



Jonathan Shaffer, M.B.A., Ph.D.  
Associate Director  
Research & Development

# The underestimated role of rRNA removal from FFPE and liquid biopsy samples

Generating consistent on-target reads for gene expression and miRNA data

## 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](http://www.qiagen.com) or can be requested from QIAGEN Technical Services or your local distributor.

# Agenda

Background

---

RNA-seq: FFPE and whole blood samples

---

miRNA-seq: Serum/plasma samples

---

Summary

---



# Agenda

## Background

---

RNA-seq: FFPE and whole blood samples

---

miRNA-seq: Serum/plasma samples

---

Summary

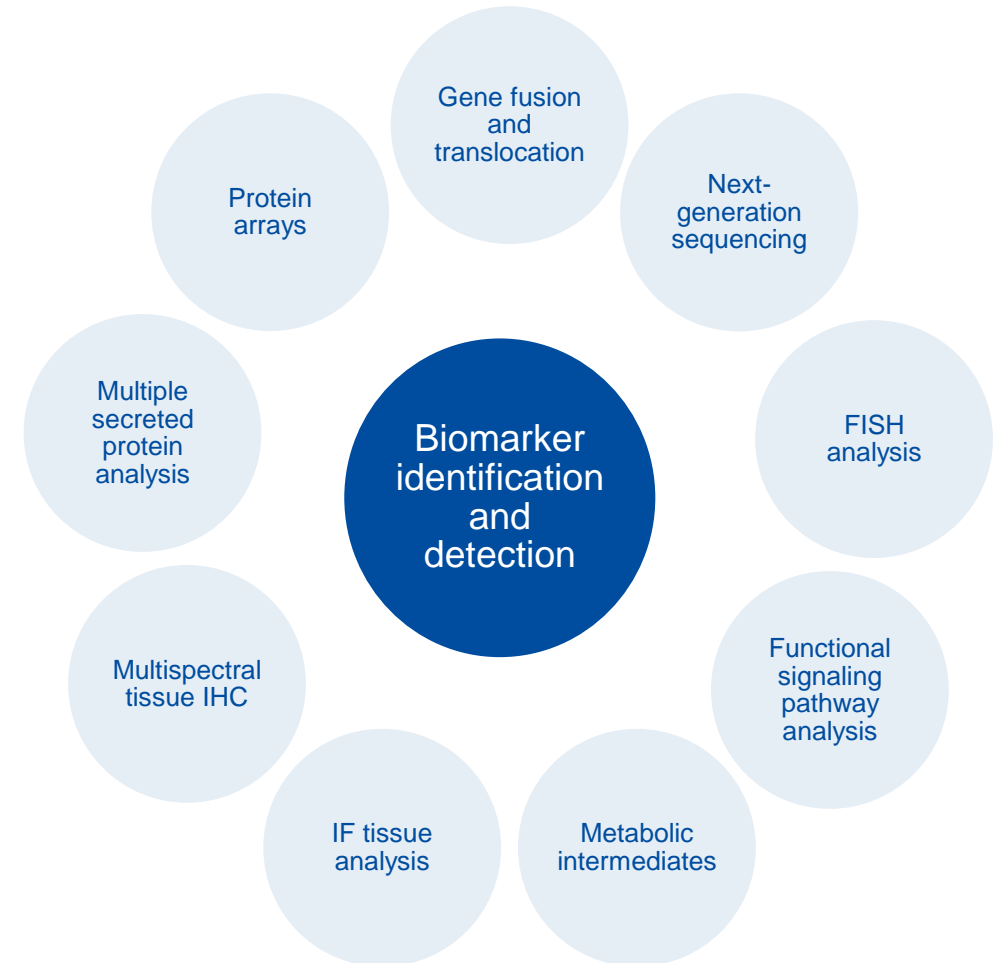
---



# What is a biomarker?

A biomarker is a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes or pharmacologic responses to a therapeutic intervention.

Characteristic(s)	Methodology
Presence of antibodies	Elisa
Abnormal BP, blood cell counts, electrolytes	Blood counts, blood pressure
Distinct histological indicators	Microscopy
Abnormal liver function markers	Biofluid assay
Presence of muscle injury protein markers	Biofluid assay
Elevated kidney marker – serum creatinine	Biofluid assay
Gene status or gene expression status	qPCR, NGS, array, etc.



## Sample types typically used for biomarker studies



- Fresh/frozen cells and tissues
- FFPE tissues
- Serum/plasma
- Biofluids
- Whole blood

● Tip: Choose sample prep kits that provide you with the flexibility to analyze all RNAs.





# FFPE tissues: Classic yin and yang

## The dark side of FFPE

RNA is chemically modified, cross-linked and degraded

- Time is not favorable to FFPE sample quality
- Different fixation and tissue processing methods create variability in quality

Unfavorable signal-to-noise ratio

- Fragments of RNAs
- Bacteria RNA contamination
- **Perception** that gene & miRNA signatures are locked away and inaccessible

---

## The bright side of FFPE

Millions of FFPE samples exist worldwide

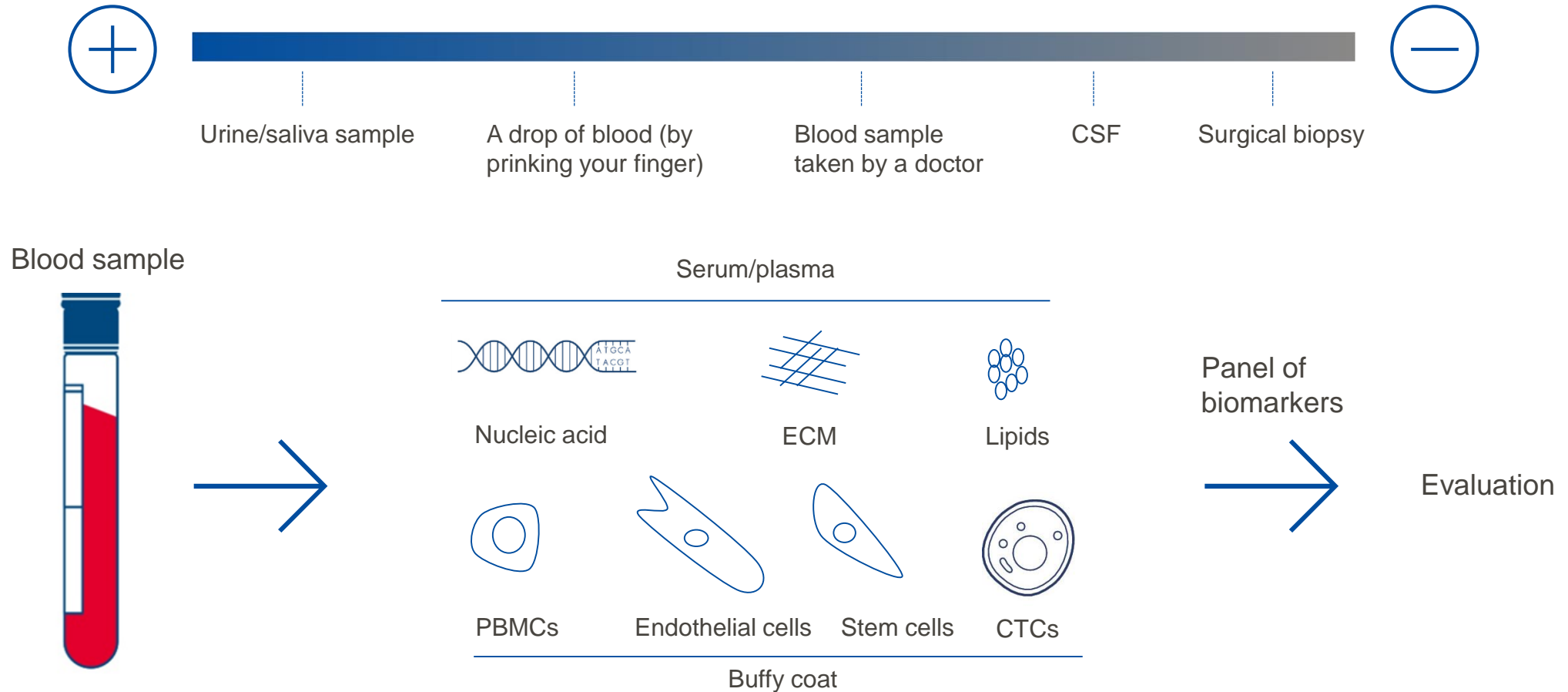
- Well annotated, containing clinical and demographic data
- Increased usage in basic and translational research
- **Reality** is that molecular analyses are possible
  - Fragments of RNAs
  - Bacteria RNA contamination

- **The challenge:** Achieve a data yield comparable to fresh frozen samples.



# Liquid biopsies

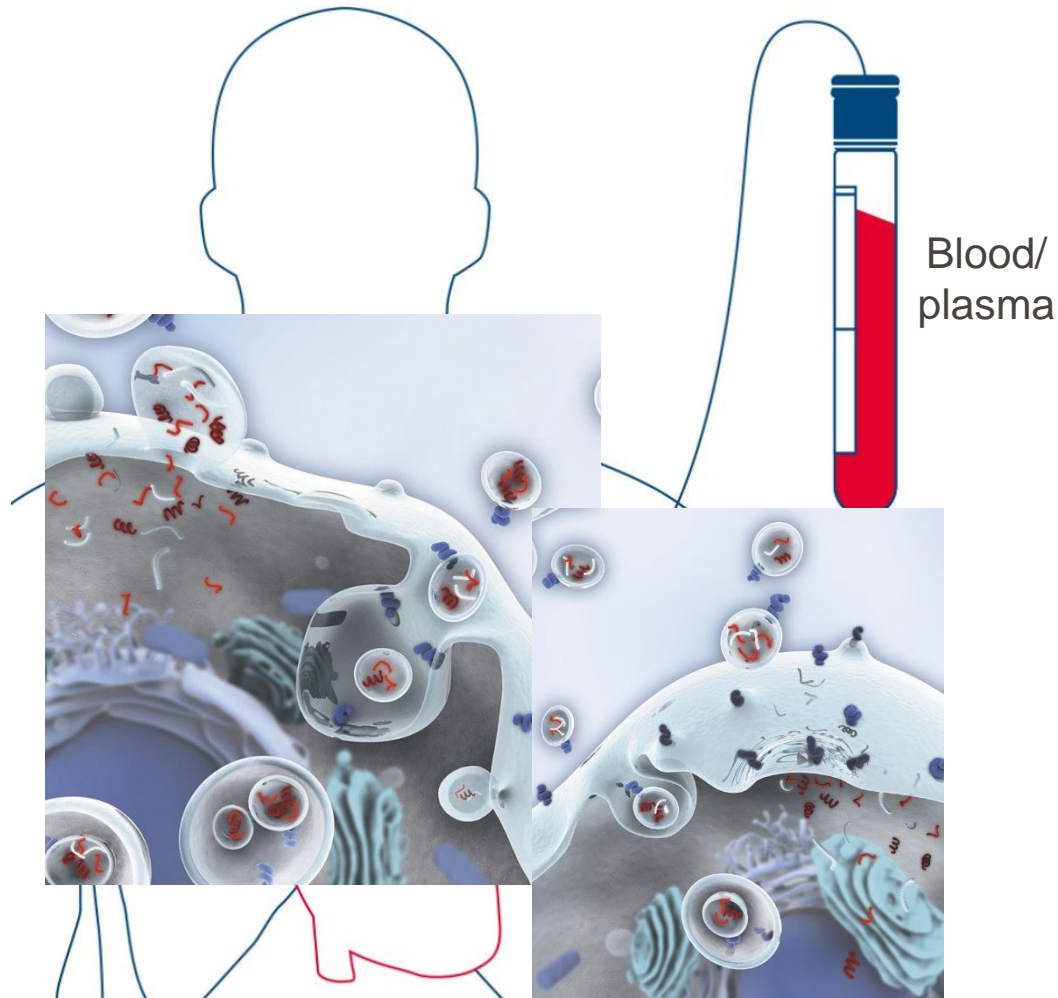
To use as a biomarker source for diagnostics, the sample must be as easy to obtain as possible



Source: Martin, K. J. et. al. (2010) A need for basic research on fluid-based early detection biomarkers. Cancer Res. 70(13): 5203–6



## Exosomes or microvesicles (MVs)



- Exosomes or MVs are ~50-200 nm small vesicles excreted by all cells
- Exosomes are found in all biofluids (e.g., blood)
- Exosomes contain stable RNA (mRNA, miRNA and other small RNAs), DNA and proteins, protected from degradation by a lipid bilayer
- Contents are specifically packaged
- Mechanism of local and distant cellular communication

## Sample preparation: A total RNA solution for every sample type

### FFPE tissue

- miRNeasy FFPE Kit
- AllPrep<sup>®</sup> DNA/RNA FFPE Kit

### Whole blood

- PAXgene<sup>®</sup> Blood miRNA Kit

### Serum/plasma

- miRNeasy Serum/Plasma Advanced Kit
- miRNeasy Serum/Plasma Kit
- QIAamp<sup>®</sup> ccfDNA/RNA Kit

### Exosome enrichment/isolation from serum/plasma

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

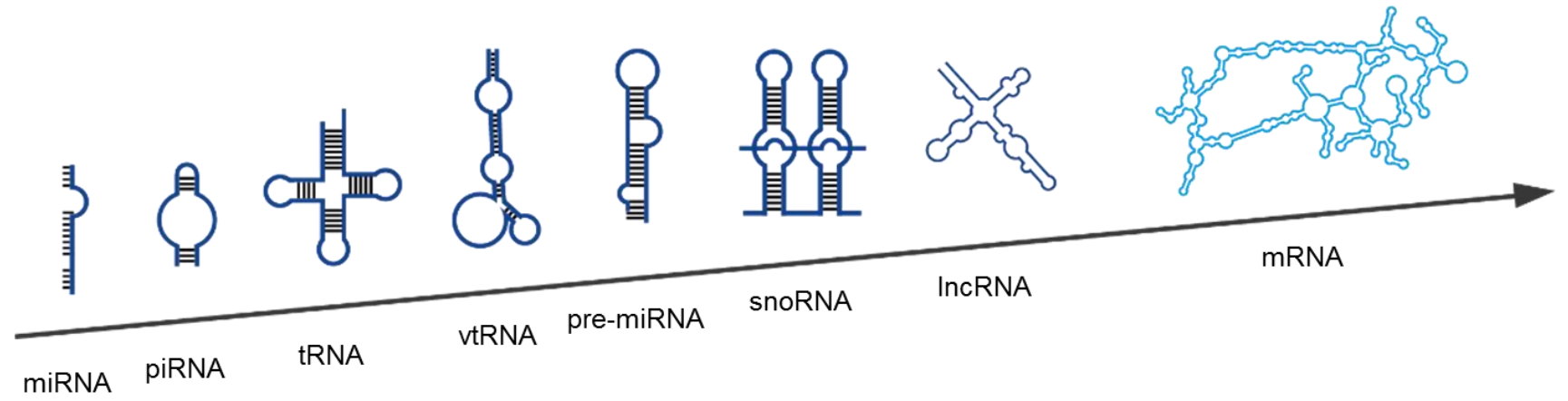
● Tip: Heparin is a potent reverse transcription inhibitor. For a custom solution from QIAGEN, drop me an email.

# RNA-focused next-generation sequencing (NGS)

## Specific applications

- Gene expression
- Pathway analysis
- Biomarker discovery
- Allele-specific expression
- Fusion genes in cancer
- Tumor heterogeneity
- Immune repertoire
- Single-cell sequencing

## Specific analytes



## All RNAs are unique, and their individual properties matter

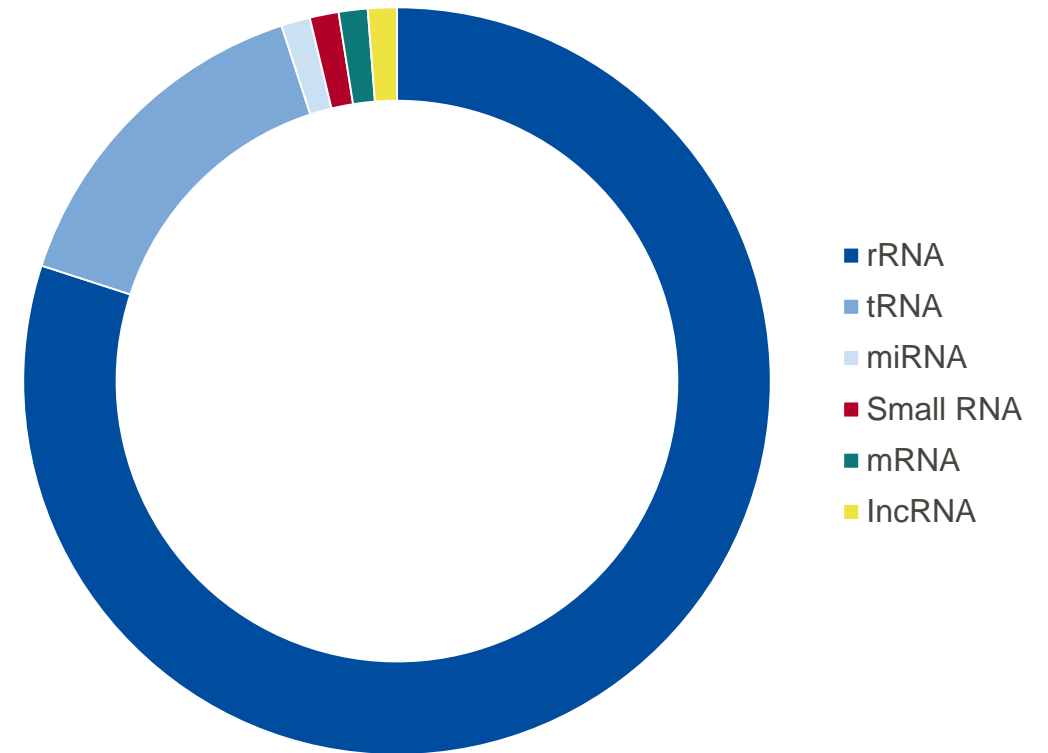
mRNA	lncRNA	miRNA/small RNA
Coding	Non-coding, regulatory functions	Non-coding, regulatory functions
200–100,000 nt	200–100,000 nt	19–25 nt
5' cap, poly(A), splicing	5' cap, may have poly(A), splicing, complex loci	No poly(A), processed from longer precursors
~21,000 human genes	~15,000 human genes (predicted 3–100 fold of mRNA in number)	2,588 human miRNAs
Low to high expression level	Very low to moderate expression level	Very low to high expression level

- Tip: The unique properties of RNAs demand specialized library prep solutions.

# Library enrichment/depletion strategies: Whole transcriptome NGS

Enrichment or depletion is necessary to maximize reads from the RNAs of interest

- Typical RNA composition in a cell: >80% ribosomal RNA (in blood cells, globin is also a major contaminant)
- Highly abundant transcripts consume a lot of reads
- Enrichment or depletion is used to obtain more reads from the RNAs of interest, such as:
  - mRNA
  - lncRNA



# Library enrichment/depletion strategies: Whole transcriptome NGS

## Enrichment/depletion strategies

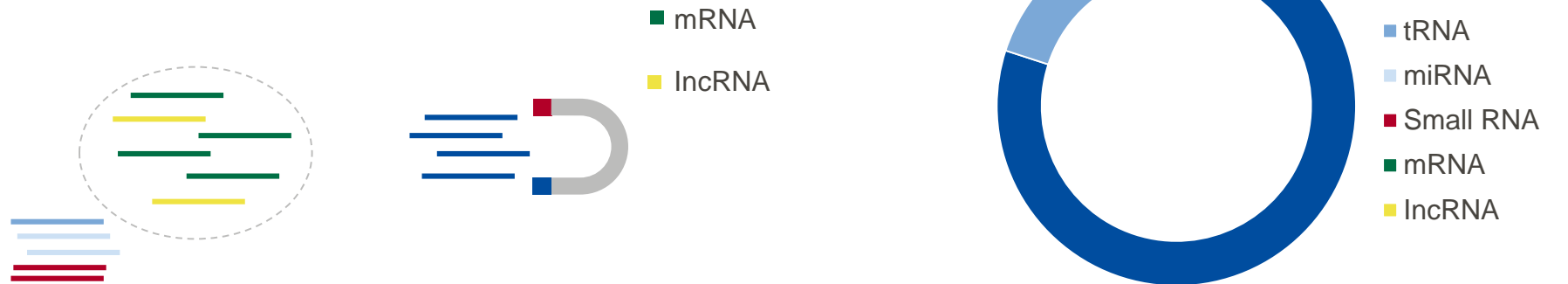
### Poly(A) enrichment

- Not useful for fragmented samples
- Not useful for prokaryotic samples



### rRNA or globin depletion

- The “catch all” method



● Strategy selection depends on the objective of your whole transcriptome sequencing.



## In miRNA-seq, hY4 Y RNA can be a major contaminant

Read set	hY4 not blocked (two replicate libraries)	
Total_reads	2,266,831	2,715,234
No_adapter_reads	98,237	120,162
Too_short_reads	346,580	383,548
UMI_defective_reads	44,259	50,209
miRNA_reads	596,675	680,215
Hairpin_reads	1,216	1,468
piRNA_reads	11,819	14,441
rRNA_reads	39,078	47,098
tRNA_reads	8,556	10,628
mRNA_reads	5,251	6,163
OtherRNA_reads	833,042	1,071,052
NotCharacterized_mappable	72,481	84,476
NotCharacterized_notmappable	209,637	245,774
miRNA mapping %	26.3	25.1
OtherRNA_reads (hY4 mapped here) %	36.7	39.4

When you do not block hY4, mapping to “OtherRNA\_reads” increases from ~2.5% to ~40%. In addition, miRNA mapping decreases from ~40% to 25%.

# Agenda

Background

---

RNA-seq: FFPE and whole blood samples

---

---

miRNA-seq: FFPE and serum/plasma samples

---

Summary

---

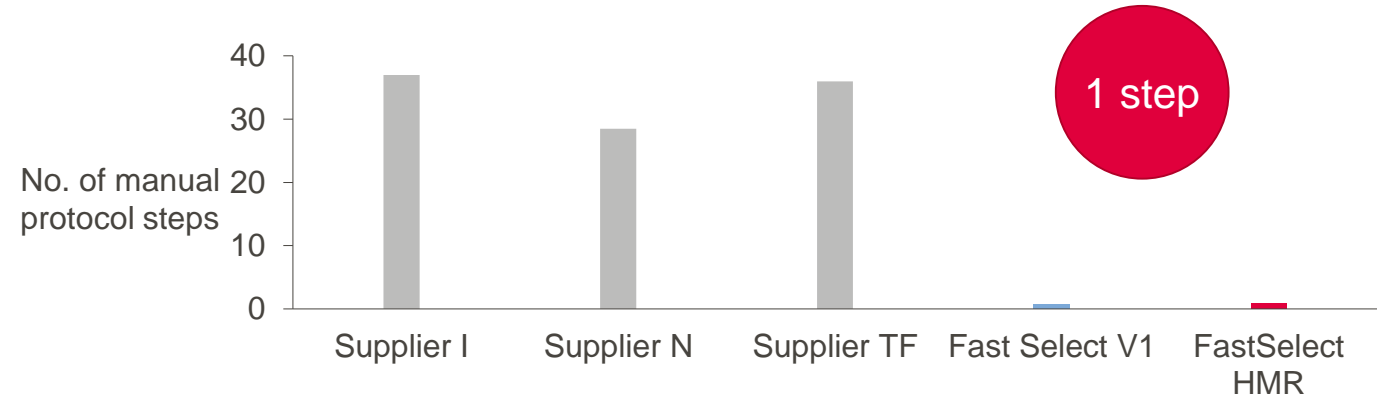


# QIAseq® FastSelect –rRNA HMR Kit

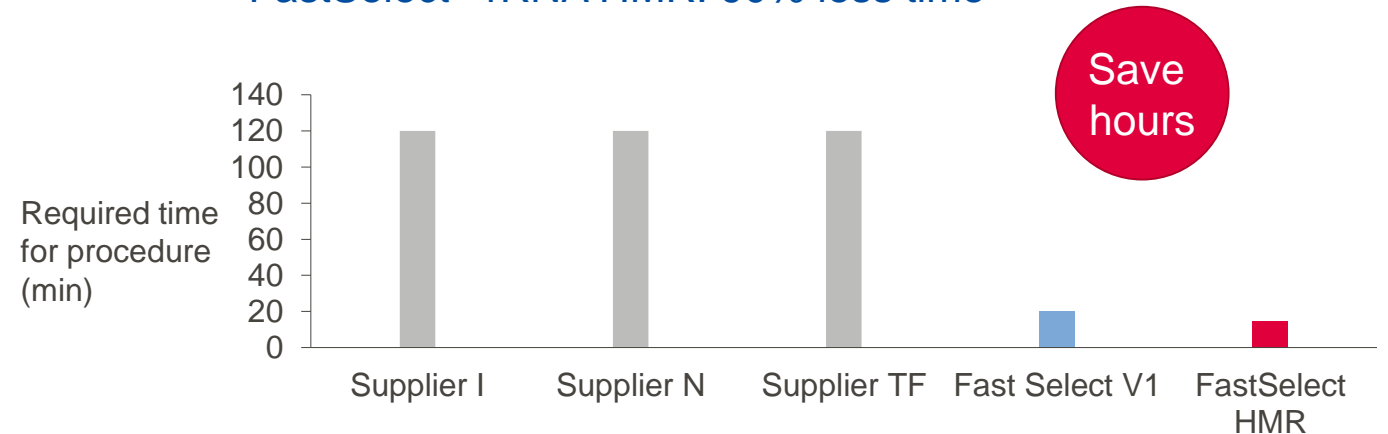
Removes rRNA in a single step requiring only 14 minutes

- Problem: rRNA is a significant NGS contaminant
  - 80–90% of RNA in samples
- Existing depletions: **Slow, tedious**
  - Number of steps: Typically 29–37
  - Time required: 2+ h
- QIAseq FastSelect: **Revolutionary**
  - Number of steps: One
  - Time required: 14 min
  - Robustness: >99% rRNA removal
  - Compatibility: Any RNA library prep kit

FastSelect –rRNA HMR: Only one step

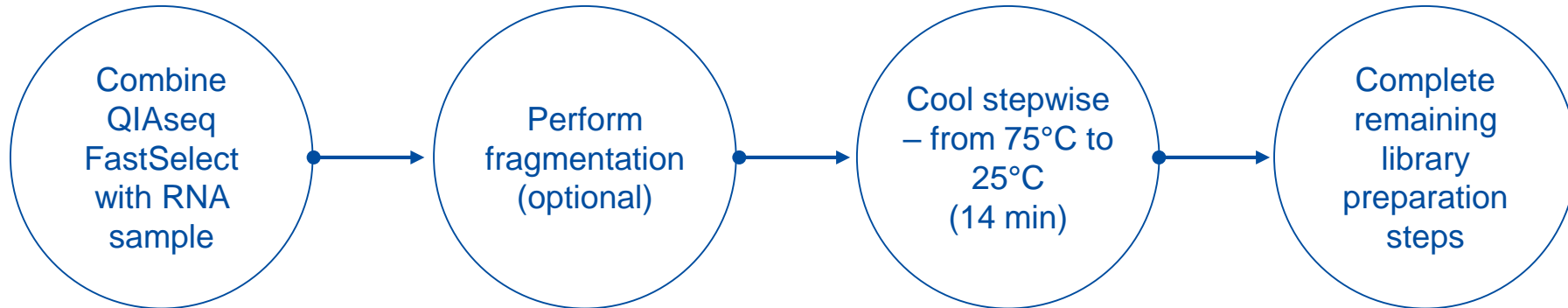


FastSelect –rRNA HMR: 90% less time



## FastSelect –rRNA HMR plus –Globin

Thirty percent faster than FastSelect V1, now in a single-tube



## FastSelect –rRNA HMR plus –Globin

### How does FastSelect –rRNA HMR plus –Globin work?

- Inhibits reverse transcription of specific targets
- Removes cytoplasmic and mitochondrial rRNA and/or globin mRNA

### Species covered in a single-tube:

- Human, mouse, rat (HMR) and other mammalian species
- HMR removes 95–99% rRNA from cow, horse, sheep and hamster samples
- HMR removes 80–90% rRNA from dog, chicken, rabbit, pig and monkey samples

### RNA compatibility:

- Total RNA: Use FastSelect –rRNA HMR (include –Globin if working with whole blood)
- Poly(A) enriched RNA: Use FastSelect –Globin if working with whole blood

### Sample compatibility:

- Cell lines, tissues (fresh/frozen), FFPE tissues, blood and biofluids

### Total RNA input:

- 1 ng – 1 µg

### Tested RNA library prep kit compatibility:

- QIAseq Stranded Total RNA Lib Kit (QIAGEN), TruSeq® Stranded (Illumina®), NEBNext® Ultra II Directional RNA Library Prep Kit (NEB®), KAPA® RNA HyperPrep Kit (Roche Group)
- FastSelect is compatible with most RNA library prep kits

# FastSelect –rRNA HMR: Robust performance with FFPE samples

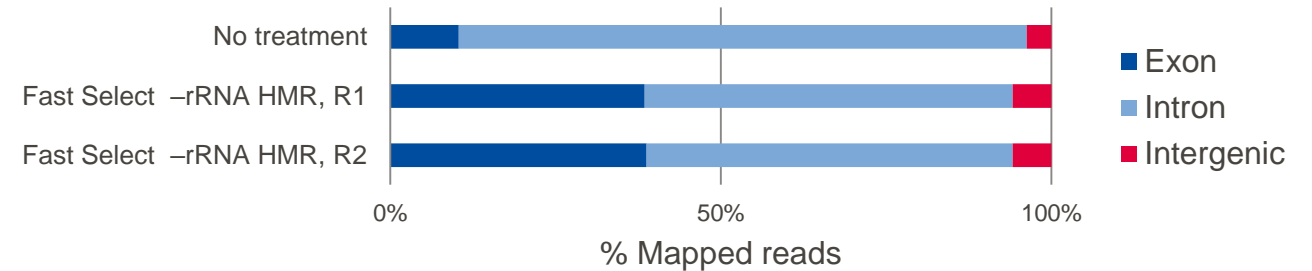
## Experimental overview

- Sample: 225 ng, Fusion RNA Positive Control (Horizon™)
- Depletion: No depletion, FastSelect
- Library prep: QIAseq Stranded Lib Kit
- Sequencing: NextSeq 550 (2 x 75 bp)
- Mapping: CLC Genomics Workbench

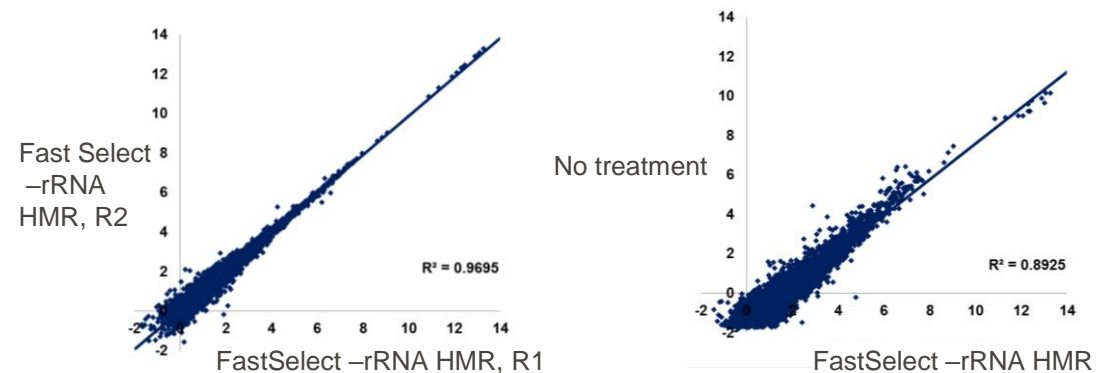
## Substantial rRNA removal

Condition	Percentage of reads mapped to rRNA
No treatment	<b>71.62</b>
Fast Select –rRNA HMR, R1	<b>2.62</b>
Fast Select –rRNA HMR R2	<b>2.83</b>

## Increased/consistent exon mapping



## Resulting gene expression profiles strongly correlate



FastSelect efficiently removes rRNA from FFPE samples, resulting in increased mapping to exons. Gene expression values from FastSelect treatments are highly correlative, without off-target effects (Log2 RPKM > 0.3).

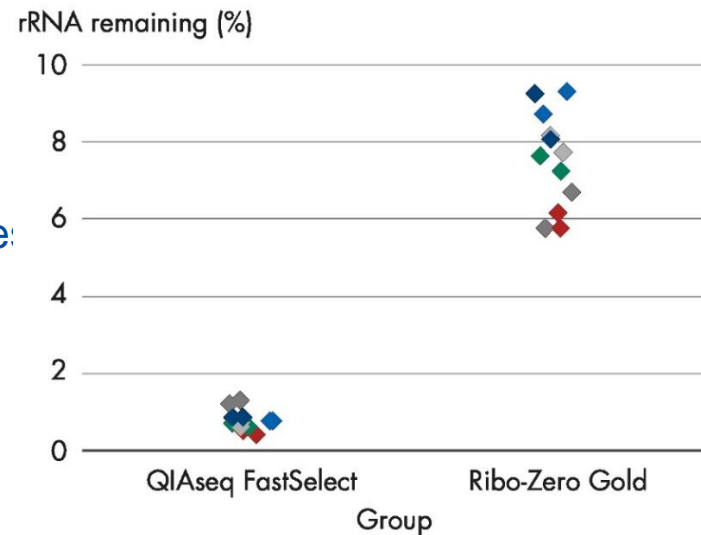


# QIAseq FastSelect: Robust performance with FFPE samples

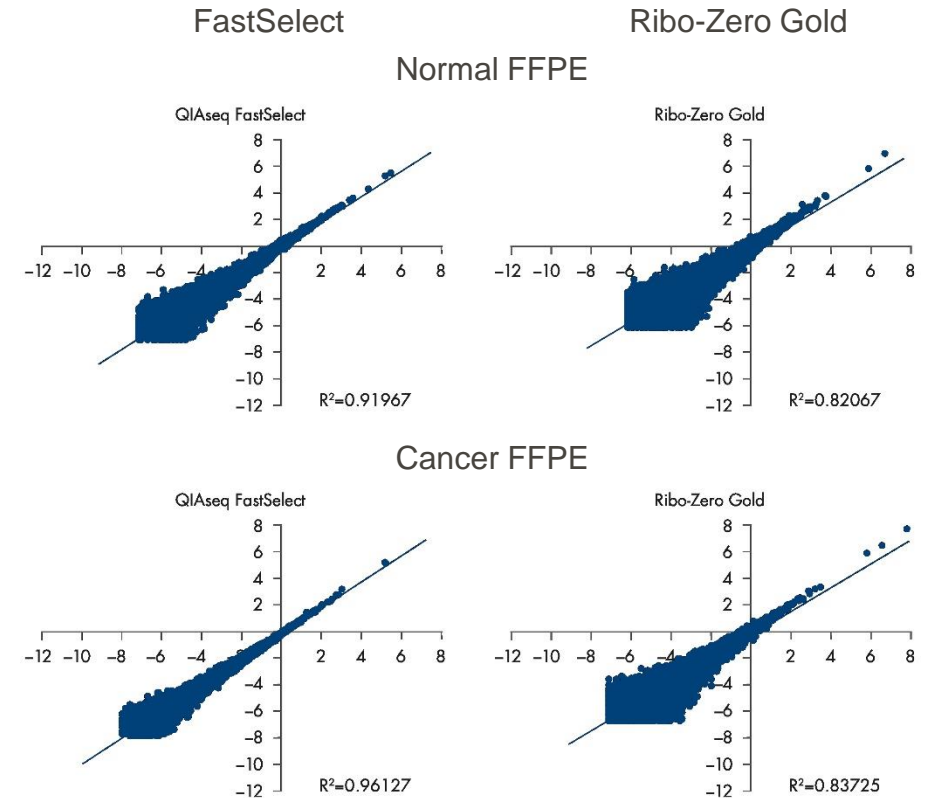
## Experimental overview

- Sample: 100 ng normal and cancer lung FFPE
- Depletion: FastSelect, Ribo-Zero Gold
- Library prep: QIAseq Stranded Lib Kit
- Sequencing: NextSeq 550 (2 x 75 bp)
- Mapping: CLC Genomics Workbench

## FastSelect robustly remove rRNA from FFPE Samples



## Replicate samples: Gene expression



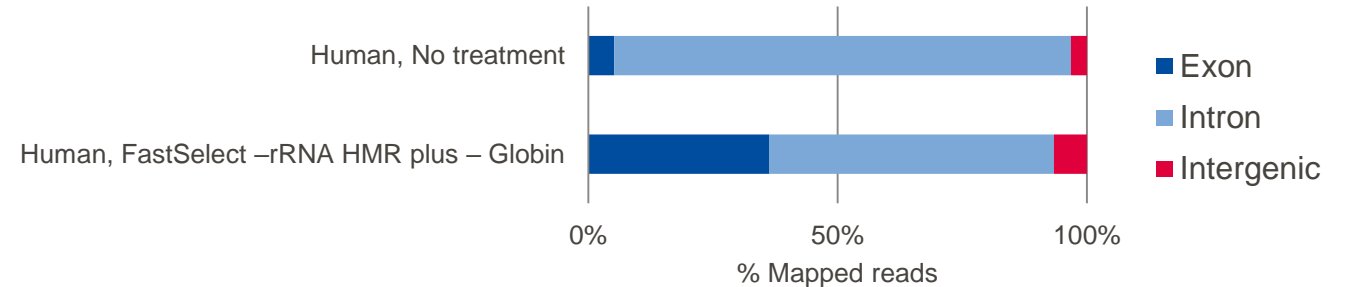
FastSelect robustly removes rRNA and enables high reproducibility of experiments. Ribo-Zero was not as effective with FFPE samples and required more amplification, suggesting loss of sample material.

# FastSelect –rRNA HMR plus –Globin: Robust removal of rRNA and Globin

## Experimental overview

- Sample: 100 ng human whole blood total RNA
- Depletion: FastSelect –rRNA HMR plus –Globin
- Library prep: QIAseq Stranded Lib Kit
- Sequencing: NextSeq 550 (2 x 75 bp)
- Mapping: CLC Genomics Workbench

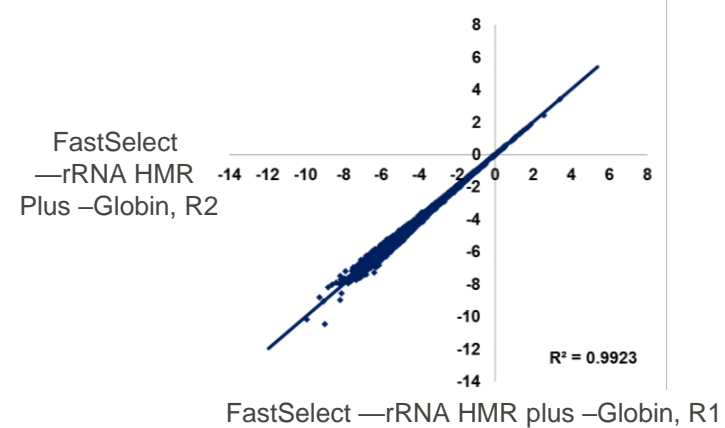
## Increased exon mapping



## Substantial removal of rRNA and Globin

Percentage of reads mapped to rRNA		Percentage of TPM mapped to Globin	
No treatment	FastSelect –rRNA HMR plus –Globin	No treatment	FastSelect –rRNA HMR plus –Globin
88.50	1.16	82.46	0.16

## Resulting gene expression profiles strongly correlate



FastSelect efficiently removes rRNA and Globin, resulting in an increased percentage of reads mapped to exons. Gene expression values from FastSelect-treated samples are highly correlative (Log2 RPKM > 0.3).

# FastSelect –rRNA HMR plus –Globin: Robust removal of rRNA and Globin (cont.)

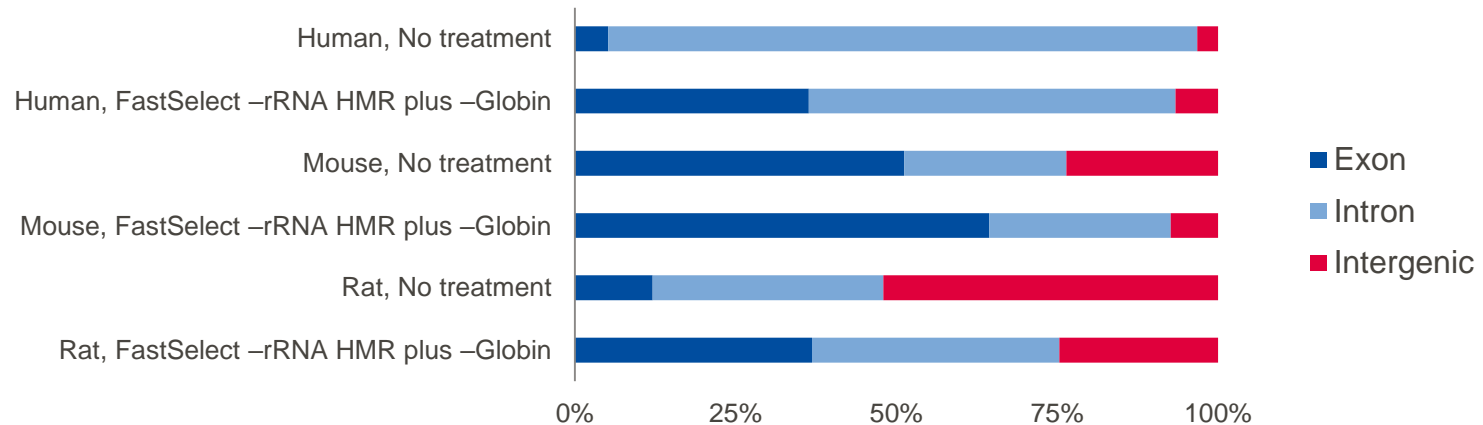
## Experimental overview

- Total RNA: Human, mouse, and rat whole blood
- Depletion: FastSelect –rRNA HMR plus –Globin
- Library prep: QIAseq Stranded Lib Kit
- Sequencing: NextSeq 550 (2 x 75 bp)
- Mapping: CLC Genomics Workbench

## Substantial removal of rRNA and Globin

Organism	Percentage of reads mapped to rRNA		Percentage of TPM mapped to Globin	
	No treatment	FastSelect –rRNA HMR plus –Globin	No treatment	FastSelect –rRNA HMR plus –Globin
Human	88.50	1.16	82.46	0.16
Mouse	94.66	6.99	20.00	0.05
Rat	91.81	2.15	23.91	0.01

## Increased exon mapping



FastSelect efficiently removes rRNA and Globin, resulting in an increased percentage of reads mapped to exons. Gene expression values from FastSelect-treated samples are highly correlative (Log2 RPKM > 0.3).

# FastSelect –Globin: Robust removal of Globin from mRNA-enriched samples

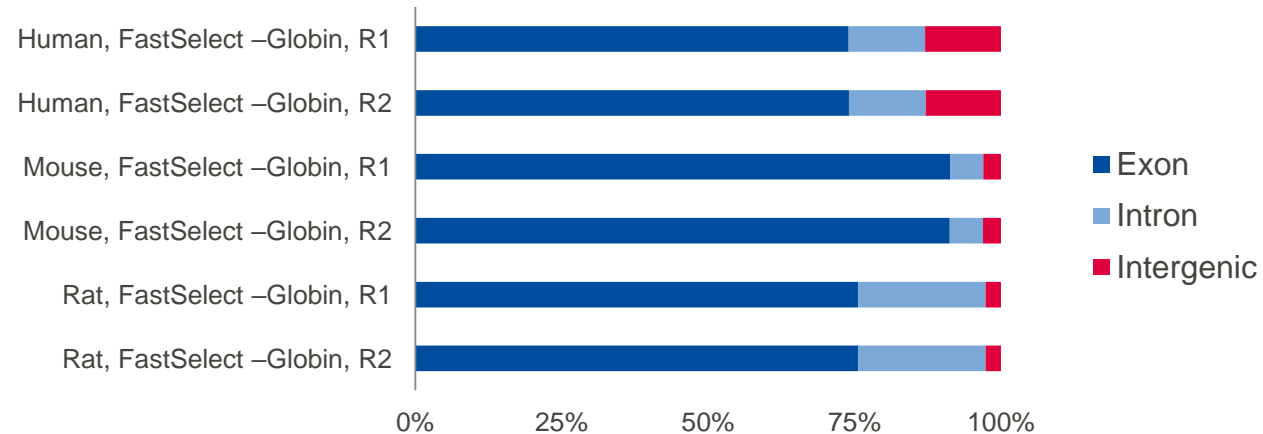
## Experimental overview

- Total RNA: Human, mouse, and rat whole blood
- Depletion: FastSelect –Globin
- Library prep: QIAseq Stranded mRNA Select Kit
- Sequencing: NextSeq 550 (2 x 75 bp)
- Mapping: CLC Genomics Workbench

## Substantial removal of rRNA and globin

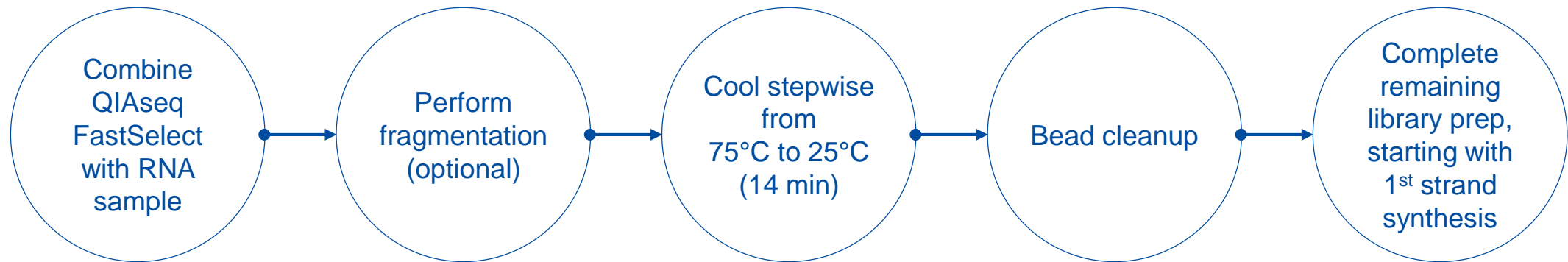
Organism	Percentage of TPM mapped to Globin
Human, R1	0.33
Human, R2	0.34
Mouse, R1	0.26
Mouse, R2	0.40
Rat, R1	0.06
Rat, R2	0.03

## Consistently high exon mapping



FastSelect efficiently removes Globin from mRNA-enriched samples. Compared to total RNA library preps, mRNA-enriched libraries exhibit a high percentage of reads mapped to exons.

## FastSelect –5S/16S/23S: An overview



# FastSelect –5S/16S/23S: An overview

## What is FastSelect –5S/16S/23S?

- Fragmentation and pan-bacterial (5S/16S/23S) rRNA depletion module

## Number of reactions:

- 24, 96 and 384

## How does it work?

- Inhibits reverse transcription of its specific targets

## Coverage:

- Designed to block **community level** cDNA synthesis of 5S, 16S and 23S rRNA
- Designed against SILVA 16S sequences (nearly 600,000 unique entries), SILVA 23S sequences (nearly unique 170,000 entries) and 5S rRNA Database (over 7,200 unique entries)
- Theoretically blocks >95% cDNA synthesis of all 5S, 16S and 23S rRNA sequences
  - In practice, results will vary, based on the exact composition of the sample

## Total RNA input:

- 20 ng to 1 µg

## Tested RNA library prep kit compatibility:

- QIAseq Stranded Total RNA Lib Kit (QIAGEN Group), TruSeq® Stranded (Illumina®, Inc), NEBNext® Ultra II Directional (New England Biolabs, Inc)
- FastSelect is compatible with most RNA library prep kits



# FastSelect –5S/16S/23S: Robust rRNA removal from bacterial communities

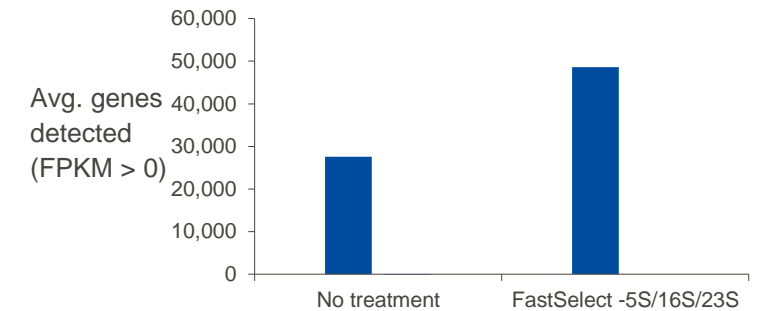
## Experimental overview

- Sample: 100 ng, 20 Strain Even Mix Whole Cell Material (ATCC) + Skin Microbiome Whole Cell Mix (ATCC) + Oral Microbiome Whole Cell Mix (ATCC)
- Depletion: No depletion, FastSelect
- Library prep: QIAseq Stranded
- Sequencing: NextSeq 550
- Mapping: CLC Genomics Workbench

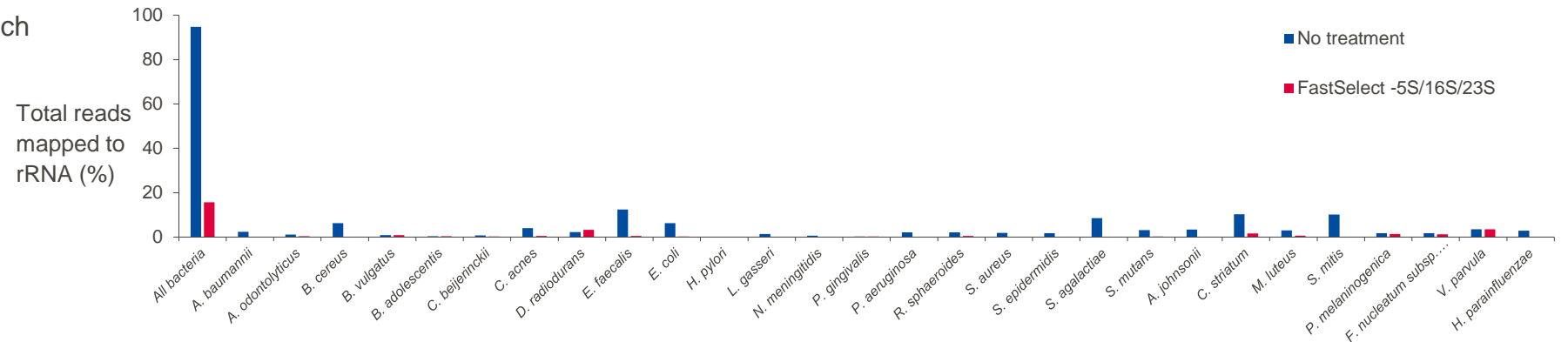
## FastSelect substantially removes rRNA

Sample	Percentage of reads mapped to bacterial rRNA (total)	
	No treatment	FastSelect – 5S/16S/23S
ATCC 3 Mix (28 bacteria), R1	<b>94.81</b>	<b>16.97</b>
ATCC 3 Mix (28 bacteria), R2	<b>94.71</b>	<b>14.45</b>

## FastSelect increases detected genes



## FastSelect robustly depletes rRNA (individual species)



FastSelect efficiently removes rRNA, freeing up substantial read budget. In turn, this read budget enables a dramatic increase in the number of genes detected.

# FastSelect –rRNA HMR seamlessly integrates with FastSelect –5S/16S/23S

## Experimental overview

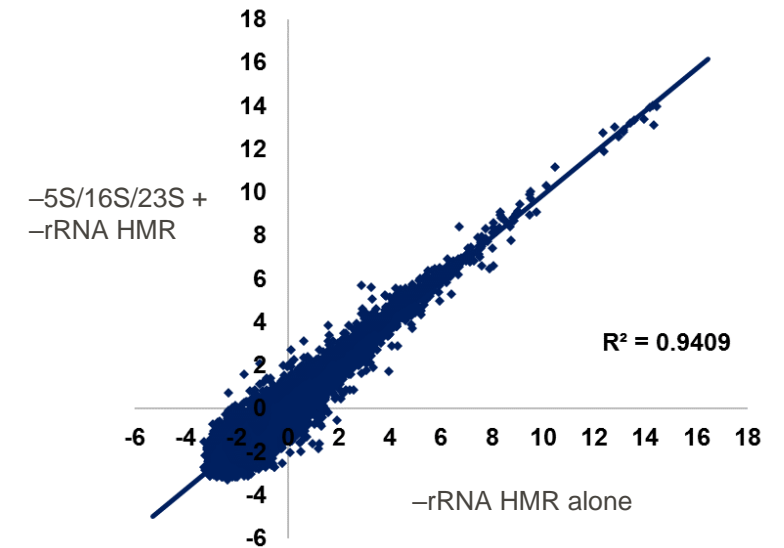
- Sample: 100 ng, different ratios of Universal Human (H) + Gut (G) RNA
- Depletion: No depletion, FastSelect –5S/16S/23S + FastSelect –rRNA HMR
- Library prep: QIAseq Stranded Total RNA Lib Kit
- Sequencing: NextSeq 550 (2 x 75 bp)
- Mapping: CLC Genomics Workbench

FastSelect –rRNA HMR + –5S/16S/23S robustly removes rRNA and matches theoretical calculations\*

Sample (Sample ratio (human [H] and gut [G]))	Percentage of reads mapped to human + bacterial rRNA (total)		
	No treatment	FastSelect (theory)	FastSelect (actual)
0 H : 100 G	<b>92.78</b>	<b>9.87</b>	<b>9.87</b>
1 H : 99 G	<b>92.74</b>	<b>9.78</b>	<b>9.76</b>
10 H : 90 G	<b>92.37</b>	<b>9.01</b>	<b>8.91</b>
25 H : 75 G	<b>92.30</b>	<b>7.73</b>	<b>8.84</b>
50 H : 50 G	<b>91.88</b>	<b>5.58</b>	<b>6.64</b>
75 H : 25 G	<b>91.84</b>	<b>3.44</b>	<b>3.49</b>
90 H : 10 G	<b>90.90</b>	<b>2.15</b>	<b>2.39</b>
99 H : 1 G	<b>90.97</b>	<b>1.38</b>	<b>1.31</b>
100 H : 0 G	<b>90.99</b>	<b>1.29</b>	<b>1.29</b>

\* Theoretical calculations based on 100% gut and 100% human values

FastSelect –rRNA HMR + –5S/16S/23S does not negatively impact human GenEx



FastSelect –rRNA HMR + –5S/16S/23S efficiently removes rRNA from combined human + bacteria samples. FastSelect –5S/16S/23S does not negatively impact human gene expression, because in the presence of –5S/16S/23S human gene expression patterns remain the same.

# Agenda

Background

---

RNA-seq: FFPE and whole blood samples

---

miRNA-seq: Serum/plasma samples

---

Summary

---



# QIAseq miRNA Library Kit: miRNA-focused library prep kit

## Distinguishing features of the QIAseq miRNA Library Kit

Gel- and adapter dimer-free workflow from 1–500 ng of total RNA

Naturally eliminates:

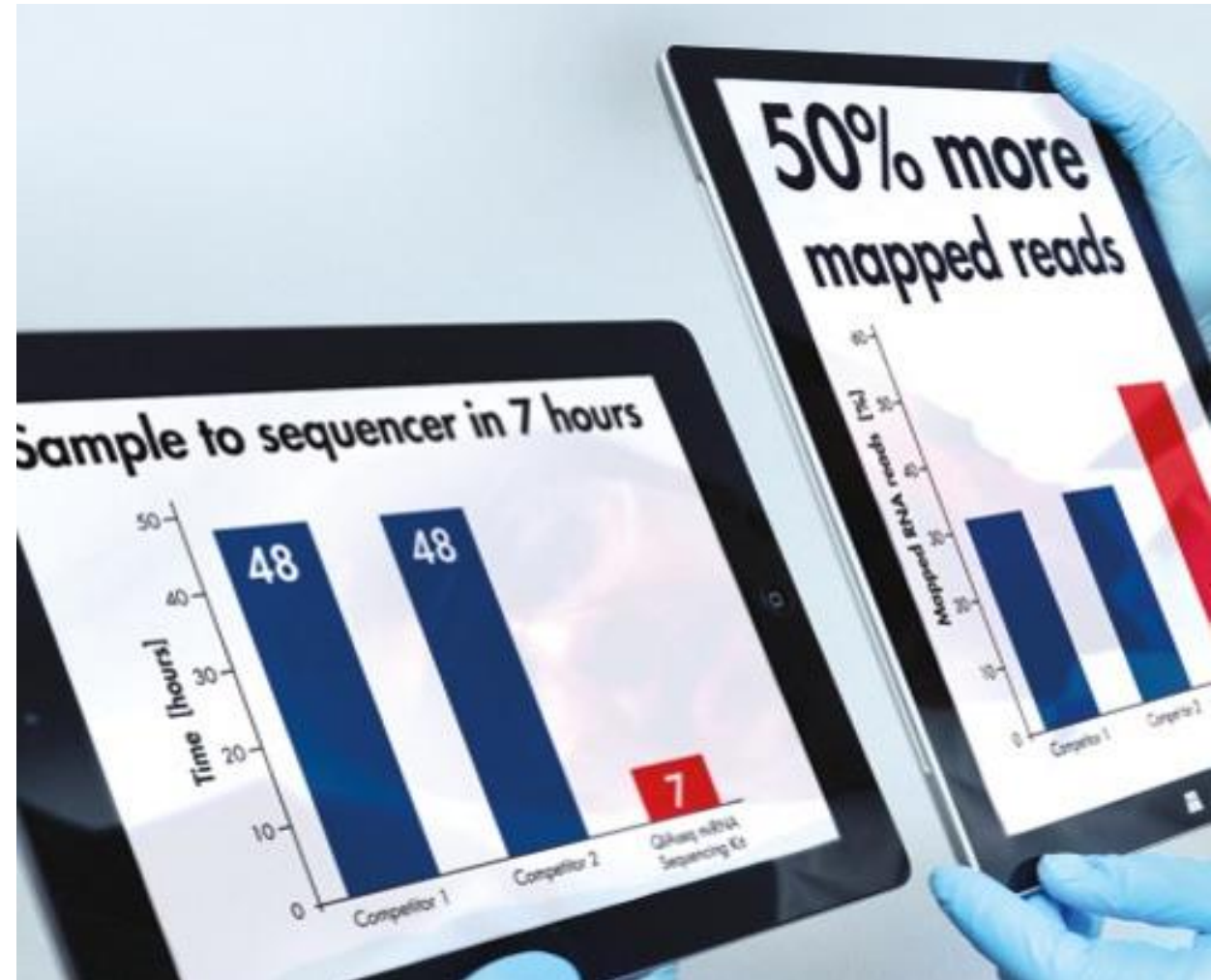
- Adapter dimer
- rRNA
- hY4 Y RNA

Integrated unique molecular index (UMI) technology

Compatible with Illumina® and Thermo Fisher Scientific® sequencers

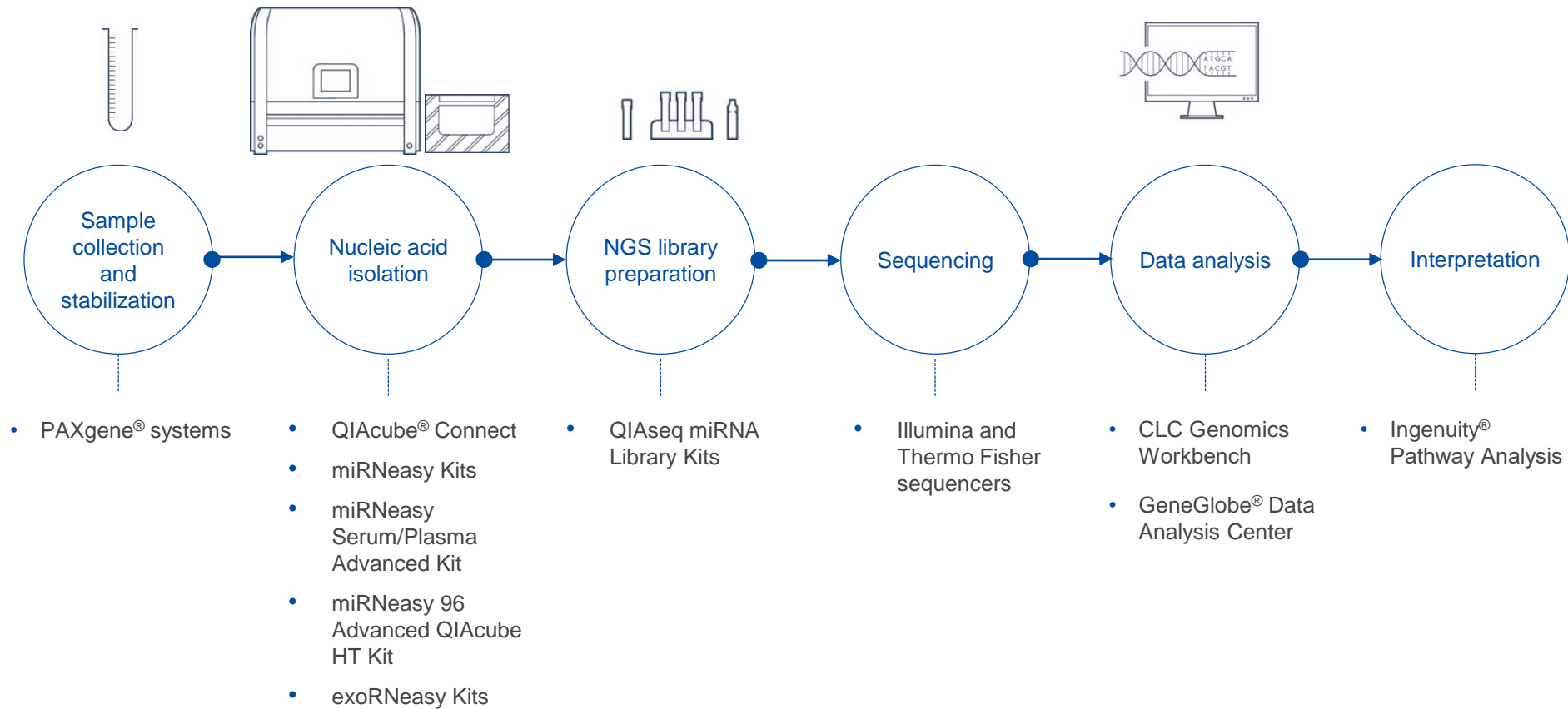
## When compared to other kits, the QIAseq Kit ranked\*:

- Highest in mapped reads from serum and plasma
- Highest in mapped reads from brain tissue
- The most efficient for biofluids and tissue

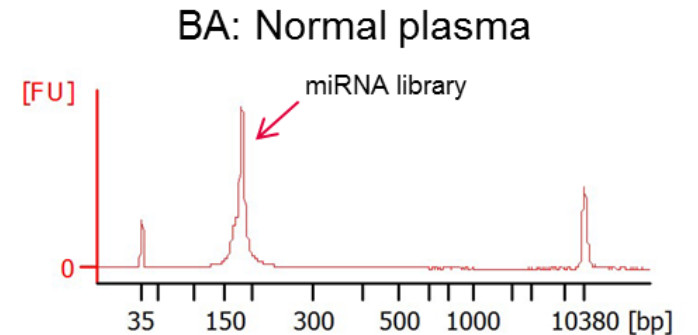
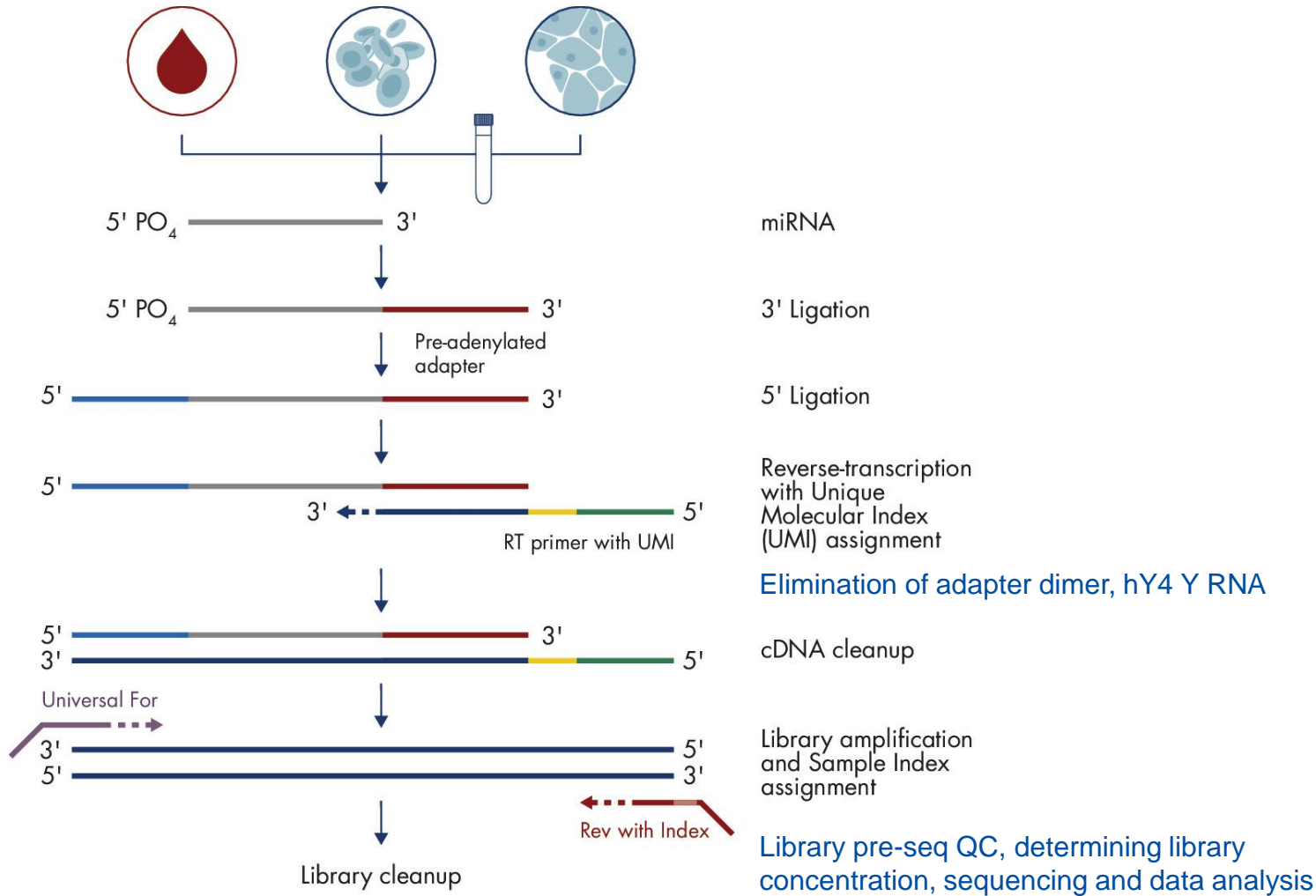


\*Source: Coenen-Stass A.M.L. et al. (2018) Evaluation of methodologies for microRNA biomarker detection by next generation sequencing, RNA Biology, 15:8, 1133–1145

# Sample to Insight solutions for miRNA sequencing



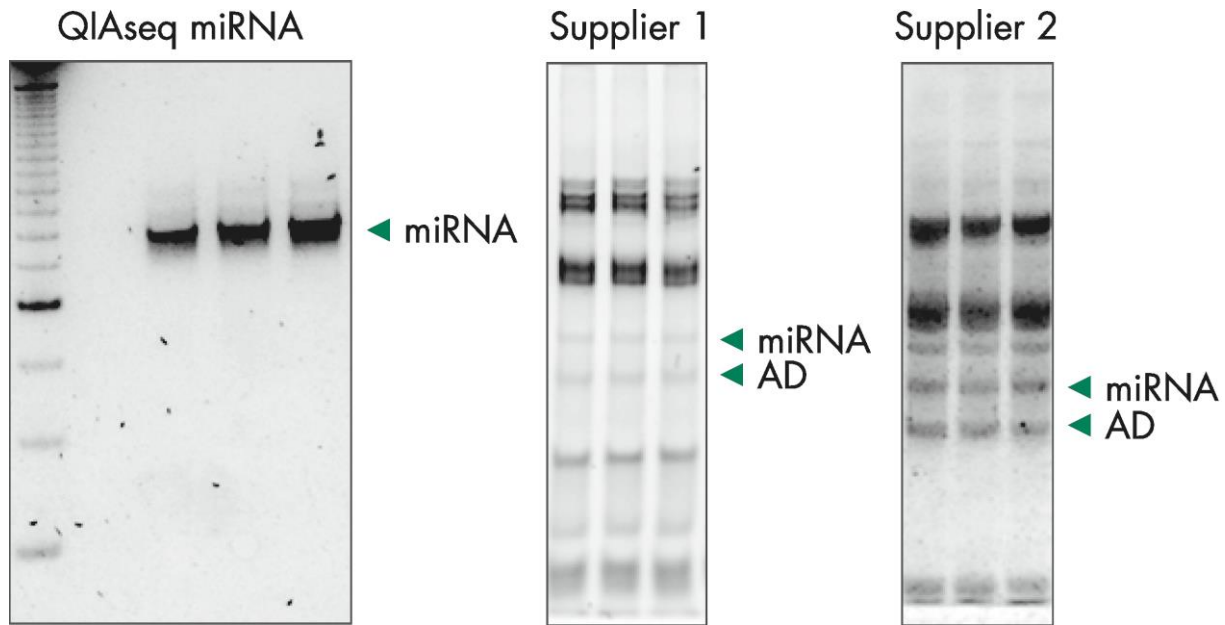
# QIAseq miRNA: Library construction





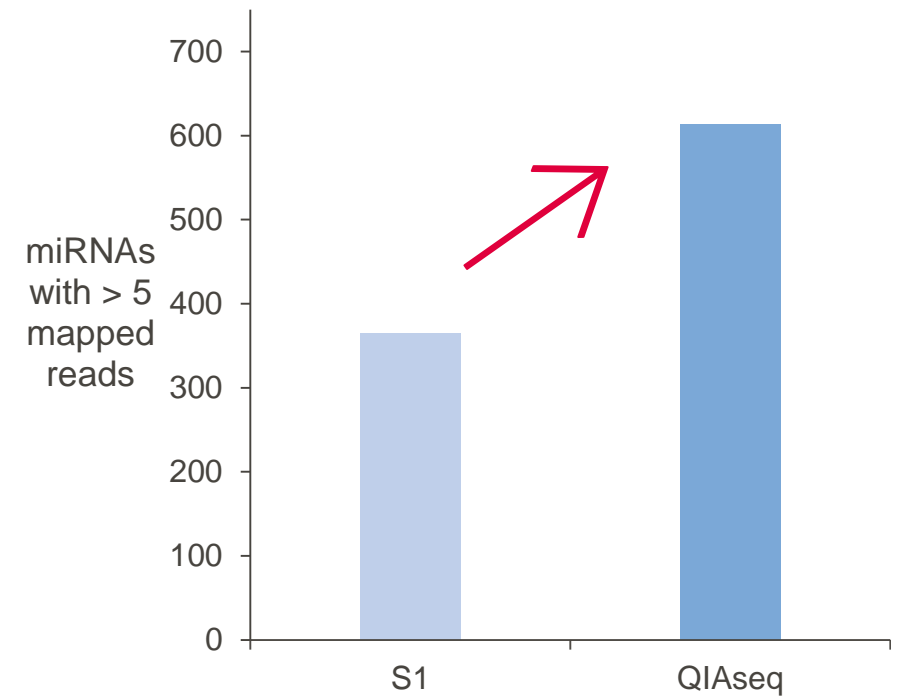
# QIAseq miRNA: Natural elimination of rRNA

PAGE gel after standard library prep protocol



RNA input: 100 ng (QIAseq miRNA), 1 µg (Supplier 1), 100 ng (Supplier 2)

2x mapped miRNAs with QIAseq



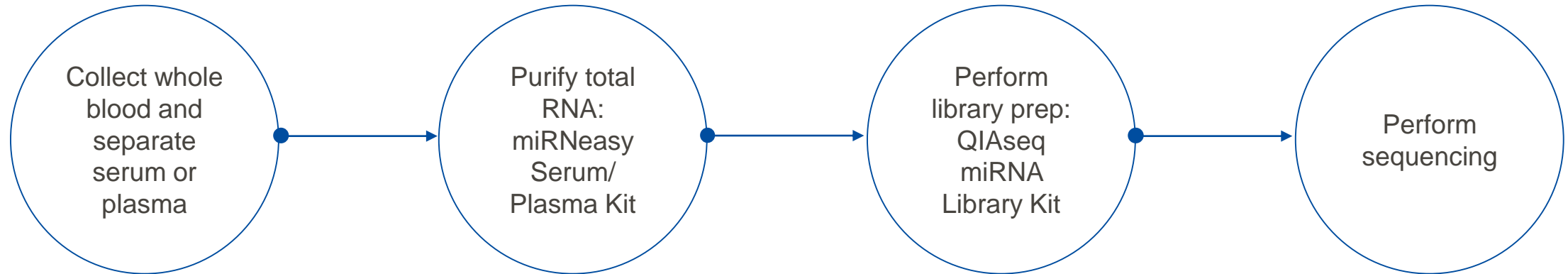
- QIAseq miRNA gives you a robust, specific miRNA library with negligible background.

## QIAseq miRNA eliminates hY4 Y RNA

Read set	hY4 not blocked (two replicate libraries)		hY4 blocked (two replicate libraries)	
Total_reads	2,266,831	2,715,234	2,293,207	3,037,150
No_adapter_reads	98,237	120,162	135,763	189,849
Too_short_reads	346,580	383,548	620,065	814,789
UMI_defective_reads	44,259	50,209	59,749	80,263
miRNA_reads	596,675	680,215	908,267	1,211,195
Hairpin_reads	1,216	1,468	1,870	2,351
piRNA_reads	11,819	14,441	19,091	24,937
rRNA_reads	39,078	47,098	57,720	76,580
tRNA_reads	8,556	10,628	12,868	16,746
mRNA_reads	5,251	6,163	8,291	10,723
OtherRNA_reads	833,042	1,071,052	60,577	81,572
notCharacterized_mappable	72,481	84,476	96,966	126,366
notCharacterized_notmappable	209,637	245,774	311,980	401,779
miRNA mapping %	26.3	25.1	39.6	39.9
OtherRNA_reads	36.7	39.4	2.6	2.7

When you block hY4, mapping to “OtherRNA\_Reads” decreases from ~40% to ~2.5%. In addition, miRNA mapping increases from ~25% to 40%.

## Workflow: Total RNA from serum and plasma



- Tip 1: Prepare 100–200  $\mu$ l for isolation
- Tip 2: Perform the optional spins to remove cellular nucleic acids attached to cell debris

- Tip: Elute in 14  $\mu$ l Nuclease-Free Water for a 12  $\mu$ l eluate
- Note: We recommend a volume equivalents approach instead of measuring RNA concentration as serum/plasma samples show low RNA concentration readings due to lack of rRNA.

- Tip: Prep library from 5  $\mu$ l of RNA eluate and perform 22 cycles of library amplification
- Note: hY4 Y RNA is blocked

- Number of reads recommended: ~10M. If you consistently see a lot of read replication, you can always decrease your allocation of reads/sample

# Serum and plasma: High mapped miRNA percentages

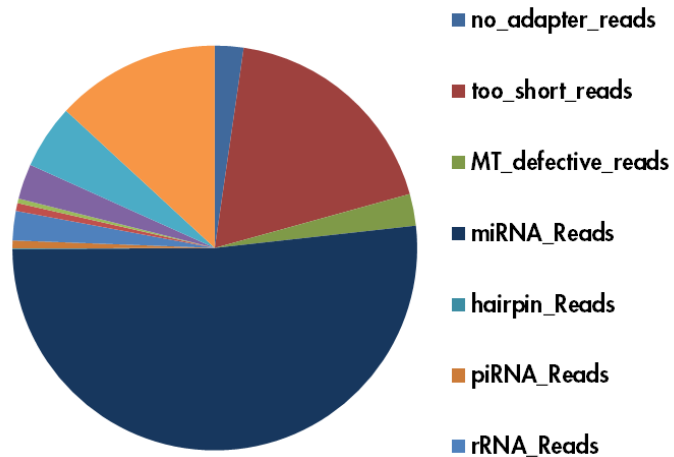
miRNeasy Serum/Plasma Kit: 200 µl input

Total RNA input: 5 µl RNA eluate (80 µl of serum equivalents)

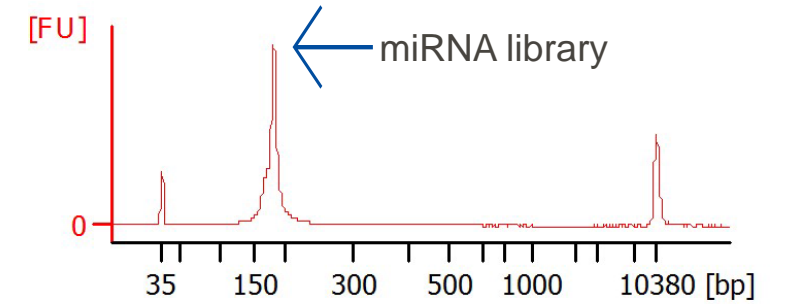
- Normal (N) plasma (n = 3)
- Breast cancer plasma (n = 3)

Sequencing: NextSeq, 75 bp Single Read

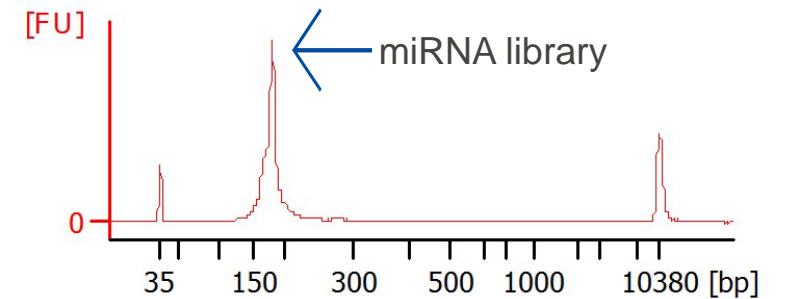
miRNA mapping %: 52 (N1), 54 (N2), 47 (N3), 49 (BC1), 41 (BC2), 57 (BC3)



BA: Normal plasma



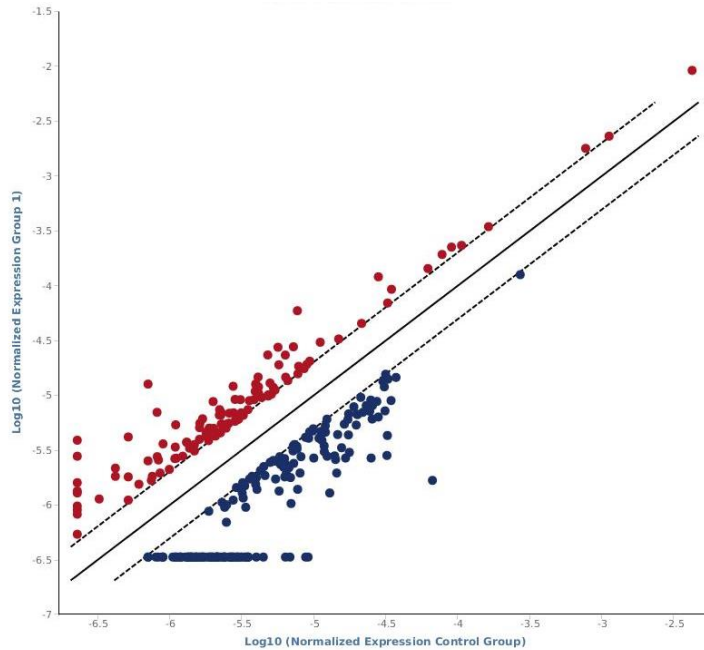
BA: Breast cancer plasma



- 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 and plasma

Scatter plot (breast cancer vs. normal)

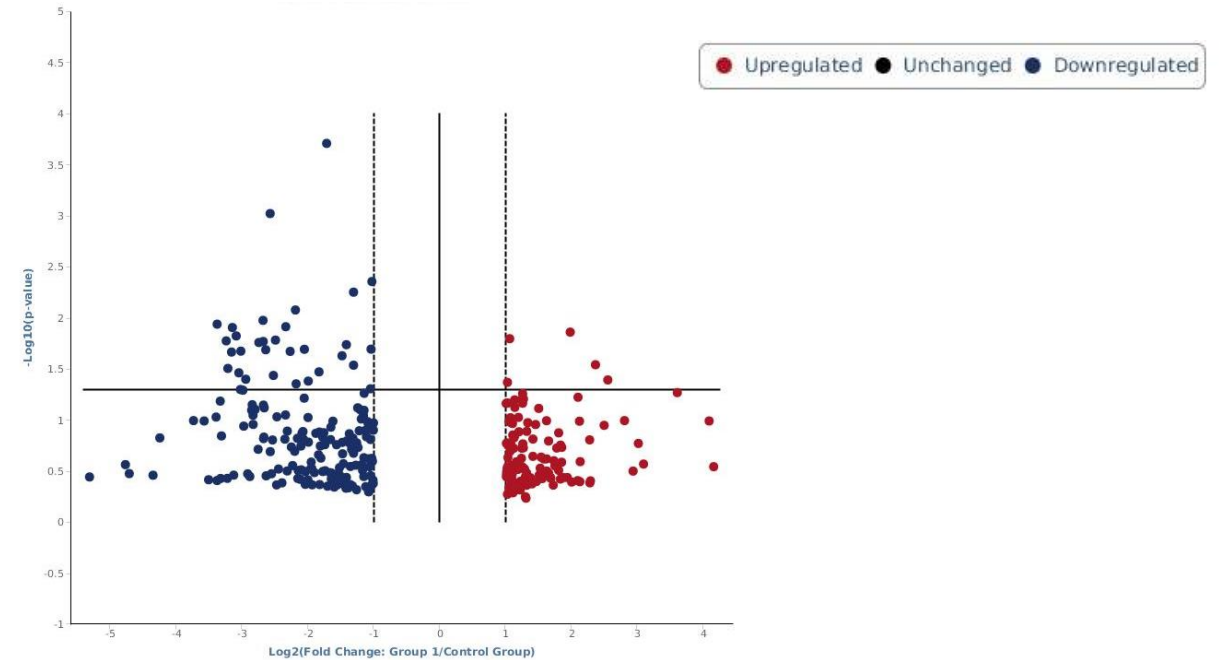


Upregulated miRNAs = 123 (6 significantly)

- miR-520g-3p: Associated with important prognostic factors in breast cancer patients

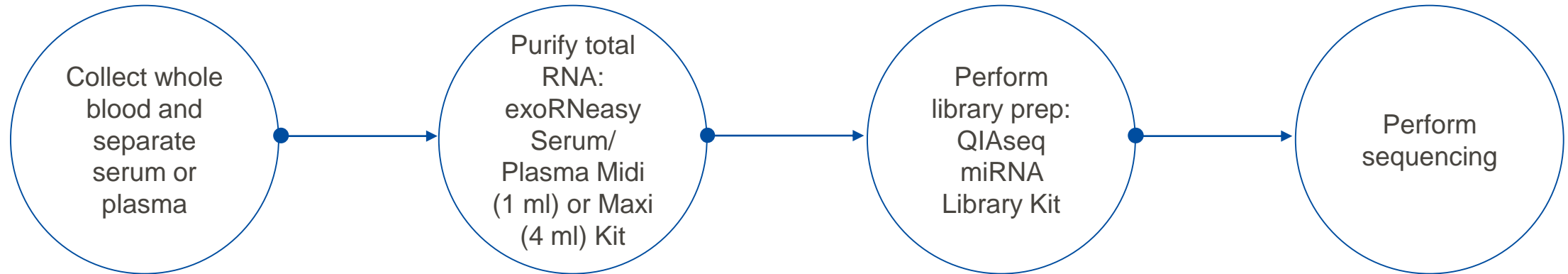
Downregulated miRNAs = 179 (31 significantly)

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



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

## Workflow: Exosomal total RNA from serum and plasma



- Tip 1: Prepare 1–4 ml for isolation (1 ml is usually sufficient)
- Tip 2: Perform the optional spins to remove cellular nucleic acids attached to cell debris

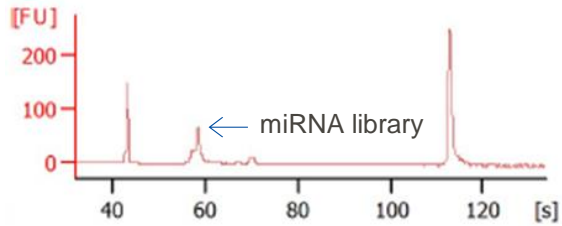
- Tip: Elute in 14  $\mu$ l Nuclease-Free Water for a 12  $\mu$ l eluate
- Note: We recommend a volume equivalents approach instead of measuring RNA concentration as serum/plasma samples show low RNA concentration readings due to lack of rRNA.

- Tip: Prep library from 5  $\mu$ l of RNA eluate and perform 22 cycles of library amplification
- Note: hY4 Y RNA is blocked

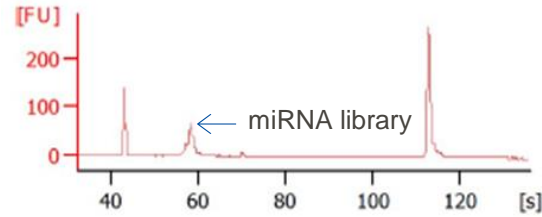
- Number of reads recommended: ~10M. If you consistently see a lot of read replication, you can always decrease your allocation of reads/sample

# Exosome samples: High mapped miRNA percentages

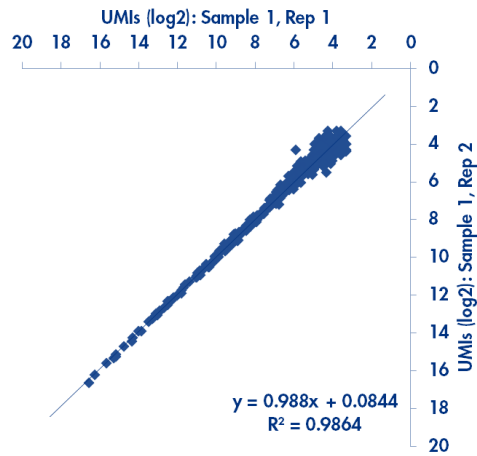
Sample 1, R1



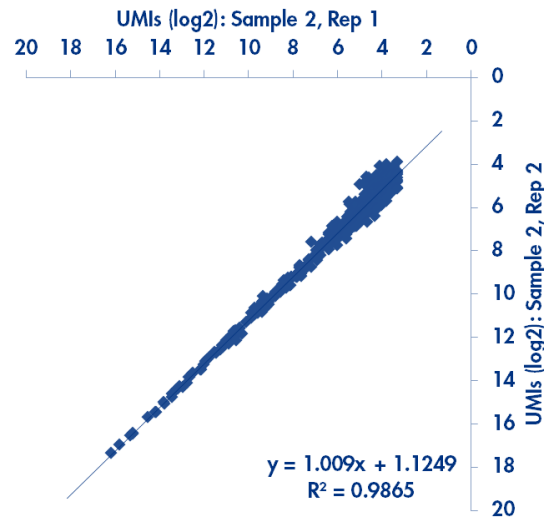
Sample 1, R2



Sample 1



Sample 1

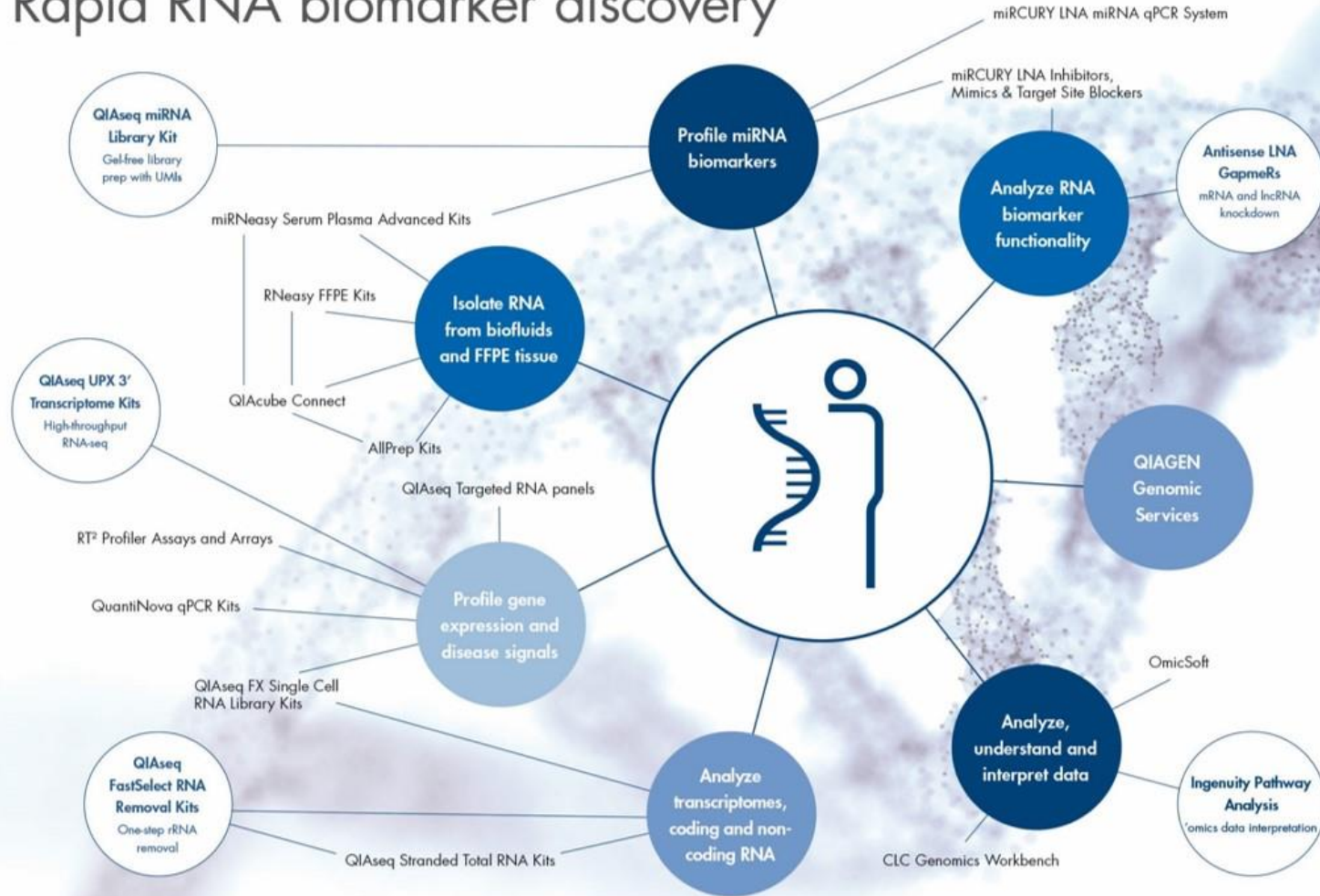


Read set	Sample 1, R1	Sample 1, R2	Sample 2, R1	Sample 2, R2
Total_reads	3,454,577	3,539,076	2,531,228	6,230,468
No_adapter_reads	321,093	276,815	345,974	562,212
Too_short_reads	737,228	799,712	461,630	1,240,571
UMI_defective_reads	192,311	154,736	194,358	398,158
miRNA_reads	1,333,379	1,424,014	913,946	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

● High mapping percentage to miRNAs; low mapping percentage to “other RNA reads” (often observed with other commercial kits).



# Rapid RNA biomarker discovery



A comprehensive portfolio for RNA biomarker discovery

# Agenda

Background

---

RNA-seq: FFPE and whole blood samples

---

miRNA-seq: Serum/plasma samples

---

Summary

---



# Summary

## RNA-seq and miRNA-seq challenges

1

FFPE and liquid biopsy samples are imperative for basic and translational research, but are often difficult to work with

Various contaminating RNAs can plague the library prep step

- rRNA, globin, hY4 Y RNA and adapter dimers

## The new FastSelect kits

2

### FastSelect–rRNA HMR and –Globin:

Removes cytoplasmic and mitochondrial rRNA and/or globin mRNA by inhibiting reverse transcription of specific targets

- Covers human, mouse, rat (HMR) and other mammalian species

**FastSelect–5S/16S/23S:** Fragmentation and pan-bacterial (5S/16S/23S) rRNA depletion module which also works by inhibiting reverse transcription of specific targets

- Blocks community level cDNA synthesis of 5S, 16S and 23S rRNA

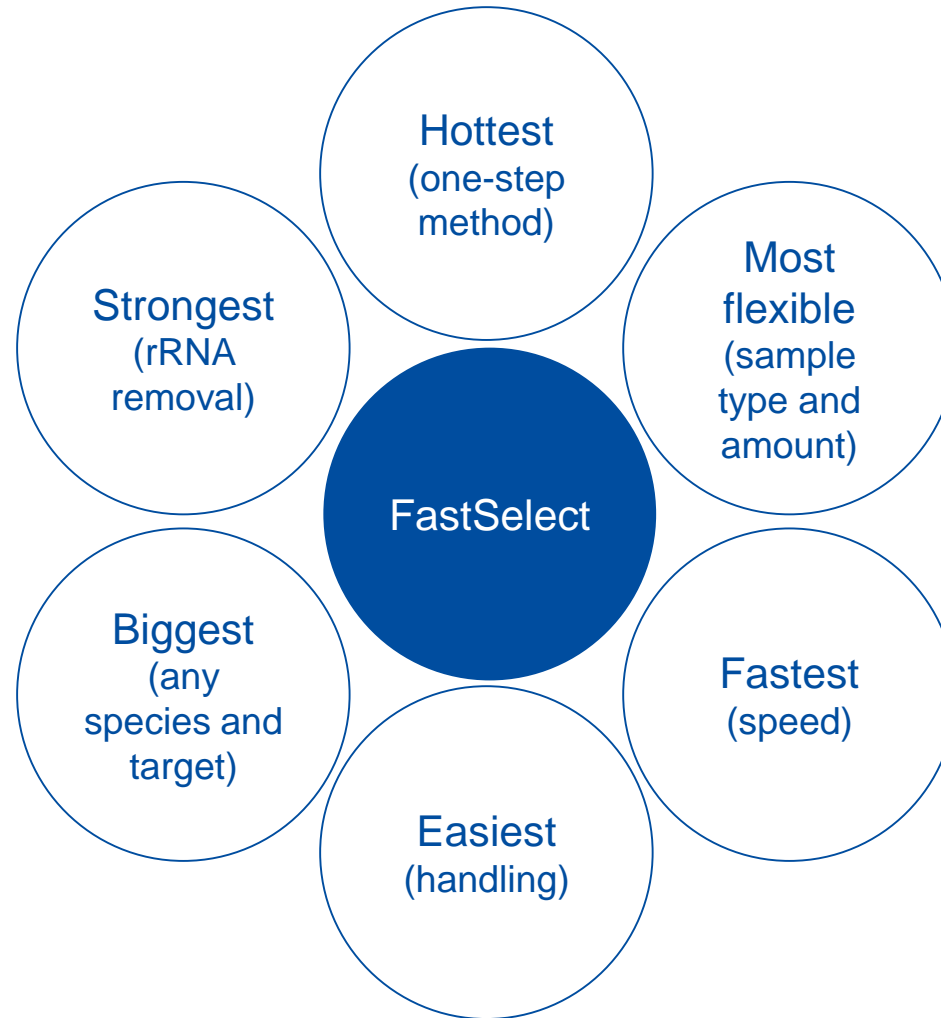
## QIAseq miRNA

3

An miRNA-focused library prep kit

- Naturally removes rRNA, hY4 Y RNA and adapter dimers
- Gel-free workflow from 1 ng to 500 ng of total RNA

## QIAseq FastSelect: An unparalleled unwanted RNA removal solution







Thank you for attending.  
Questions?

Jonathan Shaffer, M.B.A., Ph.D.  
**[Jonathan.Shaffer@qiagen.com](mailto:Jonathan.Shaffer@qiagen.com)**