microRNA-guided diagnostics for neuroendocrine tumors

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Neuroendocrine tumors

- Group of histologically similar neoplasms, likely arising from neuroendocrine cells
- Arise throughout the body, particularly in the pancreas, gastrointestinal tract, lungs, and endocrine glands, and also skin and other organs
- Hard to diagnose, often metastatic at presentation, and hard to monitor
- field desperately needs tissue and circulating biomarkers to guide clinical decision-making

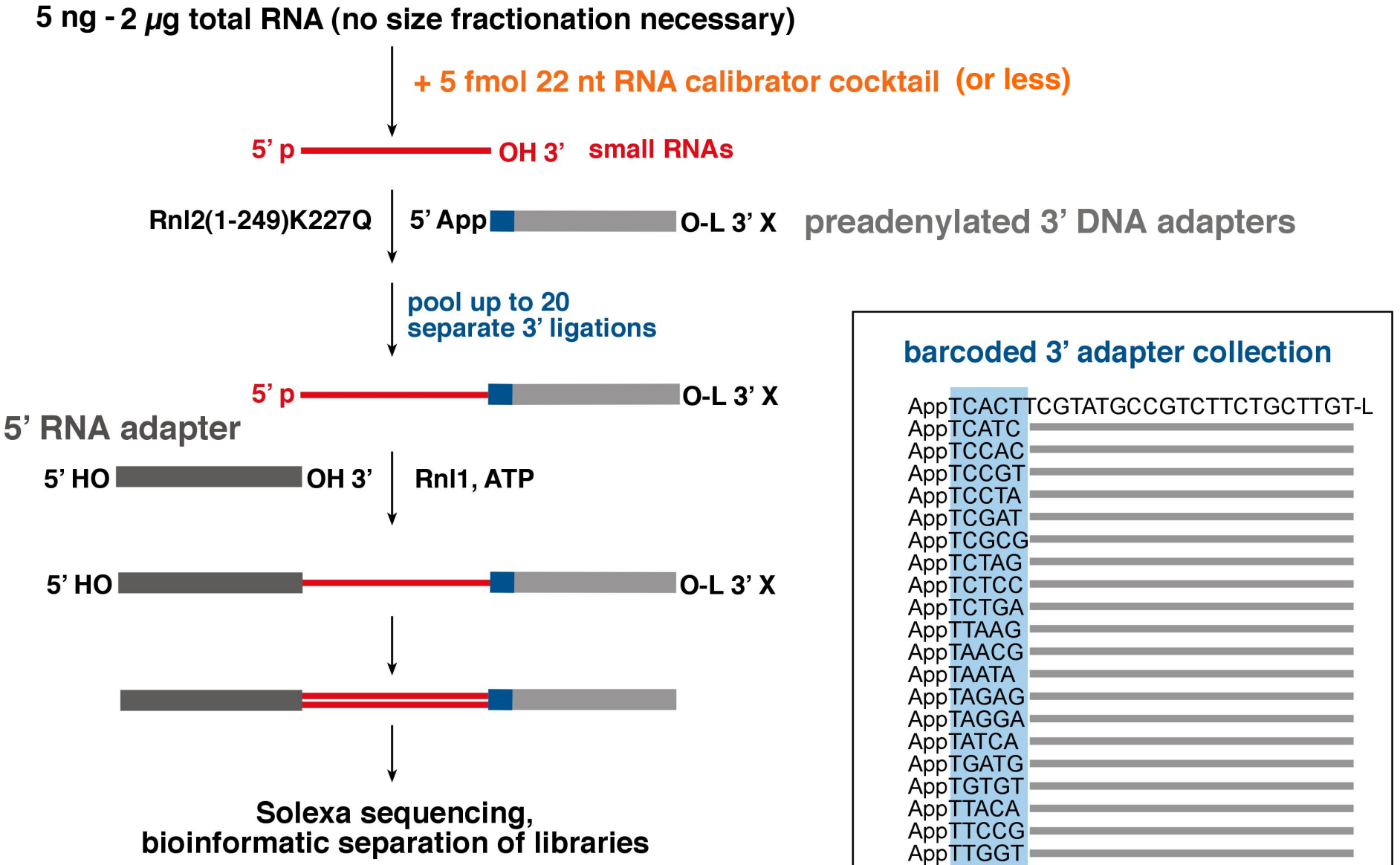
microRNAs

- are small RNA regulatory molecules
- are abundantly expressed
- exhibit cell-type and disease-stage specificity
- Are stable in solid (fresh and archived tissues and cell lines) and liquid (serum and plamsa) clinical samples

Proof-of-concept: GEP-NET study

- Obtained 81 tissue blocks from 27 pancreatic, 31 ileal, 15 appendiceal, and 8 rectal NETs
- Isolated and quality controlled total RNA, generated microRNA profiles through small RNA sequencing
- Performed data preprocessing, removing three samples from further analysis
- Performed high expression analyses
- Generated a microRNA-based classifier for classifying and (almost) grading NETs

Bar-coded small RNA library preparation with ext. standard

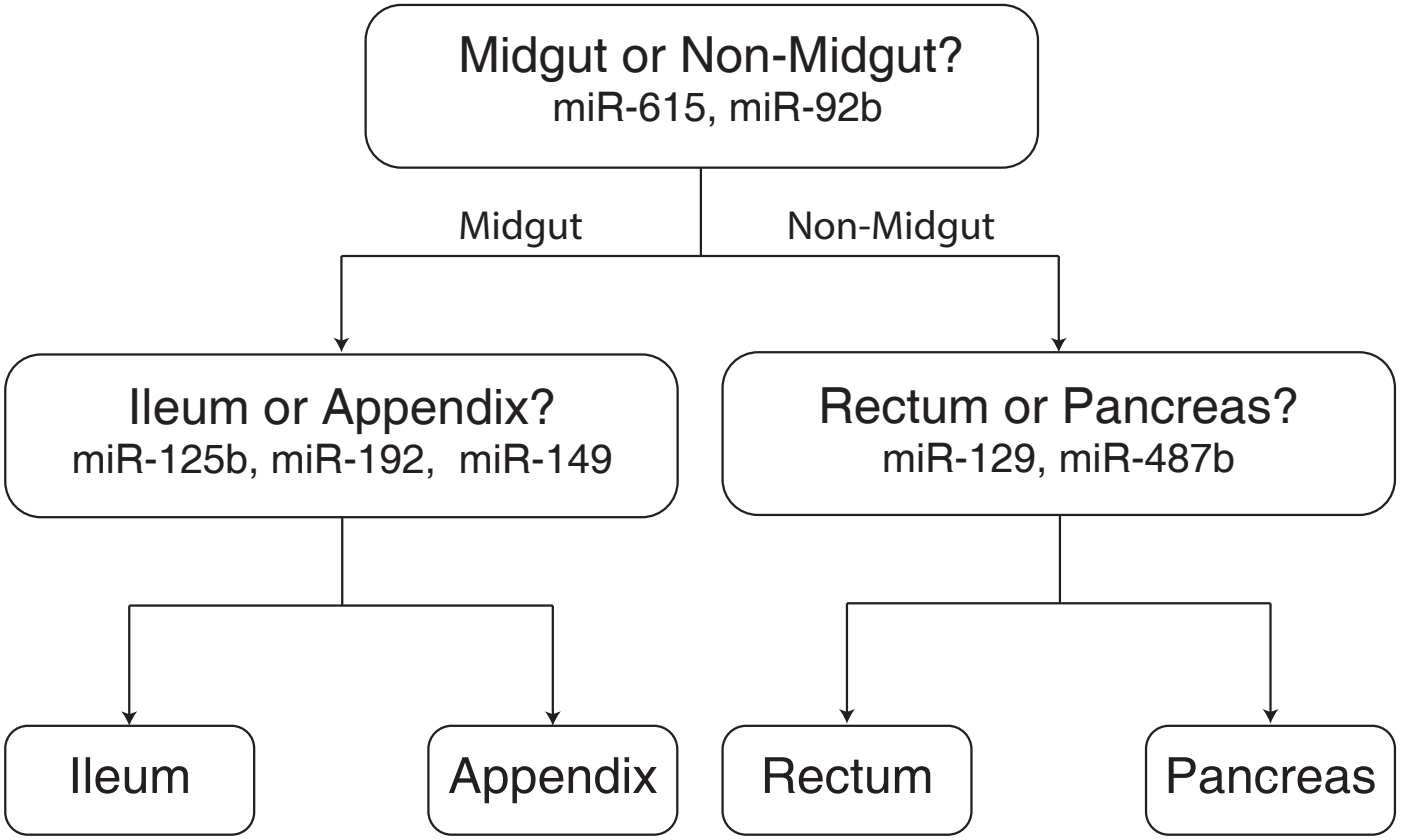


High expression analyses for GEP-NETs

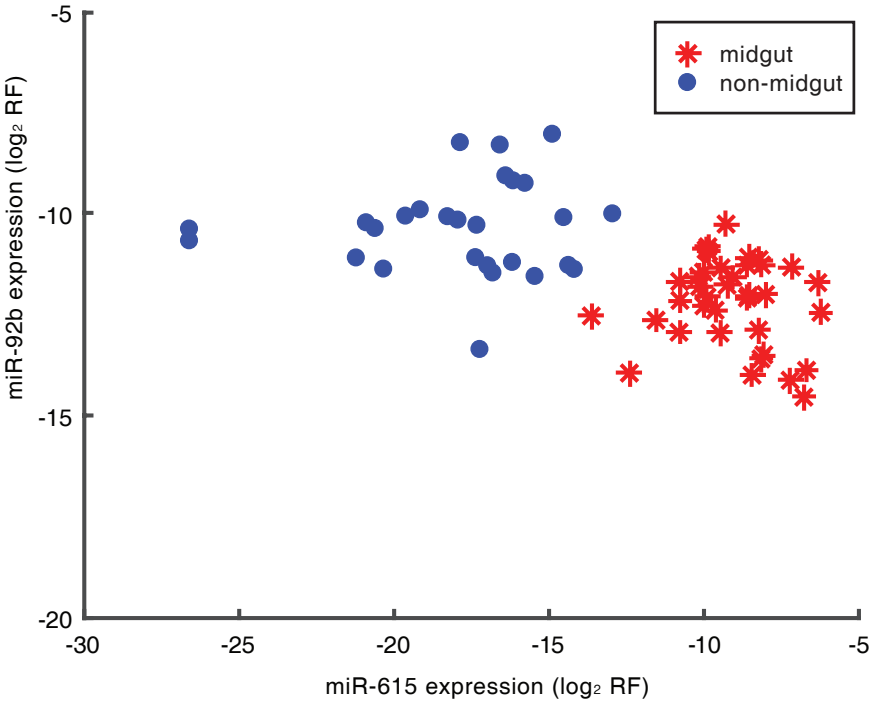
Discovery set		Validation set	
miRNA	median % of miRNA in all samples	miRNA	median % of miRNA in all samples
miR-375	16.6	miR-375	20.2
miR-143	4.9	miR-143	5.3
miR-7	3.9	miR-21	3.9
miR-21	3.3	miR-7	3.2
miR-26a	2.0	miR-192	1.9
miR-125b	1.4	miR-200a	1.4
miR-192	1.4	miR-141	1.4
let-7a	1.3	miR-26a	1.4
miR-29a	1.3	miR-125b	1.2
miR-101	1.2	miR-194	1.2
miR-125a	1.1	miR-27b	1.2
miRNA cistron	median % of miRNA cistron in all samples	miRNA cistron	median % of miRNA cistron in all samples
cluster-mir-375(1)	16.6	cluster-mir-375(1)	20.2
cluster-mir-98(13)	6.9	cluster-mir-143(2)	5.7
cluster-mir-143(2)	5.4	cluster-mir-98(13)	5.5
cluster-mir-7-1(3)	3.9	cluster-mir-21(1)	3.9

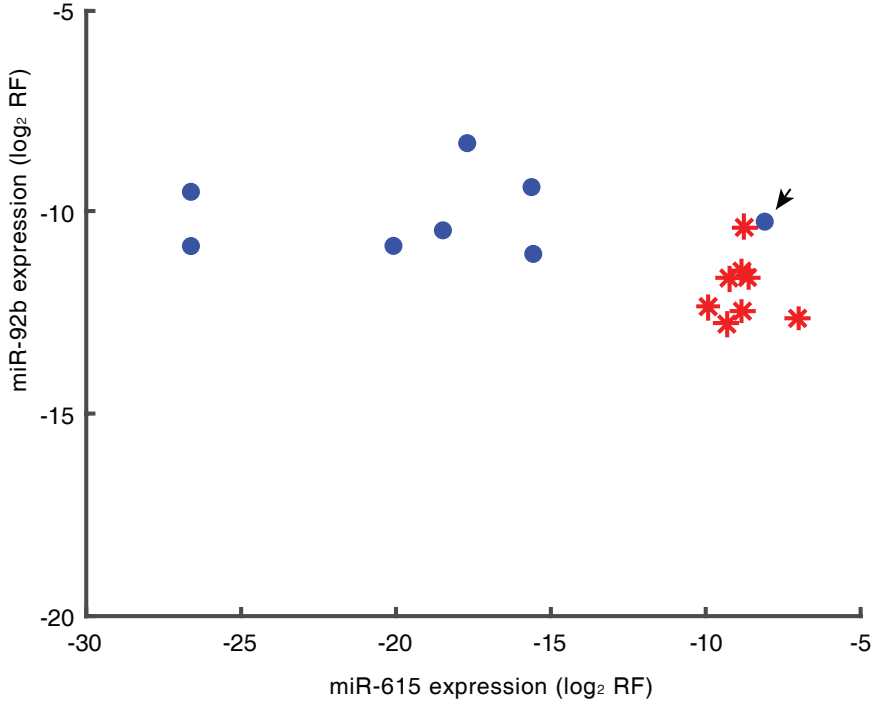
Figure 1

miRNA-based classification of GI-NETs

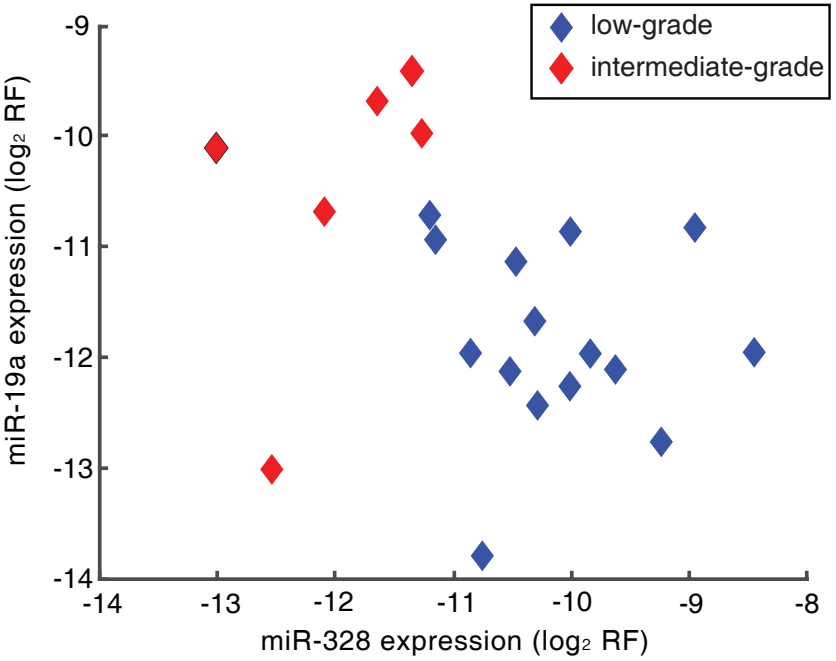


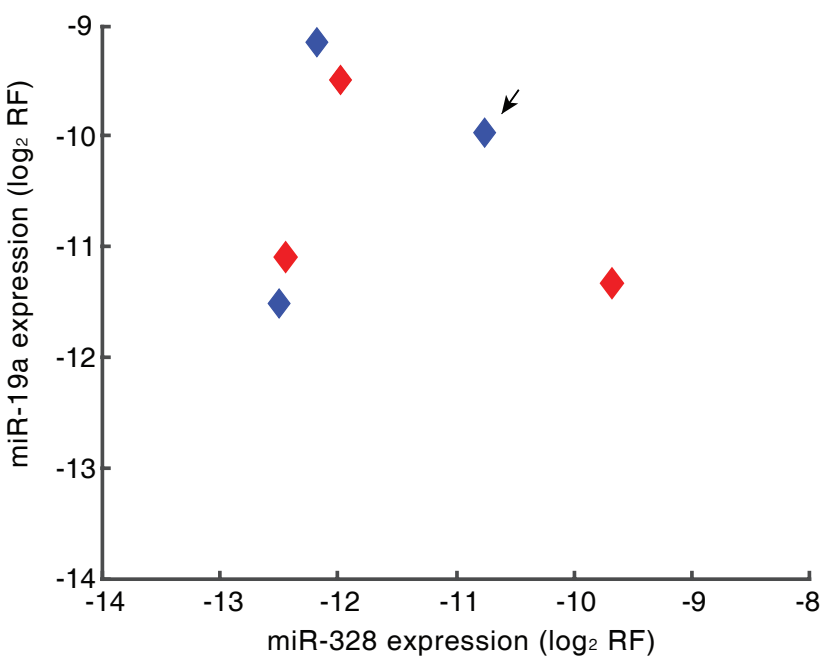
Midgut vs non-midgut NETs





Low- vs intermediate-grade pancreatic NETs



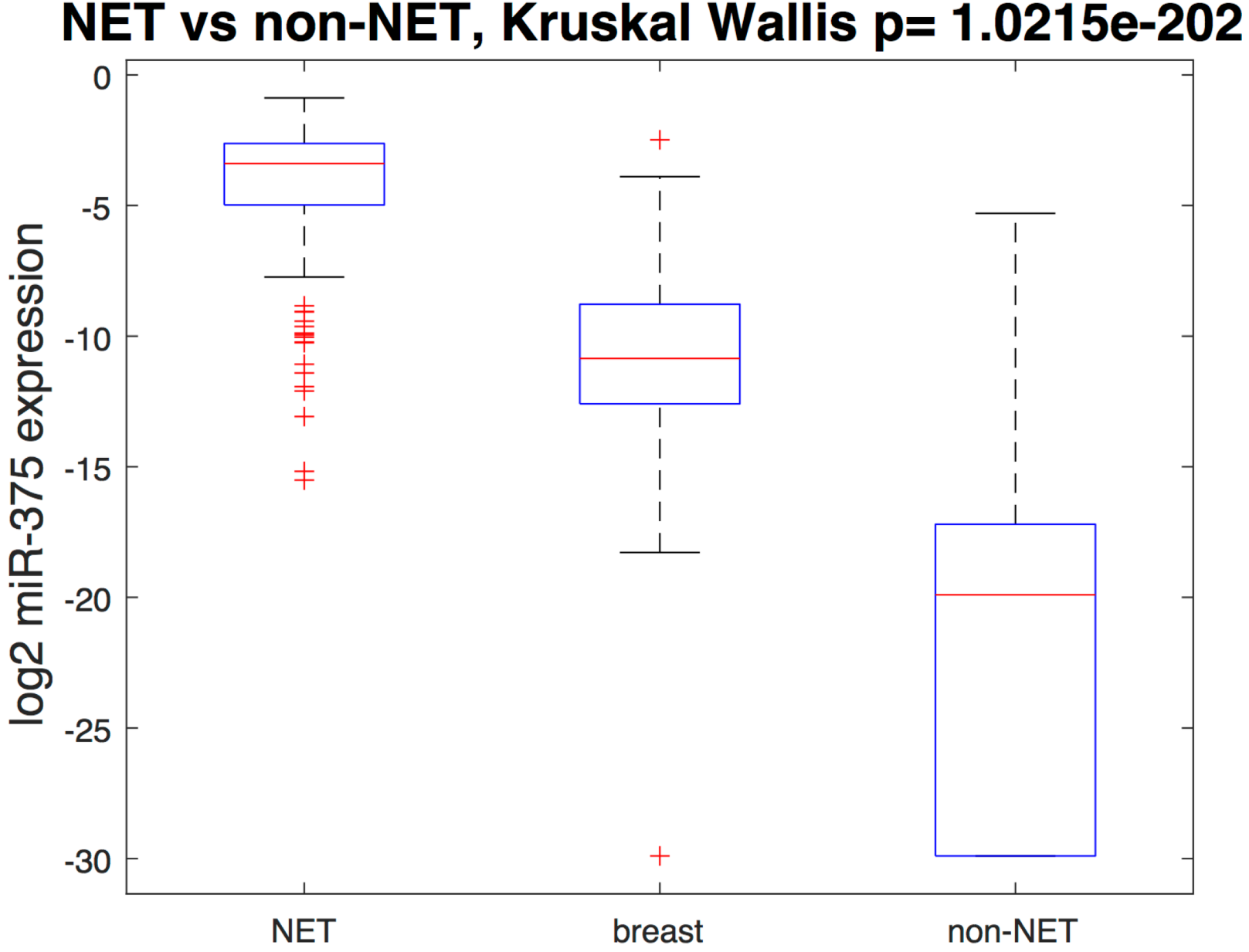


Summary

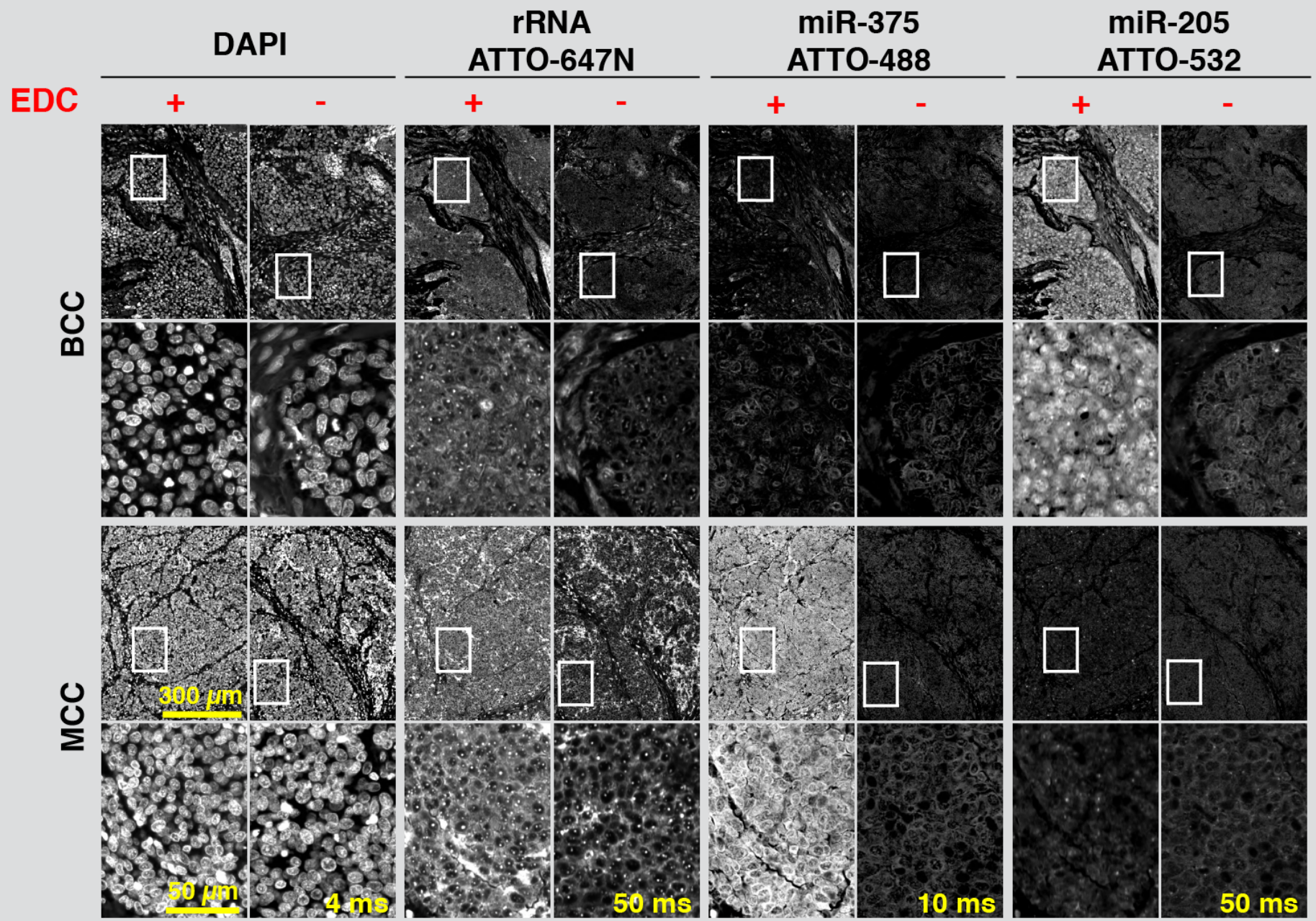
- microRNAs can be used to classify GEP-NETs by anatomic site-of-origin
- microRNAs can likely be used to grade pancreatic NETs but further testing is required
- miR-375 is abundantly expressed in all GEP-NETs and may be valuable tissue and circulatory biomarkers for this disease

Showcase

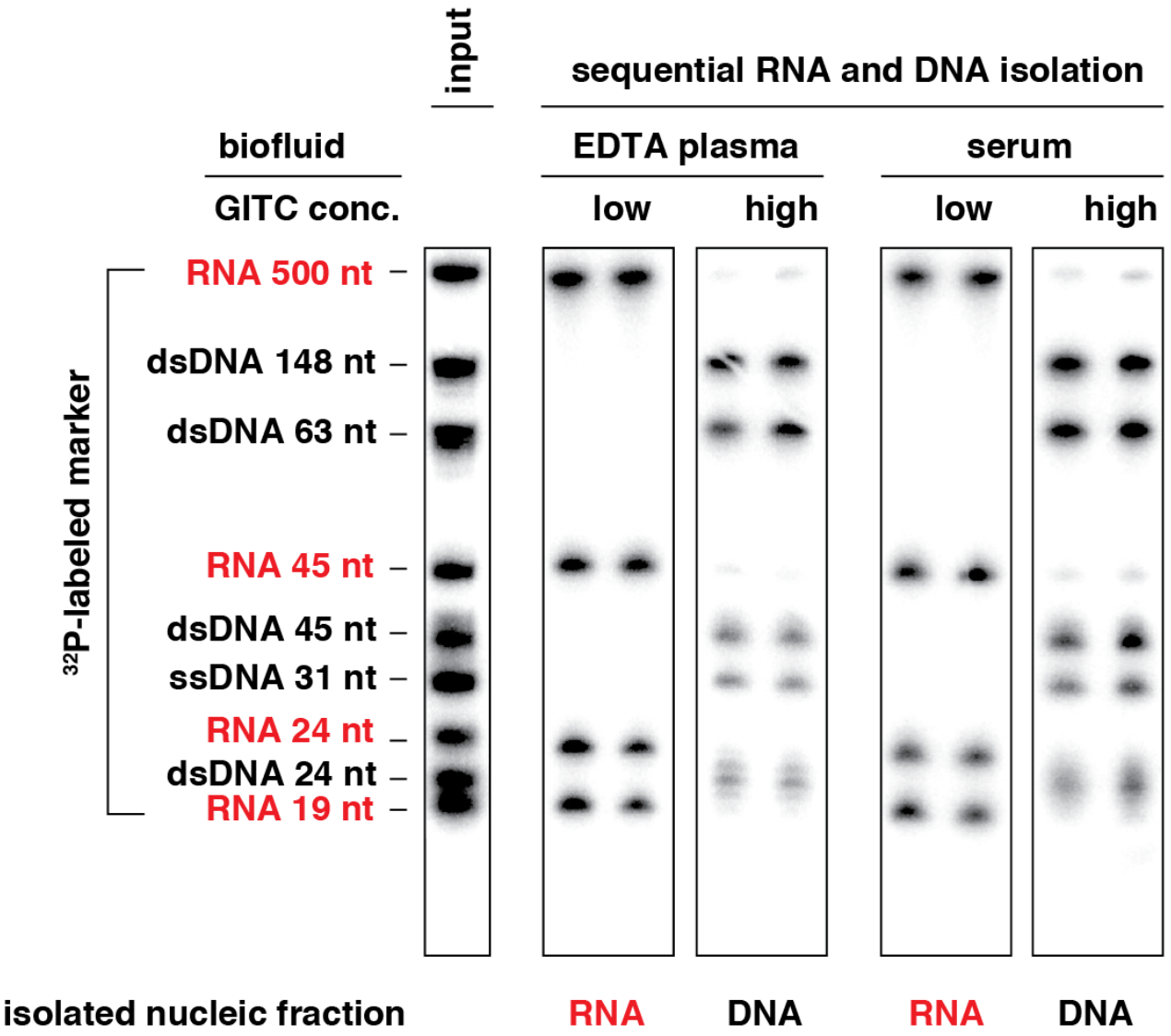
- **small RNA profiling of all NETs**
- **microRNA fluorescence *in situ* hybridization**
- **plasma microRNA profiling**
- **microRNA sequence reannotation**
- **functional characterization of relevant microRNAs**


NET vs non-NET, Kruskal Wallis p= 1.0215e-202
log2 miR-375 expression
0
-5
-10
-15
-20
-25
-30
NET
breast
non-NET

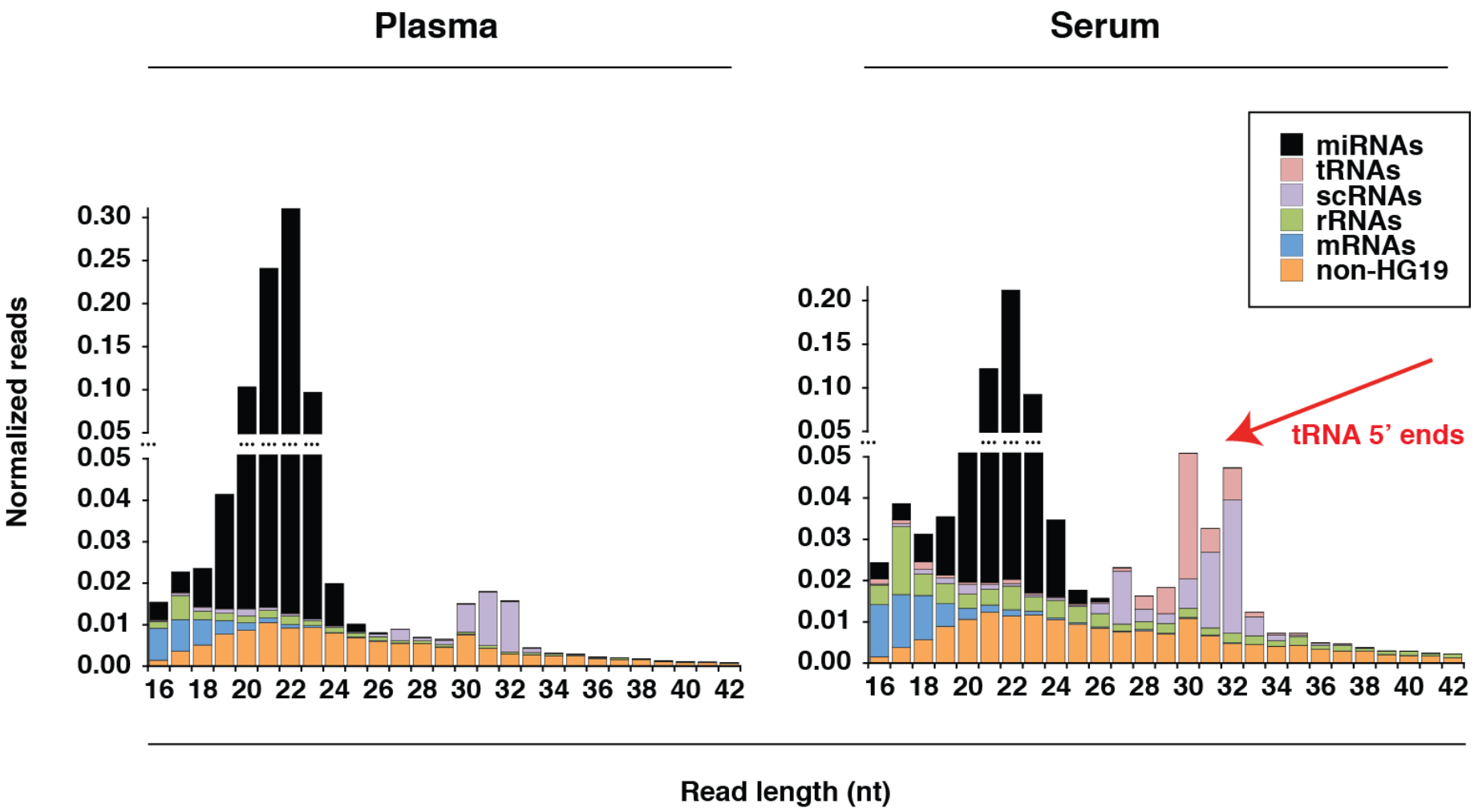
EDC fixation improves retention of long and short RNAs



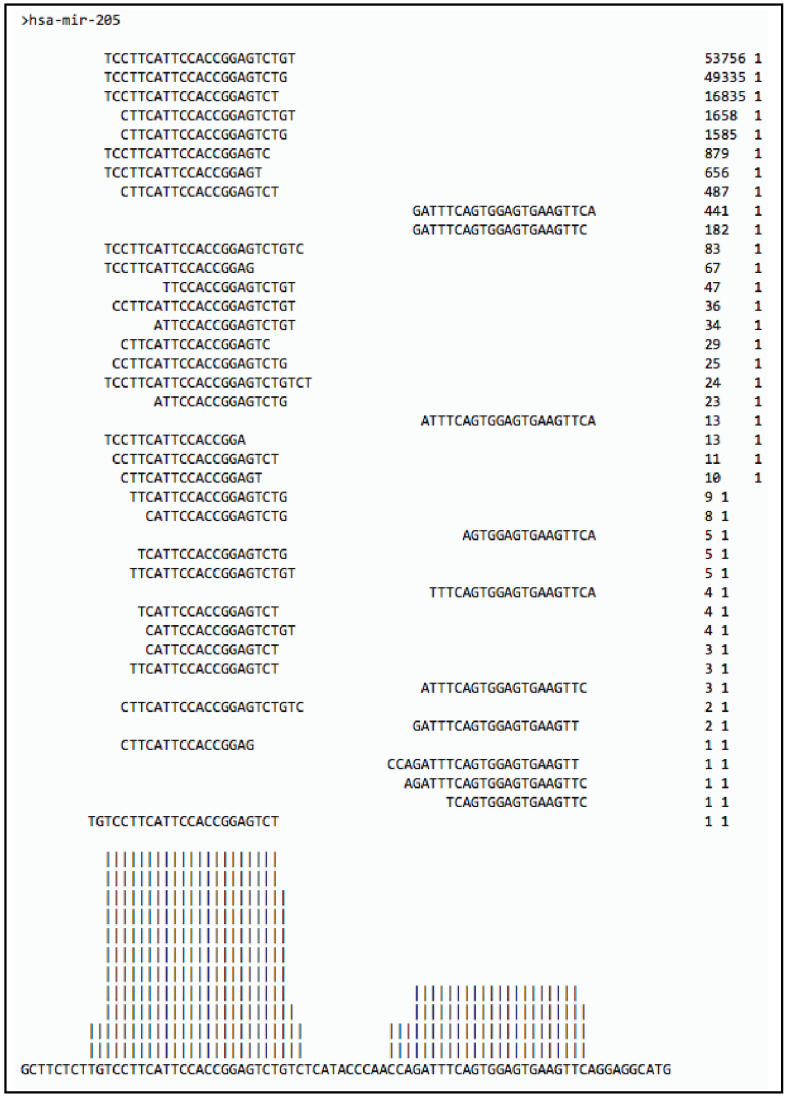
Quality control of RNA and DNA recovery and separation



Average sample composition in normal study subjects



microRNA reannotation



sequence evidence criteria supporting microRNA existence include:

non-mapping to other non-coding RNAs
sufficent read mapping
bimodal read distribution
supportive RNA folding
5’ end uracil
5’ end processing
evolutionary conservation

Acknowledgments

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