somagenics

RealSeq®-AC libraries from total RNA input as low as 1 ng

Protocol improvements now allow high quality libraries to be generated from input quantities as low as 1 ng total RNA (~100 cells). When samples are limited, RealSeq®-AC can be used to prepare libraries with 1-10 ng of high quality total RNA (RIN > 7) resulting in good quality data that is strongly correlated with data obtained from higher input amounts (**Figs. 1, 2 and Table 1**).

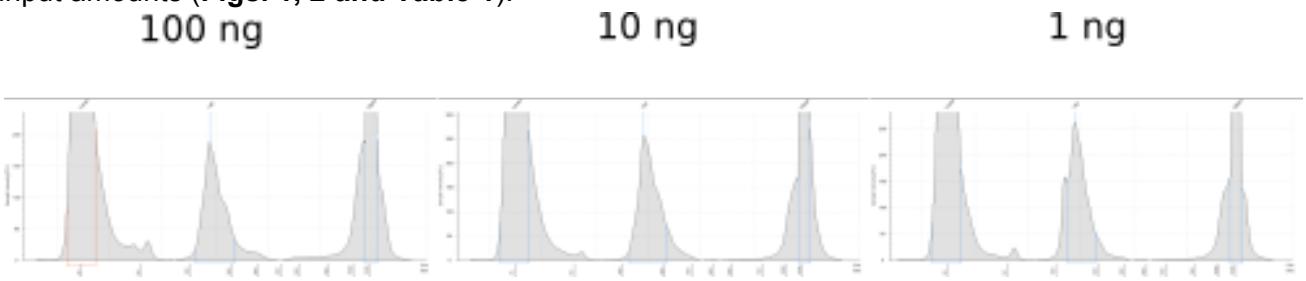

Figure 1. TapeStation profiles of three libraries prepared with 100, 10 or 1 ng of human reference total RNA (Agilent).

Libraries prepared from 1 ng of input amplified by 19 cycles of PCR generate enough material for successful sequencing on any Illumina platform. Although the percentage of reads shorter than 15 bp is increased in the libraries generated from 1 ng of total RNA as compared to libraries generated from 100 ng, the correlation of miRNA quantification is extremely high across all input levels tested (100 ng vs 1 ng, r = 0.956) (**Table 1 and Fig. 2**). This high correlation across different input amounts allows the selection of input based on sample availability without compromising results.

Input	PCR cycles	Reads	Pass filters (reads >15 bp)	Align hg19	miRNAs identified
100 ng	13	800,000	88.3%	97.9%	460
10 ng	16	800,000	76.5%	95.8%	381
1 ng	19	800,000	66.8%	84.2%	327

Table 1. Sequencing metrics for RealSeq-AC libraries prepared with a serial dilution of a human reference total RNA (Agilent). To normalize to differences in sequencing coverage, 800,000 reads were sampled at random for each experiment.

RealSeq®-AC now allows the accurate profiling of miRNAs from samples with limited availability. Additionally, RealSeq®-AC library preparation from 1 ng of total RNA requires only 19 cycles of PCR. RealSeq-AC's accuracy in detection across a wide range of input amounts is shown by the strong miRNA quantification correlation between 100, 10 and 1 ng of input material (**Fig. 2**).

Note: We recommend increasing the sequencing coverage for low input libraries compared to high input libraries since there is a higher percentage of reads with inserts < 15 nt and a lower number of total miRNAs identified when sequencing libraries created with 1 ng of input material.

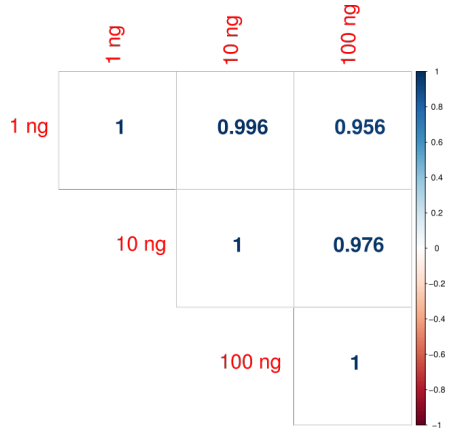

Figure 2. Correlation of miRNA reads between libraries created with 100, 10 or 1 ng of human reference total RNA (Agilent) Raw reads mapping to miRNAs were used to calculate the Pearson correlation between libraries.