure 26	MspI exhibited non-specific nuclease activity when acting within the micromixer chip.	p.59
Figure 27	No non-specific nuclease activity was observed when EcoRI or MspI was incubated with glass beads.	p.60
Figure 28	Ligation within the microfluidic chip was not as efficient as ligation within the microtube.	p.61
Figure 29	Concentrations of re-ligated products were affected by the stringency of the EcoRI inactivation steps for ligation performed in microtubes but not in the microreactor chip.	p.63

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