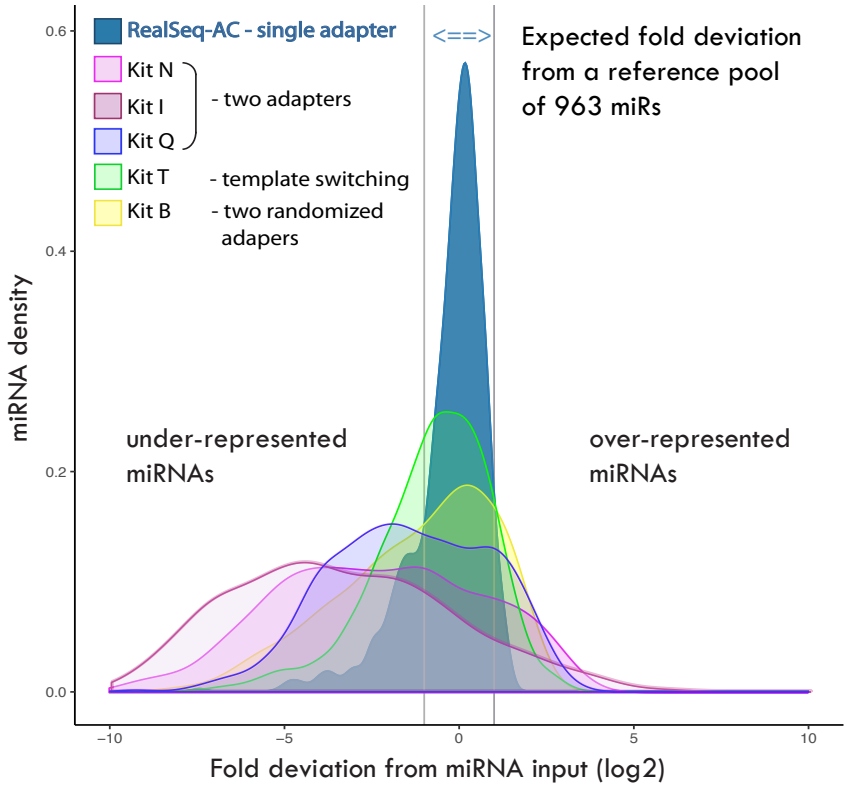
RealSeq BIOSCIENCES

Small RNA sequencing library preparation from total RNA and biofluids

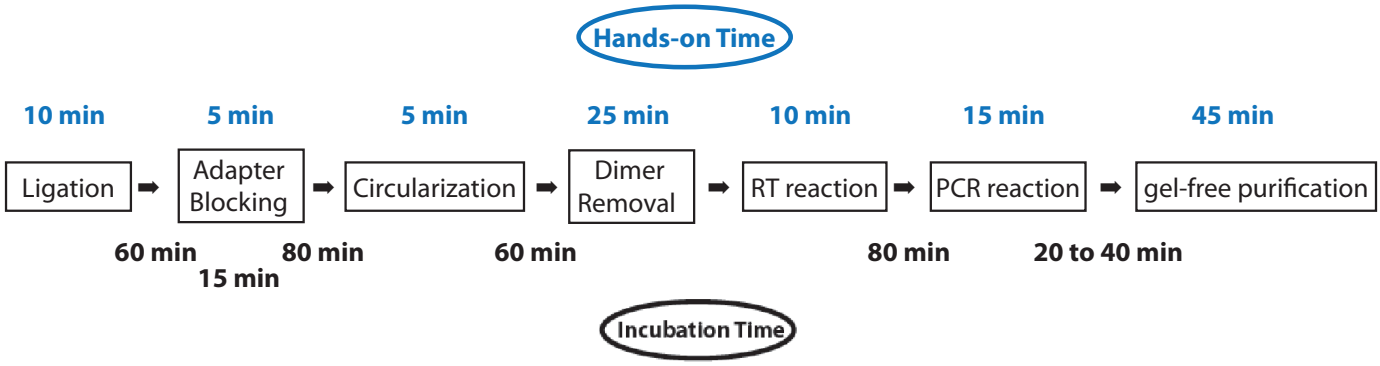
RealSeq®-AC: Reducing bias in small-RNA sequencing



- 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 1 µg of total RNA

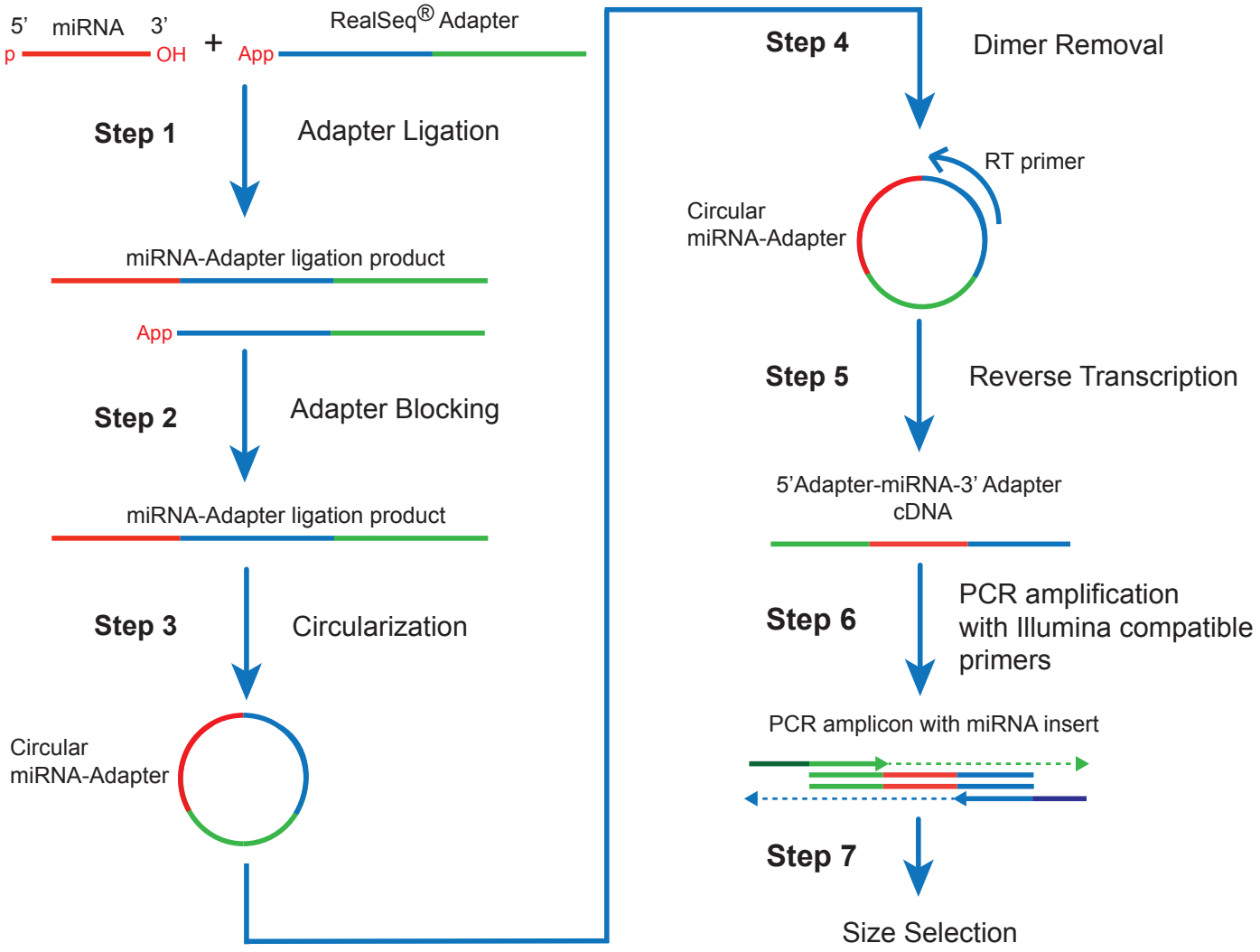
One pmole of miRXPlore Universal Reference pool (Miltenyi Biotec) was used to compare incorporation bias in six different commercially available library preparation kits. Purified libraries were sequenced on the Illumina MiSeq platform. Trimmed sequencing reads were aligned to a custom miRNA reference. 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



Small RNA sequencing library preparation from total RNA and biofluids

RealSeq®-AC schematic

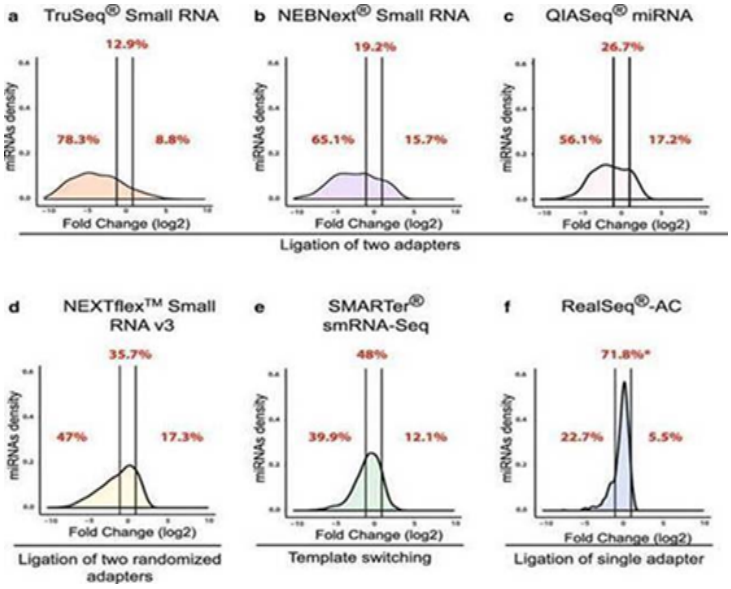


RealSeq®-AC is highly efficient, detecting more miRNAs in total RNA samples

	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.

*Elimination of biais using RealSeq® - Methods comparison from Barberan-Soler et al., 2018**



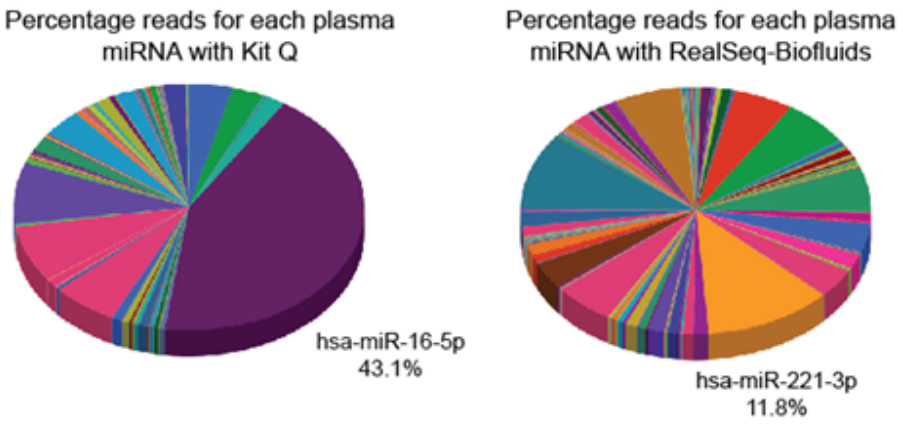
Results obtained with the miRXplore Universal Reference pool (963 miRs)

- RealSeq®-AC detects 71,8% of miRs without bias
- Other kits detect only between 12,9% and 48% of miRS

*** Decreasing miRNA sequencing bias using a single adapter and circularization approach**. Sergio Barberán-Soler, Jenny M. Vo, Ryan E. Hogans, Anne Dallas, Brian H. Johnston and Sergei A. Kazakov. Genome Biology 2018 19:105

Lire la publication

Detection of a large diversity of miRs in biofluids



Due to low sequencing biais, RealSeq®-biofluids allow more accurate analysis of biological samples

- Kit Q identifies miR-16-5p as the most abundant plasma miRNA with 43.1% of all reads
  - *However; miRXPlore pool sequencing showed that Kit Q over-represents this miRNA by >5 fold*

- In contrast RealSeq®-biofluids identifies miR-221-3p as the the most abundant miRNA with 11.8% of all reads
  - *miRXPlore pool sequencing showed that RealSeq® detects this miRNA without biais*

- In both experiments, miRs purification has been carried out with Quick-cfRNA Serum & Plasma Kit (Zymo)

Liste des produits

Kits de préparation de banques

	Référence	Désignation	Conditionnement
A partir d'ARN total 1 ng à 1 µg Billes de purification non incluses*	REA500-00012*	RealSeq-AC small RNA Library kit for Illumina sequencing	12 libraries
	REA500-00024*	RealSeq-AC small RNA Library kit for Illumina sequencing	24 libraries
	REA500-00048*	RealSeq-AC small RNA Library kit for Illumina sequencing	48 libraries
A partir de cfRNA isolés de 50 µl de plasma ou sérum Billes de purification incluses	REA600-00012	RealSeq-Biofluids small RNA Library kit for Illumina sequencing	12 libraries
	REA600-00024	RealSeq-Biofluids small RNA Library kit for Illumina sequencing	24 libraries
	REA600-00048	RealSeq-Biofluids small RNA Library kit for Illumina sequencing	48 libraries

*Billes de purification complémentaires des kits RealSeq-AC

Billes de purification Beckman	REA510-00012	RealSeq-AC size select beads	12 libraries
	REA510-00024	RealSeq-AC size select beads	24 libraries
	REA510-00048	RealSeq-AC size select beads	48 libraries

Kits de purification des acides nucléiques pour détecter une grande diversité de miRs

	Référence	Désignation	Conditionnement
Purification à partir de cellules/tissus	ZR1057	Quick-RNA MiniPrep Plus Kit	50 preps
	ZR1057T	Quick-RNA MiniPrep Plus Kit	10 Preps
	ZR1050	Quick-RNA MicroPrep Kit	50 preps
	ZR1051	Quick-RNA MicroPrep Kit	200 preps
	ZD7005	Quick-DNA/RNA Microprep Plus Kit	50 prep
	ZD7005T	Quick-DNA/RNA Microprep Plus Kit	5 prep
Purification à partir de sérum/plasma	ZR1059	Quick-cfRNA Serum & Plasma Kit	50 preps
	ZR1072	Quick-cfDNA/cfRNA Serum & Plasma Kit	50 preps
	ZR1072S	Quick-cfDNA/cfRNA Serum & Plasma (Trial Size)	1 kit

Nous contacter

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