Solvent-Resistant Elastomeric Microfluidic Devices and Applications

Thesis by

Robert Michael van Dam

In Partial Fulfillment of the Requirements

for the Degree of

Doctor of Philosophy

California Institute of Technology

Pasadena, California

2005

(Defended August 30, 2005)
For my parents, Laurie and Erling, for their love, support, guidance, inspiration, encouragement, and the values they have taught, and for instilling in me a drive to “do my best” in whatever I choose to do.
Acknowledgements

This work would not have been possible without the help and support of many individuals.

First, I would like to thank my advisor, Steve Quake, for guidance, support, and ideas, and for the opportunity to work in an exciting multi-disciplinary lab on a variety of interesting projects. The past several years have enabled me to grow as a scientist, to explore several new fields of study, and to participate in many interesting collaborations. Steve’s teaching style, approach to problem solving, and focus on the “big picture” have all been inspirations throughout my time at Caltech. I would also like to thank the other members of my committee—Marc Bockrath, Bob Grubbs, and Rob Phillips—for insightful questions and suggestions at my candidacy and thesis defense. Financial support for my work was generously provided the Caltech Applied Physics department, NSERC (Natural Sciences and Engineering Research Council of Canada), the Whittier Foundation, the Norton Simon Foundation, and by the NSF (National Science Foundation) through several programs: NanoCEMMS, MRSEC, and PREM.

During my graduate program, I have had the good fortune of working closely with many truly gifted and dedicated individuals: Julius Su, Jim Brody, Todd Thorsen, Tony Stephen, Jason Rolland, Saurabh Vyawahare, Isba Silba, George Maltezos, Rebecca Shafee, Mike Toepke, Arkadij Elizarov, and Young Shik Shin. Their tremendous contributions to this work are recognized at the close of each chapter.

I am indebted to my colleagues in Steve’s lab who have helped me to complete my research by taking the time to share their lab expertise or to engage in interesting discussions and brainstorms. They have helped to make grad student life more enjoyable and some have become good friends. Thank you to Saurabh Vyawahare, Todd Thorsen, Robert Bao, Sebastian Maerkl, Heun Jin Lee,
Marc Unger, Markus Enzelberger, Josh Marcus, Carl Hansen, Guillaume Lessard, Charles Spence, Jong Wook Hong, Jordan Gerton, Vincent Studer, Alex Groisman, Ido Braslavsky, Michael Diehl, Emil Kartalov, Mark Adams, Matt Reese, Chris Lacenere, Megan Anderson, Frank Lee, Jian Liu, Larry Wade, Anne Fu-Brody, Hou-Pu Chou, Rafael Gómez-Sjöberg, Frederick Balagadde, Jerrod Schwartz, Scott Driggs, Luigi Warren, Alejandra Torres, Christina Morales, and others. I was also fortunate to have had the support and friendship of many people outside the lab over the years. Thanks especially to Stephanie Chow, Mike Fleming, Ling Lin, and Don Morgan, as well as members of the Caltech Shotokan karate club.

I am grateful to Barbara Wold and Brian Williams for research guidance during my first couple of years, for instruction in all aspects of microarray analysis, and for providing access to equipment and resources needed for several critical experiments. Many thanks also to Ali Ghaffari for training in numerous microfabrication procedures, for interesting discussions, and for keeping the equipment running smoothly. I am also very grateful to Connie Rodriguez for help with countless administrative tasks, for advice, for the push to get my thesis finished, and for providing food at group meetings, mini celebrations, and in the bottomless cookie-tin in her office. I would also like to thank Irene Loera for assistance with purchases.

I would like to acknowledge Steve Quake, Julie Matrat, and the Caltech proofreader for valuable feedback, comments, and corrections on my manuscript. Robert Bao, Heun Jin Lee, and Julie Matrat provided helpful comments to improve my defense presentation. Thanks also to my new boss, Jim Heath, and colleague, Arkadij Elizarov, for their understanding of the delay in my start date as a result of my thesis taking longer to write than anticipated.

Finally, but most importantly, I would like to extend my deepest thanks to my parents, my brother Mark, and the rest of my family for their constant support, encouragement, and confidence in me throughout my life, and to Julie Matrat, for her tremendous love and support during the past two years—especially during the marathon writing of this thesis—and for reminding me what is truly important in life.
Abstract

Microfluidics is increasingly being used in many areas of biotechnology and chemistry to achieve reduced reagent volumes, improved performance, integration, and parallelism, among other advantages. Though early devices were based on rigid materials such as glass and silicon, elastomeric materials such as polydimethylsiloxane (PDMS) are rapidly emerging as a ubiquitous platform for applications in biotechnology. This is due, in part, to simpler fabrication procedures and to the ability to integrate mechanical microvalves at vastly greater densities. For many applications in the areas of chemical synthesis and analysis, however, PDMS cannot replace glass and silicon due to its incompatibility with many solvents and reagents.

Such areas could benefit tremendously from the development of an elastomeric microfluidic device technology that combines the advantages of PDMS with the property of solvent resistance. Simplified fabrication could increase the accessibility of microfluidics, and the possibility of dense valve integration could lead to significant advances in device sophistication. Applications could be more rapidly developed by design re-use due to the independence of mechanical valves on fluid properties (unlike electrokinetic pumping), and the property of permeability could enable novel fluidic functions for accessing a broader range of reactions than is possible in glass and silicon.

The first half of this thesis describes our strategies and efforts to develop this new enabling technology. Several approaches are presented in Chapter 3, and two particularly successful ones, based on new elastomers (FNB and PFPE), are described in Chapters 4 and 5. Chapter 6 describes a novel method of fabricating devices from 3D molds that could expand the range of useful elastomers.

The second half of this thesis discusses microfluidic combinatorial synthesis and high throughput screening—applications that take particular advantage of the ability to integrate thousands of
individual valves and reaction chambers. Chapter 7 introduces several scalable device architectures
and presents results of preliminary steps toward the synthesis of combinatorial DNA and peptide
arrays. A novel method of performing universal gene expression analysis with combinatorial DNA
arrays is described in Chapter 8 and an algorithm for predicting relationships among genes from
gene expression array data is presented in Chapter 9.
# Contents

Acknowledgements iv

Abstract vi

1 Introduction 1
  1.1 Background ....................................................... 1
  1.2 Organization ..................................................... 3
  1.3 Contributions ................................................... 4

2 Introduction to Microfluidics 7
  2.1 Introduction ....................................................... 7
  2.2 Microfluidics ...................................................... 8
    2.2.1 Benefits of size reduction .................................. 8
    2.2.2 Benefits of automation and integration ......................... 9
    2.2.3 Application areas ............................................ 10
  2.3 PDMS microfluidics .............................................. 12
    2.3.1 Elastomeric microvalves ..................................... 13
    2.3.2 Multilayer device fabrication ................................. 17
    2.3.3 Advantages of PDMS devices .................................. 20

3 Solvent-Resistant Microfluidics 24
  3.1 Introduction ..................................................... 24
  3.2 Prior work ...................................................... 25
3.3 Organic solvents and elastomers ........................................... 31
  3.3.1 Adverse interactions .................................................. 31
  3.3.2 The problem with PDMS .............................................. 32
  3.3.3 Alternative materials ............................................... 34

3.4 Solvent-resistant device principles ...................................... 38
  3.4.1 Two-layer architectures ............................................. 38
  3.4.2 Coated devices ....................................................... 38
  3.4.3 Membrane architecture .............................................. 40
  3.4.4 Summary ............................................................... 42

3.5 Research results .......................................................... 43
  3.5.1 Modified PDMS devices .............................................. 45
    3.5.1.1 Viton coating ................................................... 45
    3.5.1.2 CYTOP coating ................................................ 47
    3.5.1.3 Chemraz coating .............................................. 53
    3.5.1.4 Teflon AF coating ............................................ 53
    3.5.1.5 Parylene coating ............................................. 54
    3.5.1.6 Plastic coating ............................................... 54
    3.5.1.7 Metal coating ................................................ 54
    3.5.1.8 Teflon lubricant spray coating .............................. 55
    3.5.1.9 CF\textsubscript{4} plasma-treatment ......................... 55
    3.5.1.10 Fluorosilanization of PDMS surface ....................... 55
    3.5.1.11 Incorporation of fluorinated additives during polymerization .... 56
  3.5.2 Alternative elastomeric device materials .......................... 56
    3.5.2.1 SIFEL ............................................................ 57
    3.5.2.2 New materials for CLiPP synthesis .......................... 58
    3.5.2.3 Fluorosilicones ............................................... 59
    3.5.2.4 Other materials ............................................... 60
3.5.3 Membrane devices .................................................. 60
   3.5.3.1 Architecture validation with PDMS membrane .......... 61
   3.5.3.2 CYTOP-coated PDMS membrane .......................... 62
   3.5.3.3 PFPE membrane .............................................. 63
   3.5.3.4 Kalrez sheet .................................................. 63
   3.5.3.5 Chemraz sheet .............................................. 63
   3.5.3.6 Teflon PFA film ........................................... 64
   3.5.3.7 Teflon tape .................................................. 64

3.6 Summary ............................................................... 65

4 Solvent-Resistant Fluorinated-Norbornene (FNB) Microfluidic Devices 67
   4.1 Introduction ...................................................... 67
   4.2 Chemistry ........................................................ 68
   4.3 First-generation devices: CYTOP coating .................... 69
      4.3.1 Development of CYTOP coating procedure ............... 70
      4.3.2 Fabricating the thin (fluid) layer ....................... 74
      4.3.3 Fabricating the thick (control) layer ................... 75
      4.3.4 Bonding layers .............................................. 77
      4.3.5 Testing microfluidic devices ............................ 78
   4.4 Second-generation devices: FNB fluid layer ............... 79
      4.4.1 Solvent compatibility tests ............................... 82
      4.4.2 Device fabrication ....................................... 84
      4.4.3 Device testing ............................................ 86
   4.5 Third-generation devices: All FNB ............................ 92
      4.5.1 Towards repeatable layer thickness .................... 92
      4.5.2 Additional improvements ................................. 95
   4.6 Summary .......................................................... 96
5 Solvent-Resistant Perfluoropolyether (PFPE) Microfluidic Devices

5.1 Introduction .......................................................... 98

5.2 Proof of principle ...................................................... 100
  5.2.1 Materials synthesis and characterization ....................... 100
  5.2.2 Device fabrication .............................................. 102
  5.2.3 Methods ......................................................... 104
    5.2.3.1 Materials ............................................... 104
    5.2.3.2 Preparation of PFPE DMA ............................... 109
    5.2.3.3 Photocuring of PFPE DMA ............................... 109
    5.2.3.4 Device fabrication with PFPE DMA ....................... 109
    5.2.3.5 Swelling experiments ................................... 110
    5.2.3.6 Rheometry ............................................... 110
    5.2.3.7 Dynamic mechanical analysis ............................ 111
    5.2.3.8 Dynamic mechanical thermal analysis ................... 111
    5.2.3.9 Contact angle measurements ............................. 112

5.3 Improvements in mechanical properties .......................... 112

5.4 Improvements in device bonding ................................ 112
  5.4.1 First-generation PFPE ....................................... 113
    5.4.1.1 Layer bonding .......................................... 113
    5.4.1.2 Substrate bonding ....................................... 116
  5.4.2 Second-generation PFPE ..................................... 116
    5.4.2.1 Layer bonding .......................................... 116
    5.4.2.2 Substrate bonding ....................................... 117
  5.4.3 Third-generation PFPE ....................................... 118
  5.4.4 Fourth-generation PFPE ..................................... 121

5.5 Summary ............................................................ 122
### 6 3D-Molding of Microfluidic Devices

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.1 Introduction</td>
<td>123</td>
</tr>
<tr>
<td>6.2 Fabrication technologies for 3D microfluidics</td>
<td>124</td>
</tr>
<tr>
<td>6.2.1 Layered fabrication</td>
<td>125</td>
</tr>
<tr>
<td>6.2.2 Direct 3D fabrication</td>
<td>128</td>
</tr>
<tr>
<td>6.2.2.1 Stereolithography</td>
<td>129</td>
</tr>
<tr>
<td>6.2.2.2 Micromachining and photolithography</td>
<td>132</td>
</tr>
<tr>
<td>6.2.3 3D molding</td>
<td>135</td>
</tr>
<tr>
<td>6.3 Device fabrication from 3D wax molds</td>
<td>138</td>
</tr>
<tr>
<td>6.3.1 Mold fabrication</td>
<td>138</td>
</tr>
<tr>
<td>6.3.2 Device fabrication</td>
<td>142</td>
</tr>
<tr>
<td>6.4 Results</td>
<td>143</td>
</tr>
<tr>
<td>6.4.1 Test patterns</td>
<td>145</td>
</tr>
<tr>
<td>6.4.2 Microvalves</td>
<td>147</td>
</tr>
<tr>
<td>6.4.2.1 Tube valve architecture</td>
<td>147</td>
</tr>
<tr>
<td>6.4.2.2 Crossed-channel valve architecture</td>
<td>148</td>
</tr>
<tr>
<td>6.4.3 Fully suspended structures</td>
<td>155</td>
</tr>
<tr>
<td>6.5 Discussion</td>
<td>158</td>
</tr>
<tr>
<td>6.5.1 Summary</td>
<td>158</td>
</tr>
<tr>
<td>6.5.2 The future of 3D fabrication</td>
<td>159</td>
</tr>
</tbody>
</table>

### 7 Microfluidic Combinatorial Chemistry

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.1 Introduction</td>
<td>164</td>
</tr>
<tr>
<td>7.2 Introduction to solid-phase synthesis</td>
<td>167</td>
</tr>
<tr>
<td>7.2.1 Cycle efficiency</td>
<td>169</td>
</tr>
<tr>
<td>7.2.2 DNA synthesis chemistry</td>
<td>170</td>
</tr>
<tr>
<td>7.2.3 Peptide synthesis chemistry</td>
<td>173</td>
</tr>
<tr>
<td>7.3 Synthesizing DNA and peptide arrays</td>
<td>176</td>
</tr>
</tbody>
</table>
7.3.1 Array replication .................................................. 178
7.3.2 Array fabrication by deposition ................................. 179
7.3.3 Ink-jet and robotic synthesis of arrays ....................... 183
7.3.4 Light-directed synthesis ........................................ 185
7.4 Microfluidic combinatorial synthesis ............................ 189
  7.4.1 Principle of operation ........................................ 189
  7.4.2 Microfluidic architecture ..................................... 192
  7.4.3 Individually-addressable arrays .............................. 197
  7.4.4 Related work .................................................... 200
  7.4.5 Advantages of microfluidic synthesis ....................... 201
7.5 DNA array synthesis .............................................. 202
  7.5.1 Early experiments .............................................. 202
  7.5.2 Hybridization optimization .................................. 205
  7.5.3 Surface derivatization ....................................... 207
  7.5.4 Synthesis on substrates with a DNA synthesizer ............ 208
  7.5.5 Millifluidic synthesis and detection ...................... 213
  7.5.6 From millifluidics to microfluidics ......................... 214
7.6 Peptide array synthesis ........................................... 214
  7.6.1 Manual synthesis .............................................. 216
  7.6.2 Millifluidic synthesis ....................................... 217
  7.6.3 Microfluidic devices ......................................... 221
7.7 On-bead array synthesis ........................................... 222
7.8 Summary ............................................................ 227

8 Universal Gene Expression Analysis with Combinatorial Arrays 229
  8.1 Introduction ........................................................ 229
  8.2 Results and discussion ......................................... 232
    8.2.1 Basic analytical model .................................... 232
8.2.2 Accounting for mismatches .............................................. 233
8.2.3 Truncation of transcripts ................................................. 237
8.2.4 Estimating $n$ .......................................................... 237
8.2.5 Redundancy .............................................................. 241
8.2.6 Conclusions .............................................................. 243

8.3 Methods ................................................................. 245
8.3.1 Mathematical analysis of gene expression ......................... 245
8.3.2 Source of sequence data .............................................. 246
8.3.3 Degeneracy calculations .............................................. 246
8.3.4 Predicting the fraction of solvable expression levels ........... 248

9 A Probabilistic Method for Determining Gene Relationships from Expression Data ......................................................... 249
9.1 Introduction .............................................................. 249
9.2 Analyzing expression data .............................................. 250
9.3 Guilt by Association ..................................................... 254
9.4 Extension of GBA to expression ratio data ....................... 258
  9.4.1 Algorithm .......................................................... 259
  9.4.2 Implementation .................................................... 261
  9.4.3 Combining datasets .............................................. 265
  9.4.4 Results .............................................................. 269
9.5 Related Work .......................................................... 270
9.6 Future Directions .......................................................... 271

A Methods ............................................................................ 273
A.1 Fabrication of microfluidic molds .................................... 273
  A.1.1 Photomask preparation ........................................... 273
  A.1.2 Mold patterning .................................................... 273
A.3 Polymer film protocols ...................................................... 278
  A.3.1 Measurement of polymer film thickness ......................... 278
  A.3.2 Calibrating spin curve for a new polymer ...................... 279

Bibliography ............................................................................. 280
# List of Figures

2.1 Schematic of two common PDMS microvalve architectures .................. 15  
2.2 Photograph of an elastomeric microfluidic valve .............................. 16  
2.3 Fabrication of 2-layer microfluidic devices by replication molding ........ 18  
3.1 Swelling of PDMS in various solvents ........................................ 33  
3.2 Exposure of device layers to solvents in different valve architectures .... 39  
3.3 Novel membrane architecture for crossed-channel microvalves .............. 41  
3.4 Viton-coated PDMS microfluidic device ....................................... 46  
3.5 Structure of CYTOP perfluoropolymer coating ................................ 47  
3.6 Microvalve actuation in solvent-resistant CYTOP-coated PDMS devices .... 50  
3.7 Effect of CYTOP coating on the swelling of PDMS in dichloromethane .... 52  
3.8 Chemical structure of SIFEL perfluoroelastomer .............................. 57  
4.1 Ring-opening metathesis polymerization (ROMP) ............................. 68  
4.2 Effect of dichloromethane exposure on surface profile of CYTOP-coated device 72  
4.3 Fabrication of thick device layers from Materia resin ....................... 76  
4.4 Schematic of fluid delivery jig .................................................. 80  
4.5 Fluid delivery jig used for testing early Materia devices .................... 81  
4.6 Sulphuric acid resistance of FNB ............................................ 84  
4.7 Schematic and photographs of NanoPort connectors .......................... 87  
4.8 Dichloromethane compatibility and partially closing valves in early FNB microfluidic device ....................................................... 89
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.9</td>
<td>Successful valve actuation in FNB device</td>
<td>90</td>
</tr>
<tr>
<td>4.10</td>
<td>Dead-end channel filling in FNB device</td>
<td>91</td>
</tr>
<tr>
<td>4.11</td>
<td>Viscosity profile of purified FNB</td>
<td>94</td>
</tr>
<tr>
<td>5.1</td>
<td>Synthesis and crosslinking of photocurable PFPEs</td>
<td>100</td>
</tr>
<tr>
<td>5.2</td>
<td>Dynamic mechanical thermal analysis of PDMS and PFPE</td>
<td>102</td>
</tr>
<tr>
<td>5.3</td>
<td>PFPE device fabrication procedure</td>
<td>103</td>
</tr>
<tr>
<td>5.4</td>
<td>PFPE microchannels are not swelled shut by solvents</td>
<td>104</td>
</tr>
<tr>
<td>5.5</td>
<td>Microvalve actuation in PFPE microchannels containing solvents</td>
<td>105</td>
</tr>
<tr>
<td>5.6</td>
<td>Extreme wetting leads to air bubbles in fluid channels</td>
<td>106</td>
</tr>
<tr>
<td>5.7</td>
<td>Valve test pattern used to evaluate PFPE valves</td>
<td>107</td>
</tr>
<tr>
<td>5.8</td>
<td>Design of a primitive combinatorial array synthesizer</td>
<td>108</td>
</tr>
<tr>
<td>5.9</td>
<td>Viscosity vs. shear rate for PFPE DMA and Sylgard 184 precursors</td>
<td>111</td>
</tr>
<tr>
<td>5.10</td>
<td>PFPE sealing of off-chip connections</td>
<td>113</td>
</tr>
<tr>
<td>5.11</td>
<td>Adjusting crosslinking density of PFPE</td>
<td>114</td>
</tr>
<tr>
<td>5.12</td>
<td>Chemical layer-bonding mechanism in second-generation PFPE</td>
<td>117</td>
</tr>
<tr>
<td>5.13</td>
<td>Delamination in third-generation PFPE devices</td>
<td>120</td>
</tr>
<tr>
<td>6.1</td>
<td>3D laminated ceramic microfluidic device</td>
<td>128</td>
</tr>
<tr>
<td>6.2</td>
<td>Schematic of stereolithography process</td>
<td>129</td>
</tr>
<tr>
<td>6.3</td>
<td>Solidscape T66 3D ink-jet printer</td>
<td>138</td>
</tr>
<tr>
<td>6.4</td>
<td>Fabrication of microfluidic devices from 3D wax molds</td>
<td>142</td>
</tr>
<tr>
<td>6.5</td>
<td>Fabrication defects during protocol development</td>
<td>144</td>
</tr>
<tr>
<td>6.6</td>
<td>Test of minimum vertical gap in wax molds</td>
<td>146</td>
</tr>
<tr>
<td>6.7</td>
<td>3D tube valve</td>
<td>148</td>
</tr>
<tr>
<td>6.8</td>
<td>Design and printed molds for crossed-channel valve tests</td>
<td>151</td>
</tr>
<tr>
<td>6.9</td>
<td>PDMS devices cast from 3D crossed-channel valve test mold</td>
<td>152</td>
</tr>
<tr>
<td>6.10</td>
<td>Design and testing of “H” valve</td>
<td>154</td>
</tr>
</tbody>
</table>
6.11 Microfluidic device for $4 \times 4$ combinatorial array synthesis ........................................ 156
6.12 Schematic of controlled control channel operation ..................................................... 161
7.1 Schematic of solid-phase synthesis .................................................................................. 168
7.2 Chemistry of DNA synthesis ......................................................................................... 171
7.3 Fmoc peptide synthesis chemistry .................................................................................. 175
7.4 Microfabricated stainless steel trench pens .................................................................... 180
7.5 Hybridization to an array printed by stainless steel trench pens ..................................... 181
7.6 Principle of in situ solid-phase synthesis by surface striping ......................................... 190
7.7 Pattern of nucleotide coupling steps to build all DNA 6-mers in 6 steps ......................... 191
7.8 Scheme for synthesizing all DNA 6-mers with passive microfluidic devices .................. 193
7.9 Switching the flow direction (row or column) in a grid of microchannels ....................... 195
7.10 Design of an active DNA 6-mer synthesis device ........................................................... 196
7.11 Design and operation of an individually-addressable microfluidic array synthesizer ....... 199
7.12 DNA sequences used for hybridization optimization ..................................................... 205
7.13 Silane linker for DNA synthesis ..................................................................................... 207
7.14 Beckman Coulter Oligo 1000M DNA synthesizer ........................................................... 210
7.15 Teflon flow cell for millifluidic solid-phase DNA synthesis .......................................... 211
7.16 DNA sequences used in millifluidic synthesis experiments ............................................ 212
7.17 Demonstration of hybridization specificity onto synthesized DNA stripes ..................... 213
7.18 Demonstration of DNA extension when intersecting stripes of DNA are synthesized ..... 215
7.19 Design of a solid-phase synthesis chip ......................................................................... 225
7.20 Details of solid-phase synthesis chip operation .............................................................. 226
8.1 Typical experimental setup for gene expression profiling ............................................. 230
8.2 Comparison of predicted and actual degeneracy histograms ........................................ 234
8.3 Comparison of predicted and actual average degeneracy ............................................. 238
8.4 Minimum degeneracy histograms for the mouse genome, assuming 1 mismatch .......... 240
8.5 Fraction of transcripts having minimum degeneracy equal to 1 . . . . . . . . . . . . . 242
8.6 Comparison of predicted and actual redundancy . . . . . . . . . . . . . . . . . . . . . . . 244
9.1 Example of performing Fisher’s exact test . . . . . . . . . . . . . . . . . . . . . . . . . 258
9.2 Analysis to determine p-value representing the threshold of significance . . . . . . 263
9.3 Example of bias problem when combining expression datasets . . . . . . . . . . . . 267
List of Tables

5.1 Swelling of PFPE in various solvents .............................................. 101
5.2 Static contact angles for PFPE and PDMS ........................................ 102

8.1 Comparison of average degeneracy predictions with actual data ............. 235
8.2 Predicted and actual fraction of genes that can be trivially solved for several useful array sizes ................................................................. 241

9.1 Example of discretized expression of genes in cDNA libraries .................. 255
9.2 Example of co-expression pattern of genes in cDNA libraries .................. 256
9.3 Example of discretized gene expression ratios in expression datasets .......... 260
9.4 Example of co-expression pattern of genes from discretized ratio data .......... 260
9.5 Human microarray datasets used in GBA analysis ................................ 261