Finding the association of mRNA and miRNA using Next Generation Sequencing data of Kidney renal cell carcinoma

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Abstract MicroRNAs (miRNAs) are a class of 22-nucleotide endogenous noncoding RNAs, plays important role in regulating target gene expression via repressing translation or promoting messenger RNAs (mRNA) degradation. Numerous researchers have found that miRNAs have serious effects on cancer. Therefore, study of mRNAs and miRNAs together through the integrated analysis of mRNA and miRNA expression profiling could help us in getting a deeper insight into the cancer research. In this regards, High-Throughput Sequencing data of Kidney renal cell carcinoma is used here. The proposed method focuses on identifying mRNA-miRNA pair that has a signature in kidney tumor sample. For this analysis, Random Forests, Particle Swarm Optimization and Support Vector Machine classifier is used to have best sets of mRNAs-miRNA pairs. Additionally, the significance of selected mRNA-miRNA pairs is tested using gene ontology and pathway analysis tools. Moreover, the selected mRNA-miRNA pairs are searched based on changes in expression values of the used mRNA and miRNA dataset.

Key words: mRNA, miRNA, Next Generation Sequencing data, Particle Swarm Optimization, Random Forest

1 Introduction

MicroRNAs (miRNAs) are small non-coding RNAs of approximately 19-22 nucleotides act as post-transcriptional gene expression regulators, bind with comple-

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mentary messenger RNAs (mRNAs) for stimulating their degradation or repressing translation. In this regards, innovation of Next-Generation Sequencing (NGS) [6] technology provides improved solution to the understanding of an entire human genome sequence than compared to the previously used technologies, i.e., Sanger sequencing or Capillary sequencing. Next-Generation Sequencing technologies can also be applied to the RNA sequencing (RNA-seq) to directly sequence complementary DNAs (cDNAs). RNA sequencing permits the quantitative analysis of gene expression and transcript variant discovery. The primary aim of RNA-sequencing is to find out differentially expressed genes when applied to multiple samples at different biological conditions.

Numerous statistical approaches have been proposed over the years to analyze differentially expressed RNA-seq data, e.g., edgeR [15], DESeq [1], and sSeq [20]. In this connection, algorithms like [14, 17] can be used to rank and select the differentially expressed miRNAs. Apart from this, hypothesis test [10], classifier based method [7] and information theory based measures [13] are used in gene ranking as well as in miRNA ranking. Parametric method like [18] is developed by using the expression overlapping between different classes. Nonparametric ReDiscovery Curve (RDCurve) based method [12] study the stability of various ranking methods.

Reviewing all these methods, here we have proposed an integrated analysis of the gene expression profiling of both mRNAs and miRNAs by using Kidney renal clear cell carcinoma data that includes tumor and control samples. The proposed method is a feature selection wrapper, consisting of Random Forests [5], Particle Swarm Optimization (PSO) [8] and Support Vector Machine (SVM) [4] classifier. Moreover, emphasis is given here to identified the differentially expressed mRNAs and miRNAs in-order to find out mRNA-miRNA pairs. In particular, this kind of observations might be significant in the cancer diagnosis. Moreover, we validate some of our most interesting findings in different biological significance analysis.

The paper is organized as follows: Section 2 briefly describes the proposed method. Section 3 shows the empirical results. Finally, Section 4 concludes this paper with an additional note of future work.

2 Proposed Method

The proposed wrapper based feature selection method starts with 1135 number of samples having 20,531 mRNAs. The mRNA sample includes 534 tumors and 601 control patients whereas miRNA include 254 tumor and 455 control sample. Since this experiment is an integrated analysis of mRNA and miRNA data. Hence, common tumor and control samples among mRNA and miRNA data are considered here, that consist of 186 tumor and 71 control samples. Steps of the proposed method are described below:

The primary goal of feature selection is to avoid any kind of over fitting as well as to improve the model performance. To gain a deeper insight into the underlying processes of feature selection Random forest is used here. Several measures of variable importance can be identified by Random forest. Hence, by iteratively fitting Random Forest at each iteration smallest set of informative mRNAs are identified. In this regards, smallest *OOB* error rate is considered to be the underline criteria. Because of the iterative approach, the *OOB* error is biased down and mRNAs with smallest *OOB* error are selected as the reduced set of features. For this analysis, normalized transcript reads of mRNAs are considered.

After feature selection using Random forest, in the next step of this work t-test and fold change concepts are applied in order to segregate differentially expressed mRNAs from normal and malignant samples. For this purpose, cut-off like p-value < 0.05 and the fold change > 1 or < -1 are used as in [21]. In particular, change in expression profile is considered to be the underline criteria to find out differentially expressed mRNAs. Moreover, the differentially expressed mRNAs are mapped to their normalized mRNA expression dataset of tumor and control samples.

The Particle Swarm Optimization (PSO) and Support Vector Machine (SVM) provide a wrapper based approach for feature selection. It can effectively identify significant mRNAs associated with Kidney renal cell carcinoma. For that, initial particles of PSO are created using mRNA indices. Thereafter, the swarm is prepared from a number of such particles. Moreover, encoded indices of these particles are used to make a subset of data that are taken part during classification by SVM. Here 5 Fold Cross Validation (FCV) is applied and accuracy is considered to be the underline criteria for fitness evaluation. Block diagram of the proposed approach is presented in Fig. 1. Moreover, the optimal signature refers to be the best set of differentially expressed mRNA that provides highest classification accuracy. In this regards, most appeared mRNAs concerning all runs of the proposed methods are considered.

In this experiment, the expression datasets comprising the profiles of mRNAs and miRNAs are considered. For this analysis, expression values of 1047 miRNA in 186 tumor and 71 control sample are used. The selected mRNAs by PSO+SVM are considered to find their corresponding miRNA targets. For this validation miRDB [19] database is used. Shortlisted target miRNAs are used to find their expression profiles from the normalized miRNA expression dataset of 186 tumor and 71 control samples. Any further analysis of miRNA is done using this reference expression vector in-order to find out differentially expressed miRNAs.

Likewise, the mRNAs, based on p-value and fold change in expression levels, up and down regulated miRNAs are identified. The miRNAs are known for suppressing the expression level of mRNAs. Hence, our method selected up and down regulated mRNAs and miRNAs are used to make mRNA-miRNA pairs. For that, combination of up-regulated mRNAs with down-regulated miRNAs and vice versa are used.

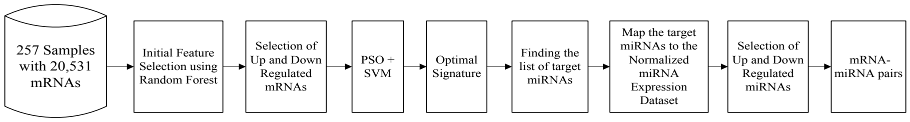

257 Samples with 20,531 mRNAs
Initial Feature Selection using Random Forest
Selection of Up and Down Regulated mRNAs
PSO + SVM
Optimal Signature
Finding the list of target miRNAs
Map the target miRNAs to the Normalized miRNA Expression Dataset
Selection of Up and Down Regulated miRNAs
mRNA-miRNA pairs

Fig. 1 A block diagram representation of the proposed workflow

3 Experimental Results

3.1 Datasets

The dataset comes from The Cancer Genome Atlas (TCGA)[1]. It features mRNA and miRNA expression levels for Kidney renal cell carcinoma (KIRC) of different tumor, and control samples. For KIRC, normalized expression mRNA and miRNA datasets consisting of 186 common tumors, and 71 common control samples are considered.

3.2 Results

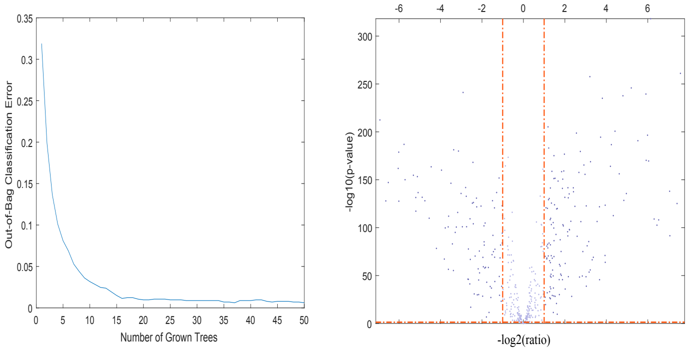
Here, the problem is to identify mRNA-miRNA pairs for the investigated dataset. For this purpose, the expression profiles of $20,531$ mRNAs over 186 kidney renal cell tumor samples and 71 control samples are considered. In order to find reduced set of features, Random forest is used here. Because of it's iterative approach, the *OOB* error is biased down and mRNAs with smallest *OOB* error are selected as the reduced set of features. This has been shown in Fig. 2 (a). By this process, 511 informative mRNAs are selected. In order to identify the up and down regulated mRNAs, significant changes in the expression profiles are measured. For this analysis, the volcano plot in Fig. 2 (b), illustrate the differentially expressed mRNAs among the 511. By this process, 242 up and 191 down regulated mRNAs are identified. Now, these shortlisted mRNAs are process, using PSO+SVM. For this process, the parameters used in the proposed method are summarized in Table 1. Here, PSO+SVM executed 30 times (N_{exe}) on these differentially expressed mRNAs. According to the experimental results, PSO+SVM achieved 92.60% average classification accuracy for mRNA data. The average values of precision, sensitivity, specificity, F-measure and MCC are $92.57\%, 92.58\%, 92.62\%, 92.58\%$ and 0.920, respectively. For this process, 10 mRNAs are considered by PSO+SVM during each run. Moreover, as PSO+SVM was executed 30 times hence, in each run of the proposed method a new set of differentially expressed mRNAs are identified. After the end of 30 executions, one list is prepared that include all the appeared mRNAs. Thereafter, from that list most appeared mRNAs covering all the runs of the proposed method are identified. These mRNAs are considered to be optimal up and down regulated mRNAs associated with Kidney renal cell cancer subtypes.

Thereafter, these selected mRNAs are used to find their corresponding miRNA targets and for this validation miRDB [19] database is used. Moreover, these shortlisted target miRNAs find their expression profiles from the normalized miRNA expression dataset of 186 tumor and 71 control samples. A total of 450 miRNAs targets are identified from the miRGate database. Out of which, 417 miRNAs matches their expression profile in the normalized miRNA expression dataset. Hence, the

[1] https://tcga-data.nci.nih.gov/tcga/

miRNAs for which expression profile information is known are only taken at this stage. Therefore, out of the 450 miRNAs in the list, 33 have been excluded because they did not have the expression profile information in our used normalized miRNA expression dataset. Thereafter, p-value and fold change analysis of the shortlisted 417 differentially expressed miRNAs, identified the up and down regulated one. By this process 14 informative miRNAs are identified, that includes 12 down regulated and 2 up regulated miRNAs. Method selected down regulated miRNAs are *hsa-mir-200c, hsa-mir-181a-2, hsa-mir-196a-1, hsa-mir-183, hsa-mir-194-2, hsa-mir-196a-2, hsa-mir-10b, hsa-mir-138-1, hsa-mir-182, hsa-mir-192, hsa-mir-135b, hsa-mir-199b* whereas *hsa-mir-141, hsa-mir-196b* are up regulated miRNAs.

Studies on miRNAs found that it can suppress the expression level of mRNAs. As miRNAs and mRNAs belonging to same sample (Tumor and Control) of KIRC dataset is used in this experiment. Hence, differentially expressed miRNAs and mRNAs of the same sample (Tumor and Control), can be paired. Moreover, up regulated miRNAs can be mapped with down-regulated mRNAs and vice versa. In the process, many of the selected mRNAs have been excluded from the list while miRNAs remain same. Final list of mRNA-miRNA pair include 98 up-regulated mRNA and 12 down-regulated miRNA pair and 62 down-regulated mRNA and 2 up-regulated miRNA pair. Details of which are given in Table2.



(a) *OOB* errors

(b) Volcano plot

Fig. 2 (a) Variation of *OOB* errors with the number of grown trees in Random forest, (b) Volcano plot depicts 242 up and 191 down regulated mRNAs

Table 1 Parameters used in the experiment

Symbol	Value	Description	Symbol	Value	Description
N_{par}	50	Number of Particles	φ_2	2	Social Constant
N_{itr}	50	Number of Iterations	ω	0.9	Inertia Weight
L	10	Length of a Particle	C	0.01	SVM C Constant
φ_1	2	Cognitive Constant	N_{exe}	30	Number of Executions

Table 2 Method selected up and down regulated miRNA and mRNAs

Down Regulated miRNA	Up Regulated miRNA	Up Regulated mRNA							Down Regulated mRNA				
hsa-mir-200c	hsa-mir-141	ABCB7	BCL2L12	C9orf117	CHTF8	DUSP23	FGF2	HIST1H3E	B3GALT5	CHURC1	DNAH17	GEMIN7	ID4
hsa-mir-181a-2	hsa-mir-196b	ABCD4	BCL7C	CA11	COL4A6	DUT	FSTL5	HSPA12A	BSND	CIITA	DPYSL5	GIN1	IDH1
hsa-mir-196a-1		ACAD11	BICC1	CACNA2D2	CPD	EDEM1	FTL	IGSF3	CCNT2	CKAP2L	E2F3	GIPC1	
hsa-mir-183		ADCY2	BMPR1B	CALB2	CREG2	ENGASE	FZD3	IL17REL	CDC42	CLNK	ELL	GIT2	
hsa-mir-194-2		ADRA2C	BPGM	CCDC141	CRNKL1	EPB41L1	GABRE	IMMP2L	CDH10	CLUL1	ENOSF1	GLUD1	
hsa-mir-196a-2		AHCYL1	BRI3BP	CCDC152	CRTAM	EPPK1	GALNT6	IMPA1	CLNK	CNOT6L	FAM172A	GNAS	
hsa-mir-10b		ALCAM	BST1	CCDC85C	CRTAP	EXT1	GATM	INCENP	DNAH17	CNR1	FAM19A5	GNPTAB	
hsa-mir-138-1		ANKRD13A	BTBD2	CCDC88A	CSF2RB	FAIM2	GDF2	IQSEC1	DPYSL5	CPEB2	FBXO48	GPR37	
hsa-mir-182		ANKRD34B	BVES	CCND2	CYBRD1	FAM120C	GLT8D2		E2F3	CPEB3	FCGR3A	GRM5	
hsa-mir-192		ARGFX	C17orf107	CCNT1	CYLD	FAM126B	GPKOW		ELL	CWF19L1	FLRT2	GSTM2	
hsa-mir-135b		ARHGAP20	C19orf70	CDK10	CYP8B1	FAM189A1	GPR161		ENOSF1	CYP1A2	GAB2	HAGHL	
hsa-mir-199b		ARHGEF18	C4orf32	CGNL1	DIRAS1	FAM196A	GTPBP8		EPN1	DAZAP2	GAL3ST1	HBS1L	
		ARMC10	C4orf45	CHML	DNAJC14	FAM198B	H6PD		GIT2	DISC1	GBP2	HEY1	
		ATG2A	C5AR1	CHST5	DPP9	FBXL18	HACE1		HDDC3	DKK1	GDF11	HIVEP3	
		ATP2B2	C9orf114	CHST6	DRP2	FCHSD1	HEPH		CHST7	DLG5	GDF7	ID3	

3.3 Biological Significance

Table 3 Most significant Gene Ontology terms concerning selected genes for Biological Process (P), Cellular Component (C) and Molecular Function (F) obtained through Enrichment analysis via Enrichr [9]

	Term	P-value	Genes
GO Biological Process (P)	cellular iron ion homeostasis (GO:0006879)	3.68e-05	HEPH, ABCB7, GDF2, CYBRD1, FTL
	N-acetylglucosamine metabolic process (GO:0006044)	5.16e-05	CHST6, CHST7, CHST5
	sulfur compound metabolic process (GO:0006790)	9.36e-05	CHST6, CHST7, CHST5
	positive regulation of cyclin-dependent protein serine (GO:0045737)	4.64e-04	CCNT2, CCND2, CCNT1
	negative regulation of cytoplasmic translation (GO:2000766)	8.66e-04	CPEB3, CPEB2
GO Cellular Component (C)	cyclin/CDK positive transcription elongation factor complex (GO:0008024)	1.60e-03	CCNT2, CCNT1
	messenger ribonucleoprotein complex (GO:1990124)	1.60e-03	CPEB3, CPEB2
	Golgi membrane (GO:0000139)	1.66e-03	EXT1, CDC42, GALNT6, CHST6, CHST7, HACE1, GNPTAB, EDEM1, CHST5
	CCR4-NOT complex (GO:0030014)	6.59e-03	CNOT6L, CPEB3
	neuron projection (GO:0043005)	1.36e-02	CALB2, CDC42, CPEB3, CPEB2
GO Molecular Function (F)	N-acetylglucosamine 6-O-sulfotransferase activity (GO:0001517)	1.53e-05	CHST6, CHST7, CHST5
	7SK snRNA binding (GO:0097322)	8.66e-04	CCNT2, CCNT1
	mRNA 3'-UTR AU-rich region binding (GO:0035925)	2.55e-03	CPEB3, CPEB2
	transforming growth factor beta receptor binding (GO:0005160)	2.87e-03	GDF11, GDF2, GDF7
	translation repressor activity, nucleic acid binding (GO:0000900)	3.10e-03	CPEB3, CPEB2

Biological significance analysis of the mRNAs-miRNAs pairs have been carried out by KEGG pathway enrichment and Gene ontology analysis. In this regards, miRSystem ver. 20160502 [11] and Enrichr [9] helps is finding the associated pathways of the significant mRNA-miRNA pairs. For the Gene enrichment analysis, the biological process (P), cellular component (C) and molecular function (F) hierarchies in gene ontology (GO) are considered. The most significant GO terms, with lowest p-values (< 0.001) for the biological processes (P) associated with mRNA data are *cellular iron ion homeostasis (GO:0006879), N-acetylglucosamine metabolic process (GO:0006044), sulfur compound metabolic process (GO:0006790), positive regulation of cyclin-dependent protein serine (GO: 0045737), negative regulation of cytoplasmic translation (GO:2000766)* etc. Significant top 5 Gene Ontology terms concerning selected genes for Biological Process (P), Cellular Component (C) and Molecular Function (F) are reported in Table.3. This table also includes a list of associated genes corresponding to each ontology term. Association of the obtained mRNAs with different biological pathways are been identified by KEGG pathway enrichment analysis. It has been observed that, significant mRNAs are associated with many KEGG pathways. Among them, only 10 significant pathways with lowest p-values are presented in Table.4. For this analysis, 98 up-regulated mRNA and 62 down-regulated mRNA are considered. Overall, the results signify the importance of selected mRNA-miRNA pairs.

Table 4 Top 10 KEGG pathways associated with the selected up and down regulated mRNAs

Term	**P-value**	**Genes**
TGF-beta signaling pathway: hsa04350	0.0040	ID4, ID3, BMPR1B, GDF7
Signaling pathways regulating pluripotency of stem cells: hsa04550	0.0049	FZD3, ID4, ID3, BMPR1B, FGF2
Salivary secretion: hsa04970	0.0050	BST1, GNAS, ADCY2, ATP2B2
Pancreatic secretion: hsa04972	0.0065	BST1, GNAS, ADCY2, ATP2B2
Mineral absorption: hsa04978	0.0071	HEPH, CYBRD1, FTL
Retrograde endocannabinoid signaling: hsa04723	0.0077	GRM5, CNR1, ADCY2, GABRE
Dorso-ventral axis formation: hsa04320	0.0182	CPEB3, CPEB2
Rap1 signaling pathway: hsa04015	0.0238	CDC42, CNR1, GNAS, ADCY2, FGF2
Phospholipase D signaling pathway: hsa04072	0.0253	GRM5, GNAS, ADCY2, GAB2
Adrenergic signaling in cardiomyocytes: hsa04261	0.0277	GNAS, CACNA2D2, ADCY2, ATP2B2

4 Conclusion

In this paper, the integrated analysis of mRNA and miRNA expression data identified a number of mRNAs and miRNAs that are differentially expressed in control and tumor samples. For this analysis, Random Forests is used as initial feature selector. Thereafter, with the help of Particle Swarm Optimization and Support Vector Machine classifier the proposed method is optimized to have best sets of mRNAs. In addition to that, functional miRNA targets of selected mRNAs are also identified for the expression data of Kidney renal cell. Finally, differentially expressed mRNA-miRNA pair are prepared. These selected mRNA-miRNA pairs are proven to be relevant according to gene ontology and pathway analysis tools. Hence, these mRNA-miRNA pairs are the potential biomarkers for the Kidney renal cell cancer type. In conclusion, this analysis is proven to be helpful for the joint mRNA-miRNA biomarker identification and could also be used as miRNA marker [3, 16] and gene selection [2].

References

1. Anders, S., Huber, W.: Differential expression analysis for sequence count data. Genome Biology **11**(10:R106) (2010)

2. Bhowmick, S.S., Saha, I., Maulik, U., Bhattacharjee, D.: Biomarker identification using next generation sequencing data of RNA. In: Advances in Computing, Communications and Informatics (ICACCI), 2016 International Conference on, pp. 299–303 (2016)
3. Bhowmick, S.S., Saha, I., Maulik, U., Bhattacharjee, D.: Identification of miRNA signature using next-generation sequencing data of prostate cancer. in Proceedings of the 3rd International Conference on Recent Advances in Information Technology pp. 528–533 (2016)
4. Boser, B.E., Guyon, I.M., N.Vapnik, V.: A training algorithm for optimal margin classifiers. in Proceedings of the 5th Annual Workshop on Computational Learning Theory pp. 144–152 (1992)
5. Breiman, L.: Random forests. Machine learning **45**(1), 5–32 (2001)
6. Grada, A., Weinbrecht, K.: Next-generation sequencing: methodology and application. Journal of Investigative Dermatology **133**(8), e11 (2013)
7. Guyon, I., Weston, J., Barnhill, S., Vapnik, V.: Gene selection for cancer classification using support vector machines. Machine learning **46**(1-3), 389–422 (2002)
8. Kennedy, J., Eberhart, R.: Particle swarm optimization. in Proceedings of IEEE International Conference on Neural Networks **4**, 1942–1948 (1995)
9. Kuleshov, M.V., Jones, M.R., Rouillard, A.D., Fernandez, N.F., Duan, Q., Wang, Z., Koplev, S., Jenkins, S.L., Jagodnik, K.M., Lachmann, A., et al.: Enrichr: a comprehensive gene set enrichment analysis web server 2016 update. Nucleic acids research p. gkw377 (2016)
10. Leidinger, P., Keller, A., Borries, A., Reichrath, J., Rass, K., Jager, S.U., Lenhof, H.P., Meese, E.: High-throughput miRNA profiling of human melanoma blood samples. BMC cancer **10**(1), 1 (2010)
11. Lu, T.P., Lee, C.Y., Tsai, M.H., Chiu, Y.C., Hsiao, C.K., Lai, L.C., Chuang, E.Y.: miRSystem: an integrated system for characterizing enriched functions and pathways of microrna targets. PloS one **7**(8), e42,390 (2012)
12. Lu, X., Gamst, A., Xu, R.: RDCurve: A nonparametric method to evaluate the stability of ranking procedures. IEEE/ACM Transactions on Computational Biology and Bioinformatics **7**(4), 719–726 (2010)
13. Peng, H., Long, F., Ding, C.: Feature selection based on mutual information criteria of max-dependency, max-relevance, and min-redundancy. IEEE Transactions on pattern analysis and machine intelligence **27**(8), 1226–1238 (2005)
14. Ray, S.S., Ganivada, A., Pal, S.K.: A granular self-organizing map for clustering and gene selection in microarray data (2015)
15. Robinson, M.D., McCarthy, D.J., Smyth, G.K.: edgeR: a bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics **26**(1), 139–140 (2010)
16. Saha, I., Bhowmick, S.S., Geraci, F., Pellegrini, M., Bhattacharjee, D., Maulik, U., Plewczynski, D.: Analysis of next-generation sequencing data of miRNA for the prediction of breast cancer. In: International Conference on Swarm, Evolutionary, and Memetic Computing, pp. 116–127 (2015)
17. Sehhati, M., Mehridehnavi, A., Rabbani, H., Pourhossein, M.: Stable gene signature selection for prediction of breast cancer recurrence using joint mutual information. IEEE/ACM Transactions on Computational Biology and Bioinformatics **12**(6), 1440–1448 (2015)
18. Sharma, A., Imoto, S., Miyano, S.: A top-r feature selection algorithm for microarray gene expression data. IEEE/ACM Transactions on Computational Biology and Bioinformatics (TCBB) **9**(3), 754–764 (2012)
19. Wong, N., Wang, X.: miRDB: an online resource for microrna target prediction and functional annotations. Nucleic acids research p. gku1104 (2014)
20. Yu, D., Huber, W., Vitek, O.: Shrinkage estimation of dispersion in negative binomial models for RNA-seq experiments with small sample size. Bioinformatics **29**(10), 1275–1282 (2013)
21. Zhuang, X., Li, Z., Lin, H., Gu, L., Lin, Q., Lu, Z., Tzeng, C.M.: Integrated miRNA and mRNA expression profiling to identify mRNA targets of dysregulated miRNAs in non-obstructive azoospermia. Scientific Reports **5** (2015)