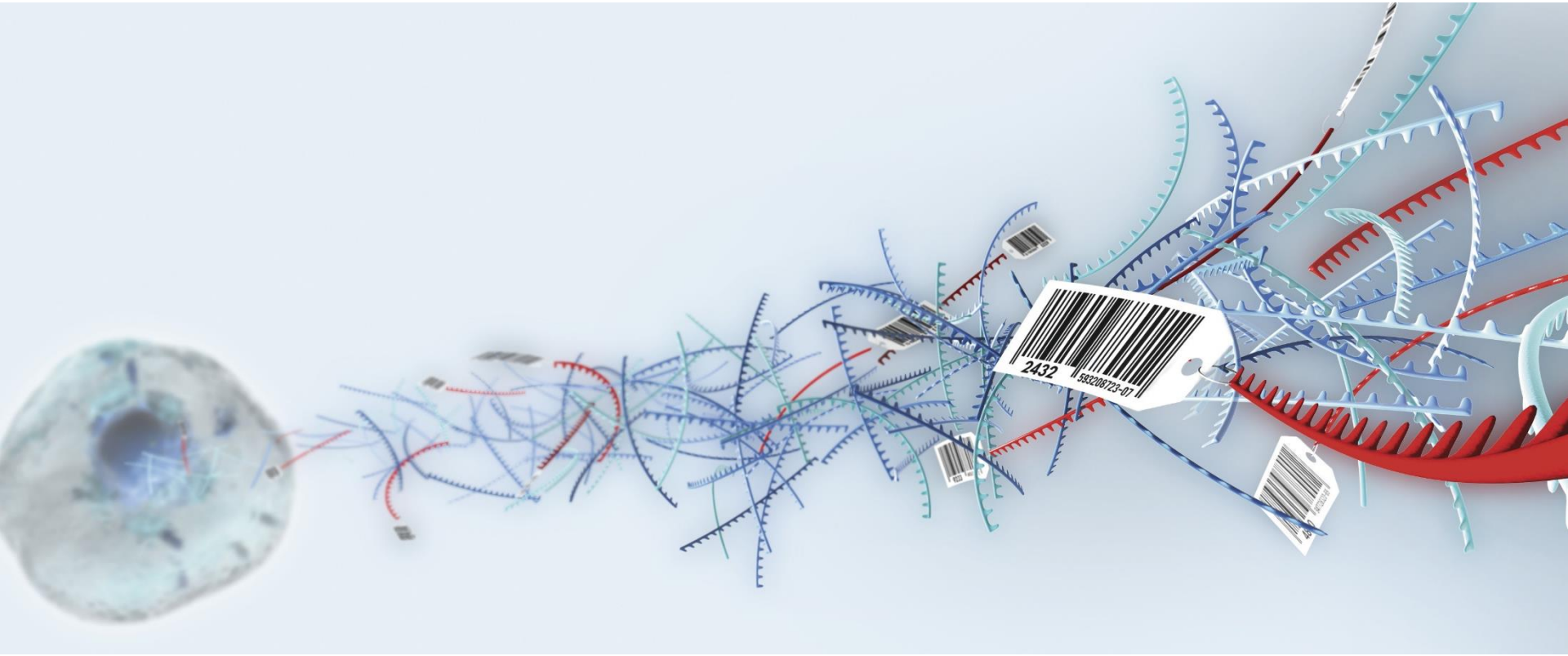
QIAseq miRNA Library Kit

The newest solution for gel-free miRNA sequencing



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Legal disclaimer

QIAGEN products shown here are intended for molecular biology applications. These products are not intended for the diagnosis, prevention or treatment of a disease.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

Agenda

1. miRNA background
2. QIAseq miRNA sequencing: Product overview
3. Data analysis overview
4. Performance and application data
5. Summary and questions

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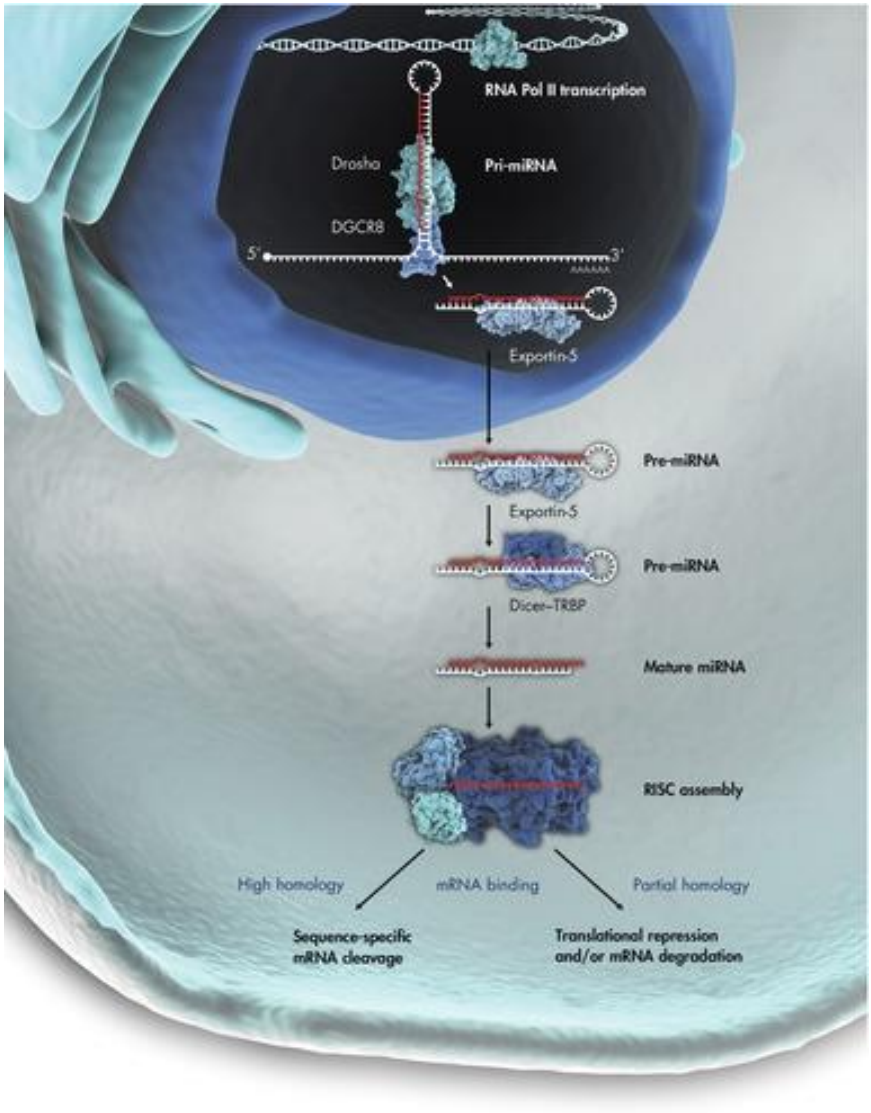
miRNAs: Master regulators of gene expression



miRNAs are ~21 nucleotide, small non-coding RNAs that are expressed in most tissues

Changes in miRNA expression can be correlated with gene expression changes in:

- Development
- Differentiation
- Signal transduction
- Infection
- Aging
- Disease

Role in cancer: miRNAs are associated with cell proliferation, resistance to apoptosis, invasiveness and differentiation in cancer cells

Why quantify miRNAs?

Using a signature of altered miRNA expression to differentiate cancer tissue from normal tissue

Using miRNA-based classifier to identify the tissue of origin for cancers of unknown primaries

Profiling circulating blood or tumor-derived exosomal miRNAs, surpassing the invasive procedures to aid in early detection of cancers

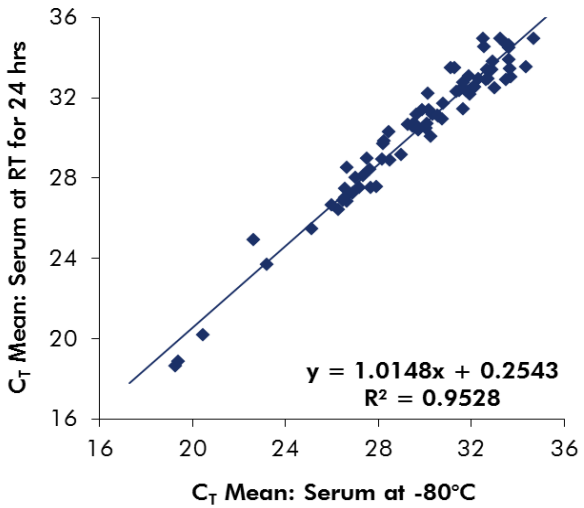
Distinguishing tumor subtypes using a panel of miRNAs that show differential expression within one cancer type

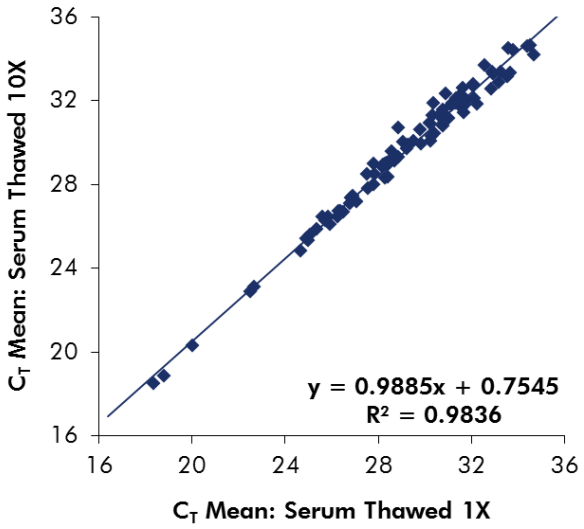
Studying SNPs in the miRNA genes, miRNA binding sites in the target mRNA genes or in the miRNA processing/machinery pathway genes to predict cancer predisposition

Source: Paranjape. et. al. (2009) Gut.

Quantifying miRNAs help identify novel expression patterns for miRNAs − both annotated (i.e. in miRBase) and non-annotated (i.e. identified using small RNA sequencing).

Why are circulating miRNAs promising biomarker candidates?





Expressed in most biofluids

- Serum, plasma, CSF, urine and saliva
- Robustly expressed in serum and plasma

Minimally invasive and easily liberated

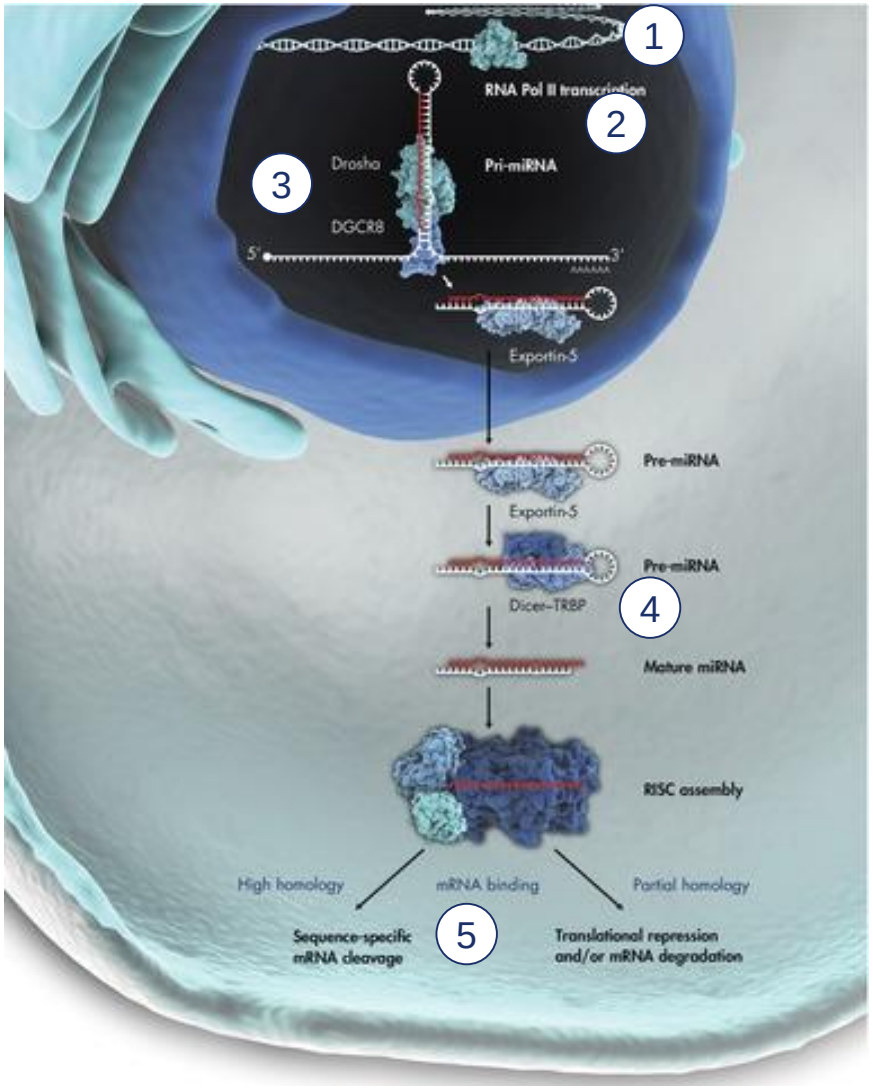
- Sensitive, specific and precise platforms exist for miRNA quantification

Protected from degradation

- Resistant to freeze-thaw cycles
- Stable at room temperature for 24 hours

miRNA expression is associated with normal and disease biology.

Disruption of miRNA–mRNA interaction



1. Genomic instability
   - Amplification or deletion
   - Translocation
   - Insertional mutagenesis
2. Altered transcription
   - Methylation
   - Histone modification
   - Transcription factor
3. Drosha processing
4. Dicer processing
5. Loss of miRNA binding site in target mRNA
   - SNP or mutation
   - Alternative splicing
   - Loss or change of 3′-UTR

miRNA expression profiling applications

miRNAs as biomarkers

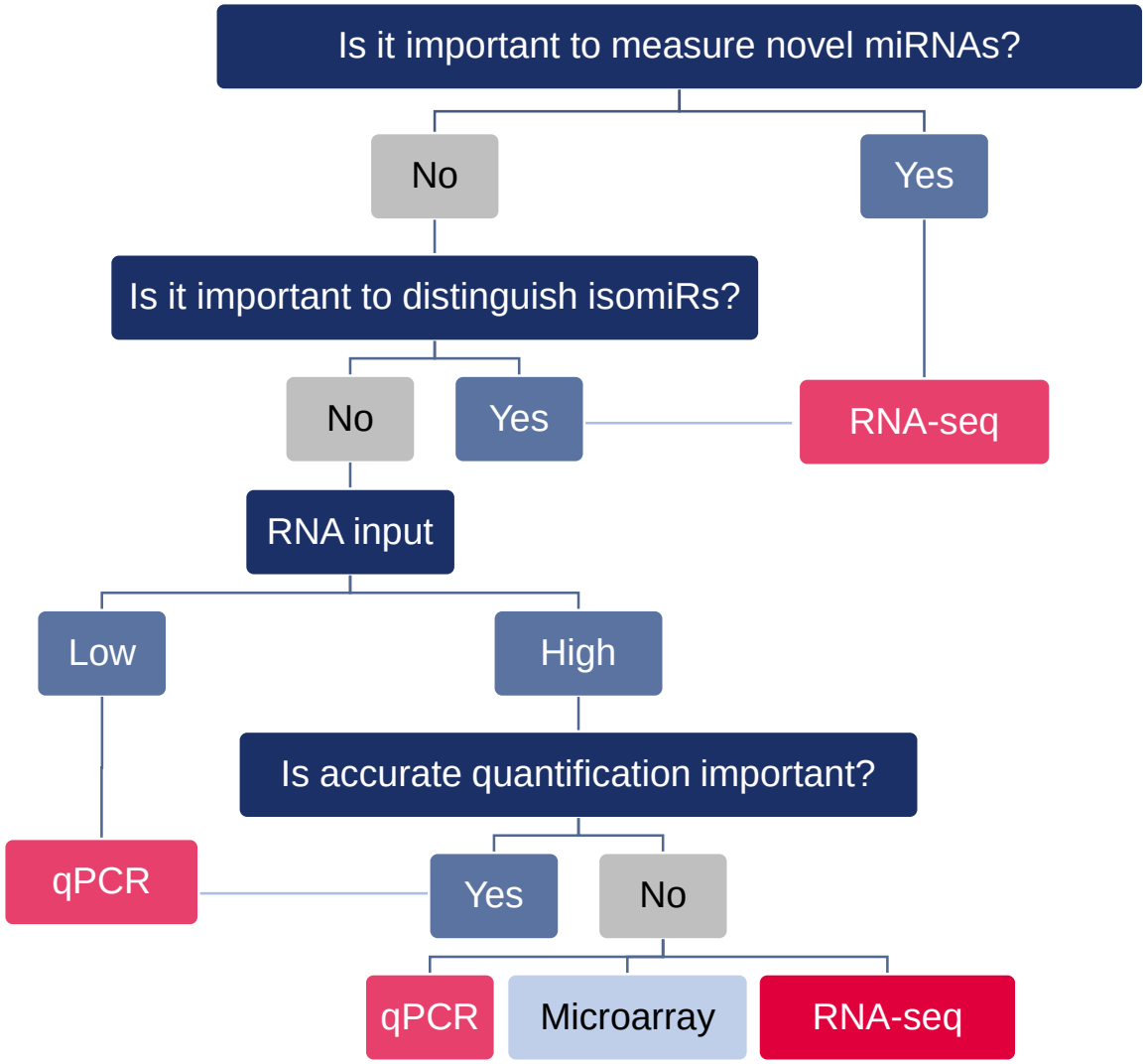
- Tissue-based miRNA profiling for biomarker discovery
- miRNA profile-based classification of tissues of unknown origin
- Circulating miRNA biomarkers
- Forensics

Understanding gene regulation

- Developmental biology
- Novel miRNA discovery
- Studying miRNA-mRNA and miRNA-protein interactions
- Total RNA discovery − integrative analyses of miRNAs in the context of gene regulatory networks

Source: Pritchard, C. C. et. al. (2012) Nature Rev. Genet. 13, 358-369

miRNA profiling − Choosing the right platform for your experiment



Source: Pritchard, C. C. et. al. (2012) Nature Rev. Genet. 13, 358-369

Current challenges facing miRNA sequencing

Inconvenient sample inputs

- Kits require up to 1 µg of sample input and not ideal with lower inputs
- May not be compatible or optimized for biofluids

Poor data quality

- On target miRNA NGS reads account for only 20−30% of total reads
- Adapter dimers eat away your read budget
- Gel band excision is non-precise (not 100% miRNA)
- Biofluid samples may be contaminated with similar-sized RNA types

Tedious workflow

- Gel-based workflows take multiple days to get to sequencing
- Large sample numbers become tedious with gel-based workflows
- Optimization of sample isolation is not done

miRNA sequencing — Novel miRNA discovery and sample screening

Real-time PCR — NGS validation and expression analysis

QIAseq miRNA sequencing

- Novel miRNA discovery
- Sample screening
- Accurate quantification
- Broad RNA input range
- No gels

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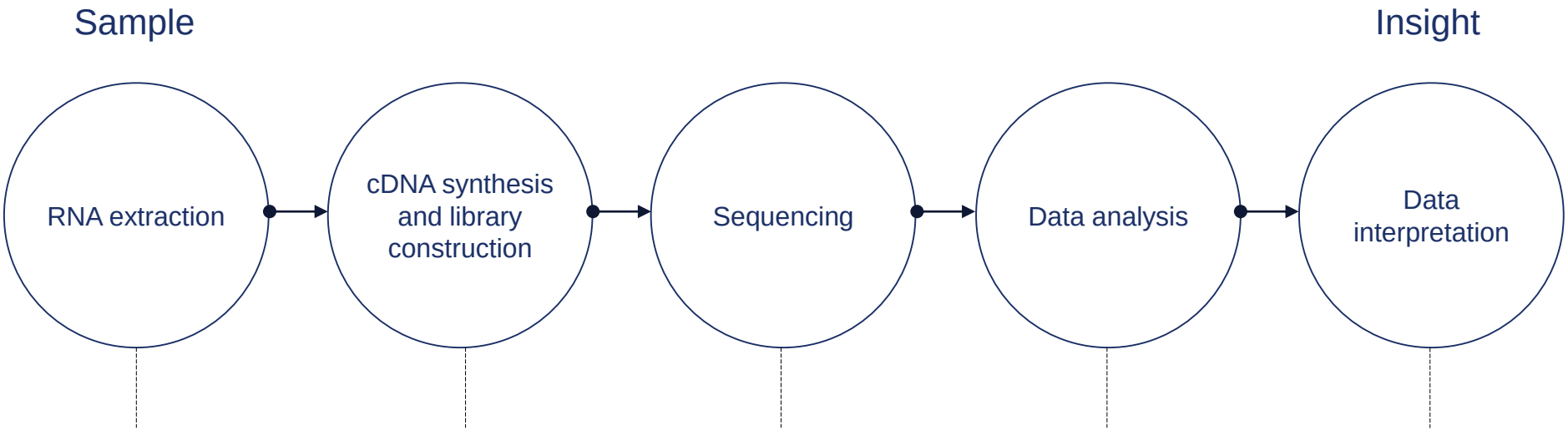
QIAseq miRNA workflow: From Sample to Insight



Sample preparation: A total RNA solution for every sample type

Cells, fresh tissue and frozen tissue

- miRNeasy Mini Kit
- miRNeasy Micro Kit
- miRNeasy 96 Kit

FFPE tissue

- miRNeasy FFPE Kit

Fluids (serum, plasma, urine, CSF and saliva)

- miRNeasy Serum/Plasma Kit

Exosome enrichment/isolation from serum/plasma

- exoRNeasy Serum/Plasma Midi Kit
- exoRNeasy Serum/Plasma Maxi Kit

PAXgene Blood miRNA Kit

PAXgene Tissue miRNA Kit

Overview of QIAseq miRNA products

QIAseq miRNA Library Kit

- 12 rxn: 331502
- 96 rxn: 331505
- What is included? 3’ ligation, 5’ ligation, reverse-transcription, cDNA cleanup, library amplification, library cleanup reagents and quality control primers

QIAseq miRNA NGS 12 Index

- 12 rxn: 331592
- What is included? Sequencing adapters, primers and indices compatible with Illumina platforms − 12 indices for 12 samples

QIAseq miRNA NGS 48 Index

- 96 rxn: 331595
- What’s included? Sequencing adapters, primers and indices compatible with Illumina platforms − two 48 indices for 96 samples

QIAseq miRNA overview

What is the kit?

miRNA-focused next-generation sequencing library prep kit and integrated bioinformatics/data analysis solution

- Compatible with Illumina sequencers

What can be done with the sequencing data?

- Differential expression calculations of miRNA from highly multiplexed samples
- Novel miRNA discovery

What are the distinguishing features of the prep kit?

- Gel-free, rapid workflow
- Broad RNA input: 1−500 ng
  - No adapter dimers at any RNA input amount
- Library prep from serum, plasma, biofluids, cells and tissues (any species)
- Integrated Unique Molecular Index (UMI) technology
- Highly optimized chemistry
- All-in-one-box solution

QIAseq miRNA Library Kit: Unparalleled miRNA-focused sequencing for robust miRNA quantification and discovery.

QIAseq miRNA mapping rates and specifications

miRNA mapping rates routinely observed

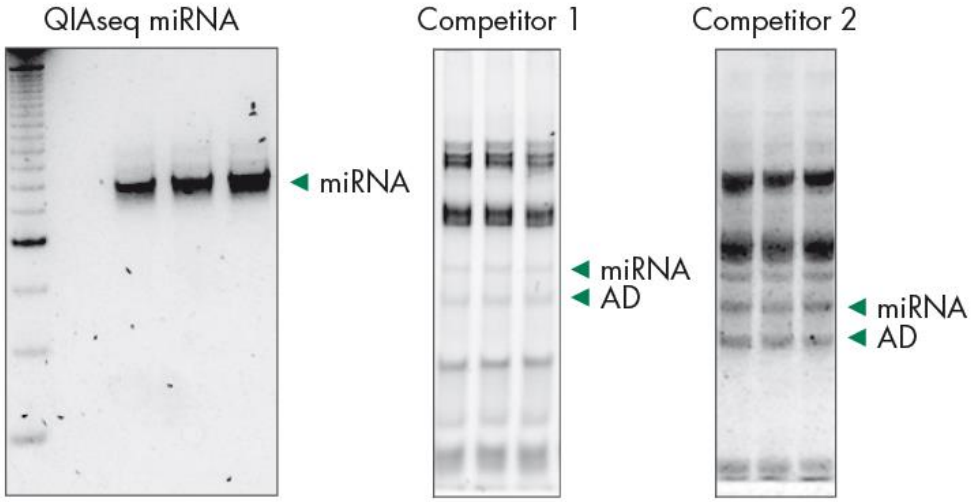
	QIAseq miRNA Kits	Competitors' kits
Cell lines	50−60% or greater	20−30% average post gel
Tissues	75% or greater	30−40% average post gel
Serum/plasma	15−30% or greater	1−15% average post gel

QIAseq miRNA Kit: Specifications

- Sample type:
  - Cells, fresh or frozen tissue, FFPE tissue, serum/plasma and other biofluids
  - Animal and plant samples
  - Any species
- Total RNA input range (cells/tissues): 1−500 ng
- Total RNA input recommendation (serum/plasma): 5 µl when RNA has been isolated from 200 µl of sample
- What RNAs are included in library prep?
  - Highly optimized for miRNA
  - piRNAs will also be efficiently sequenced
- Multiplex capability: 48 samples
- Sequencer compatibility: Illumina
- Total library construction time: 8 hours (3 hours hands-on)

- QIAseq miRNA vs. two competitor (C) kits
  - RNA amounts: 100 ng (QIAseq miRNA), 1 µg (C1) and 100 ng (C2)

PAGE gel after standard library prep protocol



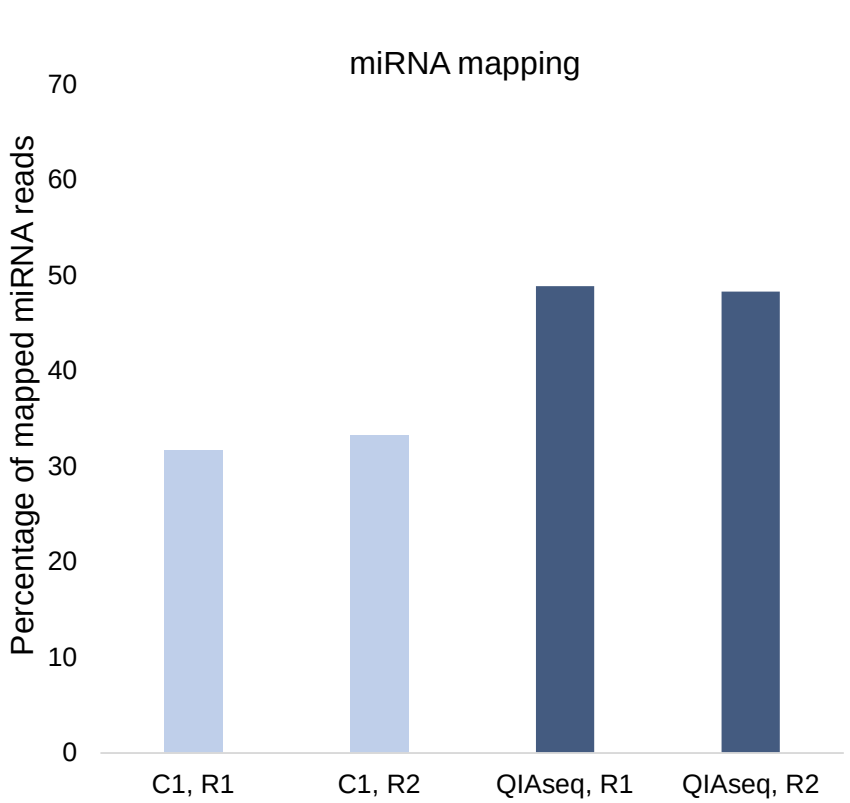
AD: Adapter dimer

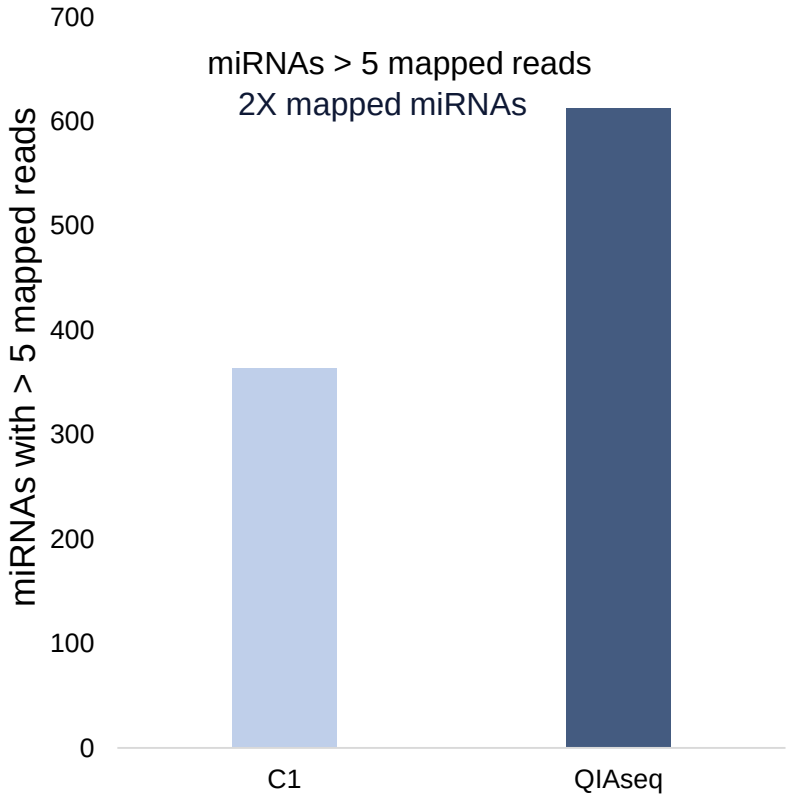
With the QIAseq miRNA standard protocol, a robust, specific miRNA library is generated with negligible background. Other commercial options are fraught with side-products, including adapter dimers.

QIAseq miRNA Library Kit outperforms the competition

Next-generation sequencing: QIAseq miRNA and competitor 1 (C1)

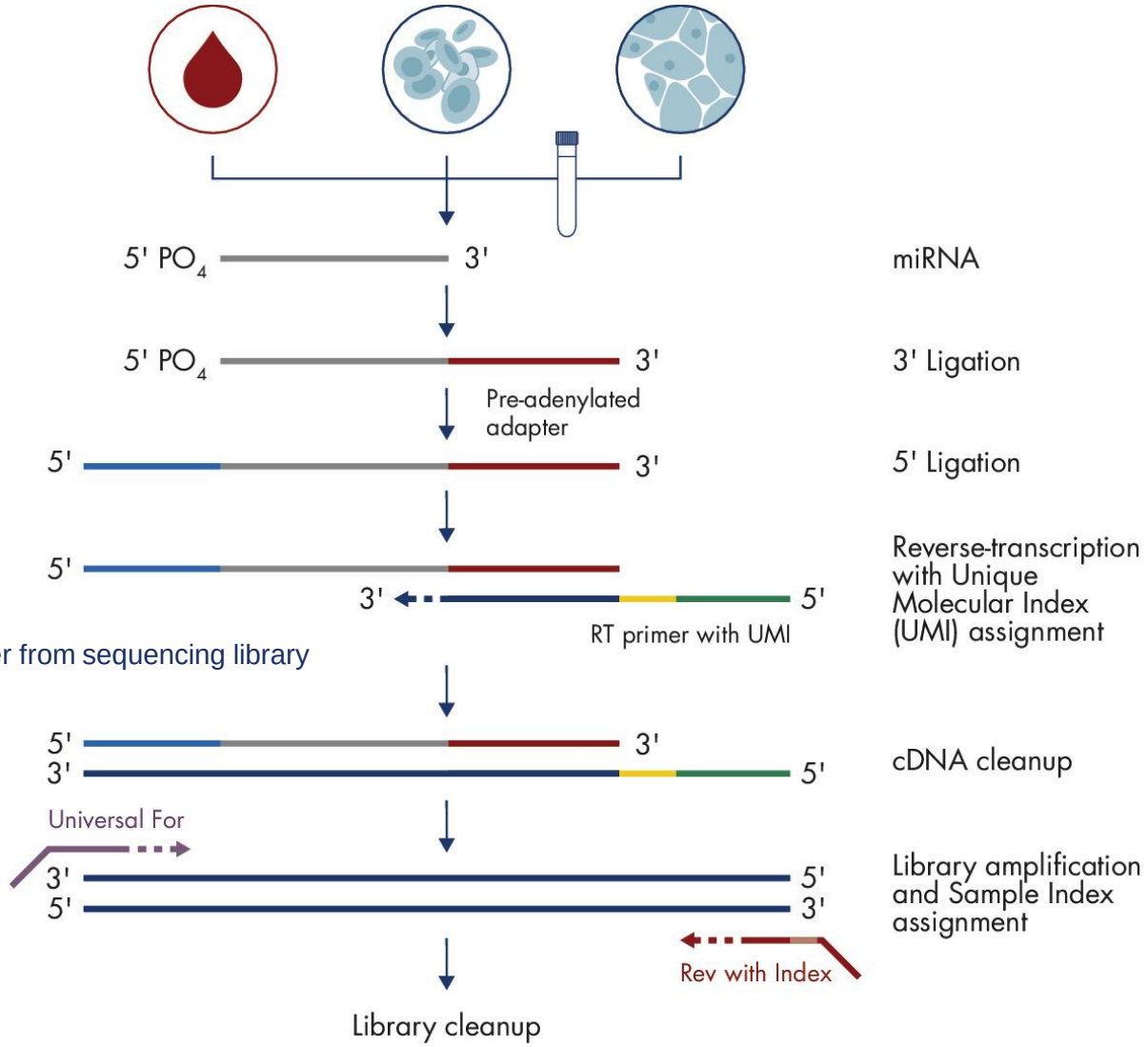
- For C1, prior to sequencing, miRNA library was excised and purified from a PAGE gel
- MiSeq: 75 bp Single-Read (QIAseq miRNA) and 50 bp Single-Read (C1)





With QIAseq miRNA, increase your mapped miRNA reads (as a result of reduced bias and improved sensitivity) while reducing your workflow time.

QIAseq miRNA: Library construction

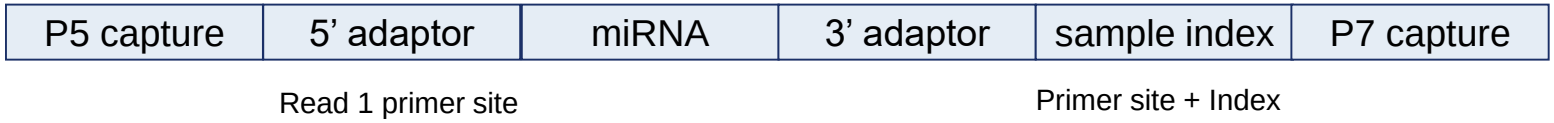


Elimination of adapter dimer from sequencing library

Library pre-seq QC, determining library concentration, preparartion for sequencing and data analysis

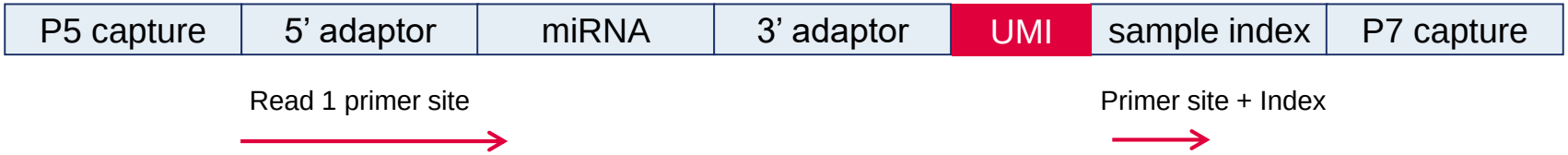
QIAseq miRNA library

Other kits



- 50 bp read
- 48 possible sample indices

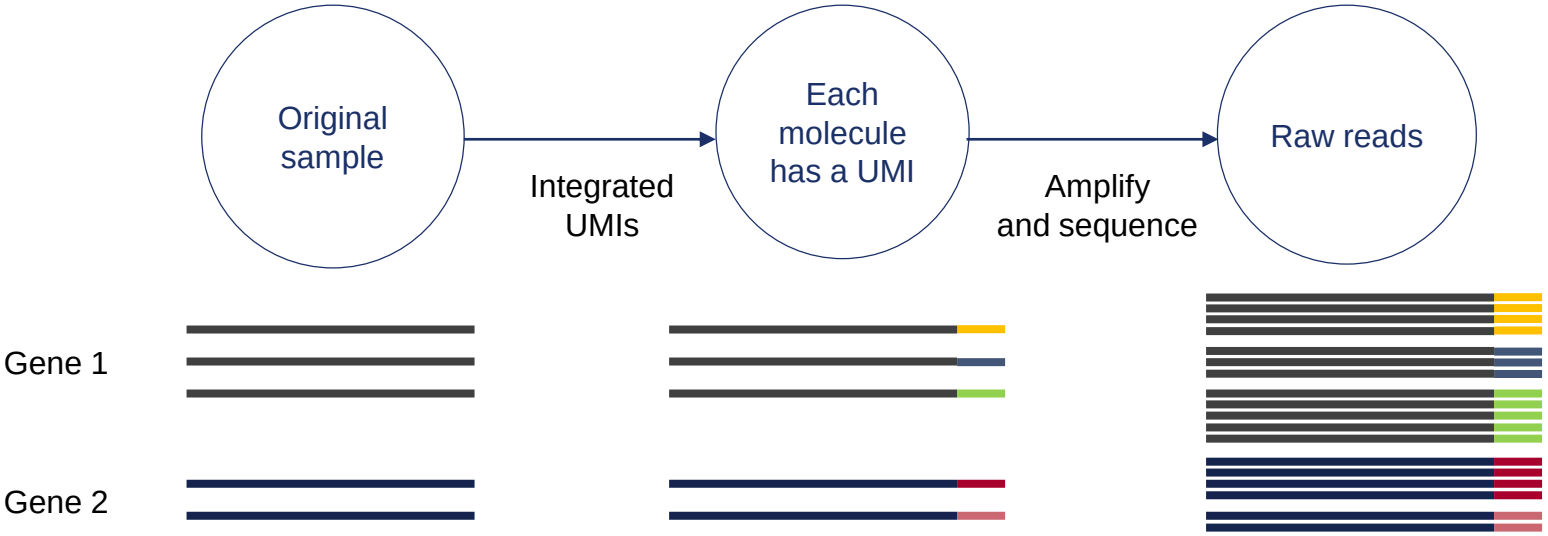
QIAseq miRNA Library Kit



- 75 bp read to make use of the UMI
  - Note: 50 bp read is possible, but the UMI will not be sequenced
- 48 possible sample indices

UMI enables precision quantification of miRNA molecules following next-generation sequencing.

The principle of Unique Molecular Indices (UMIs)



Original sample (3:2 ratio of gene 1 to gene 2)

- Gene 1: 3 molecules
- Gene 2: 2 molecules

Interpretation of raw reads (2:1 of gene 1 to gene 2)

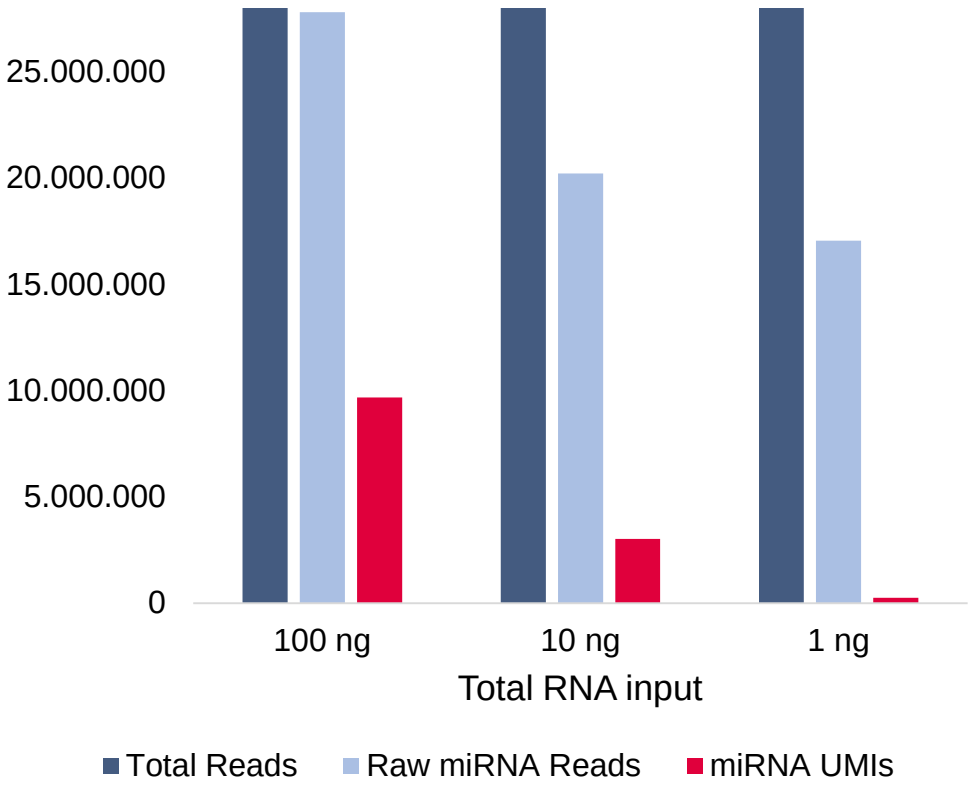
- Gene 1: 12 reads
- Gene 2: 6 reads

Interpretation of UMIs (3:2 ratio of gene 1 to gene 2)

- Reads are collapsed based on molecule counts
- Gene 1: 12 reads but 3 molecules are identified due to UMIs
- Gene 2: 6 reads but 2 molecules are identified due to UMIs

Quantification based on UMIs reflects quantities of original RNA molecules.

Value of UMIs



Assessment of raw miRNA reads

- Sequencing of the same miRNA molecule over and over results in an overestimation of miRNA expression
- The lower the RNA input, the lower the effect

Assessment of miRNA UMIs

- Individual miRNA molecules are being counted, resulting in a true assessment of miRNA expression
- The lower the RNA input, the more powerful the UMIs

UMIs give a true readout of miRNA expression.

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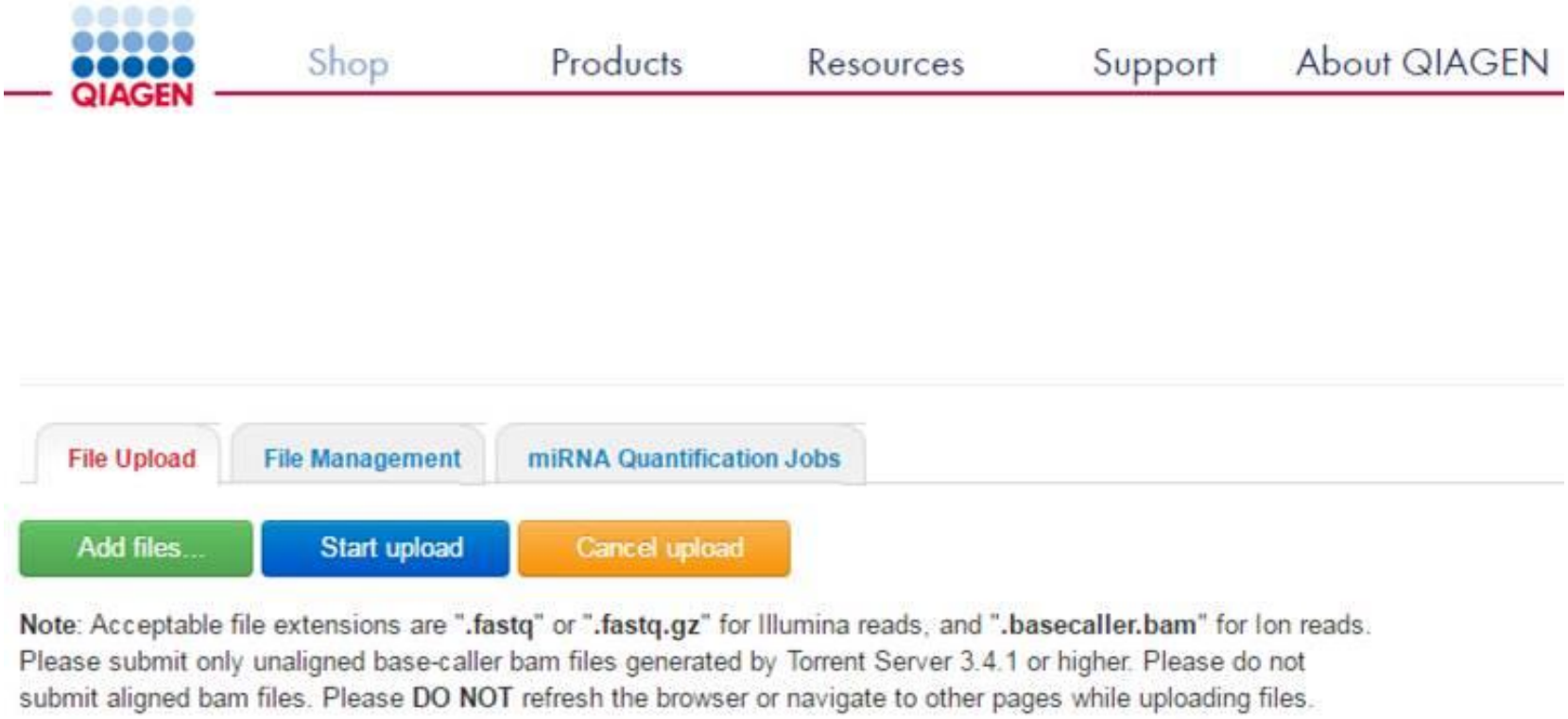
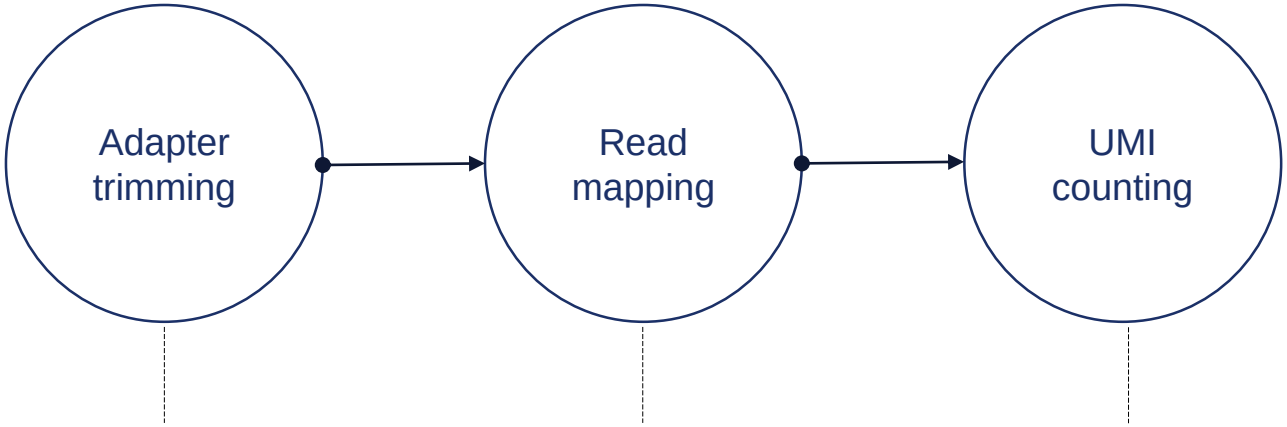
QIAseq miRNA: Primary data analysis

- Free, easy to use primary data analysis: **www.qiagen.com/geneglobe**
- Species: Human, mouse, rat or other species (all of miRBase)



QIAseq miRNA: Primary data analysis

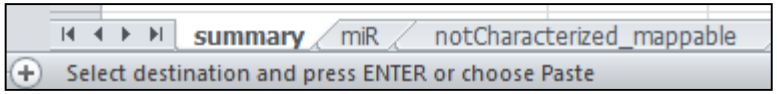


Remove the adapter sequences from the reads

- Identify the possible position of the read within the reference
- Align the read sequence to reference sequences

Merges unique UMI with mapped reads

QIAseq miRNA: Primary data analysis output



Summary tab

	A	B	C
1	read set	Sample 1, Rep 2	Sample 2, Rep 1
2	total_reads	3,485,926	3,454,577
3	no_adapter_reads	245,252	321,093
4	too_short_reads	916,139	737,228
5	UMI_defective_reads	143,102	192,311
6	miRNA_Reads	1,250,002	1,333,379
7	hairpin_Reads	3,319	2,787
8	piRNA_Reads	26,771	29,049
9	rRNA_Reads	99,637	93,880
10	tRNA_Reads	22,192	18,248
11	mRNA_Reads	12,305	12,127
12	otherRNA_Reads	115,790	149,227
13	notCharacterized_Mappable	143,611	135,715
14	notCharacterized_notMappable	507,806	429,533

miR tab

	A	B	C	D	E
1	miRNA	Sample 1, Rep 2 - UMI	Sample 2, Rep 1 - UMI	Sample 1, Rep 2 - Reads	Sample 2, Rep 1 - Reads
2	hsa-miR-16-5p	112,095	96,568	224,770	196,617
3	hsa-miR-126-3p	80,983	77,107	164,836	160,661
4	hsa-let-7a-5p	43,816	39,426	84,078	76,639
5	hsa-miR-223-3p	36,192	50,904	71,624	104,480

Features of the primary analysis report

- Summary tab: Important mapping metrics for the sequencing run
- miR tab: Mapped reads and UMI counts for each miRNA found in miRBase
- notCharacterized mappable tab: Mapped reads and UMI counts for each sequence that aligns to the genome

QIAseq miRNA primary analysis output gives a complete summary of important sequencing metrics and UMI counts for each mapped miRNA.

QIAseq miRNA: Secondary data analysis

Free, easy-to-use secondary data analysis: **qiagen.com/geneglobe**

What can you do?

- Perform gene expression analysis

How can you normalize the data?

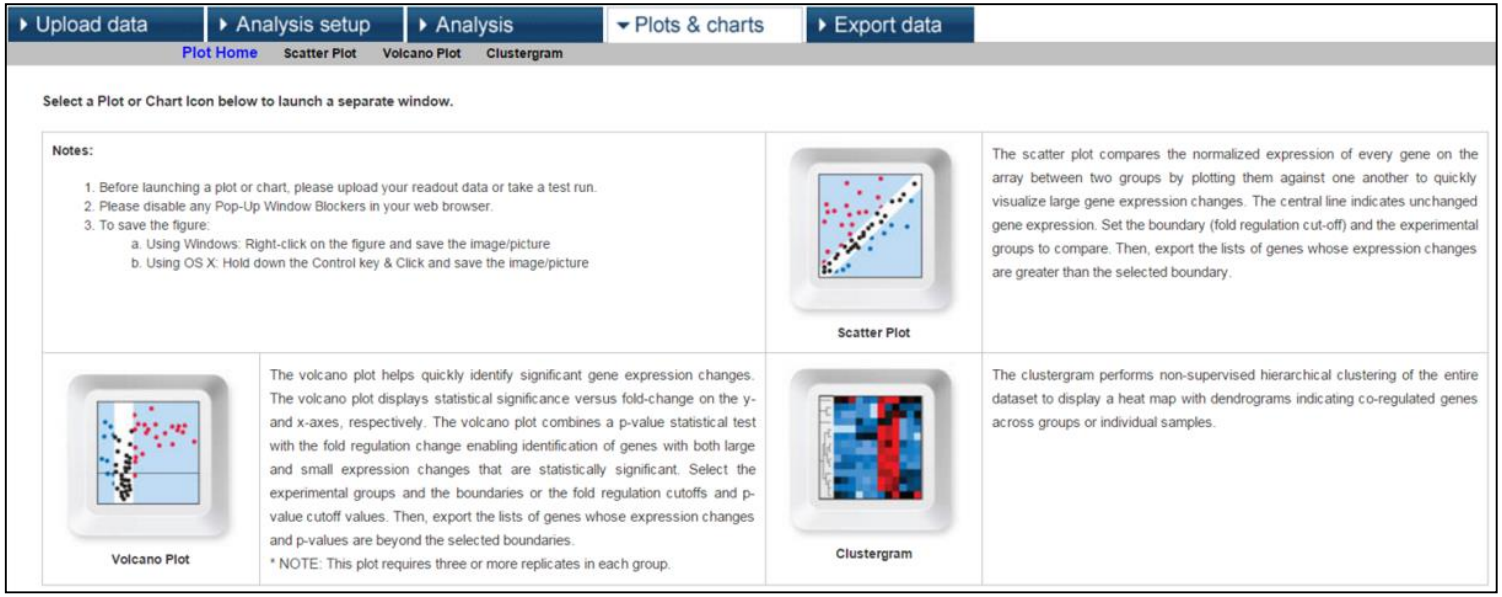
- geNorm
- Total Molecular Tag Count
- DESeq2
- Trimmed Mean of M (edgeR)

What is the output?

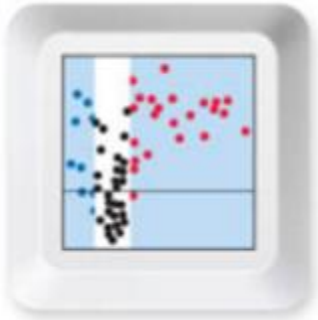
- Fold-regulation results
- Scatter plots, volcano plots and clustergrams
- Ingenuity Pathway Analysis (IPA) upload file

QIAseq targeted RNA secondary data analysis: Plots and charts

Visual representations of data



Scatter plot



Volcano plot

Clustergram

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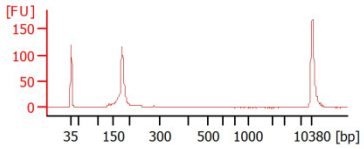
QIAseq miRNA: Input as low as 1 ng without adapter dimers

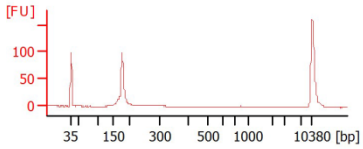
QIAseq miRNA Library Kit workflow on kidney total RNA

- RNA amounts: 500 ng, 100 ng, 10 ng and 1 ng
- Sample QC: Bioanalyzer (BA)
- Sequencing: NextSeq®, 75 bp Single Read

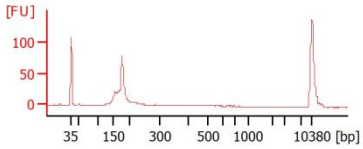
BA: 500 ng



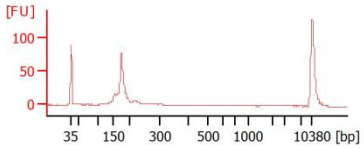
BA: 100 ng



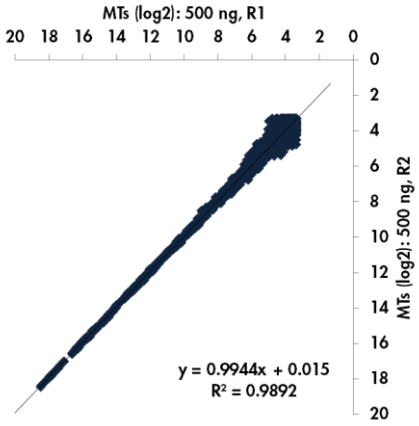
BA: 10 ng



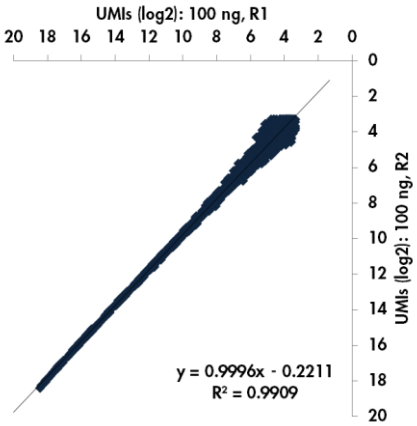
BA: 1 ng



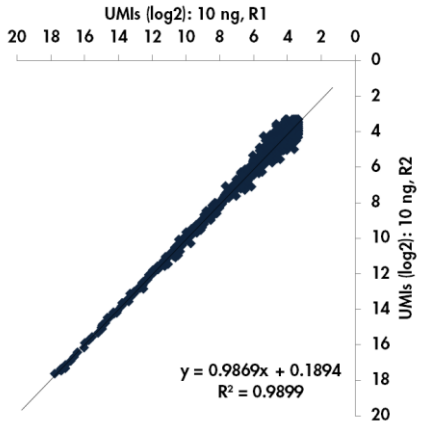
UMIs: 500 ng



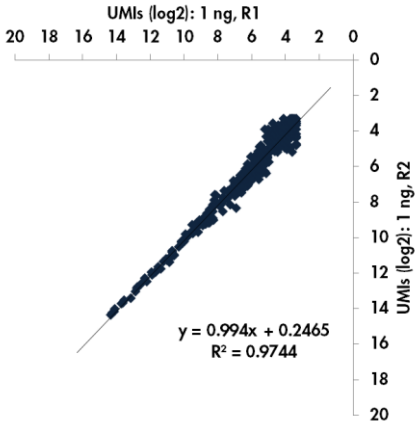
UMIs: 100 ng



UMIs: 10 ng



UMIs: 1 ng

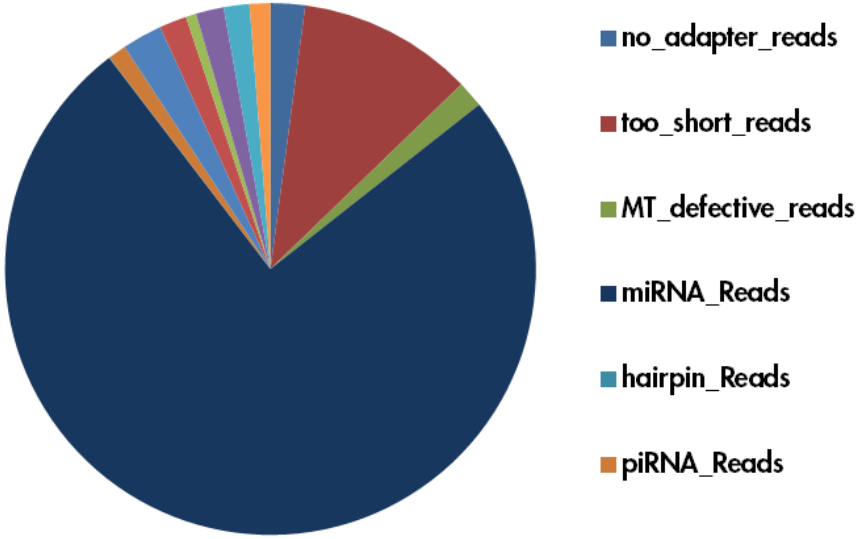


The QIAseq miRNA workflow enables robust, reproducible results from 1−500 ng.

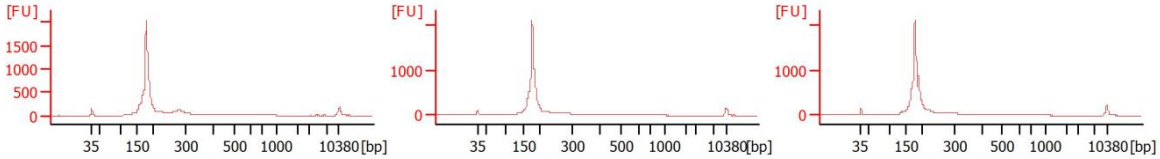
Cells and tissues: High mapped miRNA percentages

QIAseq miRNA Library Kit workflow on kidney (K) and lung (L) total RNA

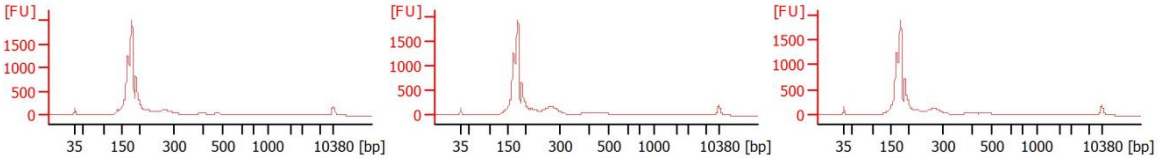
- RNA amount: 100 ng (n = 3 of each tissue)
- Sample QC: Bioanalyzer (BA)
- Sequencing: NextSeq, 75 bp Single Read



BA: Kidney libraries



BA: Lung libraries



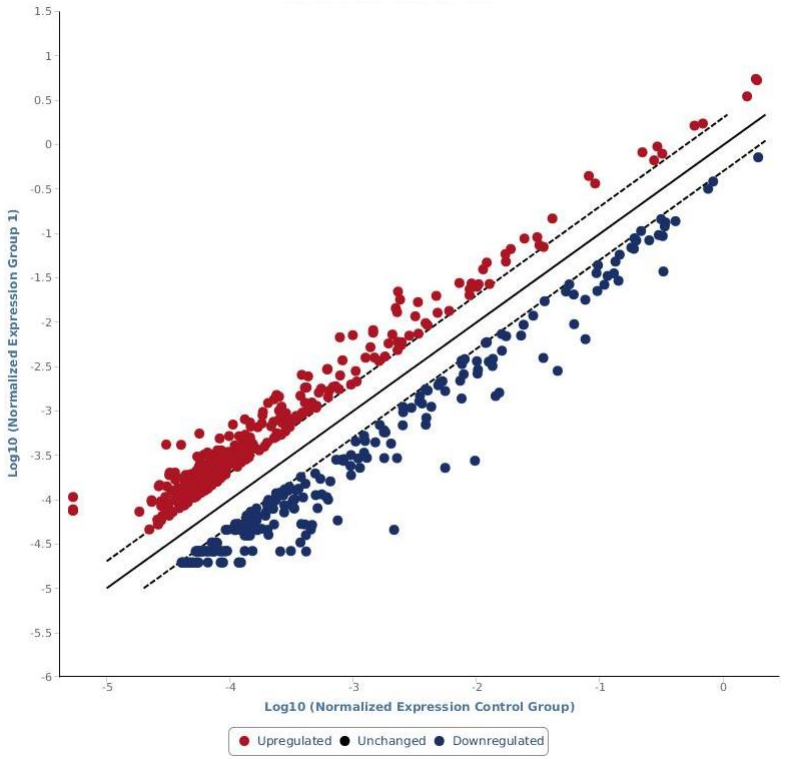
Sequencing summary

Sample	Mapped miRNA
K1	75%
K2	67%
K3	67%
L1	64%
L2	68%
L3	67%

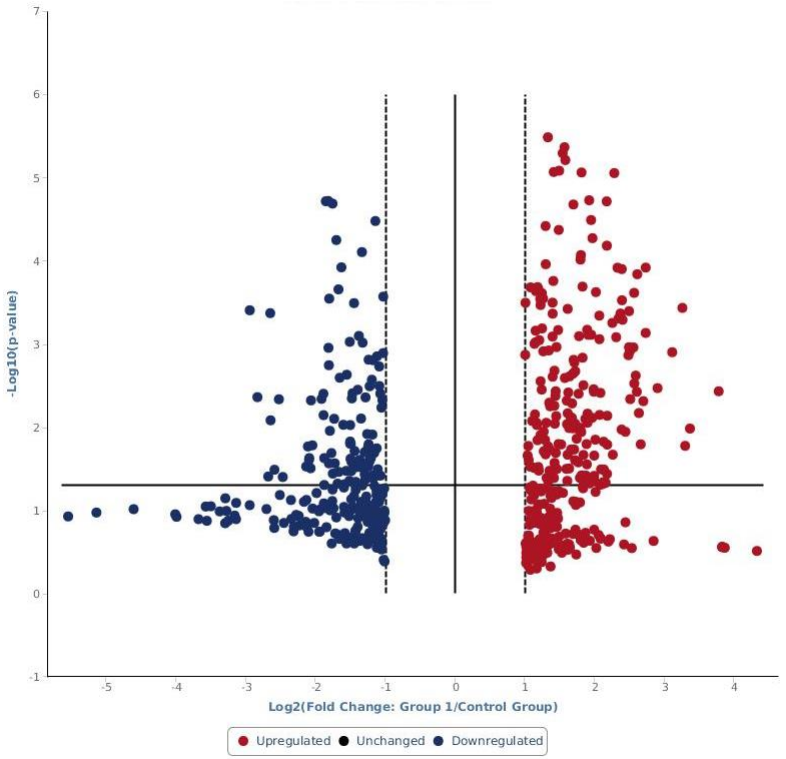
The QIAseq miRNA workflow prepares robust libraries from tissues, enabling a high percentage of miRNA reads without gel excision.

GeneGlobe Data Analysis Center to identify differential miRNA expression

Scatter plot (lung vs. kidney)



Volcano plot (fold-regulation vs. p-value)



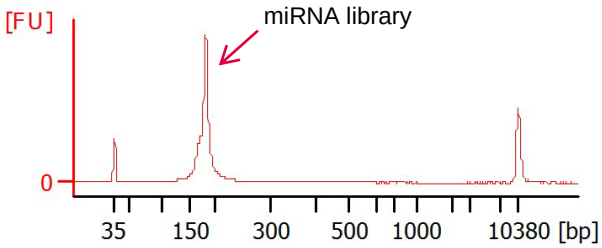
Use the GeneGlobe Data Analysis Center to easily identify differentially expressed miRNAs.

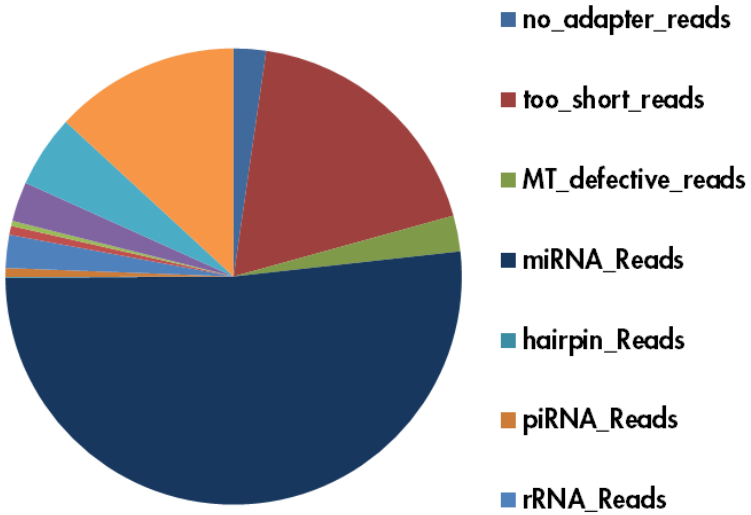
Serum and plasma: High mapped miRNA percentages

QIAseq miRNA Library Kit workflow on plasma total RNA

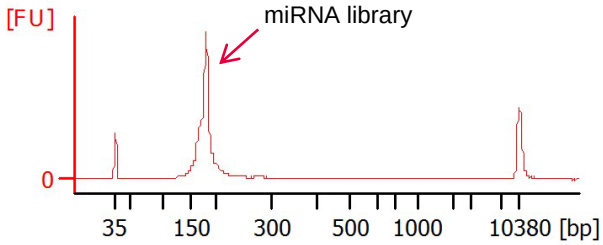
- miRNeasy Serum/Plasma Kit: 200 µl input
- Total RNA input: 5 µl RNA eluate (80 µl of serum equivalents)
  - Normal (N) plasma (n = 3)
  - Breast cancer plasma (n = 3)
- Sequencing: NextSeq, 75 bp Single Read
- miRNA mapping %: 52 (N1), 54 (N2), 47 (N3), 49 (BC1), 41 (BC2), 57 (BC3)

BA: Normal plasma





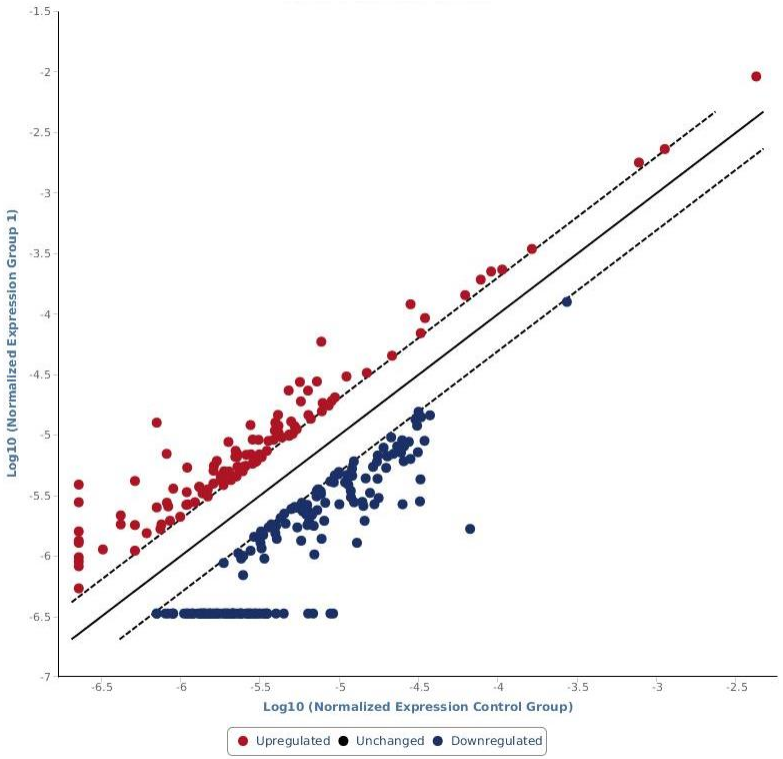
BA: Breast cancer plasma



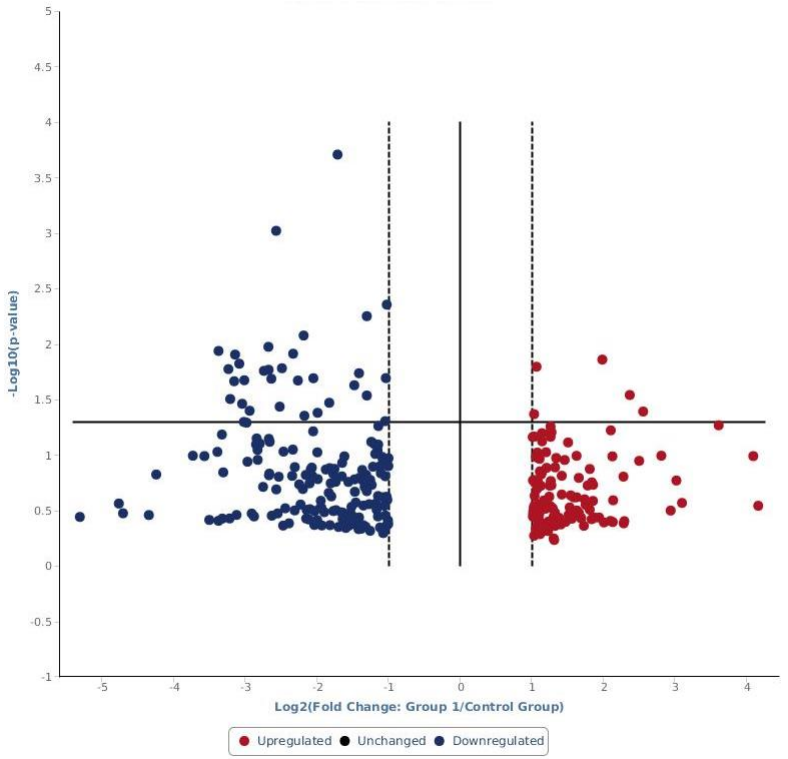
The QIAseq miRNA workflow prepares robust libraries from plasma, enabling a high percentage of miRNA reads without gel excision.

QIAseq miRNA: Optimized quantification of miRNAs from serum & plasma

Scatter plot (breast cancer vs. normal)



Volcano plot (fold-regulation vs. p-value)



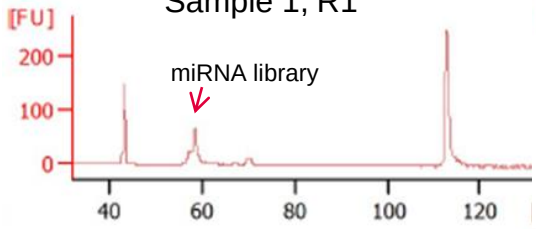
- 123 miRNAs upregulated (6 significantly)
  - miR-520g-3p: Associated with important prognostic factors in breast cancer patients
- 179 miRNAs downregulated (31 significantly)

Use the GeneGlobe Data Analysis Center to easily identify differentially expressed miRNAs.

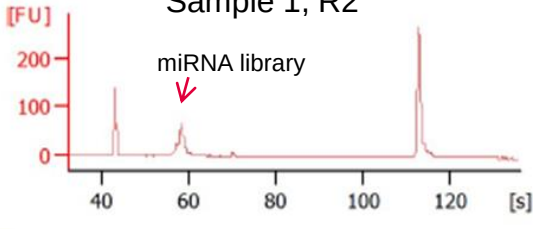
Exosome samples: High mapped miRNA percentages

- Isolation: exoRNeasy (1 ml plasma processed)
- Samples: Four total RNA samples (2 donors, 2 replicates)
- RNA input: 5 µl of RNA eluate

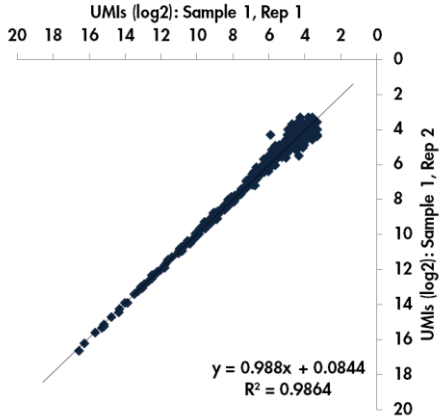
Sample 1, R1



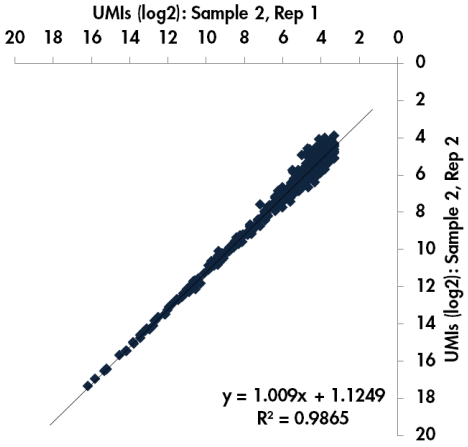
Sample 1, R2



Sample 1



Sample 2



read set	Sample 1, Rep 1	Sample 1, Rep 2	Sample 2, Rep 1	Sample 2, Rep 2
total_reads	3,454,577	3,539,076	2,531,228	6,230,468
no_adapter_reads	321,093	276,815	345,974	562,212
too_short_reads	737,228	799,712	461,630	1,240,571
UMI_defective_reads	192,311	154,736	194,358	398,158
miRNA_Reads	1,333,379	1,424,014	913,946	2,413,667
hairpin_Reads	2,787	2,851	2,078	6,554
piRNA_Reads	29,049	30,768	23,773	62,763
rRNA_Reads	93,880	92,517	83,114	201,736
tRNA_Reads	18,248	18,566	16,370	41,872
mRNA_Reads	12,127	12,383	9,533	24,309
otherRNA_Reads	149,227	152,885	88,306	239,613
notCharacterized_Mappable	135,715	139,635	120,375	326,720
notCharacterized_notMappable	429,533	434,194	271,771	712,293
miRNA Mapping %	38.6	40.2	36.1	38.7

Outcome: High mapping percentage to miRNAs; low mapping percentage to OtherRNA reads (often observed with other commercial kits).

Agenda

1. miRNA background
2. QIAseq miRNA sequencing: Product overview
3. Data analysis overview
4. Performance & application data
5. **Summary and questions**

Summary

QIAseq miRNA

miRNA-focused next-generation sequencing library prep kit and integrated bioinformatics/data analysis solution

What can be done with the sequencing data?

- Differential expression calculations of miRNA from highly multiplexed samples
- Novel miRNA discovery

What are distinguishing features of the QIAseq miRNA Kit?

- Gel-free, rapid workflow
- Broad RNA input: 1−500 ng
- Library prep from serum, plasma, biofluids, cells and tissues
- Integrated Unique Molecular Index (UMI) technology
- Highly optimized chemistry
- All-in-one-box solution

Thank you for attending!

Questions?

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