rRNA removal and optimization of host-bacterial expression data

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Legal disclaimer

Agenda

Background

FastSelect overview

FastSelect results: Mammalian, bacterial, mammalian + bacterial

Summary

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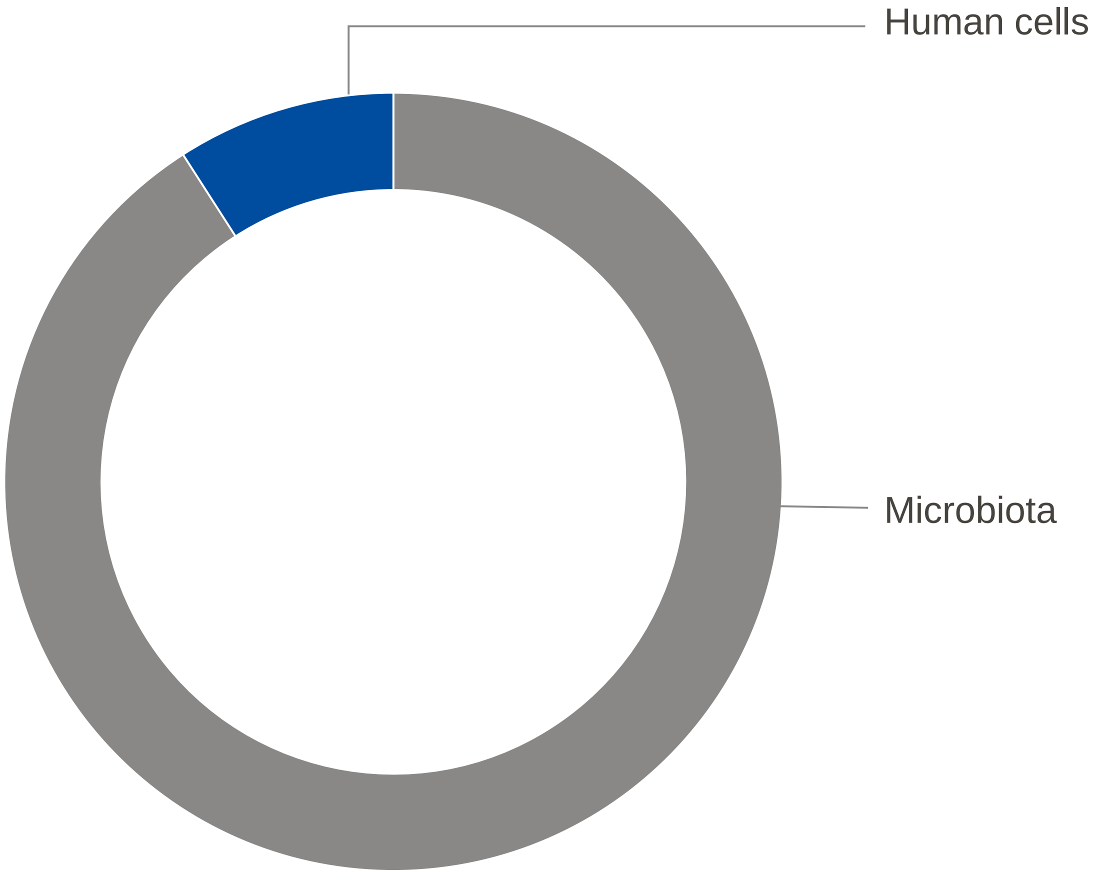
FastSelect results: Mammalian, bacterial, mammalian + bacterial

Summary

Humans or ‘superorganisms’?

Total number of cells: Human cells versus microbiota



Cellular composition of the ‘superorganism’

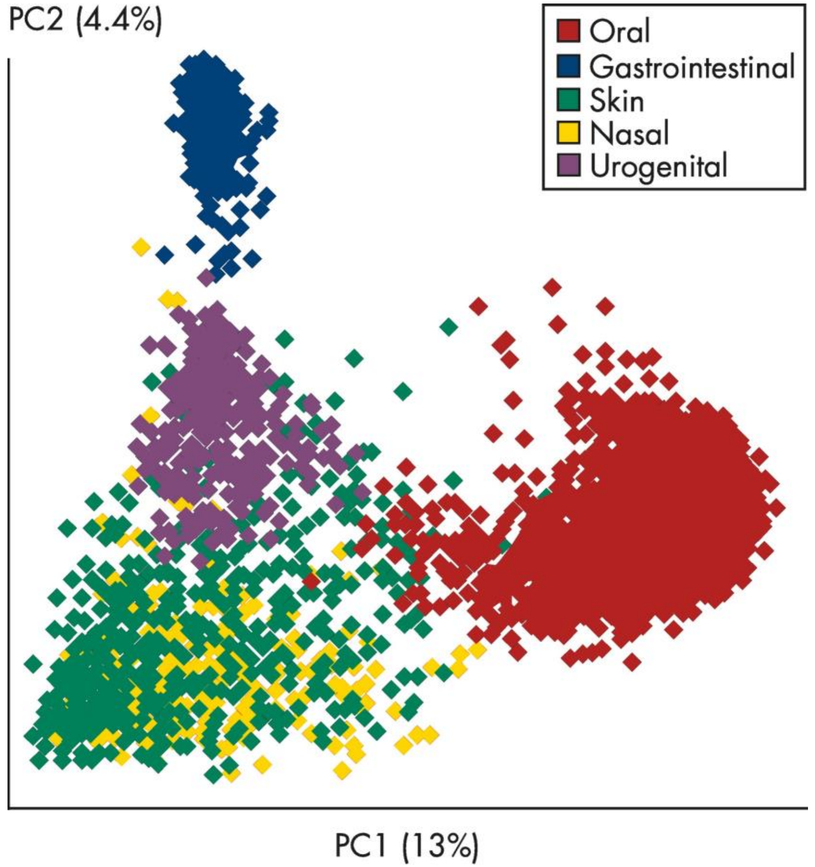
Estimation of the number of microbial cells that live in and on the human body; human cells are outnumbered by a factor of 10

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 ×10^6 genes constitute this ‘second genome’

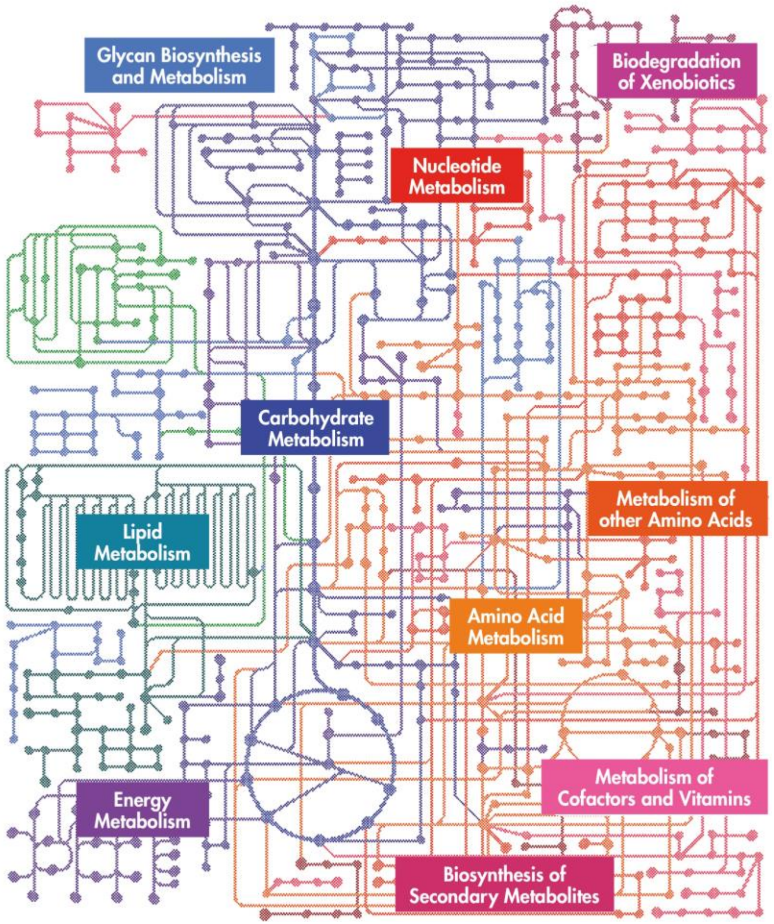
Identifying microbiota in healthy individuals revealed:

- 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

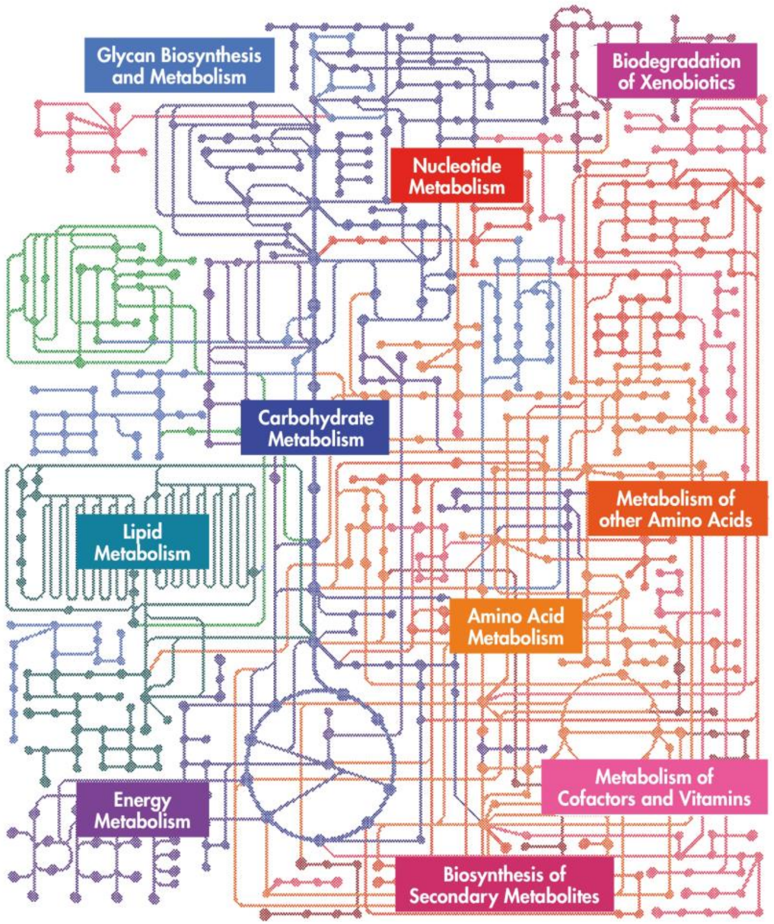


Each organism has developed its:

- 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 (continued)

Function of microbiome enables individual survival

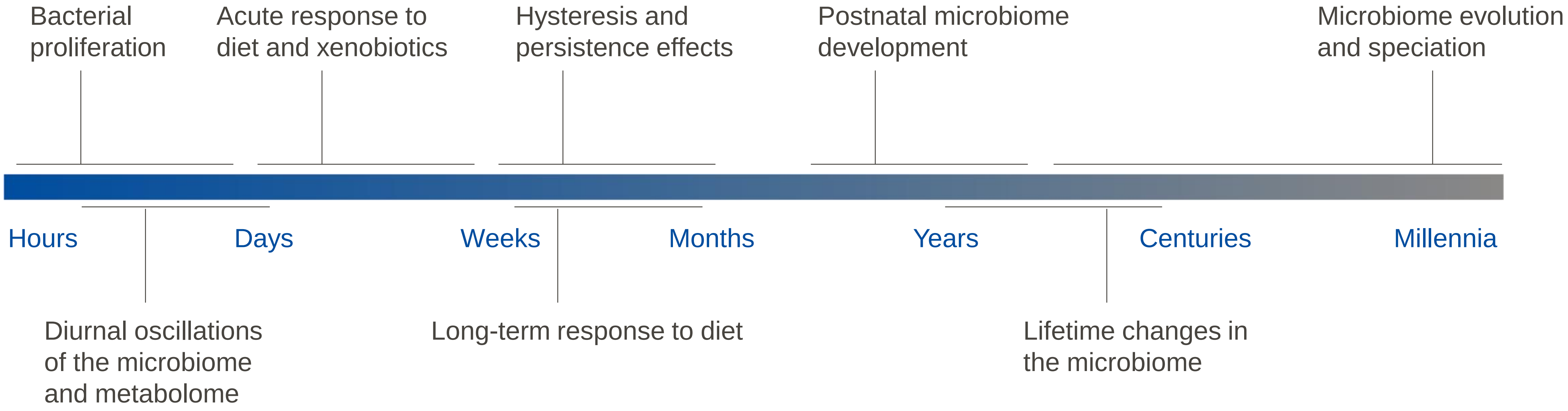


Understanding the microbiota

- 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

Microbiota dynamics important for host physiology

Function of microbiome enables individual survival



Bacterial proliferation

Acute response to diet and xenobiotics

Hysteresis and persistence effects

Postnatal microbiome development

Microbiome evolution and speciation

Hours

Days

Weeks

Months

Years

Centuries

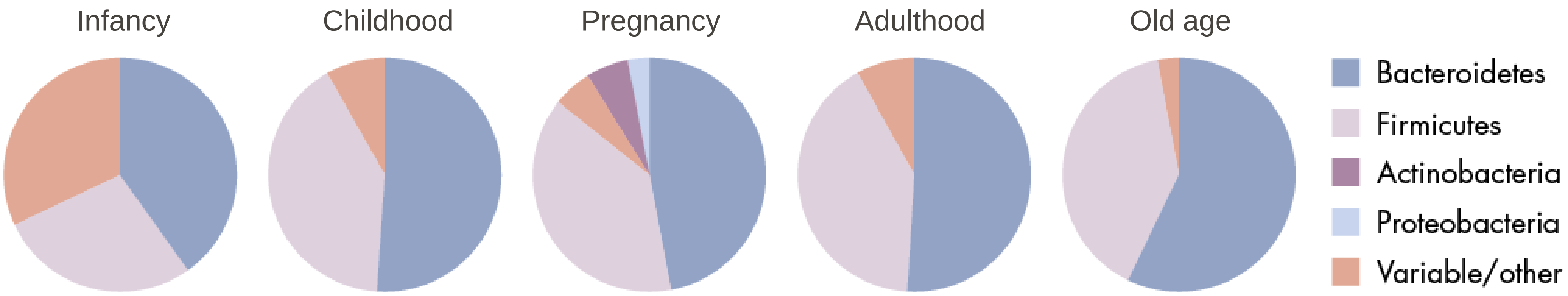
Millennia

Diurnal oscillations of the microbiome and metabolome

Long-term response to diet

Lifetime changes in the microbiome

Intestinal microbiota changes throughout the course of life



Infant
High inter-individual variability, low diversity

Toddler
Adult-like diversity and structure

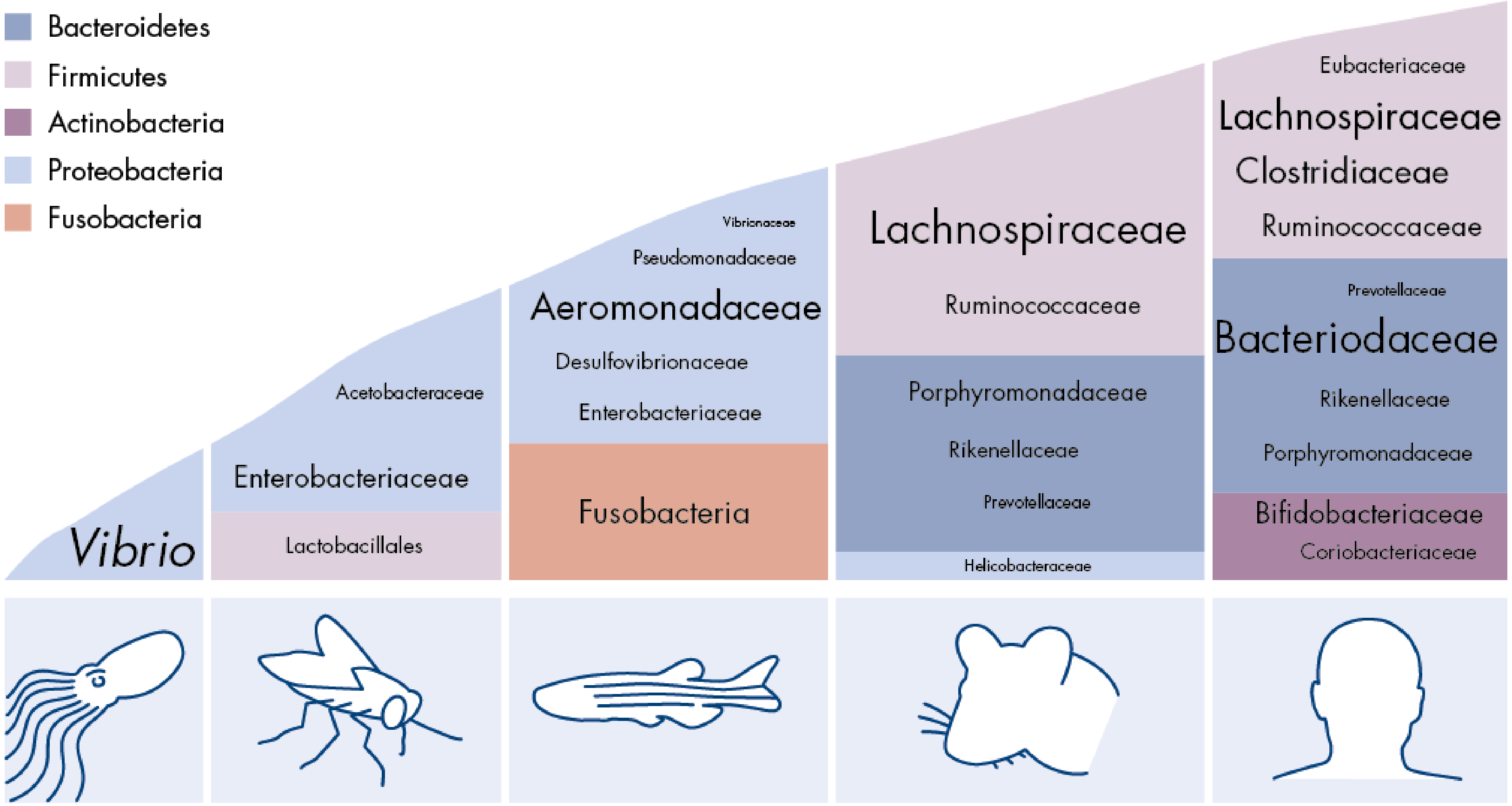
Pregnancy
Increase in certain bacteria and diversity, but returns to ‘normal’ after delivery

>65 years old
Numerous microbiota changes, increased Bacteroidetes

Source: Kostic, A.D., Howitt, M.R. and Garrett, W.S. (2013) Exploring host–microbiota interactions in animal models and humans. Genes Dev.27:701-718

Broad microbiota trends exist across species, especially at the phylum level

- **Phyla:** Represented by color
- **Abundance:** Lower taxonomic levels is indicated by font size.

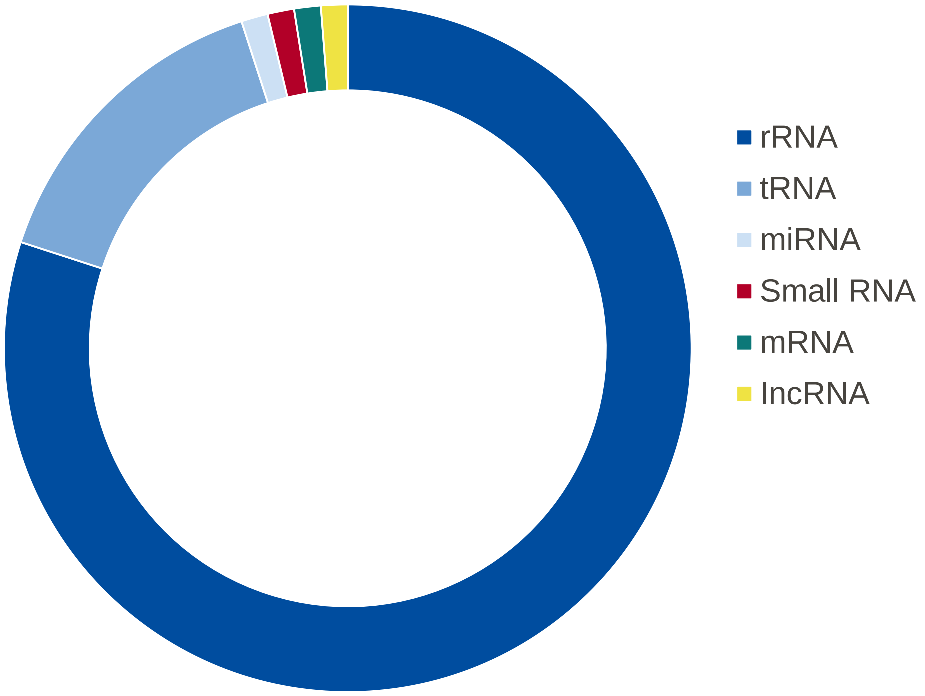


Source: Kostic, A.D., Howitt, M.R. and Garrett, W.S. (2013) Exploring host–microbiota interactions in animal models and humans. Genes Dev.27:701-718

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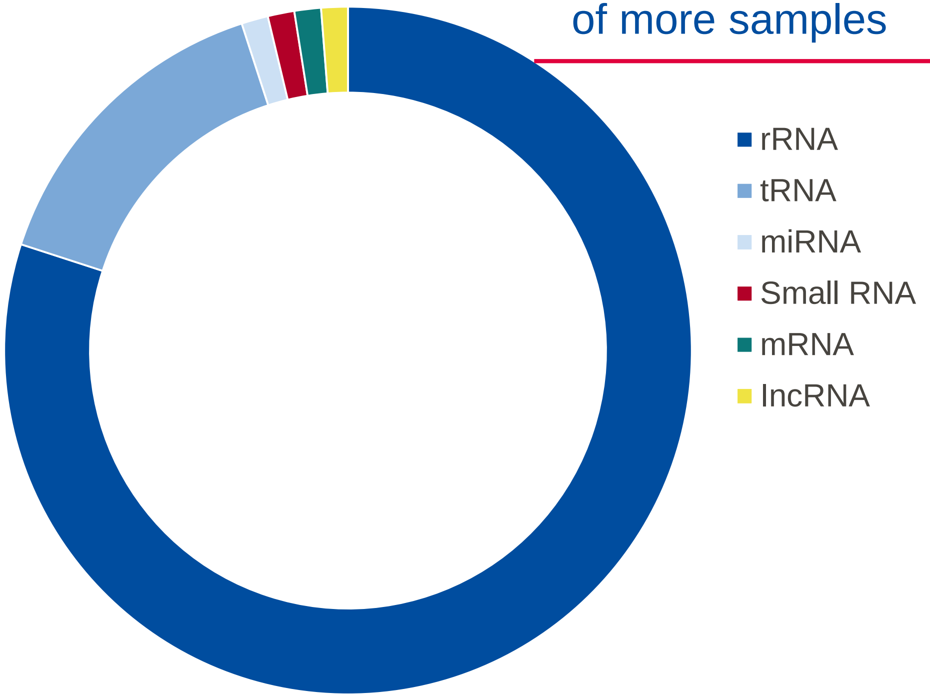
Removing rRNA will increase sensitivity and decrease the cost of sequencing, allowing analysis of more samples



Library enrichment/depletion strategies: Whole transcriptome NGS

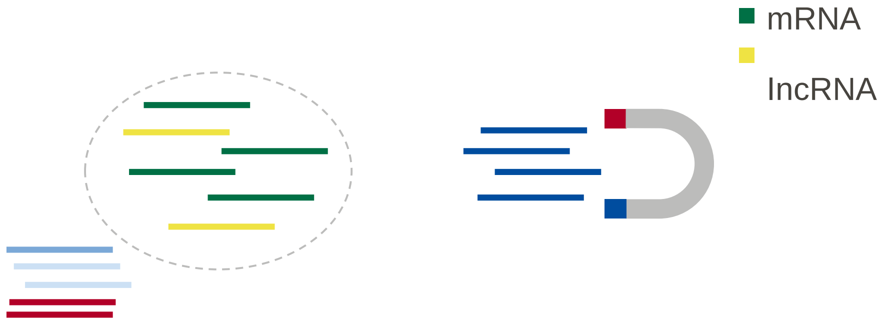
Enrichment/depletion strategies

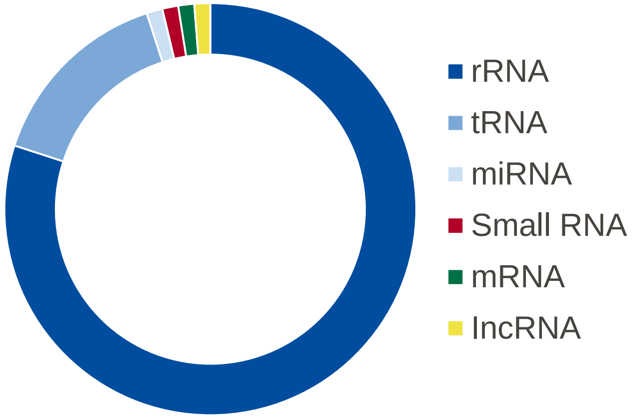
Poly(A) enrichment

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

rRNA depletion

- The ‘catch all’ method





rRNA depletion is the only choice for metatranscriptomics.

Agenda

Background

FastSelect overview

FastSelect results: Mammalian, bacterial, mammalian + bacterial

Summary

NEW

Remove bacterial rRNA only: FastSelect –5S/16S/23S 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

Remove mammalian rRNA and globin mRNA only: FastSelect –rRNA HMR and –Globin

How does FastSelect –rRNA HMR and –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

Remove both bacterial and host rRNA: FastSelect –5S/16S/23S plus –rRNA HMR

Catalog numbers

- QIAseq FastSelect –5S/16S/23S: 335925, 335927 or 335929
- QIAseq FastSelect –rRNA HMR: 334386, 334378 or 334388
- QIAseq FastSelect −Globin: 334376, 334377 or 334378
- QIAseq FastSelect −rRNA/Globin: 335376, 335377 or 335378

Where do I find the protocol?

- QIAseq FastSelect −5S/16S/23S Handbook

How long does the protocol take?

- Only 1 hour, which includes fragmentation time!

How does it work?

- Just like the individual products through inhibition of rRNA reverse transcription

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)
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Workflow for removal of both bacterial and host rRNA

Combine QIAseq FastSelect with RNA sample

Perform fragmentation (optional)

Cool stepwise from 75°C to 25°C (14 min)

Bead cleanup

Complete remaining library prep, starting with 1st strand synthesis

Mix and match ‘FastSelects’ (you can add all three or even more)

- FastSelect –5S/16S/23S: Bacterial rRNA
- QIAseq FastSelect –rRNA HMR: Mammalian rRNA
- QIAseq FastSelect −Globin: Mammalian Globin

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FastSelect overview

FastSelect results: Mammalian, bacterial, mammalian + bacterial

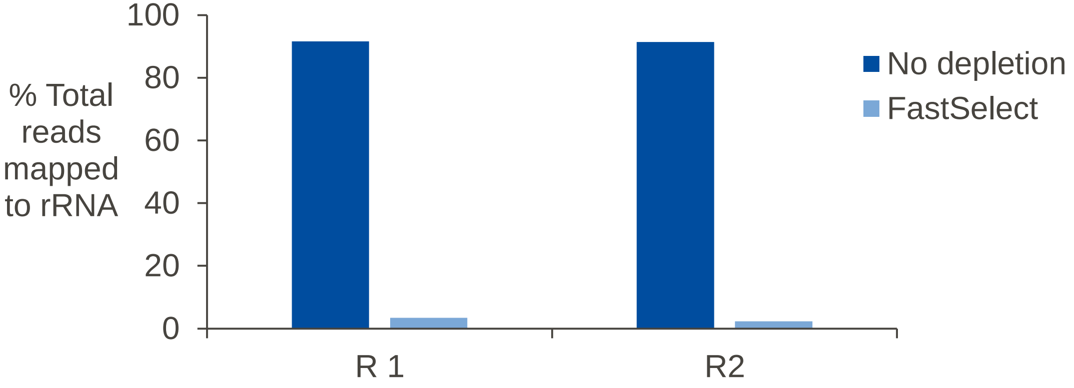
Summary

FastSelect –rRNA HMR robustly removes rRNA, freeing up reads for gene detection

Experimental overview

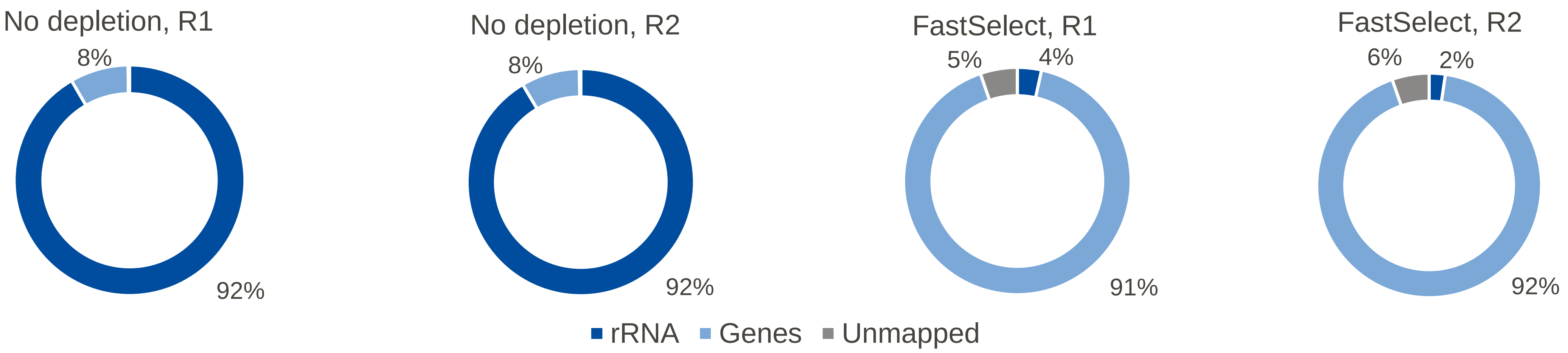
- Sample: 100 ng, Universal Human Reference RNA (UHRR)
- Depletion: No depletion, FastSelect –rRNA HMR
- Library prep: QIAseq Stranded Total RNA Lib Kit
- Sequencing: NextSeq 550 (2 x 75 bp)
- Mapping: CLC Genomics Workbench

FastSelect substantially removes rRNA



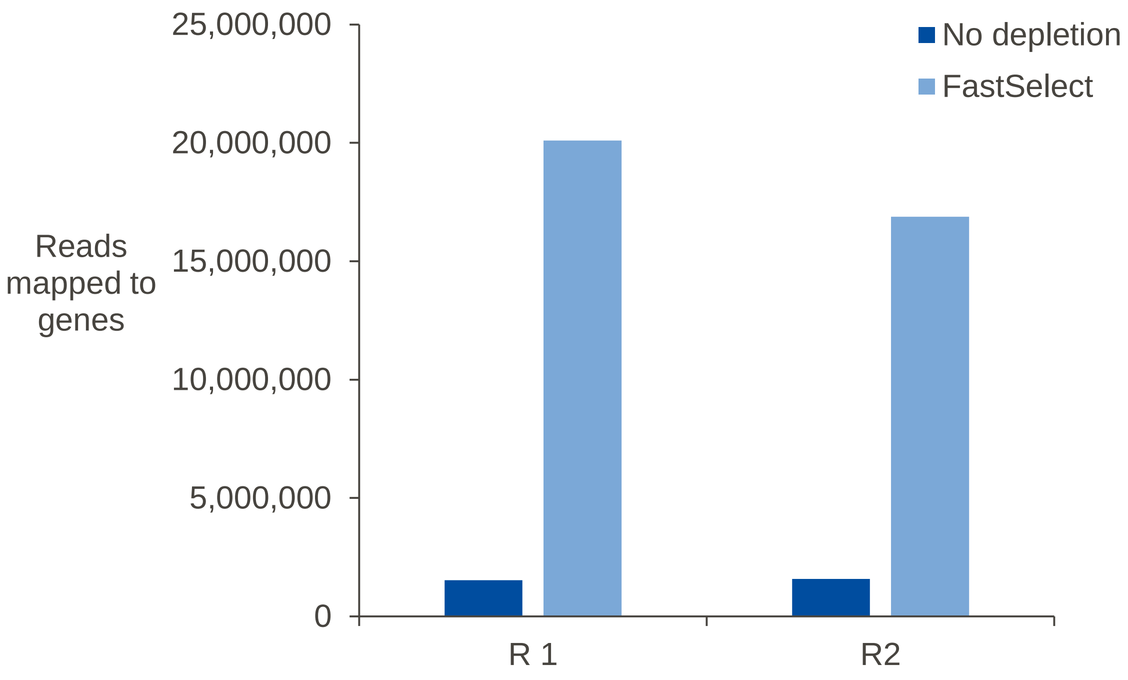
FastSelect improves read mapping metrics, removing contaminating rRNAs

Breakdown of mapped reads

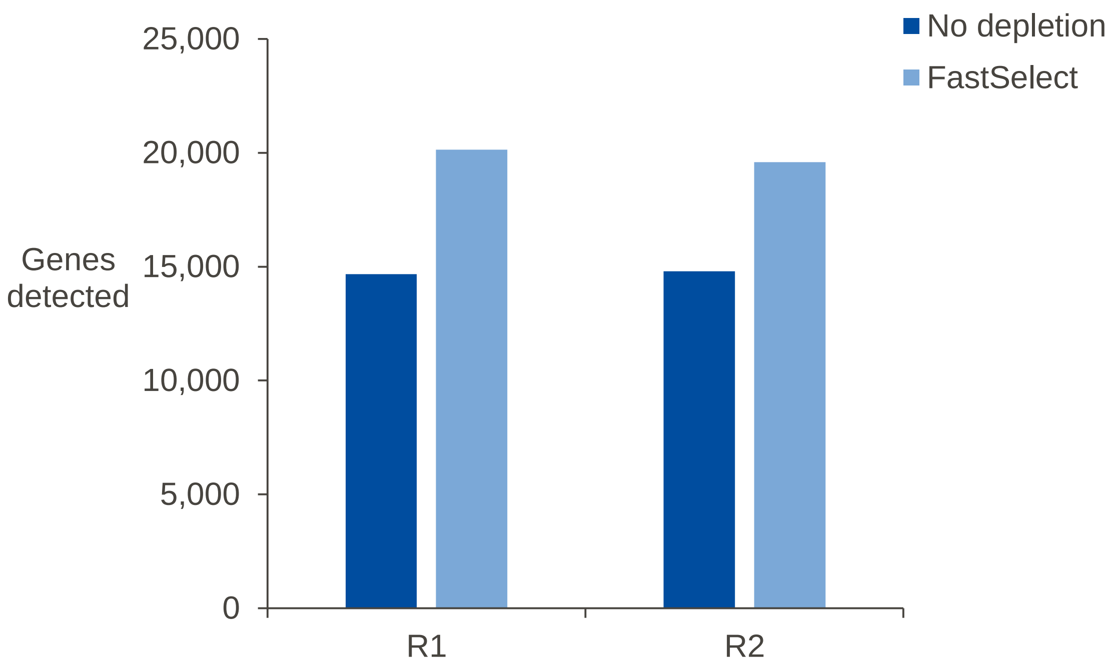


FastSelect –rRNA HMR robustly removes rRNA, freeing up reads for gene detection

FastSelect dramatically increases reads mapped to genes



FastSelect increases the number of genes detected



- FastSelect removes nearly all human rRNA, freeing up reads for robust gene detection.

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

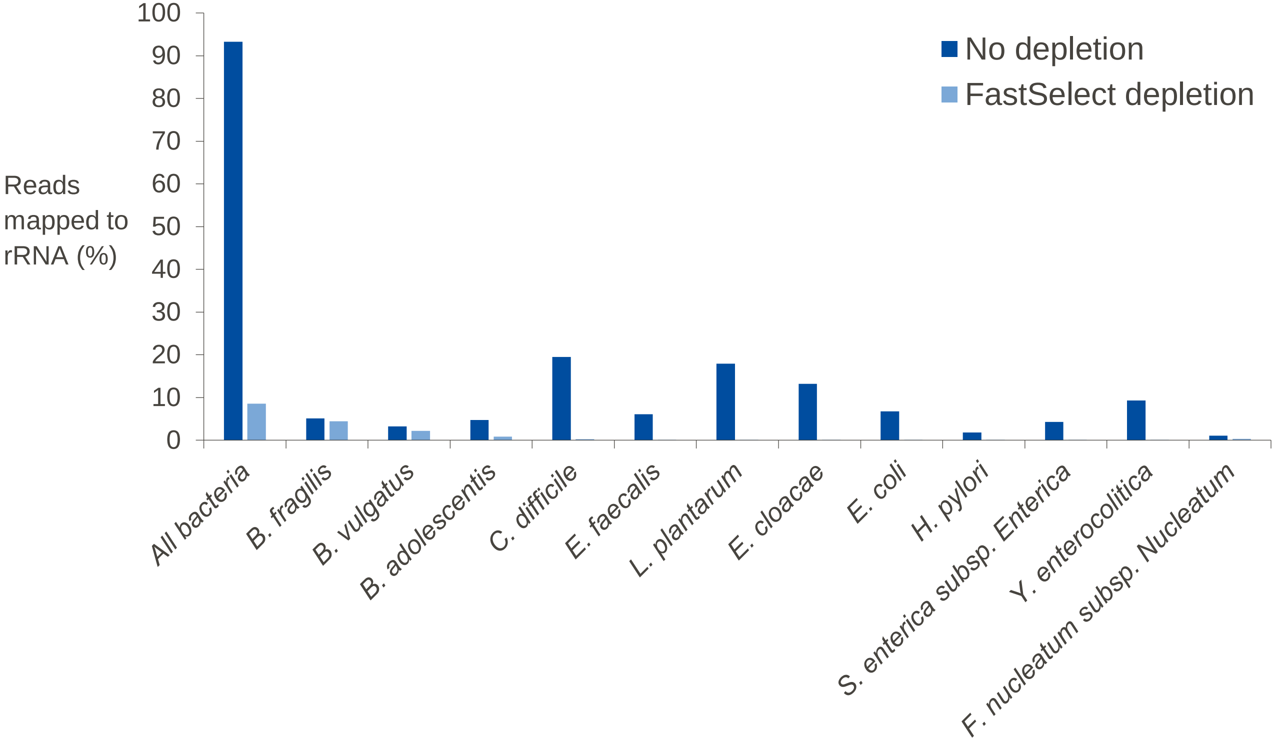
Experimental overview

- Sample: 1 µg, 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

Sample	Percentage of reads mapped to bacterial rRNA (total)	
	No treatment	FastSelect –5S/16S/23S
ATCC Gut Microbiome (12 bacteria)	93.28	8.54

Robust depletion of rRNA from individual species

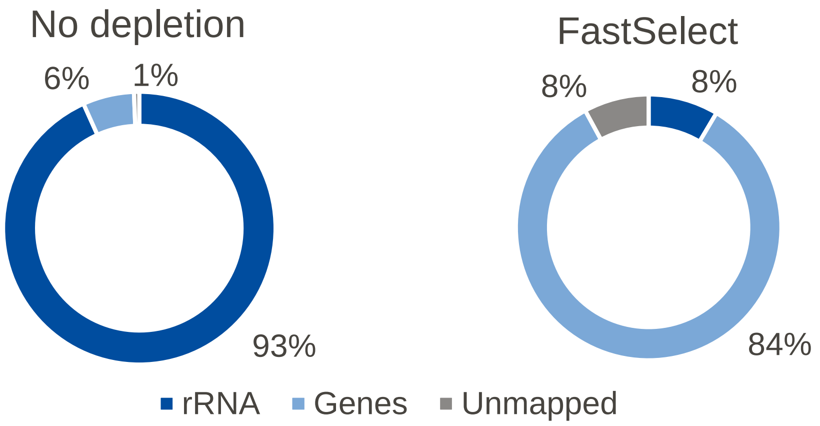


- FastSelect removes nearly 90% of all rRNA
- When individual species are mapped, FastSelect removes rRNA from a broad range of species

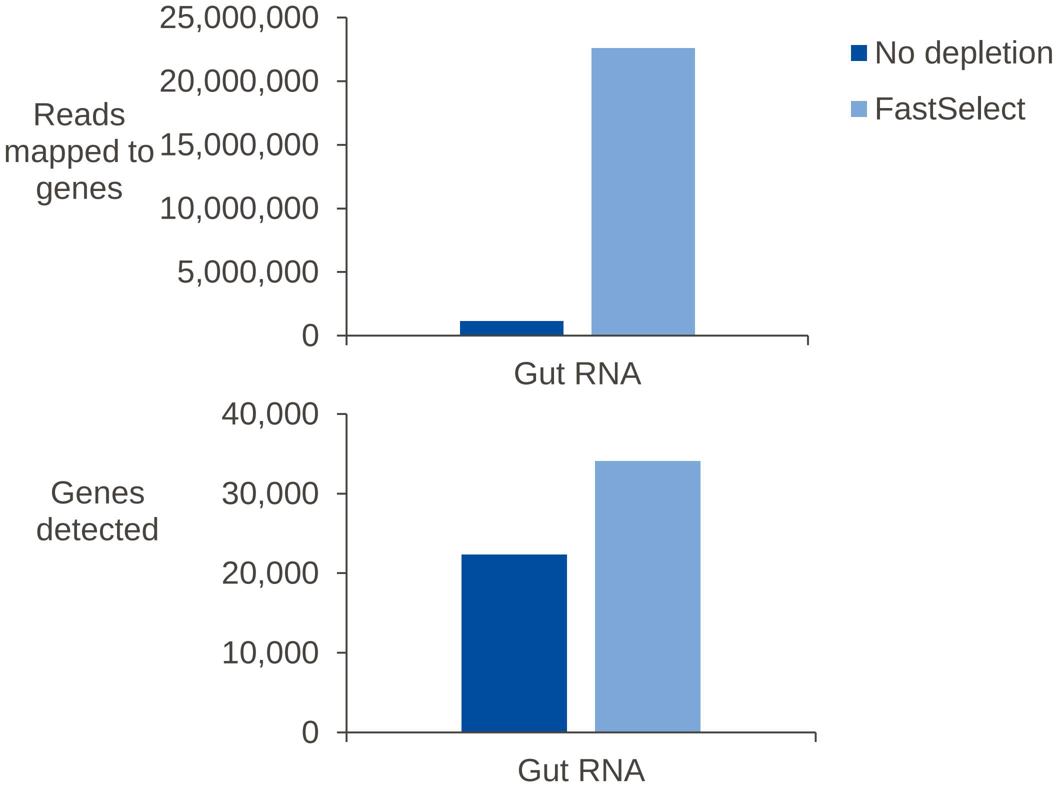
FastSelect –5S/16S/23S: Robust rRNA removal from bacterial communities

FastSelect dramatically improves read mapping metrics

Breakdown of mapped reads



FastSelect dramatically increases reads mapped to genes, resulting in a substantial increase in genes detected



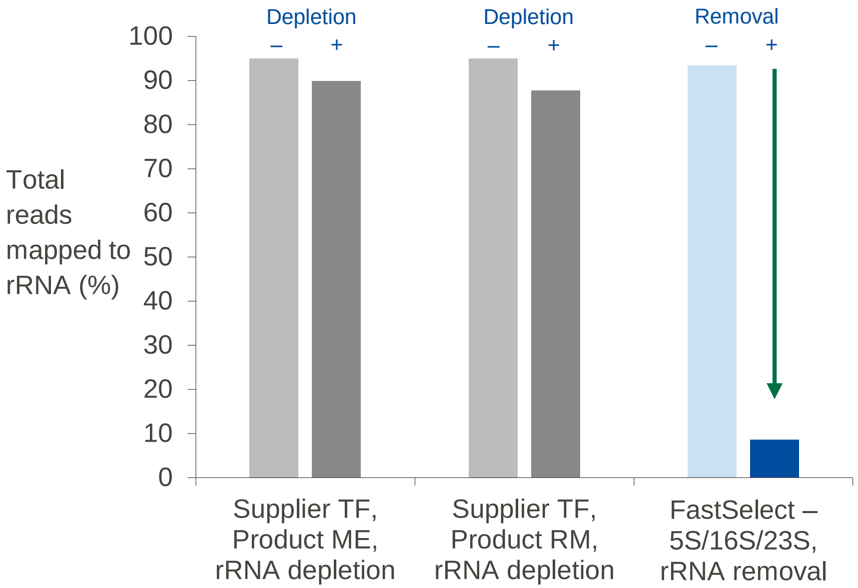
- FastSelect removes substantial amounts of bacterial rRNA, even from communities, freeing up reads for gene detection.

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

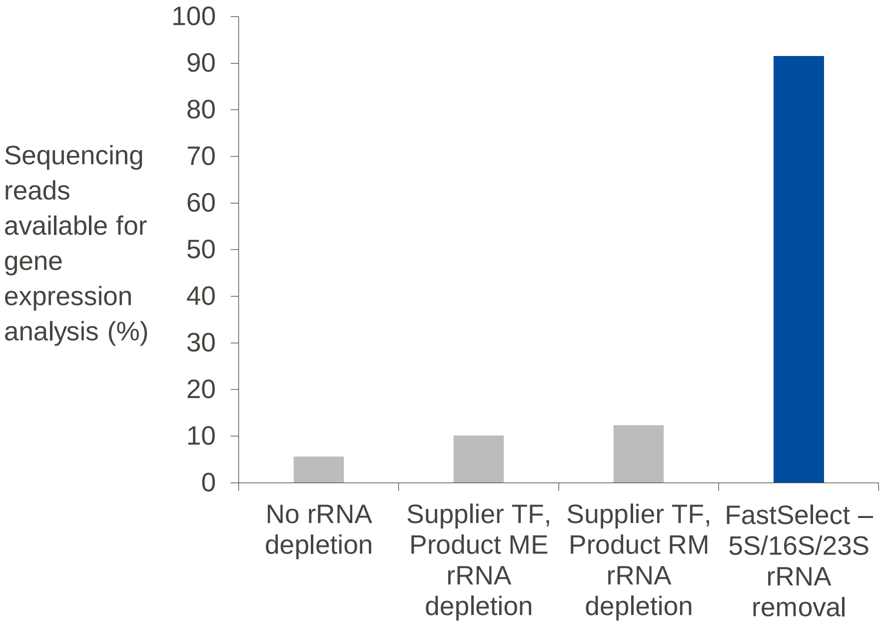
Experimental overview

- 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



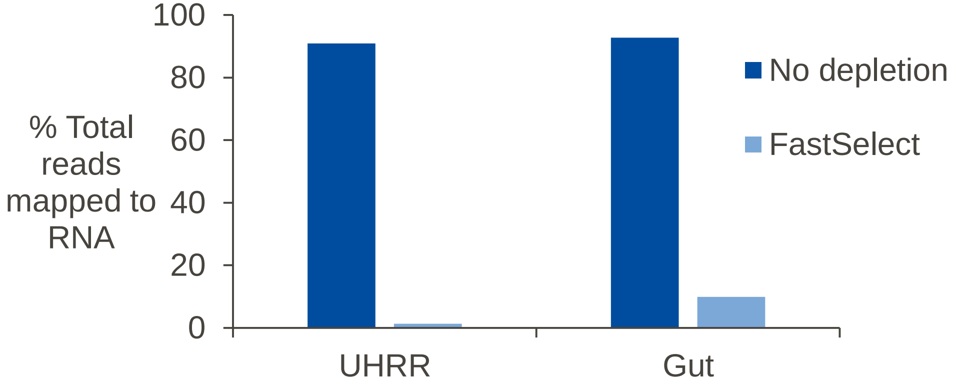
- FastSelect efficiently removes rRNA, while Supplier TF's rRNA depletion products do not
- The robust rRNA removal frees up a substantial amount of sequencing reads (9x compared to the other supplier's kits) for gene expression analysis

FastSelect –5S/16S/23S plus –rRNA HMR remove bacterial and host rRNA

Experimental overview

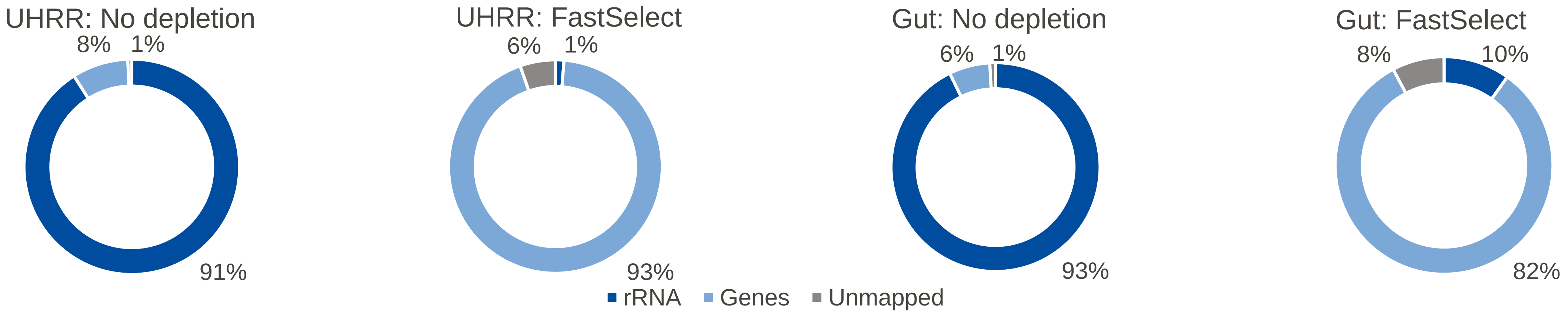
- Sample: 100 ng, different ratios of Universal Human (H) + Gut (G) RNA
- Depletion: No depletion, FastSelect –5S/16S/23S + FastSelect –rRNA HMR
- Library prep: QIAseq Stranded Total RNA Lib Kit
- Sequencing: NextSeq 550 (2 x 75 bp)
- Mapping: CLC Genomics Workbench

FastSelect substantially removes rRNA



FastSelect improves read mapping metrics, removing contaminating rRNAs

Breakdown of mapped reads

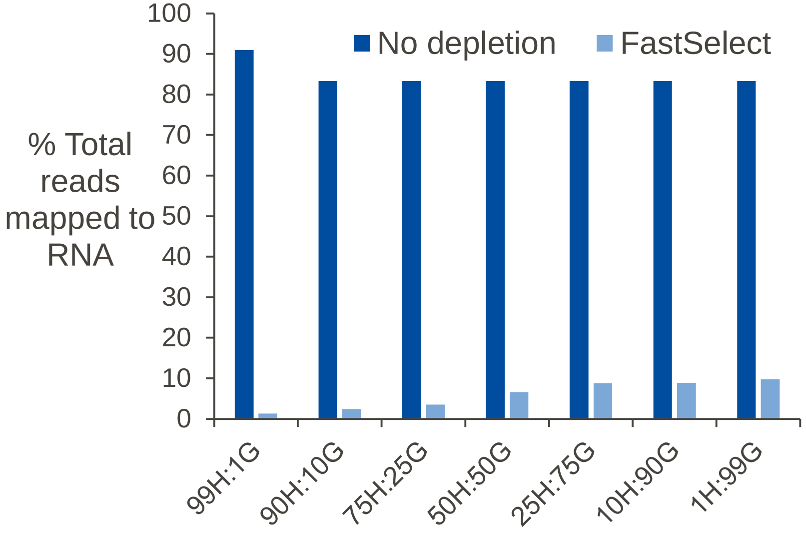


FastSelect –5S/16S/23S plus –rRNA HMR remove bacterial and host rRNA

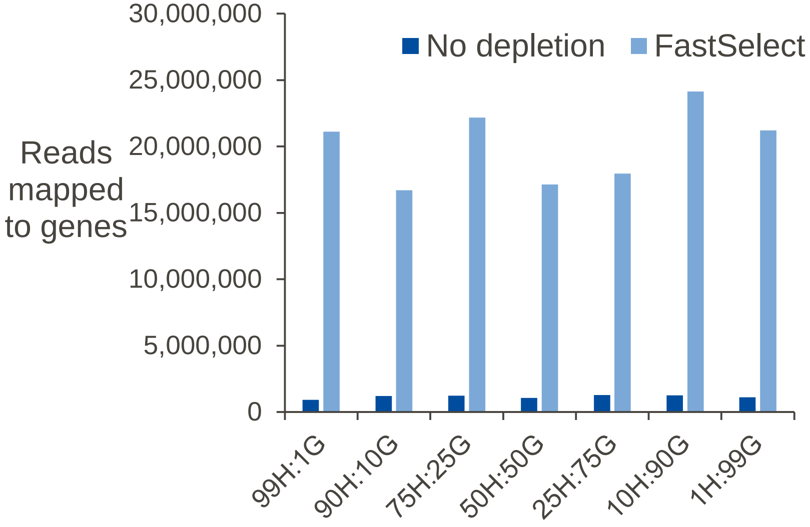
Mixed samples

- 99% Human, 1% Gut (99H:1G)
- 90% Human, 10% Gut (90H:10G)
- 75% Human, 25% Gut (75H:25G)
- 50% Human, 50% Gut (50H:50G)
- 25% Human, 75% Gut (25H:75G)
- 10% Human, 90% Gut (10H:90G)
- 1% Human, 99% Gut (1H:99G)

FastSelect –5S/16S/23S plus –rRNA HMR robustly removes rRNA



FastSelect dramatically increases reads mapped to human + bacterial genes



- FastSelect –rRNA HMR + –5S/16S/23S efficiently removes rRNA from mixed human and bacteria samples
- This dramatically increases the reads mapped to human and bacterial genes (13- to 23-fold increase)

Agenda

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FastSelect overview

FastSelect results: Mammalian, bacterial, mammalian + bacterial

Summary

FastSelect is compatible with the QIAseq Stranded Total RNA Lib Kit

Other compatible kits:

- TruSeq Stranded (Illumina)
- NEBNext Ultra II Directional (NEB)
- KAPA RNA HyperPrep (Roche)

FastSelect is compatible with most RNA library prep kits.

Summary

FastSelect –rRNA HMR and –Globin

1

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

- Thirty percent faster than FastSelect V1, now in a single-tube
- Covers human, mouse, rat (HMR) and other mammalian species
- Compatible with several sample types, RNA types and input amounts and RNA library prep kits

FastSelect –5S/16S/23S

2

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
- Compatible with a range of RNA input amounts and most RNA library prep kits

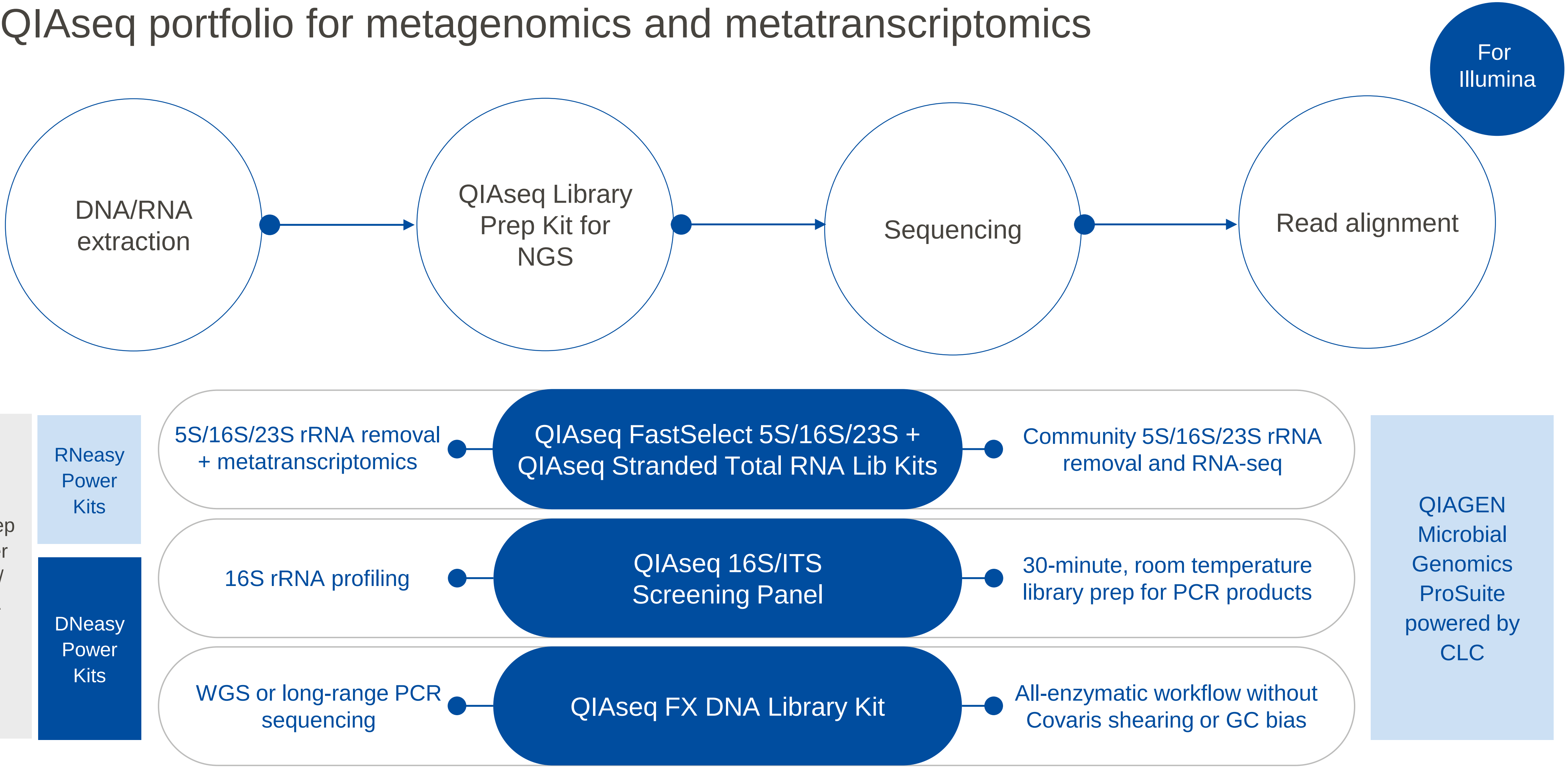
FastSelect –rRNA HMR plus –5S/16S/23S

3

The FastSelect –rRNA HMR plus –5S/16S/23S protocol removes both human and bacterial rRNA

- One-hour protocol
- Expandable to include any species
- Dramatically improves sequencing read budgets by robustly removing rRNA

Full QIAseq portfolio for metagenomics and metatranscriptomics



For Illumina

DNA/RNA extraction

QIAseq Library Prep Kit for NGS

Sequencing

Read alignment

All-Prep Power RNA/ DNA Kits

RNeasy Power Kits

DNeasy Power Kits

5S/16S/23S rRNA removal + metatranscriptomics

QIAseq FastSelect 5S/16S/23S + QIAseq Stranded Total RNA Lib Kits

Community 5S/16S/23S rRNA removal and RNA-seq

16S rRNA profiling

QIAseq 16S/ITS Screening Panel

30-minute, room temperature library prep for PCR products

WGS or long-range PCR sequencing

QIAseq FX DNA Library Kit

All-enzymatic workflow without Covaris shearing or GC bias

QIAGEN Microbial Genomics ProSuite powered by CLC

Thank you for attending.

Questions?

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