

Small non-coding RNA profiling from prostate cancer plasma by deep sequencing

Xianghua Liu, Andrew W. Hunter, Qing Zhang, Quanyuan He, Annemarie Benton, Gerri Ortiz, Vaishali Pannu, Nick Xiao, Mark R. Miglarese, David B. Spetzler
Caris Life Sciences, Phoenix, AZ, USA

Introduction

Prostate cancer (PCa) is the most common non-skin cancer among American men. MicroRNAs (miRNAs) are critical post-transcriptional regulators and involved in prostate cancer tumorigenesis. The aim of this study is to identify a PCa-specific expression profile of miRNAs from plasma to guide prostate cancer diagnosis and therapeutic treatment.

Workflow



Figure 1. Plasma was collected from 5 PCa patients and 5 normal men. Circulating RNA was extracted from 1) 200ul plasma or 2) the pellets of anti-Ago2 immunoprecipitations from 500ul plasma using the miRNeasy Serum/Plasma kit (Qiagen), with addition of glycogen as a carrier. Small RNA libraries were constructed using the NEBNext Multiplex Small RNA Prep Set for Illumina® (New England, BioLabs). The cDNA library fragments were purified by Blue Pippin (Sage Science) for extraction of 140–160 bp size fraction containing small RNA inserts. Equimolar amounts of cDNA library samples were pooled and were sequenced in a single flowcell on an Illumina HiSeq2500 with 50 cycle kit and rapid run model.

Small RNA Sequencing Data Process Flow

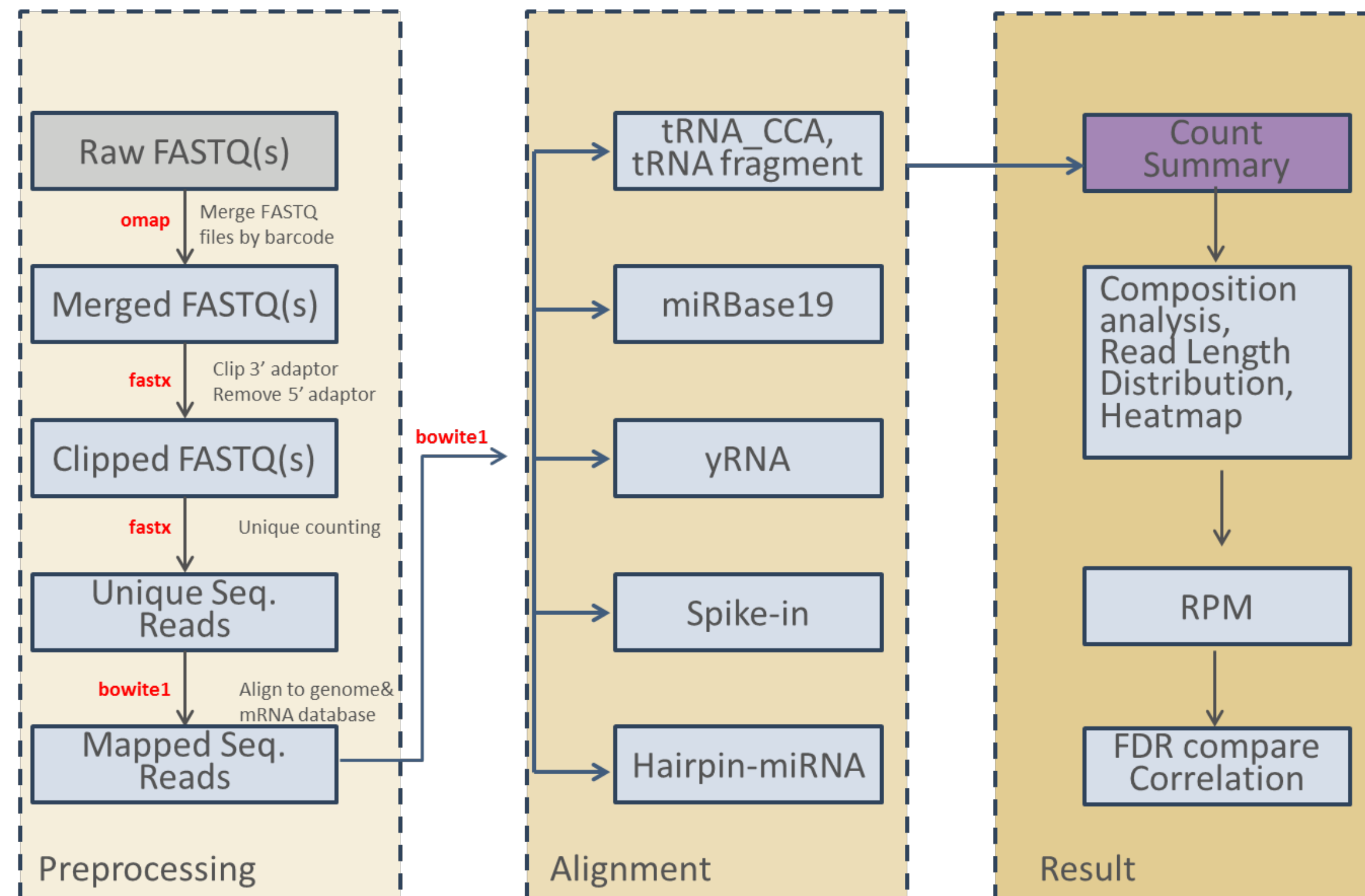


Figure 2. Adaptor was firstly removed from the raw reads, and the sequences were mapped to several small RNA databases by using bowtie1 with 1 mismatch. The multiple aligned reads were weighted to the mapped small RNAs based on their unique mapped reads counts. We then calculated the RPM (reads per million) as indicator of the expression levels of the small RNA. To get confident analysis results, we discarded the small RNAs whose averages of the raw reads counts in cancer and normal groups are smaller than 25 and only focus on the mature microRNAs. The moderate t-test is applied to find the differently expressed (DE) microRNAs between normal and cancer group.

Composition Analysis

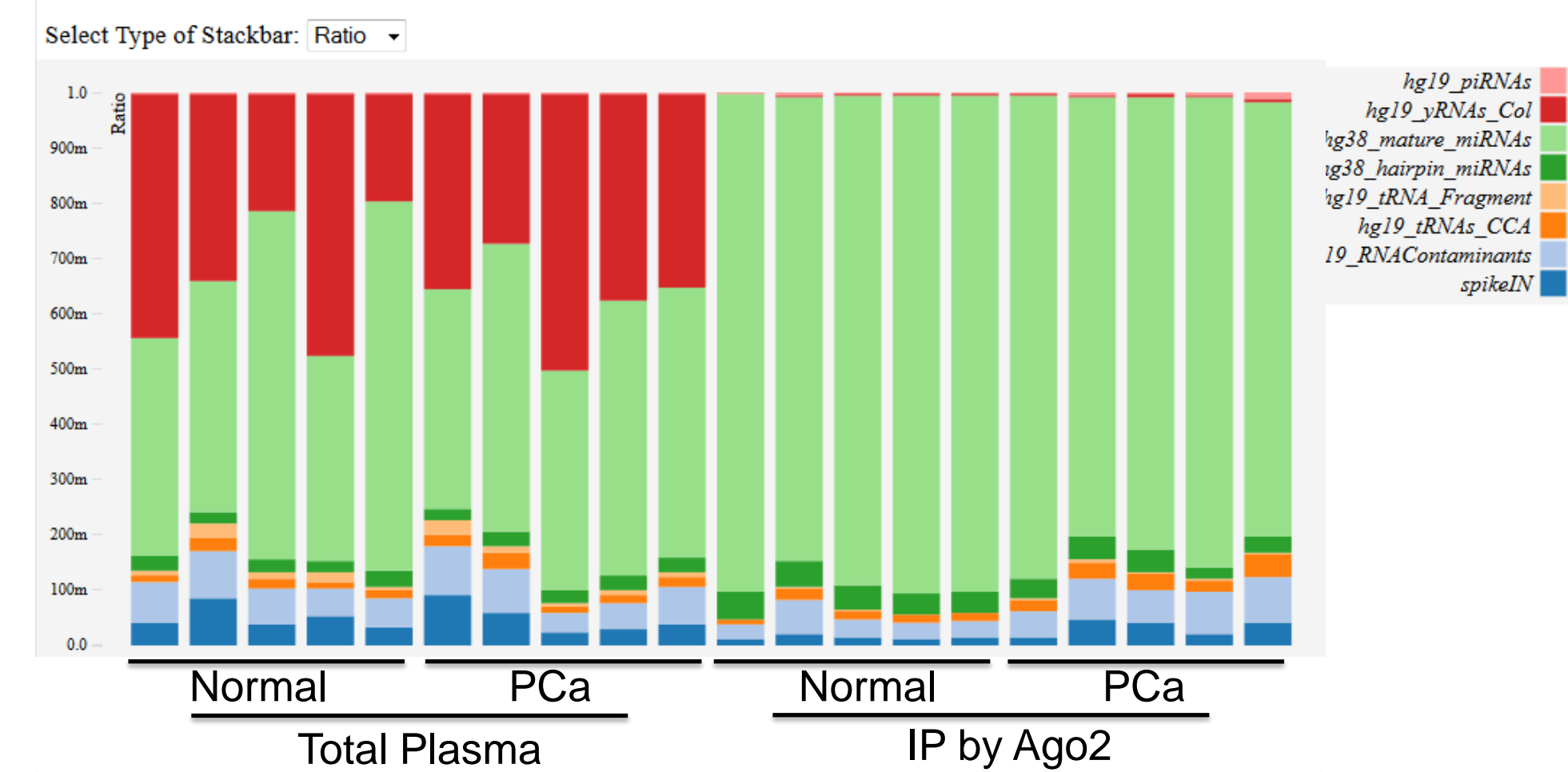


Figure 3. Composition Analysis. Two major small RNA classes identified from total plasma are miRNA (47.7%) and yRNA (35.0%). The percentage of miRNA increased to 85.3% by Ago2-IP method. The compositions of categories of small RNAs in cancer and normal samples are similar.

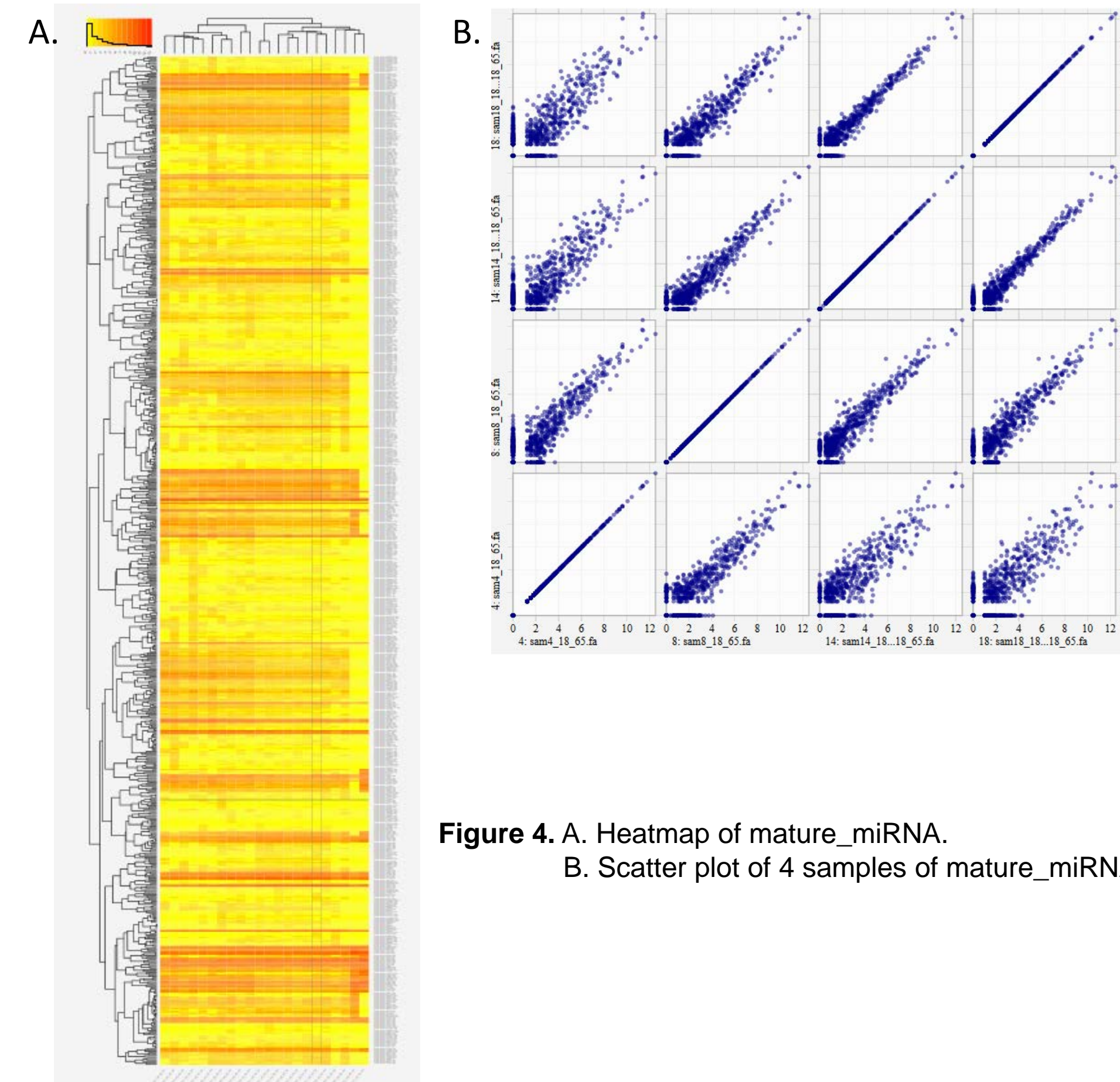


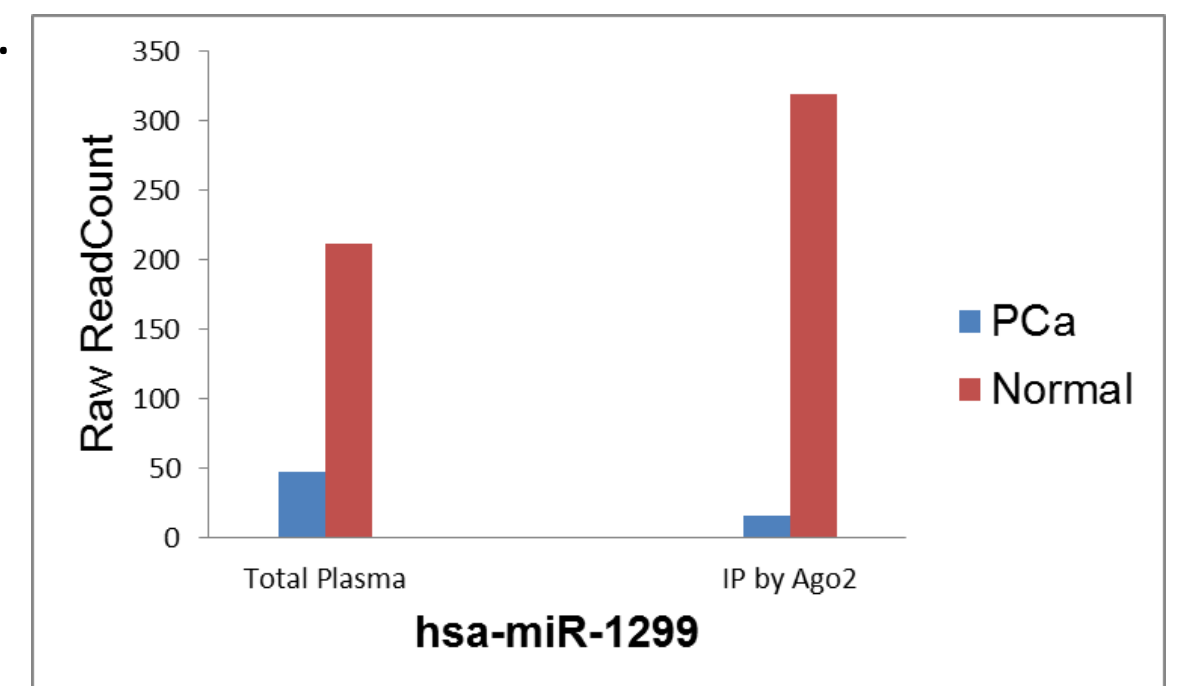
Figure 4. A. Heatmap of mature_miRNA. B. Scatter plot of 4 samples of mature_miRNA

A.

Total Plasma	logFC	PValue	FDR
hsa-miR-1299	-3.05	0.001	0.144
hsa-miR-4433b-3p	-1.73	0.023	0.296
hsa-miR-1228-5p	-1.67	0.012	0.217
hsa-miR-326	-1.50	0.038	0.345
hsa-miR-7976	-1.50	0.022	0.296
hsa-miR-23a-5p	-1.49	0.015	0.247
hsa-miR-130a-3p	1.63	0.030	0.311
hsa-miR-200a-3p	1.80	0.011	0.208
hsa-miR-126-5p	1.80	0.003	0.160
hsa-miR-15a-5p	1.96	0.001	0.144

IP by Ago-2	logFC	PValue	FDR
hsa-miR-1299	-2.59	0.008	0.199
hsa-miR-140-5p	-1.25	0.015	0.260
hsa-miR-139-3p	1.25	0.036	0.363
hsa-miR-5189-5p	1.26	0.005	0.182
hsa-miR-218-5p	1.36	0.042	0.385
hsa-miR-125a-3p	1.36	0.006	0.198
hsa-miR-184	1.47	0.003	0.126
hsa-miR-92b-5p	1.66	0.016	0.260
hsa-miR-485-5p	2.11	0.000	0.030
hsa-miR-107	2.78	0.000	0.030

B.



C.

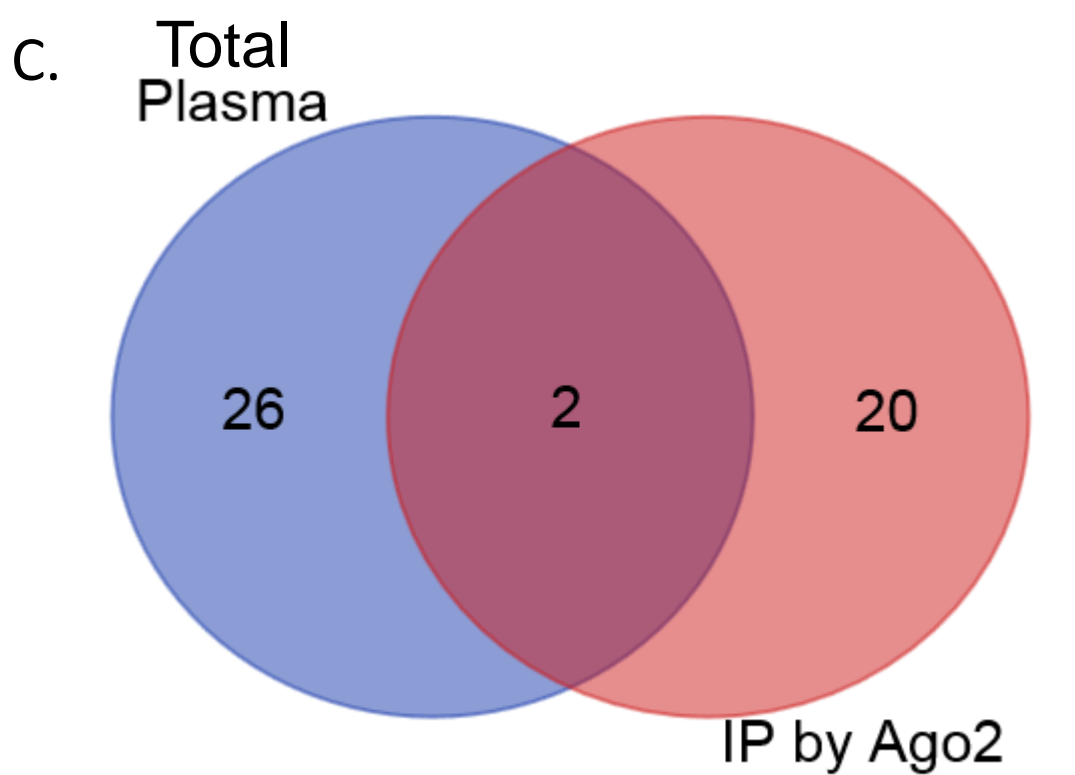


Figure 5. Top10 miRNAs (>2 fold change between cancer and normal group) by total plasma and Ago-2 IP methods respectively. B. Raw reads out of has-miR-1299 compared normal group to cancer group from total plasma and Ago-2 IP methods. C. Venn diagrams between the two methods.

Summary:

- ✓ We discovered a unique expression profile of miRNA detectable in the plasma from prostate cancer patients.
- ✓ Extracted RNA from the pellets of anti-Ago2 immunoprecipitations can enhance the detection of miRNA.
- ✓ Expand study to confirm these findings are needed.