Increasing bacterial RNA-seq sensitivity through efficient rRNA removal

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Agenda

Background

FastSelect –5S/16S/23S: An introduction

FastSelect –5S/16S/23S: Results

Summary

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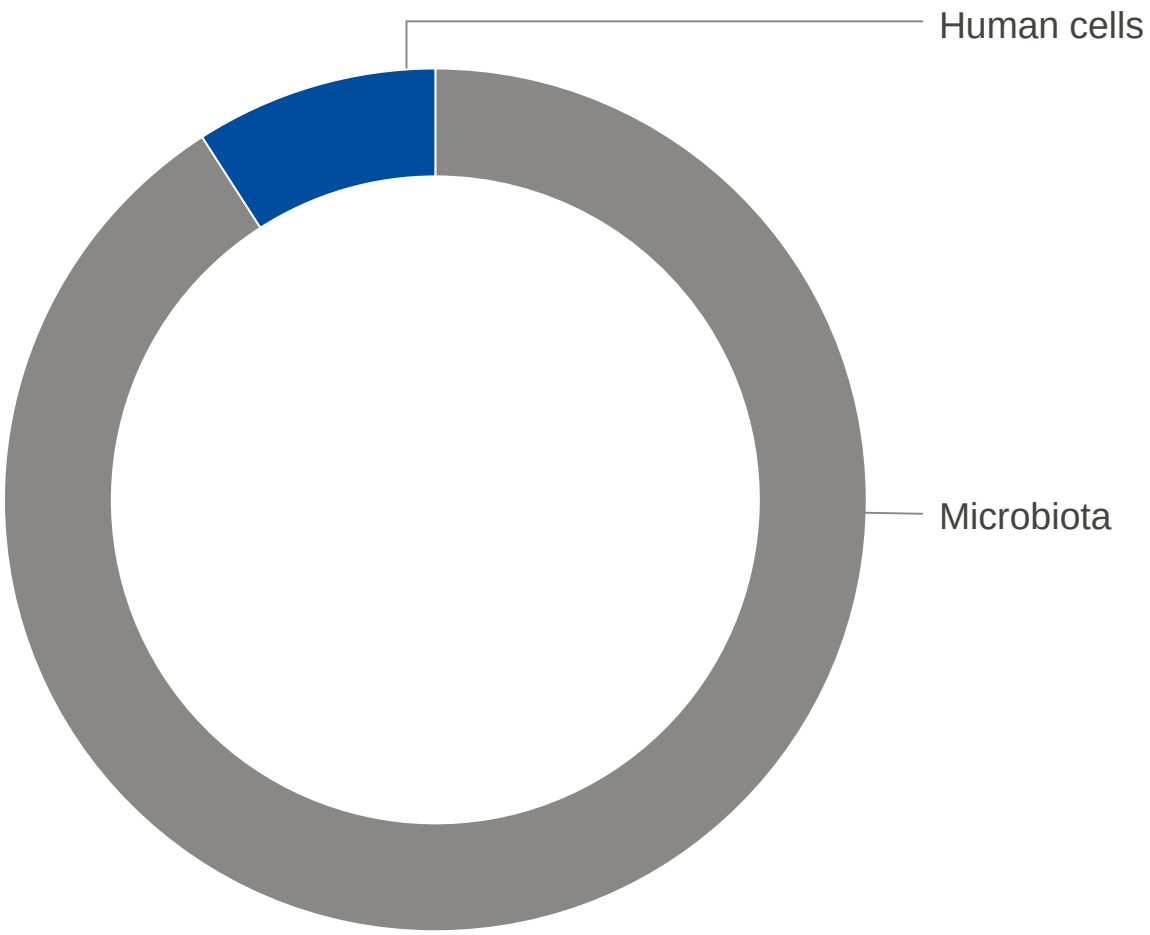
Summary

Humans or ‘superorganisms’?

Total number of cells: Human cells versus microbiota



Cellular composition of the ‘superorganism’

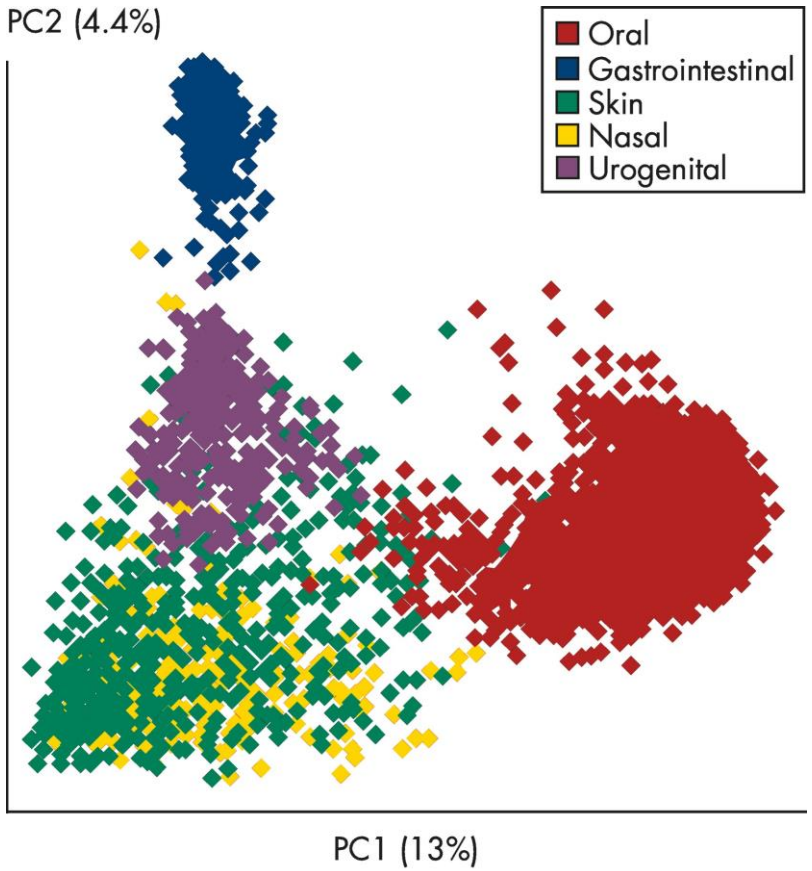
Estimation of the number of microbial cells that live in and on the human body; human cells are outnumbered by a factor of 10

Nomenclature:

- Microbiota are the microbes that live in a specific location, e.g., the human body, the gut, soil, etc.
- Metagenomics is the study of the collection of genomes derived from a specific sample or community
- Metatranscriptomics is the study of the RNA expression of genes from a community sample to interpret the physiological state of that community at that time

Microbiota composition

Microorganisms cluster by body site



Cataloguing efforts by the NIH Human Microbiome Project suggest:

- Around 10,000 organisms live with us
- Around 8 $\times 10^6$ genes constitute this ‘second genome’

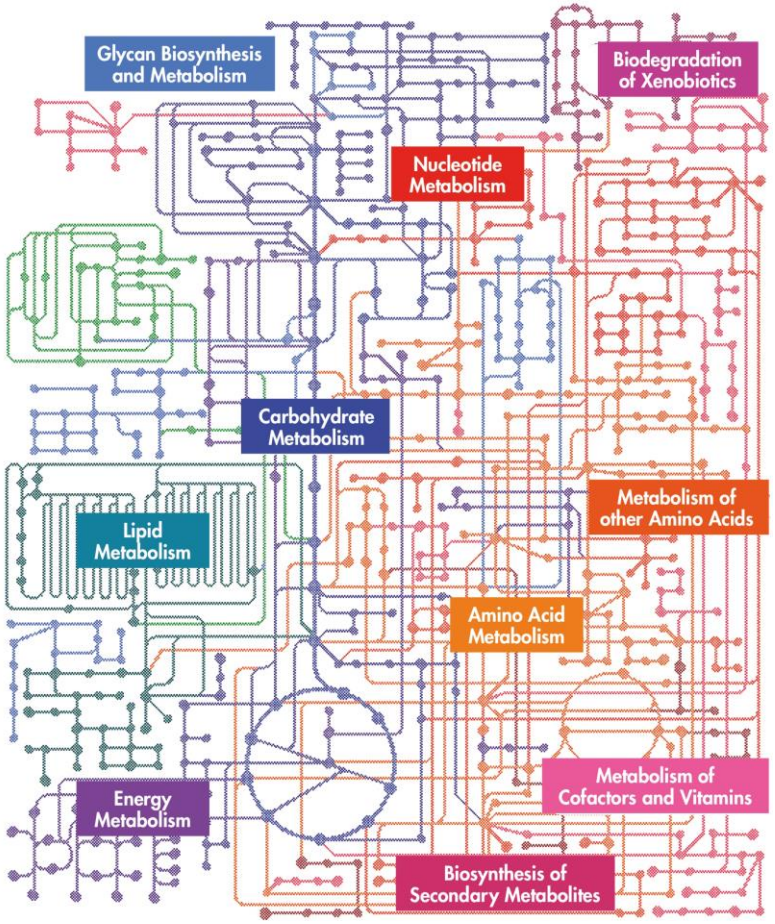
Identifying microbiota in healthy individuals revealed:

- Different body sites have unique communities
- Race, age, gender, weight or ethnicity can affect microbiota composition

Human Microbiome Project Consortium (2012). Structure, function and diversity of the healthy human microbiome. Nature, 486(7402), 207–214.

Complexity and function of genomic content

Function of microbiome enables individual survival

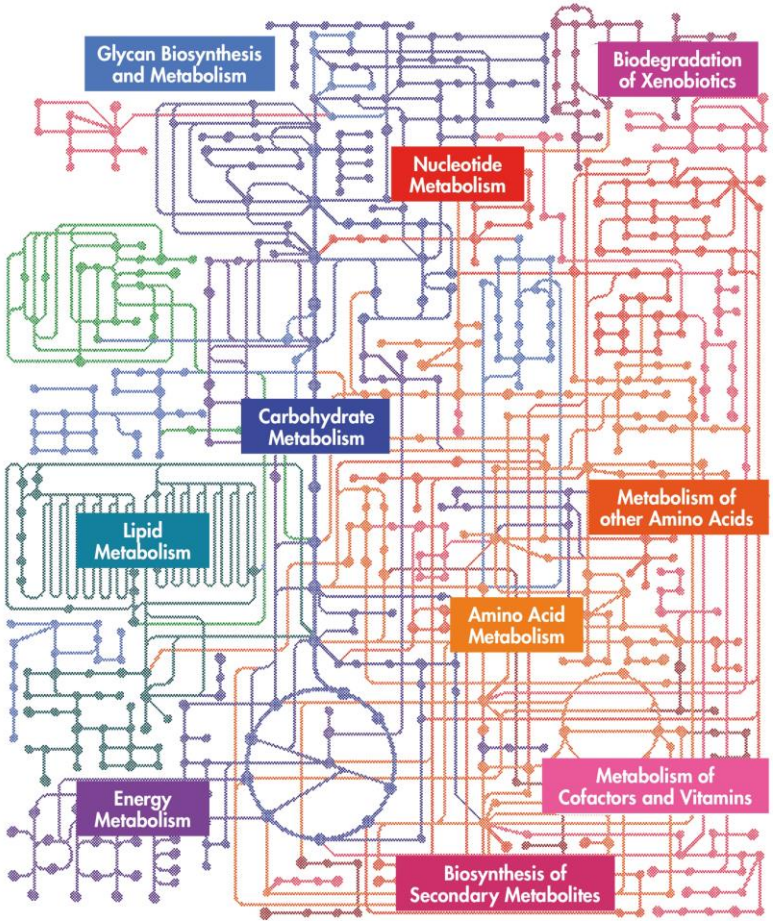


Each organism has developed its:

- Genetic content for its own survival in a specific environment
- Metabolism tuned to local nutrient sources
- Virulence factors for stable colonization
- Antibiotic resistance genes to metabolize toxins

Complexity and function of genomic content

Function of microbiome enables individual survival



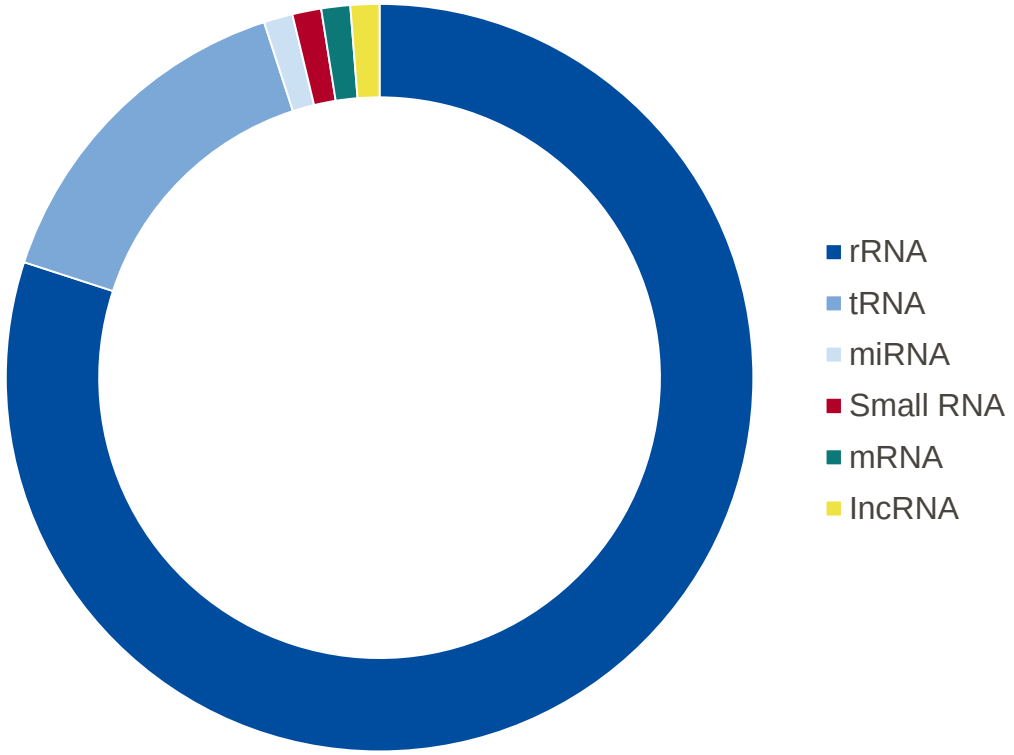
Understanding the microbiota

- DNA – who is or was there
- RNA – who is alive and what they are doing
- RNA can be used to identify:
  - Metabolic status
  - Expression of virulence factors
  - Expression of antibiotic resistance
  - Both host and microbiota

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
- 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



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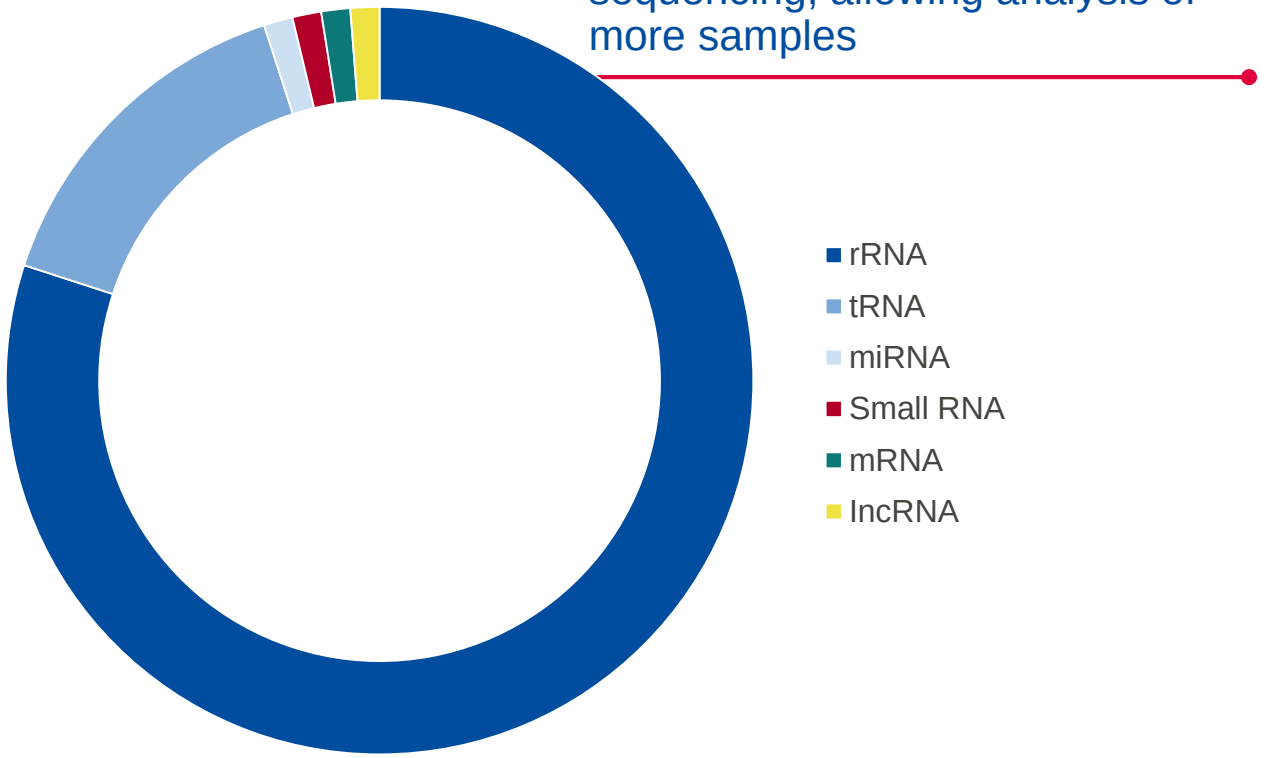
Removing rRNA will increase sensitivity and decrease the cost of sequencing, allowing analysis of more samples



Library enrichment/depletion strategies: Whole transcriptome NGS

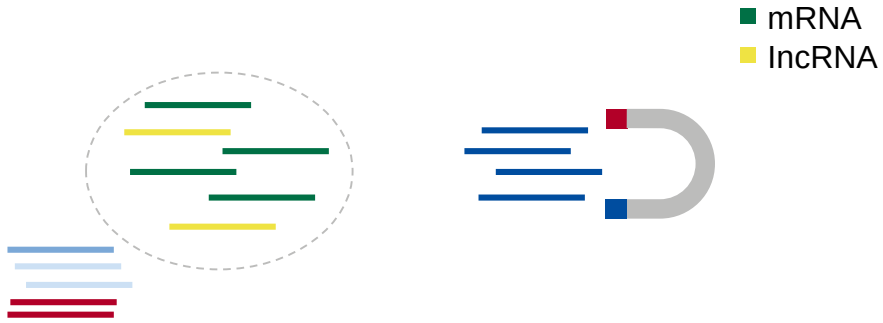
Enrichment/depletion strategies

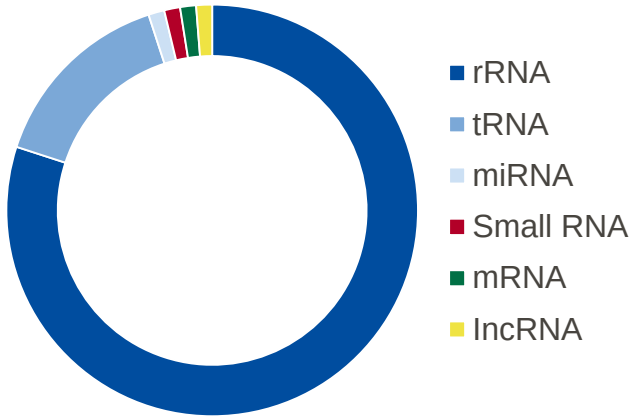
Poly(A) enrichment

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

rRNA depletion

- The “catch all” method





rRNA depletion is the only choice for metatranscriptomics.

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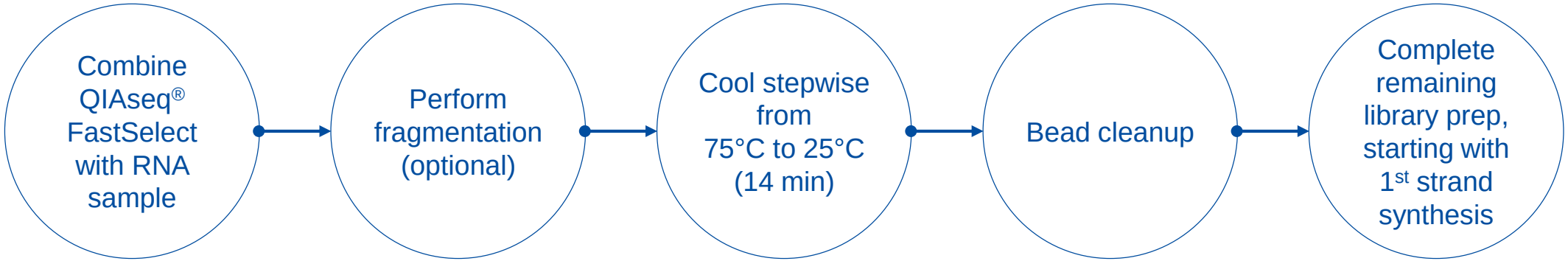
FastSelect –5S/16S/23S: An overview



NEW

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: What's in the box?

Kit sizes:

- 24 (cat. no. 335925)
- 96 (cat. no. 335927)
- 384 (cat. no. 335929)

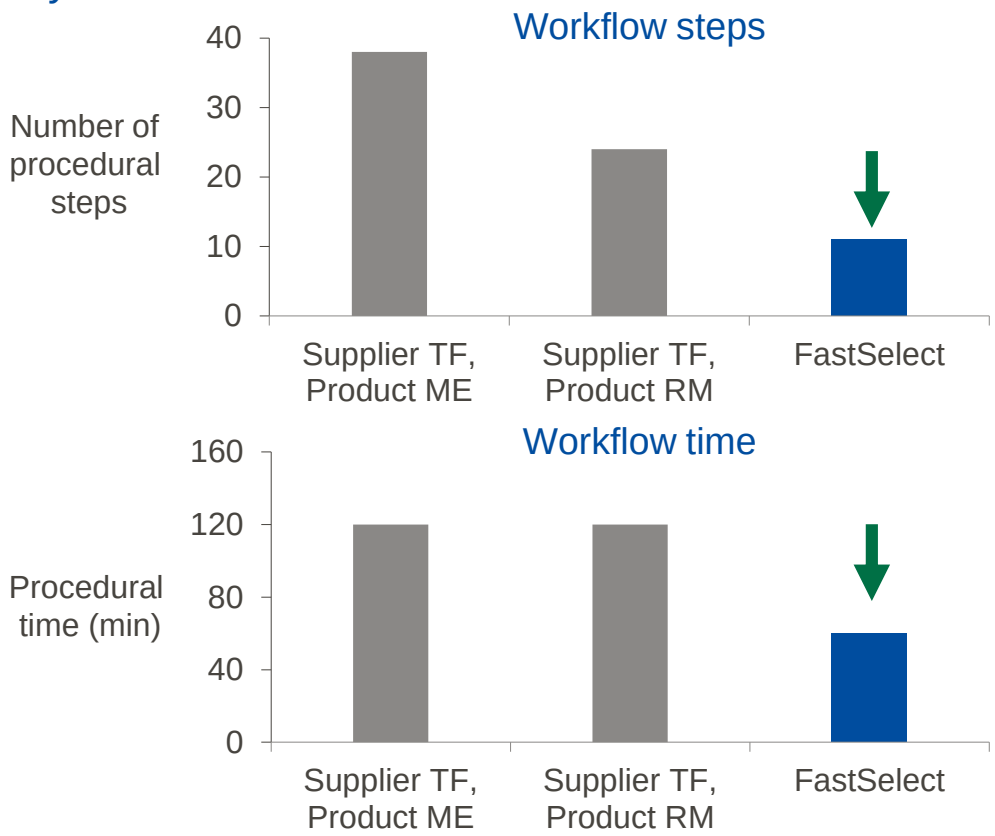
Kit components and purpose:

- FastSelect 5S/16S/23S: rRNA removal reagent
- FastSelect FH Buffer: Fragmentation and/or hybridization buffer
- Nuclease-free Water
- QIAseq Beads
- QIAseq Bead Binding Buffer

NEW

QIAseq FastSelect –5S/16S/23S: rRNA removal

Half your effort and time



Supplier TF, Product RM

Bead preparation
(7 steps)

Selective hybridization
(4 steps)

Removal of rRNA
(5 steps)

RNA purification
(8 steps)

Library Prep

FastSelect –5S/16S/23S

Add FastSelect reagent and ramp down temperature
(1 step)

Bead cleanup
(11 steps)

Library Prep

- FastSelect offers an attractive, streamlined workflow versus kits from another supplier.

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FastSelect –5S/16S/23S: An introduction

FastSelect –5S/16S/23S: Results

Summary

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

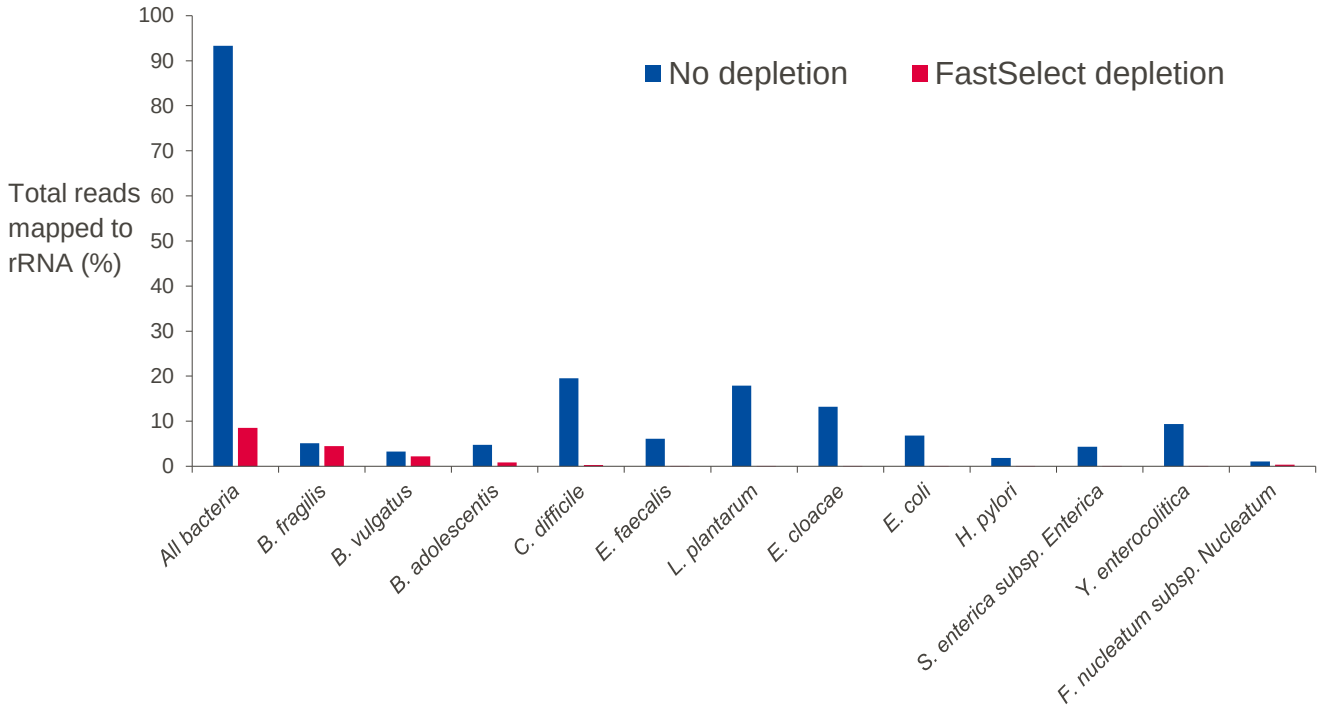
Experimental overview

- Sample: 100 ng, Gut Microbiome Whole Cell Mix (ATCC)
- Depletion: No depletion, FastSelect –5S/16S/23S
- Library prep: QIAseq Stranded Total RNA Lib Kit
- Sequencing: NextSeq 550 (2 x 75 bp)
- Mapping: CLC Genomics Workbench

FastSelect substantially removes rRNA

	Percentage of reads mapped to bacterial rRNA (total)	
Sample	**No treatment**	**FastSelect –5S/16S/23S**
ATCC Gut Microbiome (12 bacteria)	**96.35**	**12.32**

Robust depletion of rRNA from individual species



FastSelect removes nearly 90% of all rRNA. When individual species are mapped, FastSelect removes rRNA from a broad range of species.

FastSelect –5S/16S/23S: Robust rRNA removal from bacterial communities (cont.)

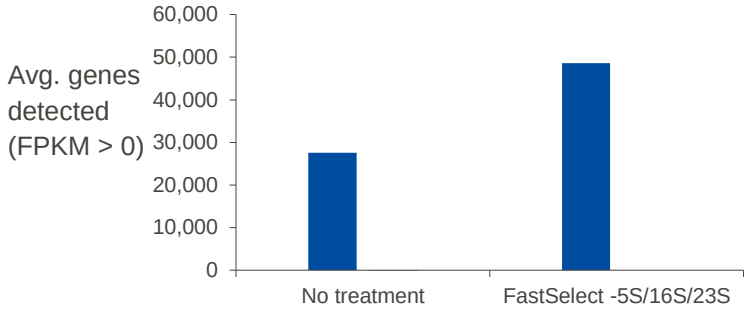
Experimental overview

- 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

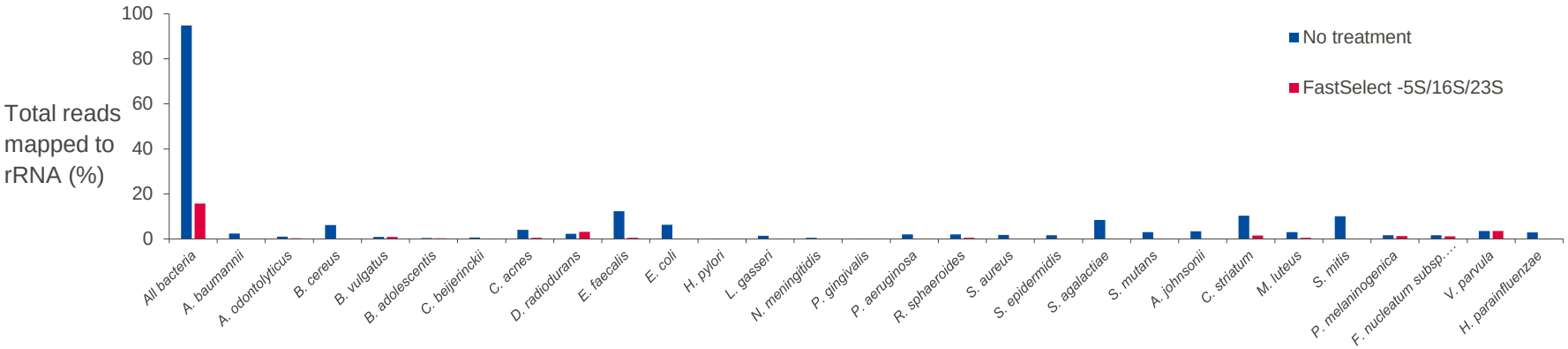
FastSelect substantially removes rRNA

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

FastSelect increases detected genes



FastSelect robustly depletes rRNA (individual species)



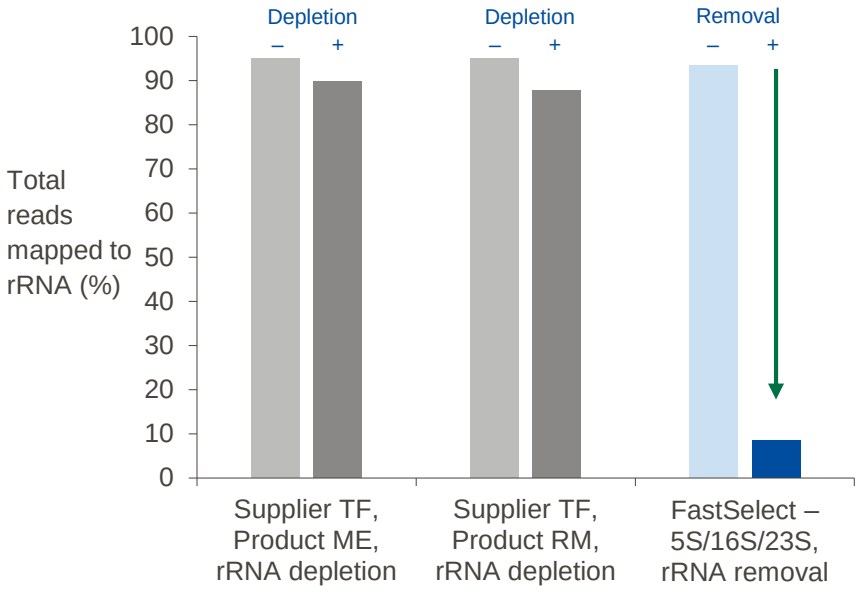
FastSelect efficiently removes rRNA, freeing up substantial read budget. In turn, this read budget enables a dramatic increase in the number of genes detected.

FastSelect –5S/16S/23S dramatically outperforms the other supplier's kits

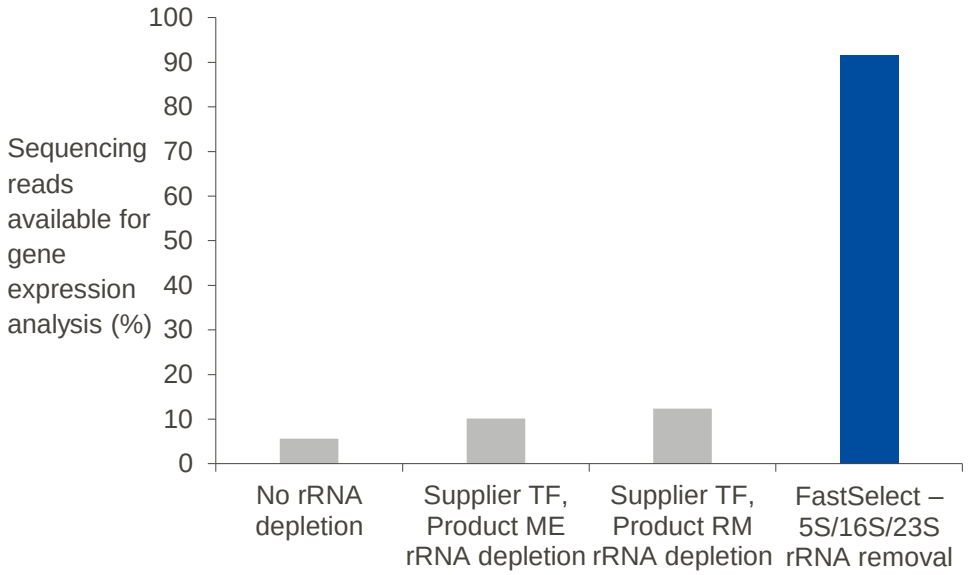
Experimental overview

- Sample: 1 µg, Gut Microbiome Whole Cell Mix (ATCC)
- Depletion: No depletion, Supplier TF – Product ME, Supplier TF – Product RM, FastSelect – 5S/16S/23S
- Library prep: QIAseq Stranded
- Sequencing: NextSeq 550
- Mapping: CLC Genomics Workbench

FastSelect robustly removes rRNA, while the other supplier's kits do not



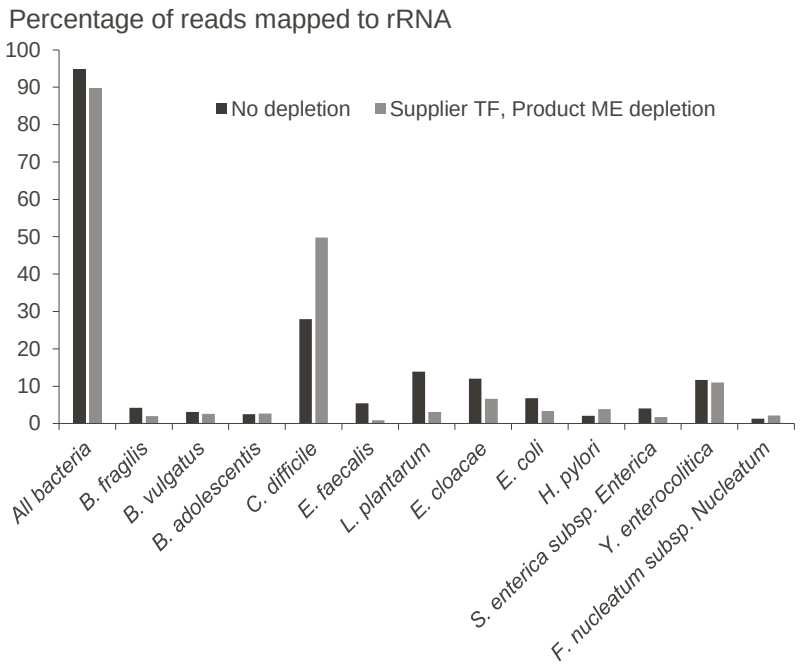
FastSelect frees up reads for gene detection



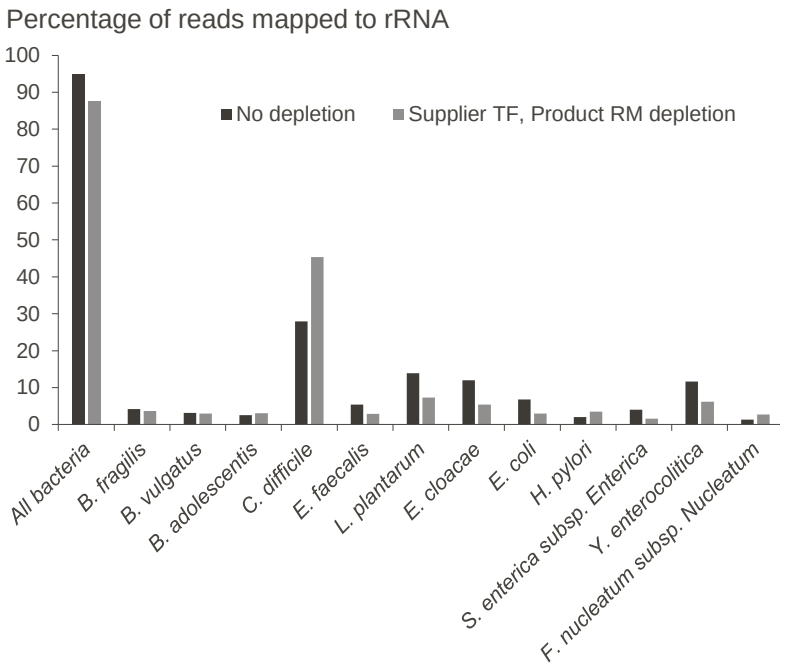
FastSelect efficiently removes rRNA, while Supplier TF's rRNA depletion products do not. The robust rRNA removal frees up a substantial amount of sequencing reads (9x compared to the other supplier's kits) for gene expression analysis.

FastSelect –5S/16S/23S dramatically outperforms the other supplier's kits

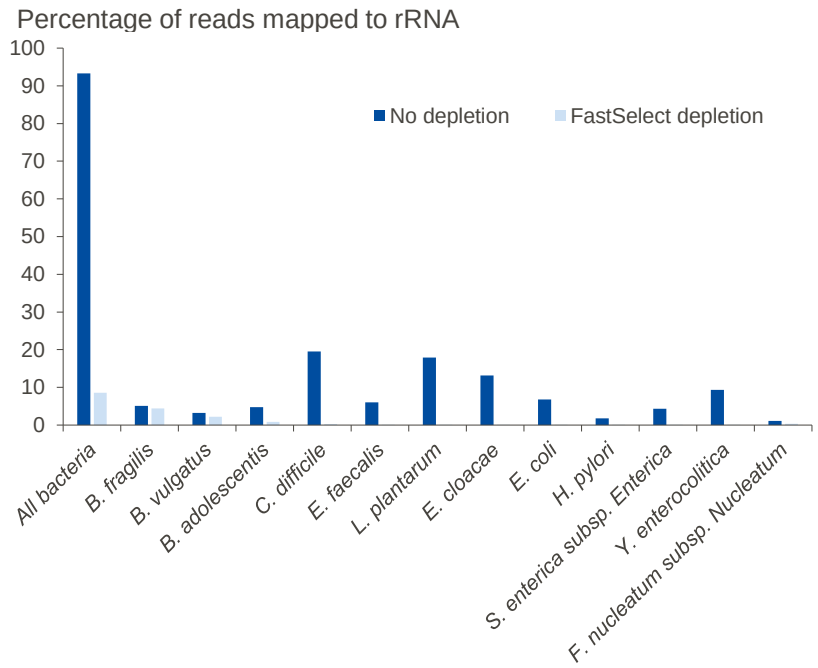
Supplier TF, Product ME



Supplier TF, Product RM



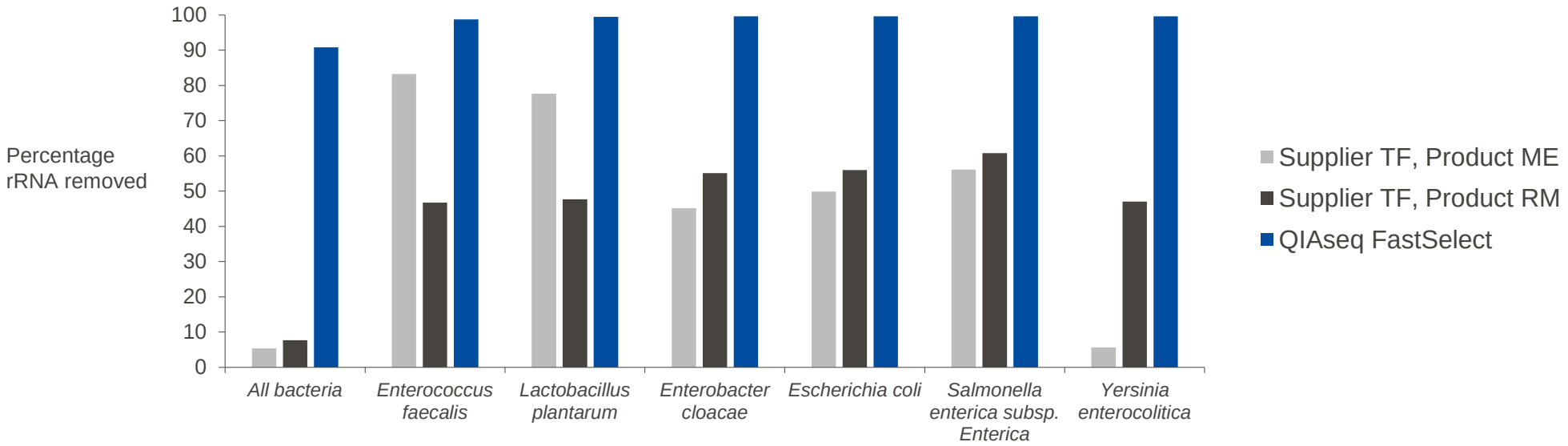
FastSelect –5S/16S/23S



The three figures depict the percentage of rRNA removed for "all bacteria" and the individual species in the community. FastSelect efficiently removes rRNA from a broad range of bacterial species, while Supplier TF, Product ME and Supplier TF, Product RM do not.

FastSelect –5S/16S/23S dramatically outperforms the other supplier's kits

FastSelect robustly removes rRNA, while the other supplier's kits do not



When "all bacteria" are analyzed, FastSelect removes greater than 90% of all rRNA, while other supplier's kits remove less than 10%. When select, individual bacteria are analyzed, the depletion varies for the other supplier's kits, while it remains consistently high for FastSelect.

FastSelect –5S/16S/23S robustly removes rRNA from single-species samples

Experimental overview

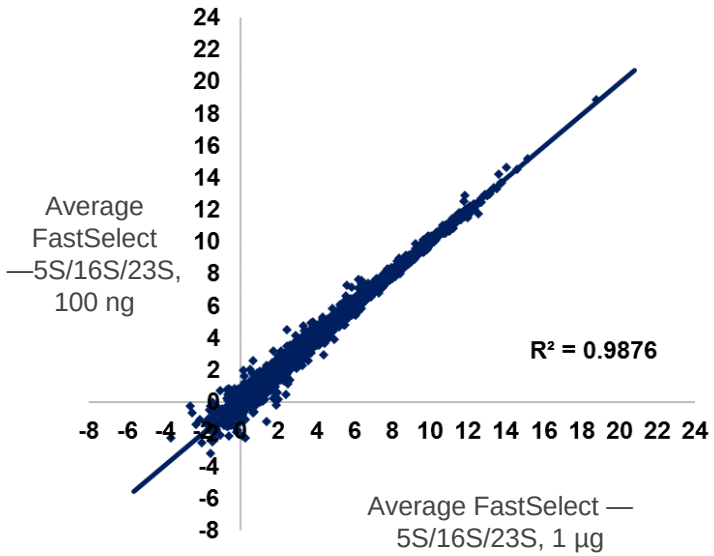
- Sample: 1 µg and 100 ng, DH5α *E. coli* total RNA (Thermo Fisher)
- Depletion: No depletion; FastSelect
- Library prep: QIAseq Stranded
- Sequencing: NextSeq 550
- Mapping: CLC Genomics Workbench

FastSelect substantially removes rRNA

Sample	Percentage of reads mapped to rRNA, FastSelect —5S/16S/23S
E. coli K12, 1 µg, R1	**2.01**
E. *coli* K12, 1 µg, R2	**1.88**
E. coli K12, 100 ng, R1	**0.55**
E. coli K12, 100 ng, R2	**2.57**

Sample	Percentage of reads mapped to bacterial rRNA	
	No treatment	FastSelect —5S/16S/23S
E. coli K12, 100 ng, R1	**97.79**	**0.55**
E. coli K12, 100 ng, R2	**97.08**	**2.57**

Strong gene expression correlation between inputs



FastSelect efficiently removes rRNA. Gene expression values from FastSelect-treated samples, even at different RNA input amounts, are highly correlative (Log2 RPKM > 0.3).

FastSelect –5S/16S/23S does not alter gene expression

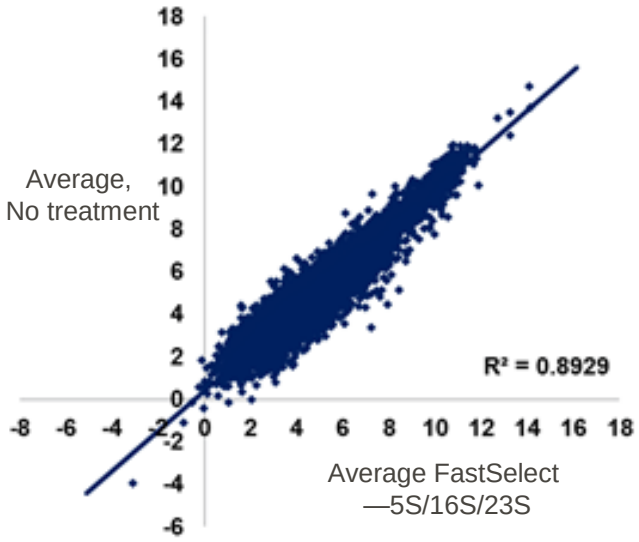
Experimental overview

- Sample: 100 ng, Gut Microbiome Whole Cell Mix
- 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 Gut Microbiome (12 bacteria), R1	**95.83**	**13.63**
ATCC Gut Microbiome (12 bacteria), R2	**95.88**	**13.44**

Strong gene expression correlation



FastSelect removes nearly 90% of all rRNA, and does not alter the expression of genes.

FastSelect is compatible with the QIAseq Stranded Total RNA Lib Kit

Other compatible kits:

- TruSeq Stranded (Illumina)
- NEBNext Ultra II Directional (NEB)

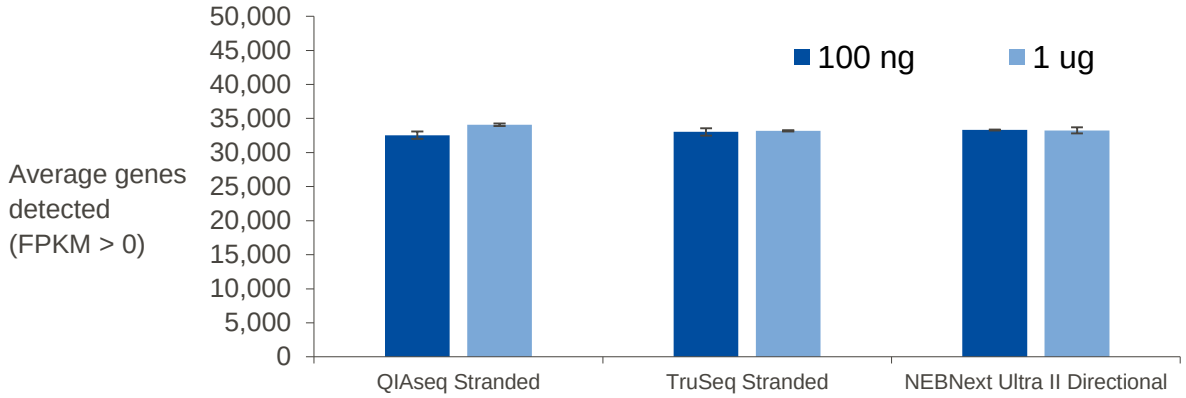
- FastSelect is compatible with most RNA library prep kits.

FastSelect –5S/16S/23S: Robust, reproducible results

Experimental overview

- Sample: 1 µg and 100 ng, Gut Microbiome Whole Cell Mix
- Depletion: No depletion, FastSelect
- Library prep: QIAseq Stranded, TruSeq Stranded, NEBNext Ultra II Directional
- Sequencing: NextSeq 550
- Mapping: CLC Genomics Workbench

Genes detected with each stranded kit



FastSelect substantially removes rRNA, regardless of the RNA library prep kit used

QIAseq Stranded

Sample	Percentage of reads mapped to bacterial rRNA (total)	
	No treatment	FastSelect – 5S/16S/23S
1 µg	**93.28**	**8.54**
100 ng	**92.46**	**11.94**

TruSeq Stranded

Sample	Percentage of reads mapped to bacterial rRNA (total)	
	No treatment	FastSelect – 5S/16S/23S
1 µg	**93.44**	**19.42**
100 ng	**92.18**	**24.11**

NEBNext Ultra II Directional

Sample	Percentage of reads mapped to bacterial rRNA (total)	
	No treatment	FastSelect – 5S/16S/23S
1 µg	**93.17**	**8.55**
100 ng	**92.17**	**8.83**

FastSelect is compatible with most RNA library prep kits.

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- 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

Total RNA input: 20 ng to 1 µg

Kit compatibility: QIAseq Stranded Total RNA Lib Kit (QIAGEN Group), TruSeq Stranded (Illumina, Inc), NEBNext Ultra II Directional (New England Biolabs, Inc)

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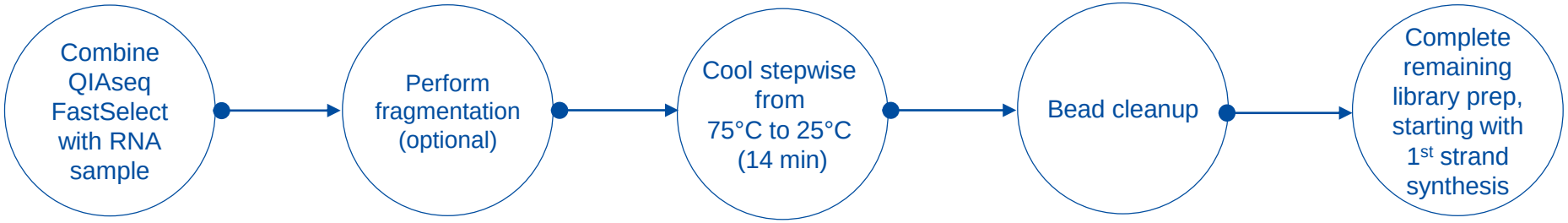

Thank you for attending

Questions?

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