## CONTENTS

Introduction
BIOSAPIENS: A European Network of Excellence to develop genome annotation resources (*D. Frishman, A. Valencia*)  

### SECTION 1: Gene definition

Chapter 1.1
State of the art in eukaryotic gene prediction (*T. Alioto, R. Guigó*)  

1 Introduction 7
2 Classes of information 10
   2.1 Extrinsic information 10
   2.2 Intrinsic information 11
      2.2.1 Signals 11
      2.2.2 Content 14
   2.3 Conservation 15
3 Frameworks for integration of information 17
   3.1 Exon-chaining 17
   3.2 Generative models: Hidden Markov models 18
      3.2.1 Basic hidden Markov models 18
      3.2.2 Generalized hidden Markov models 21
      3.2.3 Generalized pair HMMs 21
      3.2.4 Phylo-HMMs or evolutionary HMMs 23
   3.3 Discriminative learning 24
      3.3.1 Support vector machines 24
      3.3.2 Semi-Markov conditional random fields 25
   3.4 Combiners 25
4 Training 26
5 Evaluation of gene prediction methods 27
   5.1 The basic tools 27
   5.2 Systematic evaluation 28
Chapter 1.2

1 Introduction 41
2 Quality control of gene predictions 42
  2.1 Principles of quality control 42
    2.1.1 Violation of some generally valid rules about proteins 42
      2.1.1.1 Conflict between the presence of extracellular Pfam-A domain(s) in a protein and the absence of appropriate sequence signals 43
      2.1.1.2 Conflict between the presence of extracellular and cytoplasmic Pfam-A domains in a protein and the absence of transmembrane segments 43
      2.1.1.3 Co-occurrence of nuclear and extracellular domains in a predicted multidomain protein 43
      2.1.1.4 Domain size deviation 44
    2.1.2 Violation of some generally valid rules of protein-coding genes 44
      2.1.2.1 Chimeric proteins parts of which are encoded by exons located on different chromosomes 44
3 Results 44
  3.1 Validation of the MisPred pipeline 44
  3.2 Errors detected by the MisPred tools in public databases 46
    3.2.1 Analysis of the TrEMBL section of UniProtKB 46
    3.2.2 Analysis of sequences predicted by the Ensembl and Gnomon gene prediction pipelines 47
4 Alternative interpretations of the results of MisPred analyses 50
  4.1 MisPred has a low false positive rate 50
  4.2 MisPred detects errors in gene prediction 50
4.3 MisPred detects “errors” of biological processes 51
4.4 MisPred discovers exceptions to generally valid rules 51

5 Conclusions 52

SECTION 2: Gene regulation and expression

Chapter 2.1
Evaluating the prediction of cis-acting regulatory elements in genome sequences (O. Sand, J. Valéry Turatsinze, J. van Helden) 55

1 Introduction 55
2 Transcription factor binding sites and motifs 58
3 Scanning a sequence with a position-specific scoring matrix 59
   3.1 Background probability 61
   3.2 Probability of a sequence segment given the motif 62
   3.3 Scanning profiles 63
4 Evaluating pattern matching results 64
   4.1 Evaluation statistics 64
   4.2 Accuracy profiles 66
   4.3 Avoiding circularity in the evaluation 67
   4.4 Why the statistics involving TN should be avoided 67
   4.5 Difficulties for the evaluation of pattern matching 68
5 Discovering motifs in promoter sequences 69
   5.1 Example of pattern discovery result 70
   5.2 Evaluation statistics 72
   5.3 Correctness of predicted motifs for a collection of annotated regulons 73
   5.4 Distributions of motif scores in positive and negative testing sets 77
   5.5 The Receiver Operating Characteristics (ROC) curve 81
   5.6 Using ROC curves to find optimal parameters 83
6 Methodological issues for evaluating pattern discovery 83
7 Good practices for evaluating predictive tools 84
   7.1 Use comprehensive data sets 85
   7.2 Think about your negative control 85
   7.3 Ensure neutrality 85
8 What has not been covered in this chapter 86
9 Materials 87
Chapter 2.2
A biophysical approach to large-scale protein-DNA binding data
(T. Manke, H. Roider, M. Vingron) 91

1 Binding site predictions 92
2 Affinity model {XE “affinity model, TRAP”} 95
3 Affinity statistics {XE “affinity statistics”} 99
4 Applications 101
5 Summary 102

Chapter 2.3
From gene expression profiling to gene regulation (R. Coulson, T. Manke, K. Palin, H. Roider, O. Sand, J. van Helden, E. Ukkonen, M. Vingron, A. Brazma) 105

1 Introduction 105
2 Generating sets of co-expressed genes 106
3 Finding putative regulatory regions using comparative genomics 109
4 Detecting common transcription factors for co-expressed gene sets 111
5 Combining transcription factor information 114
6 “De novo” prediction of transcription factor binding motifs 115

SECTION 3: Annotation and genetics

Chapter 3
Annotation, genetics and transcriptomics (R. Mott) 123

1 Introduction 123
2 Genetics and gene function 125
   2.1 Genetic association studies in humans 125
3 Use of animal models 128
4 Transcriptomics: gene expression microarrays 130
5 Gene annotation 132

SECTION 4: Functional annotation of proteins

Chapter 4.1
Resources for functional annotation (A. J. Bridge, A. Lise Veuthey, N. J. Mulder) 139

1 Introduction 139
2 Resources for functional annotation – protein sequence databases 140

X
<table>
<thead>
<tr>
<th>Chapter</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>UniProt – The Universal Protein Resource</td>
<td>141</td>
</tr>
<tr>
<td>4</td>
<td>The UniProt Knowledgebase (UniProtKB)</td>
<td>142</td>
</tr>
<tr>
<td>4.1</td>
<td>UniProtKB/Swiss-Prot</td>
<td>142</td>
</tr>
<tr>
<td>4.1.1</td>
<td>Sequence curation in UniProtKB/Swiss-Prot</td>
<td>145</td>
</tr>
<tr>
<td>4.1.2</td>
<td>Computational sequence annotation in UniProtKB/Swiss-Prot</td>
<td>147</td>
</tr>
<tr>
<td>4.1.3</td>
<td>Functional annotation in UniProtKB/Swiss-Prot</td>
<td>147</td>
</tr>
<tr>
<td>4.1.4</td>
<td>Annotation of protein structure in UniProtKB/Swiss-Prot</td>
<td>149</td>
</tr>
<tr>
<td>4.1.5</td>
<td>Annotation of post-translational modifications in UniProtKB/Swiss-Prot</td>
<td>149</td>
</tr>
<tr>
<td>4.1.6</td>
<td>Annotation of protein interactions and pathways in UniProtKB/Swiss-Prot</td>
<td>150</td>
</tr>
<tr>
<td>4.1.7</td>
<td>Annotation of human sequence variants and diseases in UniProtKB/Swiss-Prot</td>
<td>150</td>
</tr>
<tr>
<td>4.2</td>
<td>UniProtKB/TrEMBL</td>
<td>151</td>
</tr>
<tr>
<td>5</td>
<td>Protein family classification for functional annotation</td>
<td>152</td>
</tr>
<tr>
<td>5.1</td>
<td>Protein signature methods and databases</td>
<td>152</td>
</tr>
<tr>
<td>5.1.1</td>
<td>Regular expressions and PROSITE</td>
<td>152</td>
</tr>
<tr>
<td>5.1.2</td>
<td>Profiles and the PRINTS database</td>
<td>153</td>
</tr>
<tr>
<td>5.1.3</td>
<td>Hidden Markov Models (HMM) and HMM databases</td>
<td>153</td>
</tr>
<tr>
<td>5.1.4</td>
<td>Structure-based protein signature databases</td>
<td>154</td>
</tr>
<tr>
<td>5.1.5</td>
<td>ProDom sequence clustering method</td>
<td>154</td>
</tr>
<tr>
<td>5.2</td>
<td>InterPro – integration of protein signature databases</td>
<td>154</td>
</tr>
<tr>
<td>5.3</td>
<td>Using InterProScan for sequence classification and functional annotation</td>
<td>155</td>
</tr>
<tr>
<td>5.3.1</td>
<td>InterProScan</td>
<td>155</td>
</tr>
<tr>
<td>5.3.2</td>
<td>Interpreting InterProScan results</td>
<td>156</td>
</tr>
<tr>
<td>5.3.3</td>
<td>Large-scale automatic annotation</td>
<td>159</td>
</tr>
<tr>
<td>6</td>
<td>From genes and proteins to genomes and proteomes</td>
<td>160</td>
</tr>
<tr>
<td>7</td>
<td>Summary</td>
<td>161</td>
</tr>
</tbody>
</table>

**Chapter 4.2**

**Annotating bacterial genomes (C. Médigue, A. Danchin)** 165

<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Background</td>
<td>165</td>
</tr>
<tr>
<td>2</td>
<td>Global sequence properties</td>
<td>170</td>
</tr>
<tr>
<td>3</td>
<td>Identifying genomic objects</td>
<td>172</td>
</tr>
<tr>
<td>4</td>
<td>Functional annotation</td>
<td>174</td>
</tr>
<tr>
<td>5</td>
<td>A recursive view of genome annotation</td>
<td>176</td>
</tr>
</tbody>
</table>
Chapter 4.3
Data mining in genome annotation (I. Artamonova, S. Kramer, D. Frishman) 191

1 Introduction 191
2 An overview of large biological databases 193
   2.1 Manually curated vs. automatic databases 193
   2.2 Manually curated databases: the Swiss-Prot example 196
   2.3 Automatically generated databases: the PEDANT example 198
3 Data mining in genome annotation 200
   3.1 General remarks 200
   3.2 Supervised learning 201
   3.3 Unsupervised learning 201
   3.4 Clustering 202
   3.5 Association rule mining 203
4 Applying association rule mining to the Swiss-Prot database 205
5 Applying association rule mining to the PEDANT database 207
6 Conclusion 210

Chapter 4.4

1 Homologous and non-homologous sequence methods for assigning protein functions 213
   1.1 Introduction 213
   1.2 Homologs, orthologs, paralogs 216
   1.3 The HAMAP resource for the annotation of prokaryotic protein sequences and their orthologues 219
   1.4 CATH, Gene3D & GeMMA 222
   1.5 From SMART to STRING and STITCH: diverse tools for deducing function from sequence 228
1.6 General approaches for inheriting functions between homologous proteins 230
1.7 Non-homologous methods for predicting protein function from sequence 234

Chapter 4.5

1 Introduction to protein structure and function 239
2 FireDB and firestar – the prediction of functionally important residues 241
   2.1 Introduction 241
   2.2 FireDB 242
   2.3 Firestar 244
3 Modelling local function conservation in sequence and structure space for predicting molecular function 246
   3.1 Introduction 246
   3.2 Method 246
   3.3 Application 247
4 Structural templates for functional characterization 249
   4.1 Introduction 249
   4.2 Predicting protein function using structural templates 249
   4.3 FLORA method 250
5 An integrated pipeline for functional prediction 252
   5.1 Introduction 252
   5.2 The ProFunc server 253
      5.2.1 Sequence-based searches 254
      5.2.2 Structure-based searches 255
   5.3 Case studies 257
      5.3.1 Case study 1: published function identified 257
      5.3.2 Case study 2: function unclear 259
   5.4 Conclusion 259

Chapter 4.6
Harvesting the information from a family of proteins (B. Vroling, G. Vriend) 263

1 Introduction 263
   1.1 Information transfer 264
2 Molecular class-specific information systems 265
   2.1 G-protein-coupled receptors 266
SECTION 5: Protein structure prediction

Chapter 5.1
Structure prediction of globular proteins (A. Tramontano, D. Jones, L. Rychlewski, R. Casadio, P. Martelli, D. Raimondo and A. Giorgetti) 283

1 The folding problem 283
2 The evolution of protein structures and its implications for protein structure prediction 286
3 Template based modelling 287
   3.1 Homology-based selection of the template 288
   3.2 Fold recognition 288
   3.3 Using sequence based tools for selecting the template 289
   3.4 Completing and refining the model 291
   3.5 Current state of the art in template based methods 292
4 Template-free protein structure prediction 293
   4.1 Energy functions for protein structure prediction 296
   4.2 Lattice methods 297
   4.3 Fragment assembly methods 298
   4.4 Practical considerations 299
5 Automated structure prediction 300
   5.1 Practical lessons from benchmarking experiments 302
6 Conclusions and future outlook 304

Chapter 5.2
The state of the art of membrane protein structure prediction: from sequence to 3D structure (R. Casadio, P. Fariselli, P. L. Martelli, A. Pierleoni, I. Rossi, G. von Heijne) 309

1 Why membrane proteins? 309
2 Many functions 311
3 Bioinformatics and membrane proteins: is it feasible to predict the 3D structure of a membrane protein? 311
4 Predicting the topology of membrane proteins 312
5 How many methods to predict membrane protein topology? 314
   5.1 From theory to practice 314
6 Benchmarking the predictors of transmembrane topology 316
   6.1 Testing on membrane proteins of known structure and topology 316
   6.2 Topological experimental data 317
   6.3 Validation towards experimental data 318
7 How many membrane proteins in the Human genome? 319
8 Membrane proteins and genetic diseases: PhD-SNP at work 320
9 Last but not least: 3D MODELLING of membrane proteins 322
10 What can currently be done in practice? 323
11 Can we improve? 324

SECTION 6: Protein–protein complexes, pathways and networks

Chapter 6.1
Computational analysis of metabolic networks (P.-Y. Bourguignon, J. van Helden, C. Ouzounis, V. Schächter) 329

1 Introduction 329
2 Computational resources on metabolism 331
   2.1 Databases 331
      2.1.1 KEGG 331
      2.1.2 BioCyc 332
      2.1.3 Reactome 332
      2.1.4 Querying and exporting data 333
   2.2 Reconstruction of metabolic networks 333
      2.2.1 From annotated genomes to metabolic networks 334
      2.2.2 Filling gaps 334
3 Basic notions of graph theory 335
   3.1 Metabolic networks as bipartite graphs 335
   3.2 Node degree 336
   3.3 Paths and distances 336
4 Topological analysis of metabolic networks 336
   4.1 Node degree distribution 337
      4.1.1 Robustness to random deletions and targeted attacks 339
      4.1.2 Generative models for power-law networks 340
   4.2 Paths and distances in metabolic networks 341
5 Assessing reconstructed metabolic networks against physiological data 342
5.1 Constraints-based models of metabolism 343
  5.1.1 The flux balance hypothesis 343
  5.1.2 Modelling the growth medium 344
  5.1.3 Biomass function 345
5.2 Predicting metabolic capabilities 345
  5.2.1 Predicting growth on a defined medium 345
  5.2.2 Predicting gene essentiality 346
5.3 Assessing and correcting models using experimental data 347
5.4 Structural properties of the flux cone 347
5.5 Working with constraints-based models 348
6 Conclusion 348

Chapter 6.2

1 Introduction 353
2 Experimental methods 354
3 Protein interaction databases 356
4 Data standards for molecular interactions 356
5 The IntAct molecular interaction database 360
6 Interaction networks 362
7 Visualization software for molecular networks 365
8 Estimates of the number of protein interactions 371
9 Multi-protein complexes 372
10 Network modules 373
11 Diseases and protein interaction networks 376
12 Sequence-based prediction of protein interactions 380
  12.1 Phylogenetic profiling 381
  12.2 Similarity of phylogenetic trees 383
  12.3 Gene neighbourhood conservation 384
  12.4 Gene fusion 385
13 Integration of experimentally determined and predicted interactions 385
14 Domain–domain interactions 389
15 Biomolecular docking 395
  15.1 Protein-ligand docking 396
  15.2 Protein–protein docking 398
SECTION 7: Infrastructure for distributed protein annotation

Chapter 7
Infrastructure for distributed protein annotation (G. A. Reeves, A. Prlic, R. C. Jimenez, E. Kulesha, H. Hermjakob) 413

1 Introduction 413
2 The Distributed Annotation System (DAS) 415
3 DAS infrastructure 415
   3.1 DASTY2 – a protein sequence-oriented DAS client 418
   3.2 SPICE – a protein structure-oriented DAS client 418
   3.3 Ensembl 420
   3.4 DAS servers 422
4 The protein feature ontology 422
5 Conclusion 425

SECTION 8: Applications

Chapter 8.1
Viral bioinformatics (B. Adams, A. Carolyn McHardy, C. Lundegaard, T. Lengauer) 429

1 Introduction 429
2 Viral evolution in the human population 430
   2.1 Biology and genetics 430
   2.2 Vaccine strain selection for endemic influenza 431
   2.3 Pandemic influenza 433
   2.4 Conclusion 434
3 Interaction between the virus and the human immune system 434
   3.1 Introduction to the human immune system 434
   3.2 Epitopes 436
   3.3 Prediction of epitopes 437
   3.4 Epitope prediction in viral pathogens in a vaccine perspective 441
4 Viral evolution in the human host 442
   4.1 Introduction 442
   4.2 Replication cycle of HIV 443
   4.3 Targets for antiviral drug therapy 444
   4.4 Manual selection of antiretroviral combination drug therapies 444
   4.5 Data sets for learning viral resistance 445
   4.6 Computational procedures for predicting resistance 446

XVII
Chapter 8.2


1 Introduction 453
2 Prediction of variant location 455
3 Prediction of variant function – analysis of the role of alternative splicing in changing function by modulation of functional residues 458
   3.1 Functions associated with alternative splicing 458
   3.2 Functional adaptation through alternative splicing 458
      3.2.1 Tafazzin 459
      3.2.2 Phosphoribosylglycinamide formyltransferase (GARS-AIRS-GART) 461
   3.3 Analysis across the ENCODE dataset 462
4 Prediction of variant structure 463
5 Summary of effects of alternative splicing 467
6 Prediction of principal isoforms 472
   6.1 A series of automatic methods for predicting the principal isoform 473
      6.1.1 Methods 474
      6.1.2 Evaluation of pipeline definitions 474
7 The ENCODE pipeline – an automated workflow for analysis of human splice isoforms 477
   7.1 Behind EPipe 477
   7.2 Example workflow: IFN alpha/beta receptor protein 479
   7.3 Future perspectives 480