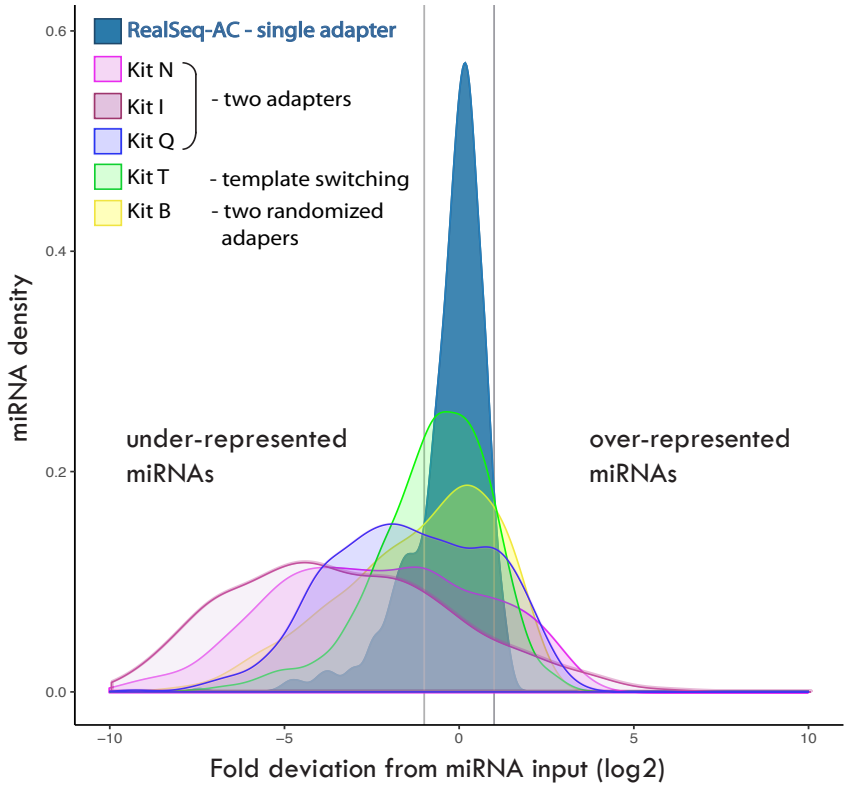
somagenics

RNA Detection

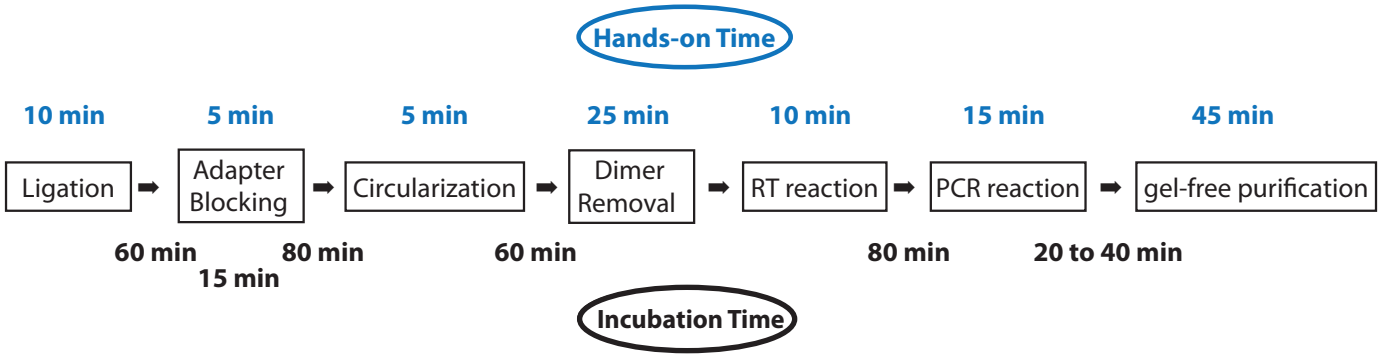
RealSeq®-AC: Reducing bias in small-RNA sequencing



- Accurately quantifies biologically relevant small RNAs
- Eliminates bias-induced over/under represented small RNAs
- Allows discovery of novel small RNAs
- Inputs between 1 ng to 100 ng of total RNA

One pmole of miRXPlore Universal Reference pool (Miltenyi Biotec) was used to compare incorporation bias in six different commercially available library preparation kits. Purified libraries were sequenced on the Illumina MiSeq platform. Trimmed sequencing reads were aligned to a custom miRNA reference. Reads mapping to miRNAs were counted and fold-deviations from the equimolar input were calculated and plotted as log2 values. Measurements of miRNA levels within a factor of two of the expected values (between vertical lines) are considered unbiased (Fuchs et al, 2015). The method of adapter attachment to the miRNAs is noted in the legend.

RealSeq®-AC sequence-ready library in one day



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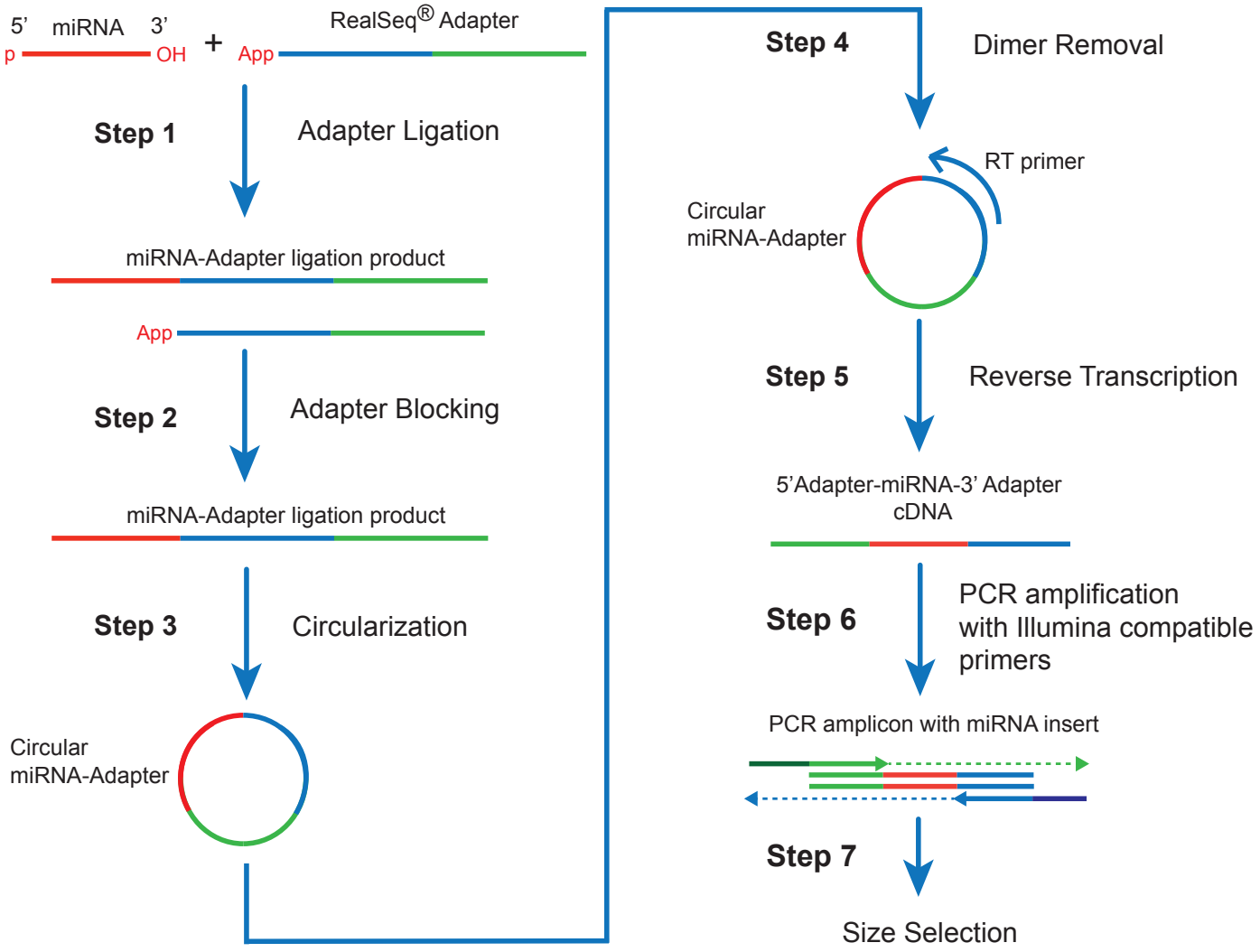
RealSeq®-AC schematic



RealSeq®-AC is highly efficient, detecting more miRNAs in total RNA samples

	Kit I	Kit N	Kit B	Kit T	RealSeq®-AC
miRNAs with >5 reads	404	452	412	324	500
miRNAs with >10 reads	328	365	352	239	385

RealSeq®-AC detects more miRNAs with over 5 or 10 reads per million, respectively, from total RNA samples compared to other kits for miRNA sequencing library preparation. RealSeq®-AC is optimized for inputs between 1 ng to 100 ng of total RNA. Lower numbers of PCR cycles are reducing PCR-induced issues.