ERCC Data Analysis Workshop

Use Case 2: Circulating miRNA Changes Associated with Alzheimer's and Parkinson's Diseases

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Thursday, 23 April, 2015
7:30 pm

Data and disease background slides kindly provided by
Kendall Jensen, Translational Genomics Research Institute

Burgos K., et al. (2014) Profiles of Extracellular miRNA in Cerebrospinal Fluid and Serum from Patients with Alzheimer's and Parkinson's Diseases Correlate with Disease Status and Features of Pathology. PLoS ONE 9: e94839.

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Use Case 2: miRNA Changes in Alzheimer's and Parkinson's Disease

The goal of this use case is to show that the Genboree Workbench and the exceRpt small RNA-seq analysis pipeline can replicate the results of Burgos et al. We have developed these pipelines because as the Extracellular RNA Communication Consortium (ERCC) begins to generate more datasets, it is vital that they be analyzed in a reproducible and comparable way.

Burgos K., et al. (2014) Profiles of Extracellular miRNA in Cerebrospinal Fluid and Serum from Patients with Alzheimer's and Parkinson's Diseases Correlate with Disease Status and Features of Pathology. PLoS ONE 9: e94839.

Use Case 2: miRNA Changes in Alzheimer's and Parkinson's Disease

Biological Question of Interest

Alzheimer's and Parkinson's are two neurodegenerative diseases that are difficult to assess by biopsy, since the affected tissue is inaccessible.

In their study, Burgos et al. assessed the miRNA content in cerebrospinal fluid (CSF) and serum from postmortem subjects with full neuropathology evaluations. The goal of the study was to identify extracellular miRNA biomarkers that correlate with disease status and progression.

In this use case, we focus on biomarkers that correlate with disease status by replicating the differential expression analysis in Burgos et al.

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Use Case 2: miRNA Changes in Alzheimer's and Parkinson's Disease

345 Biological Samples to Be Analyzed

Number of Samples	Disease	Biofluid
62	Alzheimer's	CSF
52	Alzheimer's	Serum
57	Parkinson's	CSF
50	Parkinson's	Serum
62	Control	CSF
62	Control	Serum

The dataset analyzed in this use case is available from dbGap, accession phs000727.v1.p1.

Differential Expression of miRNAs
in Cerebrospinal Fluid (CSF) – Control vs. AD

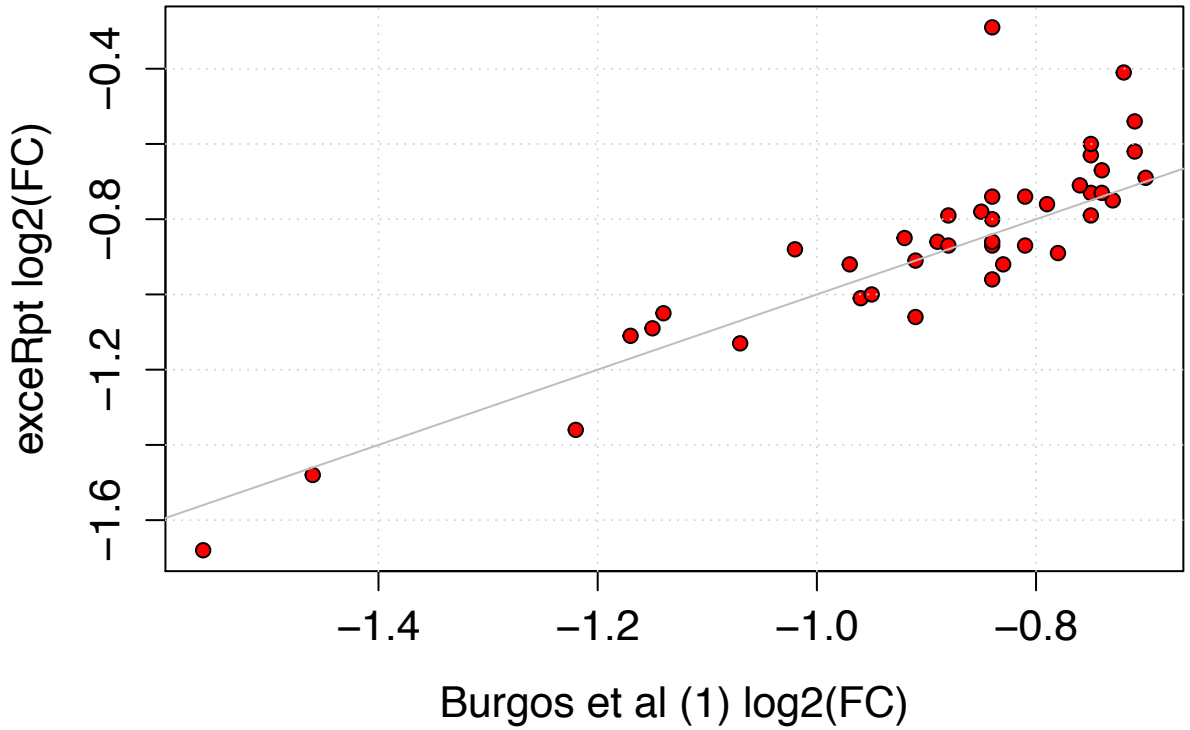
	Burgos et al		exceRpt + DESeq2	
miR Name	log2(FC)	P-value	log2(FC)	P-value
miR-124-3p	-1.56	1.00E-06	-1.68	2.00E-06
miR-138-5p	-1.46	1.00E-06	-1.48	7.00E-06
miR-127-3p	-1.17	1.90E-05	-1.11	2.55E-04
miR-132-3p	-0.89	1.90E-05	-0.86	1.29E-04
miR-127-5p	-1.15	3.10E-05	-1.09	3.07E-04
miR-136-3p	-1.02	4.00E-05	-0.88	1.16E-03
miR-381(-3p)*	-1.14	4.90E-05	-1.05	9.86E-04
miR-101-5p	-0.92	6.70E-05	-0.85	9.86E-04
miR-199b-5p	-1.22	6.70E-05	-1.36	1.29E-04
miR-136-5p	-0.91	1.91E-04	-0.91	7.73E-04
miR-184	-0.97	2.69E-04	-0.92	1.73E-03
miR-181a-5p	-0.71	3.57E-04	-0.62	3.49E-03
miR-598(-3p)*	-0.96	4.15E-04	-1.01	4.24E-04
miR-218-5p	-0.79	4.93E-04	-0.76	2.56E-03
miR-9-3p	-0.84	4.95E-04	-0.96	3.07E-04
miR-769-5p	-0.84	6.20E-04	-0.80	3.49E-03
miR-95(-3p)*	-0.95	6.20E-04	-1.00	9.86E-04
miR-760	-0.88	8.46E-04	-0.87	1.94E-03
miR-181a-3p	-0.74	1.01E-03	-0.67	7.55E-03

	Burgos et al		exceRpt + DESeq2	
miR Name	log2(FC)	P-value	log2(FC)	P-value
miR-181b-5p	-0.75	1.01E-03	-0.63	1.42E-02
miR-488-3p	-0.88	1.01E-03	-0.79	1.08E-02
miR-495(-3p)*	-1.07	1.01E-03	-1.13	2.28E-03
miR-708-3p	-0.84	1.01E-03	-0.74	1.31E-02
miR-874(-3p)*	-0.75	1.01E-03	-0.79	1.39E-03
miR-873-5p	-0.81	1.48E-03	-0.74	9.08E-03
miR-129-5p	-0.84	1.65E-03	-0.87	4.32E-03
miR-181d(-5p)*	-0.72	1.89E-03	-0.41	6.86E-02
miR-139-5p	-0.84	1.96E-03	-0.86	4.55E-03
miR-3200-3p	-0.75	2.77E-03	-0.73	9.09E-03
miR-431-3p	-0.91	3.72E-03	-1.06	1.71E-03
miR-9-5p	-0.75	4.97E-03	-0.60	3.32E-02
miR-326	-0.76	5.18E-03	-0.71	1.68E-02
miR-377-5p	-0.81	6.87E-03	-0.87	9.09E-03
miR-433(-3p)*	-0.85	7.77E-03	-0.78	2.96E-02
miR-323a-3p	-0.73	8.53E-03	-0.75	1.54E-02
miR-134(-5p)*	-0.7	8.98E-03	-0.69	1.90E-02
miR-329(-3p)*	-0.83	8.98E-03	-0.92	1.09E-02
miR-10a-5p	-0.84	1.16E-02	-0.29	4.35E-01
miR-33b-5p	-0.74	1.24E-02	-0.73	2.66E-02
miR-410(-3p)*	-0.71	1.47E-02	-0.54	8.82E-02
miR-708-5p	-0.78	1.51E-02	-0.89	1.27E-02

Burgos K., et al. (2014) Profiles of Extracellular miRNA in Cerebrospinal Fluid and Serum from Patients with Alzheimer's and Parkinson's Diseases Correlate with Disease Status and Features of Pathology. PLoS ONE 9: e94839.

Differential Expression of miRNAs
in Cerebrospinal Fluid (CSF) – Control vs. AD

Comparison of Differential Expression Results



The plot to the left compares the differential expression results from Burgos et al. (x-axis) and the exceRpt pipeline (y-axis). miRNAs with absolute $\log_2$ fold changes < 0.7 were not reported in the article.

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Differential Expression of miRNAs
in Cerebrospinal Fluid (CSF) – Control vs PD

	Burgos et al		exceRpt + DESeq2	
miR Name	log2(FC)	P-value	log2(FC)	P-value
miR-132-5p	-1.02	3.93E-04	-1.11	3.31E-04
miR-19a-3p	0.98	3.93E-04	0.97	3.77E-03
miR-19b-3p	0.92	3.93E-04	1.05	3.31E-04
miR-485-5p	-1.08	3.93E-04	-1.24	3.31E-04
miR-127-3p	-0.98	4.48E-04	-1.06	5.28E-04
miR-128(-3p)*	-0.85	3.45E-03	-0.92	3.70E-03
miR-409-3p	-0.77	3.45E-03	-0.80	4.20E-03
miR-433(-3p)*	-0.86	6.29E-03	-0.90	1.05E-02
let-7g-3p	0.82	8.41E-03	0.93	9.40E-03
miR-370(-3p)*	-0.83	8.41E-03	-0.88	1.05E-02
miR-431-3p	-0.81	8.41E-03	-0.74	2.99E-02
miR-873-3p	-0.83	8.68E-03	-0.89	1.38E-02
miR-136-3p	-0.72	1.20E-02	-0.68	3.03E-02
miR-212-3p	-0.73	2.29E-02	-0.78	3.03E-02
miR-10a-5p	-0.76	2.37E-02	-0.27	4.76E-01
miR-1224-5p	-0.76	3.29E-02	-0.73	9.88E-02

*In some cases, Burgos et al reports results for the parent form of a miRNA. The sRNAbench tool used by exceRpt always estimates which mature product of the miRNA is most likely expressed based on the position of reads in the miRNA sequence.

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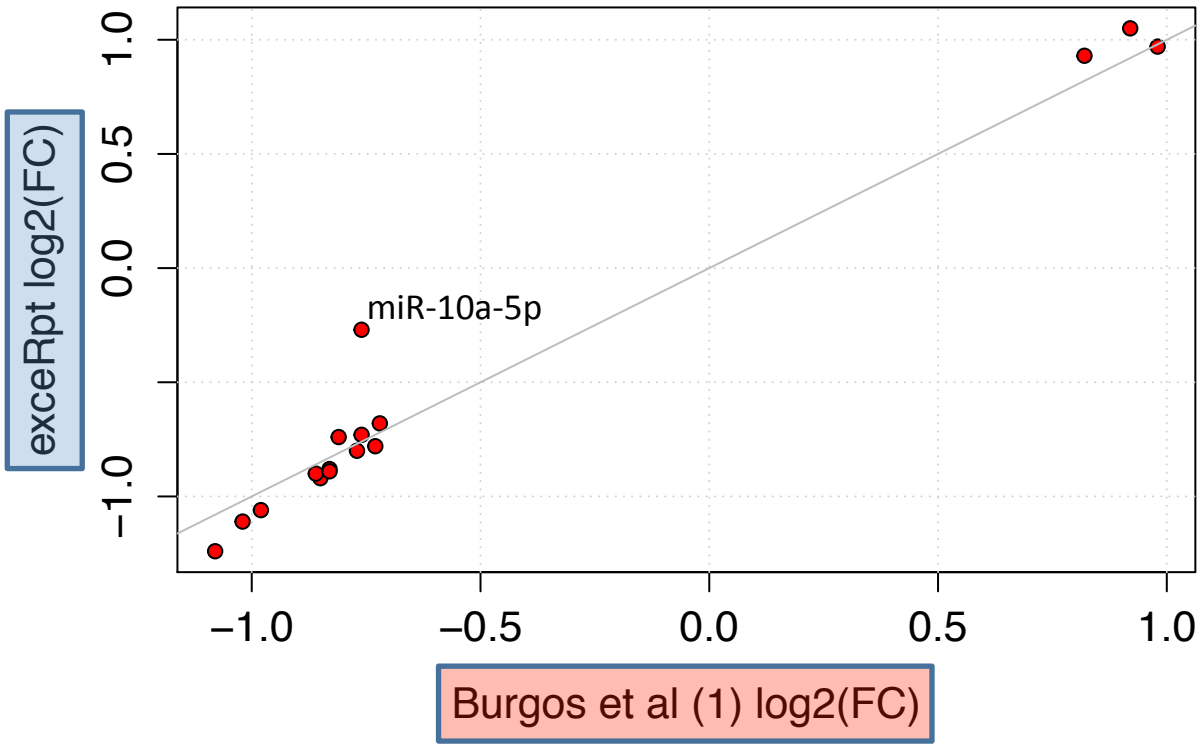
Differential Expression of miRNAs
in Cerebrospinal Fluid (CSF) – Control vs PD

Comparison of Differential Expression Results



The plot at left compares the differential expression results from Burgos et al (x axis) and the exceRpt pipeline (y axis). miRNAs with absolute $\log_2$ fold changes < 0.7 were not reported in the article.

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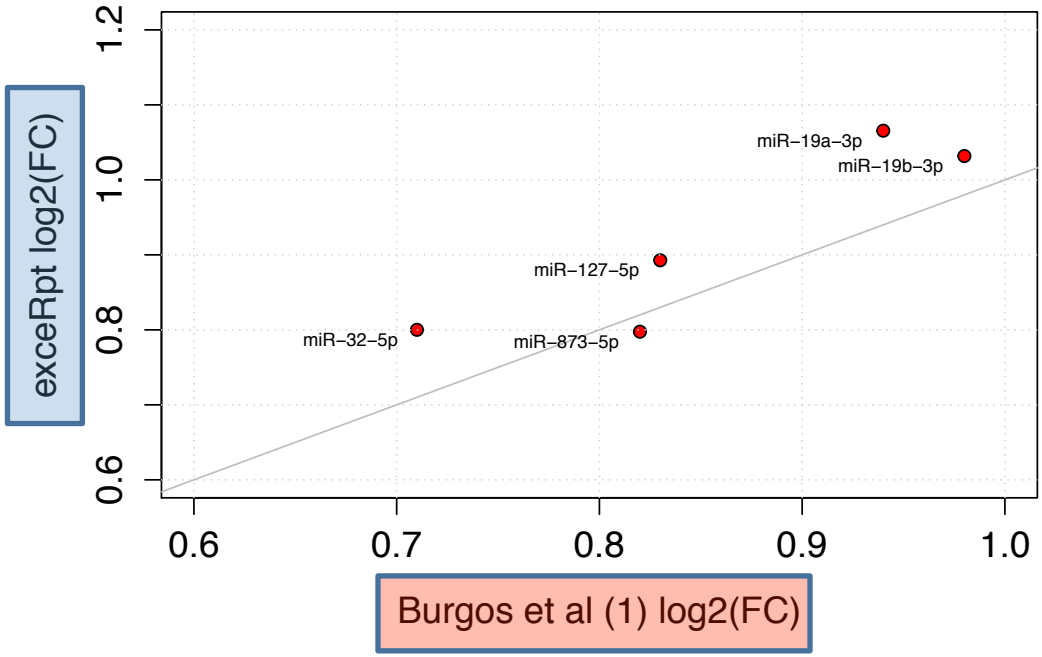
Differential Expression of miRNAs
in Cerebrospinal Fluid (CSF) – AD vs PD

	Burgos et al		exceRpt + DESeq2	
miR Name	log2(FC)	P-value	log2(FC)	P-value
miR-19b-3p	0.98	2.34E-04	1.03	7.39E-04
miR-19a-3p	0.94	6.84E-04	1.07	1.93E-03
miR-127-5p	0.83	1.57E-02	0.89	1.95E-02
miR-873-5p	0.82	1.57E-02	0.80	3.39E-02
miR-32-5p	0.71	3.06E-02	0.80	2.02E-02



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Differential Expression of miRNAs in serum – Control vs Alzheimer's

	Burgos et al		exceRpt + DESeq2	
miR Name	log2(FC)	P-value	log2(FC)	P-value
miR-34b-3p	2.36	1.90E-05	1.95	4.77E-03
miR-219-2-3p	1.93	5.37E-04	2.62	9.20E-05
miR-22-5p	1.38	5.37E-04	1.11	4.77E-03
miR-125b-1-3p	1.32	8.03E-04	1.29	1.10E-03
miR-1307-5p	1.39	1.28E-03	0.81	7.82E-02
miR-34c-5p	1.57	1.96E-03	2.04	2.52E-04
miR-34b-5p	1.71	2.57E-03	2.33	8.70E-04
miR-887(-3p)*	1.34	3.31E-03	1.58	1.06E-03
miR-182-5p	-1.01	3.82E-03	-1.14	9.36E-04
miR-135a-5p	1.66	4.84E-03	2.73	3.22E-04
miR-184	1.43	4.84E-03	2.50	5.00E-06
miR-30c-2-3p	1.22	4.84E-03	1.31	6.41E-03
miR-873-3p	1.30	4.84E-03	1.43	4.77E-03
miR-125a-3p	1.27	9.65E-03	1.74	8.70E-04
miR-671-3p	1.21	9.65E-03	0.75	1.53E-01
miR-21-5p	-0.82	2.35E-02	-0.88	1.58E-02
miR-1285-3p	1.03	2.45E-02	1.60	2.52E-04
miR-375	-0.94	3.26E-02	-1.00	2.89E-02
miR-3176	1.06	3.35E-02	1.01	6.07E-02
miR-127-3p	0.92	3.74E-02	0.94	3.49E-02

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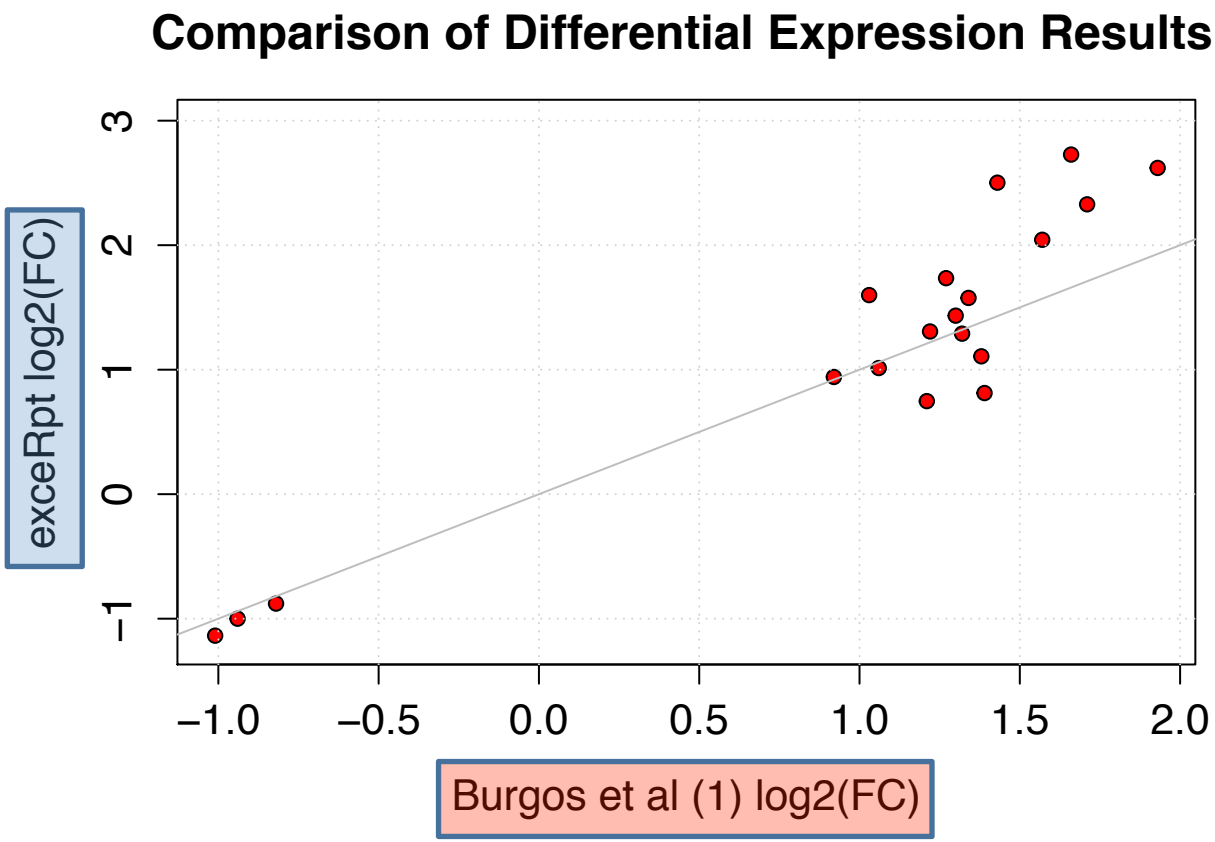
Differential Expression of miRNAs
in serum – Control vs Alzheimer's



The plot at left compares the differential expression results from Burgos et al (x axis) and the exceRpt pipeline (y axis). miRNAs with absolute $\log_2$ fold changes < 0.7 were not reported in the article.

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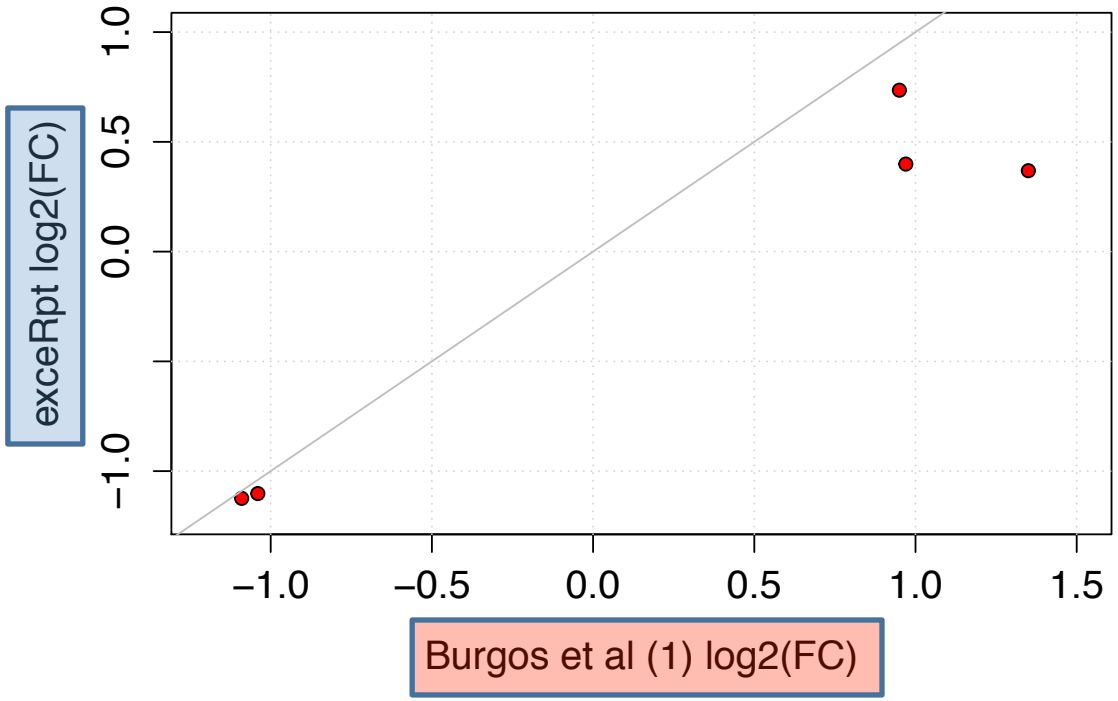
Differential Expression of miRNAs in serum – Control vs Parkinson's

	Burgos et al		exceRpt + DESeq2	
miR Name	log2(FC)	P-value	log2(FC)	P-value
miR-338-3p	1.35	1.06E-03	0.37	5.64E-01
miR-16-2-3p	-1.09	3.96E-03	-1.12	2.10E-02
miR-1294	-1.04	1.17E-02	-1.10	2.10E-02
miR-30e-3p	0.97	1.33E-02	0.40	4.70E-01
miR-30a-3p	0.95	1.54E-02	0.74	1.63E-01



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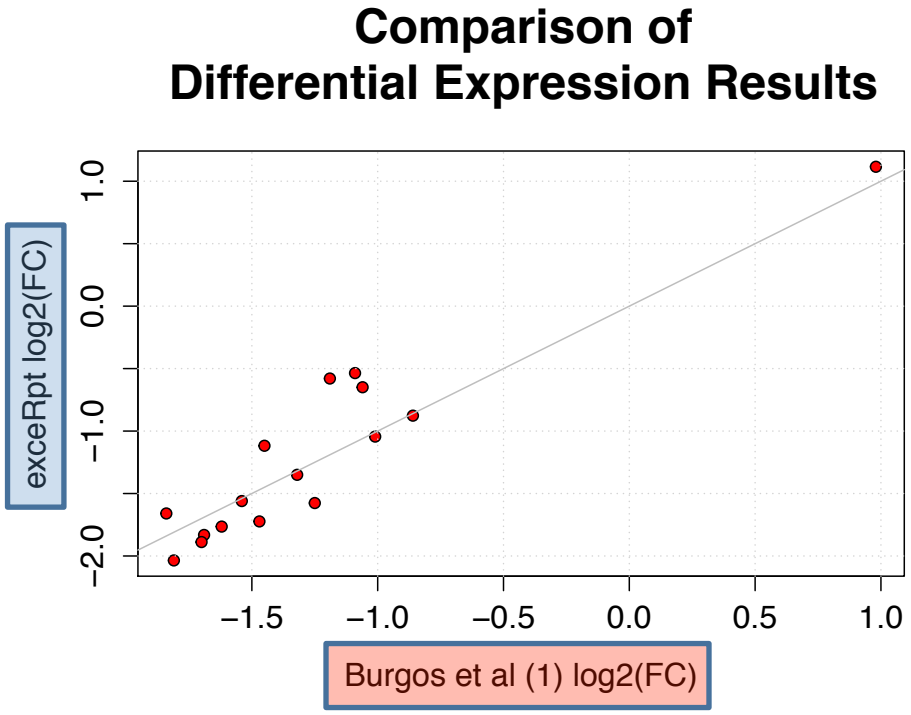
Differential Expression of miRNAs
in serum – Alzheimer's vs Parkinson's

	Burgos et al		exceRpt + DESeq2	
miR Name	log2(FC)	P-value	log2(FC)	P-value
miR-320a	-1.81	4.60E-05	-2.04	3.10E-05
miR-320b	-1.84	4.60E-05	-1.66	1.92E-03
miR-378d	-1.69	4.60E-05	-1.83	5.80E-05
miR-378f	-1.70	4.60E-05	-1.89	4.10E-05
miR-378a(-3p)*	-1.54	7.70E-05	-1.56	2.07E-04
miR-378b	-1.62	1.32E-04	-1.76	1.17E-03
miR-378c	-1.47	4.53E-04	-1.72	1.42E-04
miR-193a-5p	-1.32	2.22E-03	-1.35	3.25E-03
miR-320c	-1.45	3.48E-03	-1.12	5.60E-02
miR-1285-3p	-1.25	6.90E-03	-1.58	1.03E-03
miR-550a-3-5p	-1.09	1.36E-02	-0.54	2.25E-01
miR-874(-3p)*	-1.06	1.39E-02	-0.65	1.65E-01
miR-125a-5p	-1.01	1.69E-02	-1.04	1.54E-02
miR-21-5p	0.98	1.69E-02	1.12	6.74E-03
miR-671-3p	-1.19	1.69E-02	-0.58	3.08E-01
let-7i-3p	-0.86	2.77E-02	-0.88	2.80E-02



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Digging Deeper:
Parkinson's vs. Parkinson's with Dementia

	Burgos et al		exceRpt + DESeq2	
miR Name	log2(FC)	P-value	log2(FC)	P-value
miR-30b-5p	1.94	3.67E-04	2.10	3.71E-04
miR-30c-5p	1.79	3.67E-04	1.86	4.92E-04
miR-30a-3p	1.89	4.79E-04	2.00	3.38E-03
miR-664-3p	2.35	6.85E-04	---	---
miR-26a-5p	1.70	1.41E-03	1.84	1.66E-03
miR-34c-5p	2.12	2.26E-03	1.53	6.55E-02
miR-582-3p	1.86	2.90E-03	0.49	7.36E-01
miR-26b-5p	1.76	3.23E-03	1.04	2.53E-01
miR-1298(-3p)*	2.05	5.54E-03	1.01	5.43E-01
let-7f-1-3p	1.90	6.94E-03	2.18	1.62E-02
miR-375	-1.61	8.07E-03	-1.10	1.71E-01
miR-1247-5p	-1.82	9.22E-03	-2.20	1.62E-02
miR-1911-5p	1.98	9.22E-03	1.00	4.86E-01
miR-374a-5p	1.90	9.22E-03	2.29	1.62E-02
miR-34b-5p	2.01	9.56E-03	1.26	4.28E-01
miR-152(-3p)*	1.36	1.07E-02	1.61	9.79E-03
miR-7-1-3p	1.68	1.29E-02	1.94	1.94E-02
miR-135a-5p	1.86	1.70E-02	-0.12	9.71E-01
miR-18b-5p	1.78	1.70E-02	0.62	8.17E-01
miR-23c	1.37	1.70E-02	---	---
miR-135b-5p	1.82	1.76E-02	---	---
miR-374a-3p	1.63	2.19E-02	1.87	4.07E-02
miR-148b-3p	1.27	2.20E-02	1.32	3.88E-02
miR-18a-5p	1.70	2.20E-02	0.46	8.24E-01
miR-204-5p	1.72	2.20E-02	1.55	7.65E-02

	Burgos et al		exceRpt + DESeq2	
miR Name	log2(FC)	P-value	log2(FC)	P-value
miR-374b-5p	1.47	3.15E-02	1.64	5.61E-02
miR-3960	1.44	3.31E-02	---	---
miR-3605-5p	-1.44	3.31E-02	-0.21	8.58E-01
miR-101-3p	1.29	3.47E-02	0.71	4.91E-01
miR-34b-3p	1.71	3.48E-02	0.43	8.29E-01
miR-503(-5p)*	1.67	3.48E-02	0.21	9.31E-01
miR-132-3p	1.05	5.18E-02	1.05	9.72E-02
miR-142-5p	1.07	5.27E-02	1.21	5.61E-02
miR-582-5p	1.46	6.28E-02	0.42	8.26E-01
miR-1246	-1.43	6.60E-02	-0.34	8.29E-01
miR-1260a	1.20	6.71E-02	0.76	4.96E-01
miR-208b(-3p)*	1.42	6.71E-02	-0.79	5.26E-01
miR-196a-5p	-1.50	7.35E-02	---	---
miR-548d-5p	1.04	7.48E-02	---	---
miR-548am-5p	1.15	7.48E-02	---	---
miR-1264	1.46	7.69E-02	-0.14	9.64E-01
miR-424-5p	1.29	7.69E-02	0.03	9.84E-01
miR-98(-5p)*	1.13	7.69E-02	1.17	1.92E-01
miR-214-3p	1.22	8.20E-02	1.36	1.71E-01
miR-34c-3p	1.47	8.33E-02	0.53	8.23E-01
miR-486-3p	-1.05	8.33E-02	-1.11	1.80E-01
let-7f-2-3p	1.18	9.16E-02	1.67	6.64E-02
miR-124-3p	1.44	9.21E-02	2.03	4.85E-02
miR-199a-3p	0.99	9.79E-02	1.05	1.80E-01
miR-340-5p	1.11	9.79E-02	1.26	1.71E-01
...	...	...	...	...

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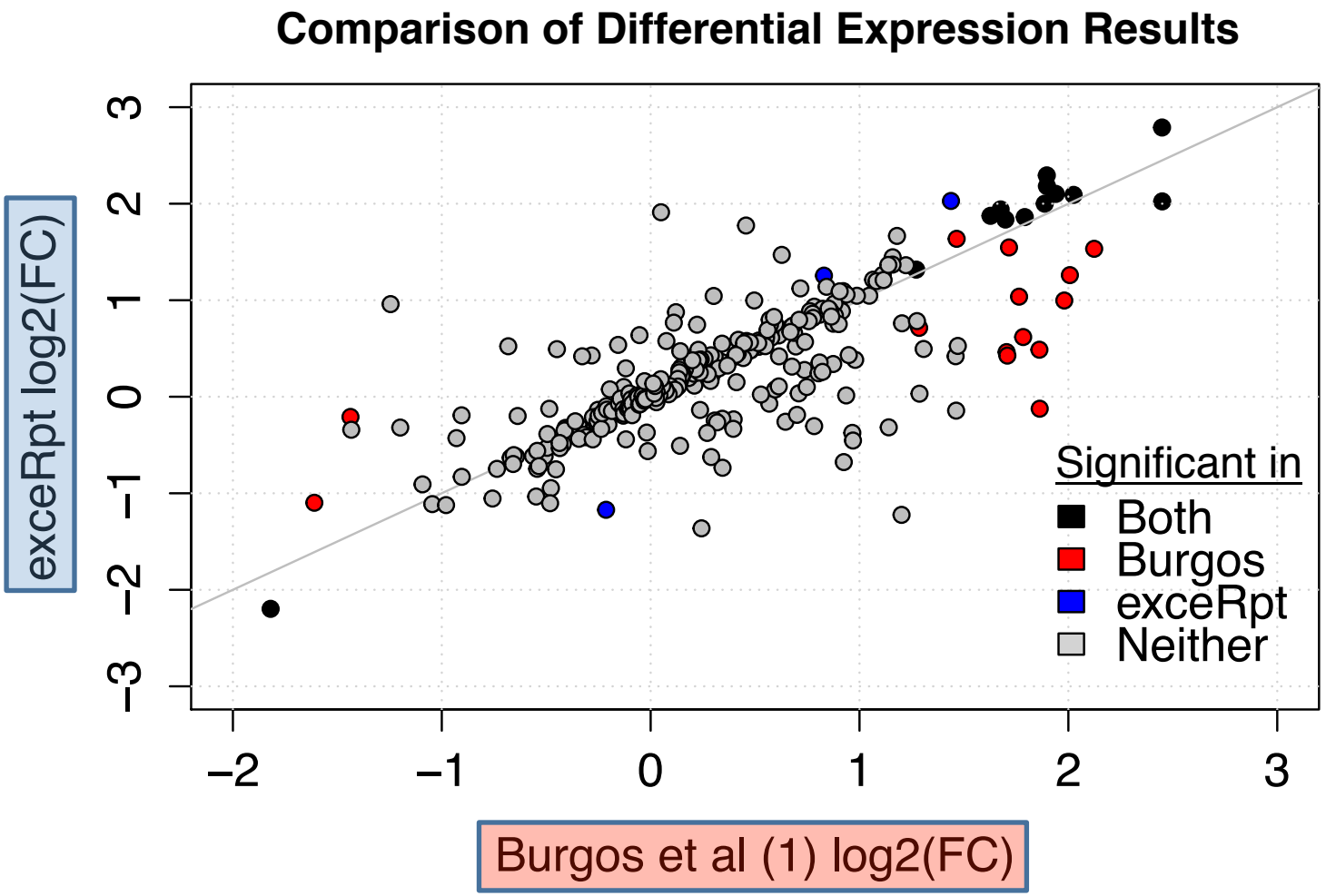
Digging Deeper:
Parkinson's vs. Parkinson's with Dementia



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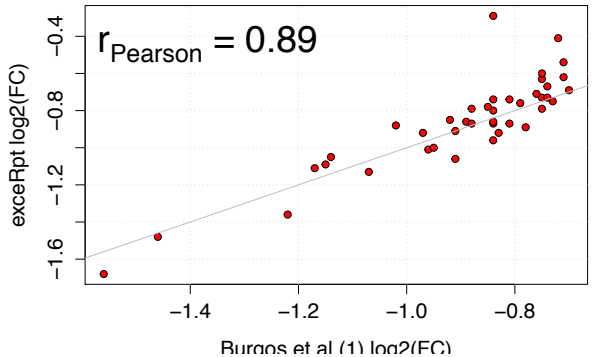
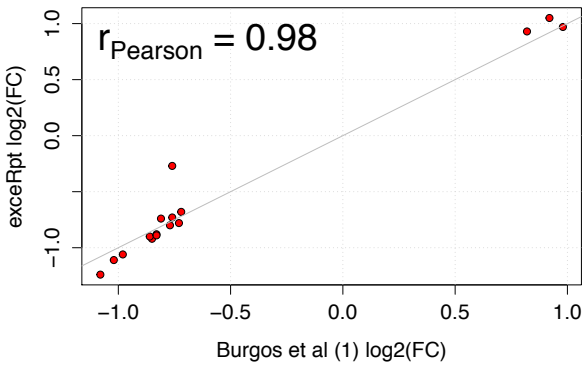
Use Case 2: Summary

Differential expression analysis of all pairwise comparisons shows that the exceRpt pipeline does a good job of reproducing the results from Burgos et al.

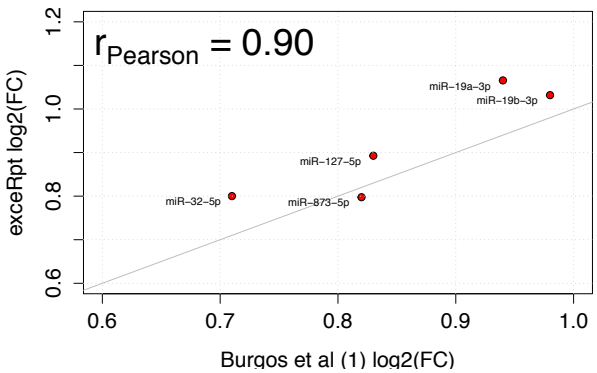
AD vs control in CSF



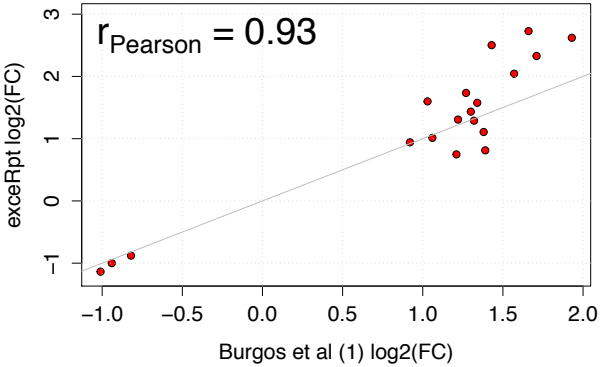
PD vs control in CSF



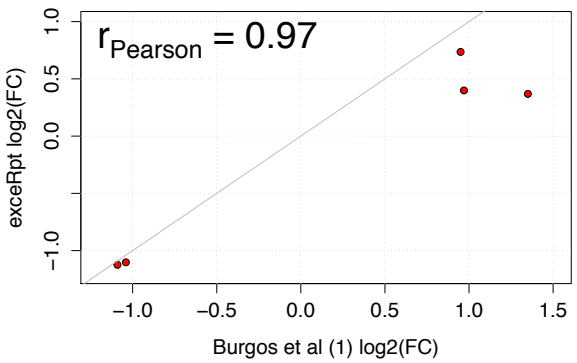
AD vs PD in CSF



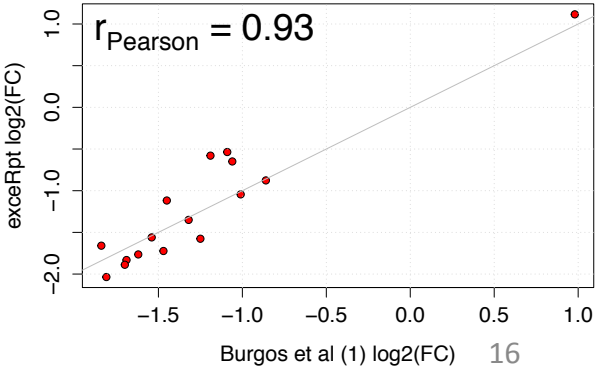
AD vs control in Serum



PD vs control in Serum



AD vs PD in Serum



Use Case 2: Summary

Alzheimer's CSF vs control: all 41 of the miRNAs identified in Burgos et al as significantly differentially expressed were also so identified by exceRpt.

Pearson correlation of $\log_2$(fold change) for those top 41 miRNAs between exceRpt and Burgos et al is 0.89.

30 / 41 (~75%) of the miRNAs have been identified in the literature as deregulated in Alzheimer's.

Thus, this set of miRNAs represent useful candidates for further study and development into clinical biomarkers of AD disease status.

See Burgos et al. for discussion of the other comparisons: AD vs PD and PD vs Control in CSF, and all serum cases.

Burgos K., et al. (2014) Profiles of Extracellular miRNA in Cerebrospinal Fluid and Serum from Patients with Alzheimer's and Parkinson's Diseases Correlate with Disease Status and Features of Pathology. PLoS ONE 9: e94839.

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Use Case 2: References

1. Burgos K., Malenica I., Metpally R., Courtright A., et al. **(2014)** *Profiles of extracellular miRNA in cerebrospinal fluid and serum from patients with Alzheimer's and Parkinson's diseases correlate with disease status and features of pathology.* **PLoS ONE** 9:e94839.
2. Barturen et al. **(2014)** *sRNAbench: profiling of small RNAs and its sequence variants in single or multi-species high-throughput experiments*. **Methods in Next Generation Sequencing**. Volume 1, Issue 1
3. Ben Langmead, Cole Trapnell, Mihai Pop and Steven L Salzberg **(2009)** *Ultrafast and memory-efficient alignment of short DNA sequences to the human genome.* **Genome Biology** 10(3):R25.
4. Kozomara A, Griffiths-Jones S. **(2011)** *miRBase: integrating microRNA annotation and deep-sequencing data*. **NAR** 39 (Database Issue)
5. Langmead B, Salzberg SL. **(2012)** *Fast gapped-read alignment with Bowtie 2*. **Nature Methods**. 9 : 357-359.
6. Love M.I., Huber W., and Anders S. **(2014)** *Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2*. **Genome Biology** 15: 550.
7. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, et al. **(2003)** *Cytoscape: a software environment for integrated models of biomolecular interaction networks.* **Genome Research** 13(11):2498-504
8. **ExRNA Portal Software Resources (http://exrna.org/resources/software)**

Useful Links

- exRNA Portal Software Resources http://exrna.org/resources/software
- exRNA Atlas – http://genboree.org/java-bin/exRNAAtlas.jsp
- Genboree Workbench - http://genboree.org/java-bin/workbench.jsp
- Data Coordination Center Wiki - http://genboree.org/theCommons/projects/exrna-mads/wiki
- exRNA Data Analysis Tools Wiki - http://genboree.org/theCommons/projects/exrna-tools-may2014/wiki
- Use Case Tutorials – exRNA Portal Data Resource http://exrna.org/resources/data/