

TABLE OF CONTENTS

1. Introduction	1
1.1 Central Dogma	1
1.2 Gene Expression	3
1.2.1 Gene Expression Data	3
1.3 Gene Expression Data Analysis	5
1.3.1 Gene Co-expression Analysis: Biclustering Approach	5
1.3.2 Differential Co-expression Analysis (DCA)	5
1.3.3 Differential Expression Analysis	6
1.3.4 Discussion	6
1.4 Motivation	8
1.5 Objectives	8
1.6 Contributions	9
1.6.1 Biclustering Analysis	9
1.6.2 Differential Expression Analysis (DEA)	10
1.6.3 Differential Co-expression Analysis (DCA)	10
1.6.4 DCA on scRNA-Seq data	11
1.6.5 Potential Biomarker Identification and Ranking	12
1.7 Organization of the Thesis	12
2. Background	14
2.1 Statistical Methods and Measures	14
2.1.1 <i>p-value</i>	14
2.1.2 False Discovery Rate	15
2.1.3 <i>q-value</i>	16
2.1.4 Local False Discovery Rate	17
2.1.5 Heirarchical Clustering	17
2.1.6 Principal Component Analysis (PCA)	18
2.1.7 Benjamini-Hochberg	19
2.1.8 t-test	20
2.1.9 Preservation Analysis	21
2.1.10 Z-summary Statistics	21
2.2 Gene Expression Analysis Programming/Tools	22
2.2.1 R	22
2.2.2 Bioconductor	23
2.2.3 DAVID	24

2.2.4	GENIE3	26
2.3	Knowledge Repositories	26
2.3.1	Entity Identifiers	27
2.3.2	Gene Ontology (GO)	29
2.3.3	Kyoto Encyclopedia of Genes and Genomes(KEGG)	30
2.3.4	hgu133plus2.db	30
2.3.5	org.Hs.eg.db	31
2.4	Biological Analysis	31
2.4.1	Functional Enrichment analysis	32
2.4.2	Regulatory Network Behavior Analysis	33
2.5	Biomarker Criterion	34
2.6	Datasets Used	35
2.6.1	Microarray Data	35
2.6.2	BulkRNAseq data	36
2.6.3	scRNAseq data	36
2.7	pre-processing of Gene Expression Data	37
2.7.1	Pre-processing of Microarray Data	37
2.7.2	Pre-processing of bulk RNA-Sequencing (Bulk RNA-Seq) Data	38
2.7.3	Pre-processing of Single Cell RNA-Sequencing (scRNA-Seq) Data	38
2.8	Discussion	39
3.	Biclustering Approach	40
3.1	Introduction	40
3.1.1	Biclustering Analysis	41
3.2	Related Works	42
3.2.1	Metric-based Biclustering Approach	43
3.2.2	Non-metric Biclustering Approach	49
3.3	Some Selected Biclustering Algorithms	50
3.3.1	Bimax	50
3.3.2	xMOTIFs (conserved gene expression Motifs)	51
3.3.3	Plaid models(PM)	52
3.3.4	Iterative Signature Algorithm (ISA)	53
3.3.5	Factor Analysis for BIcluster Acquisition (FABIA)	54
3.3.6	QUalitative BIClustering algorithm(QUBIC)	54
3.3.7	Iterative Binary Biclustering algorithm with greedy search (iBBiG)	55
3.3.8	FLexible Overlapped biClustering (FLOC)	56
3.4	BicGenesis: A Method to Identify ESCC Biomarkers Using Biclustering Approach	61
3.4.1	Pre-processing	63
3.4.2	Biclustler Analysis	63

3.4.3	DCA	64
3.4.4	Identification of DEGs	66
3.4.5	Validation	66
3.5	Experimental Results	67
3.5.1	Pre-processing	67
3.5.2	Bicluster Analysis	68
3.5.3	DCA	70
3.5.4	Preservation Analysis	75
3.5.5	Hub-gene Finding	78
3.6	Validation	82
3.6.1	Enrichment Analysis of Biclusters	82
3.6.2	Biological Analysis	83
3.6.3	Literature Trace	91
3.7	Discussion	100
3.8	Chapter Summary	101
4.	Differential Expression Analysis	103
4.1	Introduction	103
4.1.1	Differential Expression Analysis (DEA)	103
4.2	Related Works	105
4.2.1	Statistical Tests	105
4.2.2	Microarray Methods	106
4.2.3	Bulk RNA-Seq Methods	108
4.3	Identification of Potential Biomarkers using Integrative Approach: Ap- plication to ESCC	115
4.3.1	Pre-processing	115
4.3.2	DEA	116
4.3.3	DCA	117
4.3.4	Identification of TEDs	118
4.3.5	Validation	118
4.4	Experimental Results	119
4.4.1	Pre-processing	119
4.4.2	Identification of DEGs	120
4.4.3	DCA	121
4.5	Validation	128
4.5.1	Enrichment Analysis of DEGs and Modules	128
4.5.2	Biological Analysis	130
4.5.3	Literature Trace	137
4.6	Discussion	145
4.7	Chapter Summary	148

5. Differential Co-expression Analysis	150
5.1 Introduction	150
5.1.1 Differential Co-expression Analysis (DCA)	150
5.2 Related Works	152
5.2.1 Unsupervised DCA approaches	152
5.2.2 Supervised DCA Approaches	154
5.3 Basics of Centrality Measures	156
5.4 CBDCEM: An effective Centrality Based Differential Co-Expression Method for Crucial Gene Finding	161
5.4.1 Pre-processing	161
5.4.2 CEN Construction	162
5.4.3 Module Extraction	162
5.4.4 Hub-gene Finding	163
5.4.5 Validation	167
5.5 Experimental Results	168
5.5.1 Pre-processing	168
5.5.2 CEN Construction	169
5.5.3 Module Extraction	169
5.5.4 Hub-gene Finding	175
5.6 Validation	178
5.6.1 Enrichment Analysis of Modules	178
5.6.2 Biological Analysis	178
5.6.3 Literature Trace	186
5.7 Discussion	191
5.7.1 Comparison of with four other hub-gene finding algorithm . . .	194
5.7.2 CBDCEM vs. WGS	194
5.7.3 CBDCEM vs. PCO	195
5.7.4 CBDCEM Vs IMC	195
5.7.5 CBDCEM Vs Degree	197
5.8 Chapter Summary	198
6. Differential Co-expression Analysis on Single Cell RNA-Seq Data	201
6.1 Introduction	201
6.1.1 Single Cell RNA Sequencing (scRNA-Seq)	202
6.2 Related Works	203
6.3 Background	205
6.3.1 Measures for hub gene finding	205
6.3.2 Seurat	208
6.3.3 High Dimensional WGCNA (hdWGCNA)	208

6.4	scDiffCoAM: A Complete Framework to Identify Potential ESCC Biomarkers using ScRNA-Seq Data Analysis	210
6.4.1	Pre-processing	211
6.4.2	Dimensionality Reduction	211
6.4.3	Partitioning into cell types	212
6.4.4	CEN Construction and Module Extraction	212
6.4.5	Hub-gene Finding	213
6.4.6	Identification of DEGs	214
6.4.7	Validation	214
6.5	Experimental Results	215
6.5.1	Pre-processing	215
6.5.2	Dimensionality Reduction	217
6.5.3	Partitioning into cell types	218
6.5.4	CEN Construction and Module Extraction	218
6.5.5	Preservation Analysis	221
6.5.6	Hub Gene Finding	226
6.5.7	Identification of DEGs	228
6.6	Validation	229
6.6.1	Enrichment Analysis of Modules	229
6.6.2	Biological Analysis	230
6.6.3	Literature Trace	240
6.7	Discussion	252
6.7.1	Comparison with four other hub-gene finding algorithm	257
6.7.2	Biomarker Ranking	260
6.8	Chapter Summary	262
7.	Conclusion	263
7.1	Concluding Remarks	263
7.2	Future Works	266