

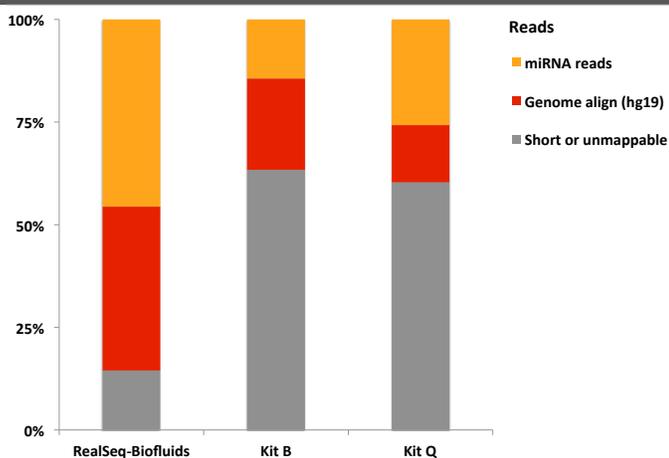
RealSeq[®] - biofluids

accuracy through circularization



- SomaGenics' patented RealSeq[®] single adapter circularization technology
- Highly sensitive and unbiased small RNA detection
 - Accurate cell-free (cf)-small RNA detection from biofluids
 - Optimized for liquid samples
- Gel-free workflow

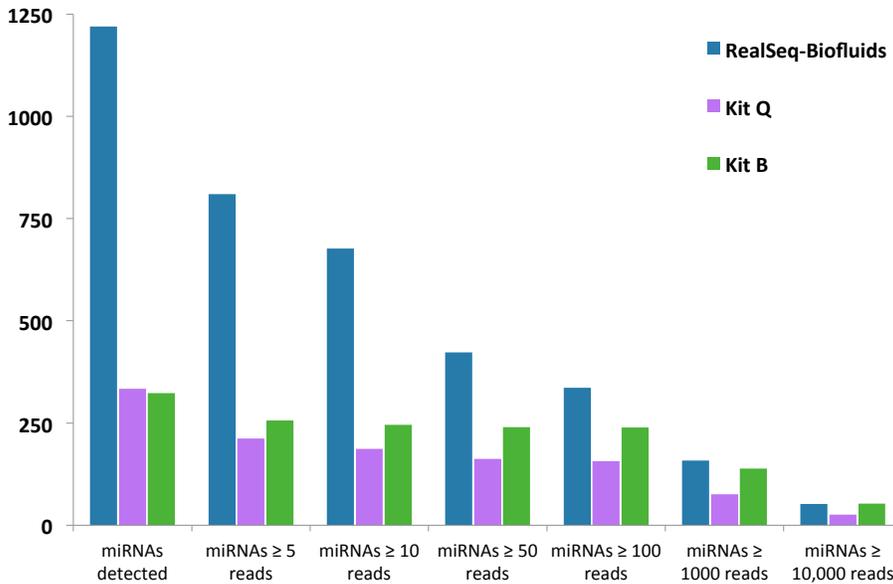
Superior detection of usable miRNA reads from low plasma input



Comparison of sequencing results obtained from commercially available library preparation kits using 200 μ l plasma samples. Short or unmappable reads (grey) correspond to reads <15 nt after adapter trimming; reads passing this filter are then aligned to a reference file with all human miRNAs (miRBase 21, orange), and reads that do not align to miRNAs are then aligned to the human genome (hg19, red).

RealSeq[®] - biofluids shows substantially more miRNA reads than the other two libraries.

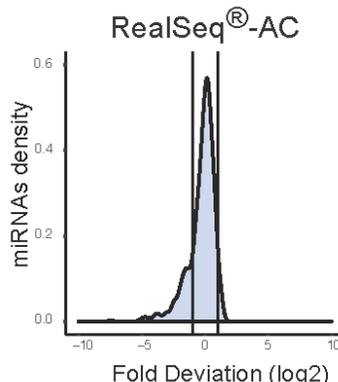
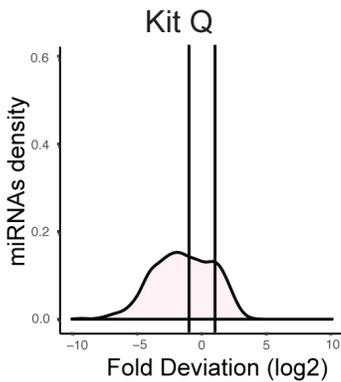
Highly accurate profiling allows identification of more cf- miRNAs



Comparison of miRNA read frequencies of three different library preparation kits (gel-free protocols) from 200- μ l plasma samples. Reads were subsampled to 10 million reads per kit and aligned to a reference that includes all human miRNAs listed in miRBase 21. The number of miRNAs detected at different coverage for each library preparation kit is shown.

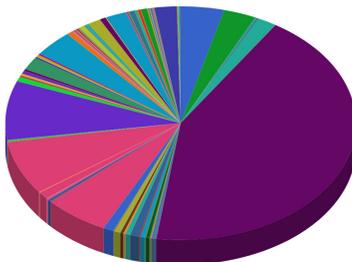
RealSeq[®] - biofluids detects significantly more miRNA species than the other two libraries, especially low copy-number

Elimination of bias-induced sequencing reads



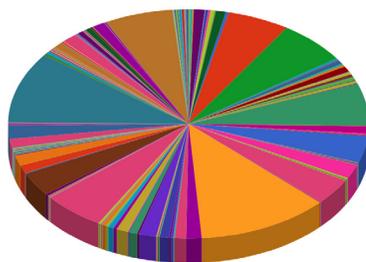
Bias of sequencing kits was assessed by sequencing the miRXplore Universal Reference pool (Miltényi Biotec), which contains equimolar amounts of 963 synthetic miRNAs. The density of miRNAs within a two-fold deviation from the expected values (area between vertical lines) is considered unbiased (Fuchs et al 2015). RealSeq[®]-AC, the platform technology for RealSeq[®]-biofluids, quantifies miRNAs significantly more accurately than Kit Q.

Percentage reads for each plasma miRNA with Kit Q



hsa-miR-16-5p
43.1%

Percentage reads for each plasma miRNA with RealSeq[®] - biofluids



hsa-miR-221-3p
11.8%

Consequence of biased miRNA analysis

- Kit Q identifies miR-16-5p as the most abundant plasma miRNA with 43.1% of the reads
- However, the sequencing miRXplore pool showed Kit Q over-represents this miRNA >5 fold bias
- In contrast, RealSeq[®]-biofluids identifies miR-221-3p as the most abundant with 11.8% of the reads
- Profiling of miR-221-3p with miRXplore pool showed that RealSeq detects this miRNA without bias

Due to low sequencing bias, RealSeq[®] - biofluids allows more accurate analysis of biological samples.