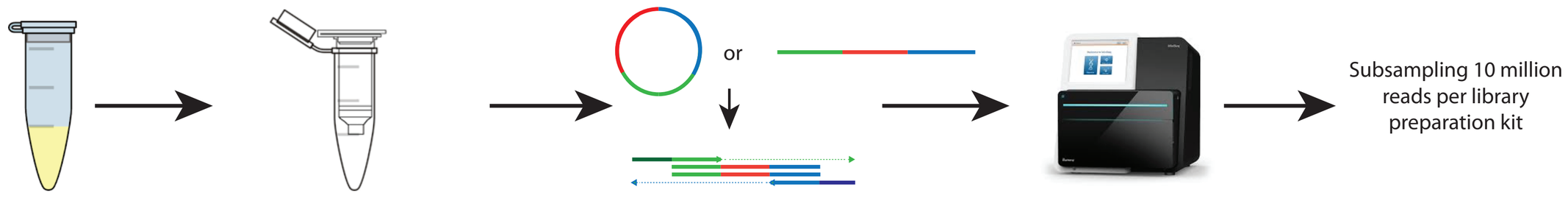
RealSeq®-Biofluids

Gel -free extracellular small RNA profiling from as low as 50 µl plasma



- Plasma and other biofluid samples contain extremely low concentrations of cf-miRNAs

- Accurate and sensitive quantification of cf-miRNAs from biofluids requires different reaction conditions compared to tissue samples

- Gel-free detection is a must for reproducible and automatable biomarker discovery pipelines

- RealSeq-Biofluids capitalizes on the accuracy of RealSeq-AC while sensitive enough to allow gel-free detection of cf-miRNAs from biofluids

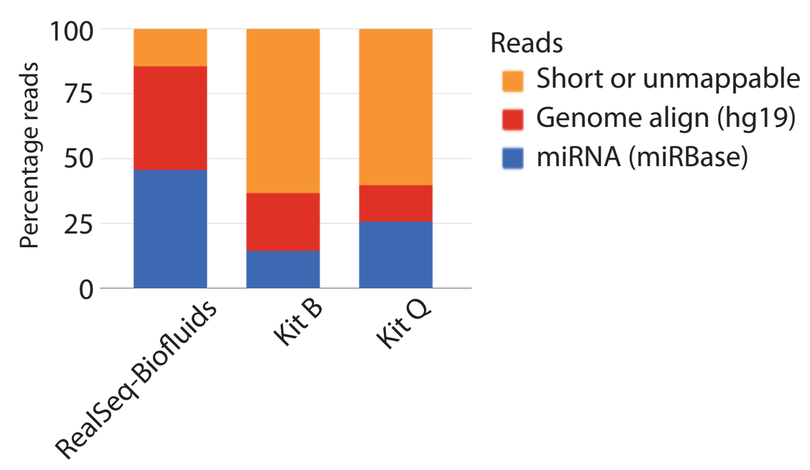
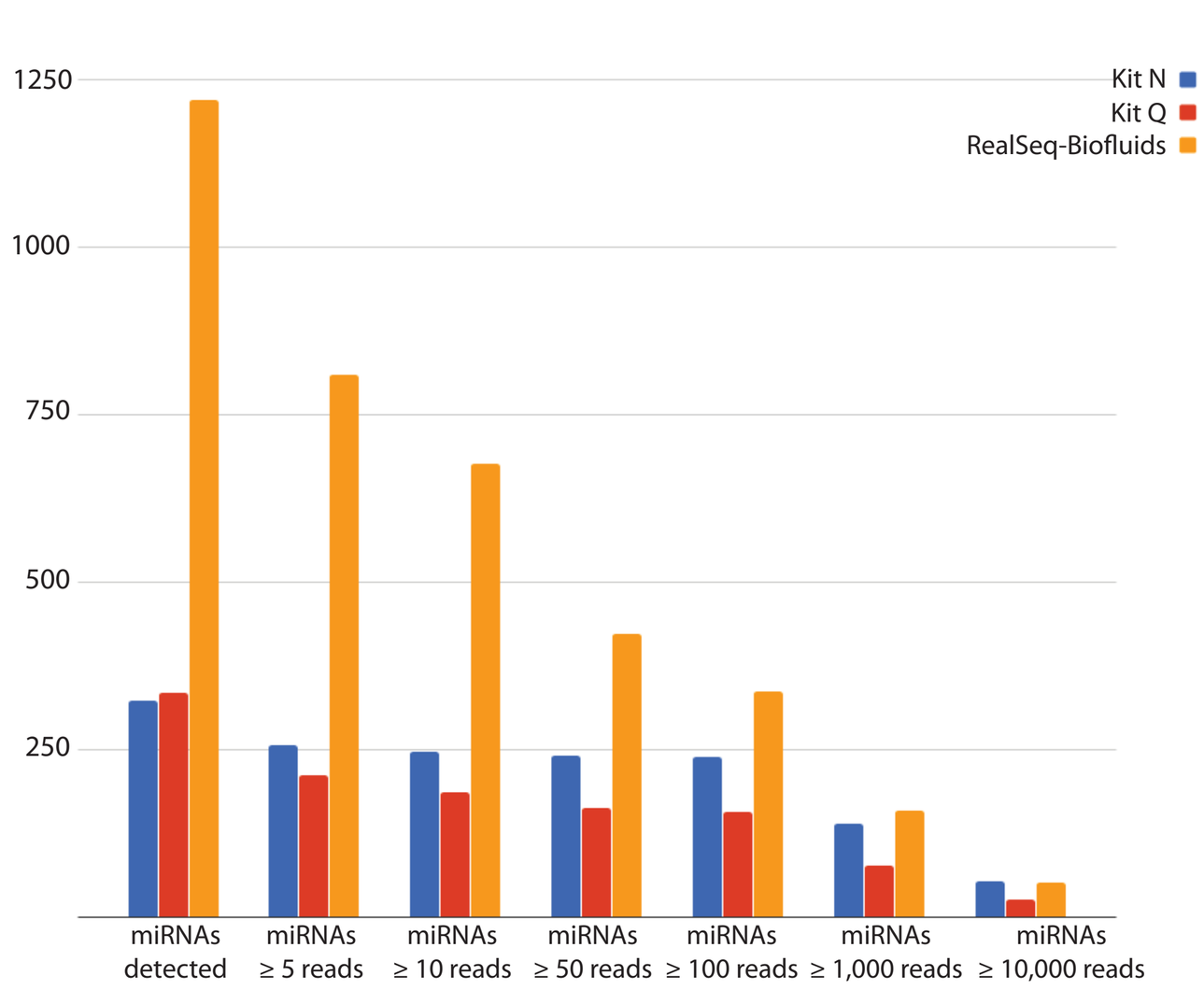
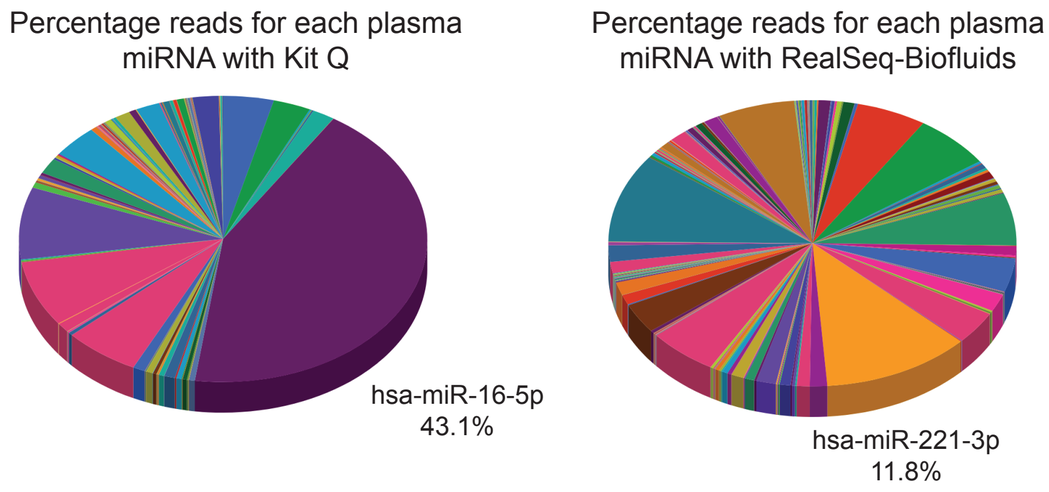


Figure 5. Outline of library preparation from plasma samples. The same plasma sample was used to prepare sequencing libraries with three different kits. Lower right panel shows sequencing metrics of libraries prepared with each kit. Short reads correspond to reads <15 nt after adapter trimming; reads passing this filter are then align to a reference file with all human miRNAs (miRBase 21), reads that do not align to miRNAs are then aligned to the human genome (hg19).

Detection of plasma miRNAs with different library preparation kits





- Kit Q identifies miR-16-5p as the most abundant plasma miRNA with 43.1% of the reads

- Profiling of miR-16-5p with miRXplore pool (Figure 2) shows that Kit Q over-represents this miRNA with >5 fold bias

- Comparatively, RealSeq-Biofluids identifies miR-221-3p as the most abundant plasma miRNA with 11.8% of the reads

- Profiling of miR-221-3p with miRXplore pool (Figure 2) shows that RealSeq detects this miRNA with no bias

Figure 6. Profiling of plasma miRNAs with three different library preparation kits. 200 ul of plasma sample from a healthy donor was used to extract RNA with Quick-cfRNA Plasma/Serum kit (Zymo research) following manufacturer recommendations. RNA from three extractions was pooled and used to prepare sequencing libraries with the three kits following manufacturer recommendations for gel-free libraries. To normalize for sequencing coverage reads were subsampled to 10 million reads per kit. Sequencing reads were processed as Figures 2-3, except that reads were aligned to a reference that includes all human miRNAs in miRBase 21. The left panel shows the number of miRNAs detected at different coverage for each library preparation kit. The right panel shows the percentage of plasma reads for each miRNA with kits Q and RealSeq-Biofluids.

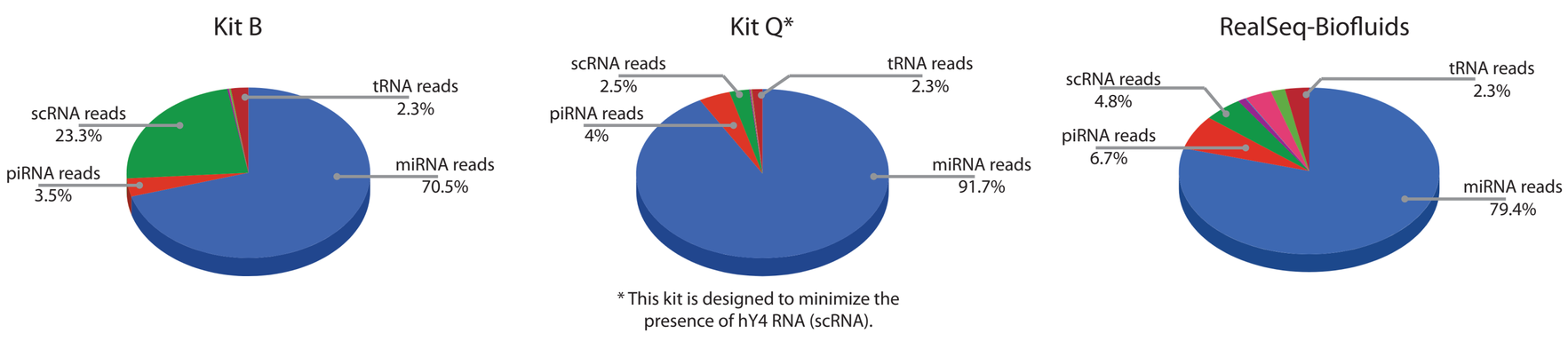


Figure 7. Percentage of reads that map to different classes of ncRNAs for each library preparation kit. Kit Q, according to the manufacturer, is specifically designed to remove reads mapping to HY4 RNA (scRNA) impeding its quantification.

Conclusions RealSeq-Biofluids

- RealSeq-Biofluids allows preparation of gel-free sequencing libraries with an RNA input obtained from only 50 µl of plasma
- RealSeq-Biofluids delivers the highest percentage of usable reads (>15 nt and that align to either miRBase or genome) of the 3 kits tested
- Highly accurate profiling allows the identification of a larger set of cf- miRNAs (Figure 6, left panel)
- Detection bias inherent in the two-adapter platforms reduces the number of miRNAs identified (Figure 6, left panel)
- Detection bias also results in the overrepresentation of a few miRNAs that consume the majority of sequencing reads (Figure 6)
- scRNAs are overrepresented in libraries prepared with the two-adapter ligation scheme (Figure 7), while they represent only 4% of the reads for RealSeq-Biofluids (single-adapter and circularization)
- RealSeq-Biofluids allows accurate and sensitive quantification of cell-free miRNAs with a gel-free protocol

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• RealSeq® technology is covered by issued and pending patents.