Introduction to rRNA removal

Evolution of new technologies for mammalian and bacterial samples

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Agenda

Background

Mammalian rRNA depletion

Bacterial rRNA depletion

Summary

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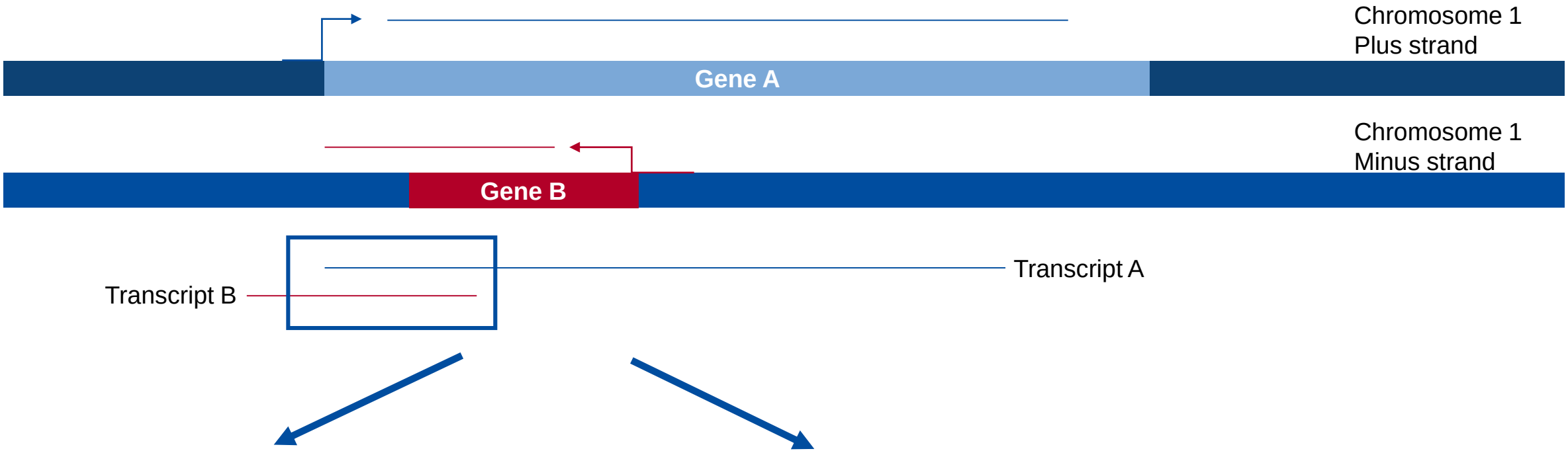
Whole transcriptome stranded RNA NGS: What does it mean?



Non-stranded RNAseq

- Transcript orientation is not retained
- You cannot say if it's A or B

Stranded RNAseq

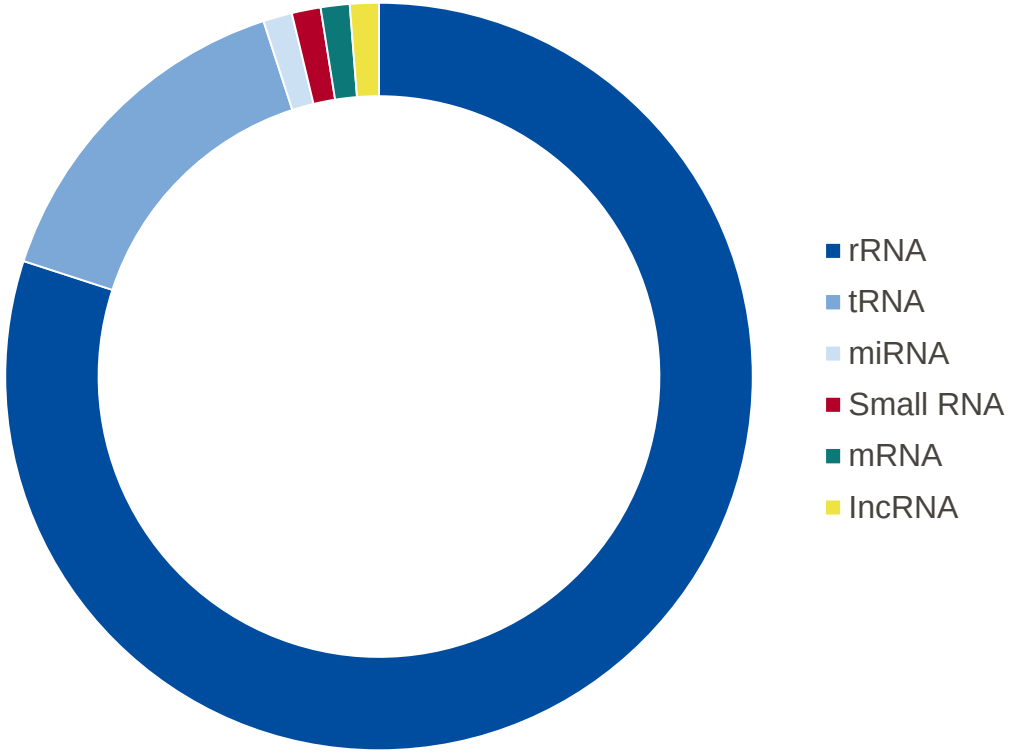
- Transcript orientation is retained
- You can say if it's A, B or both

Tip: Start with more than 100 ng of total RNA to reduce duplicates.

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



Library enrichment/depletion strategies: Whole transcriptome NGS

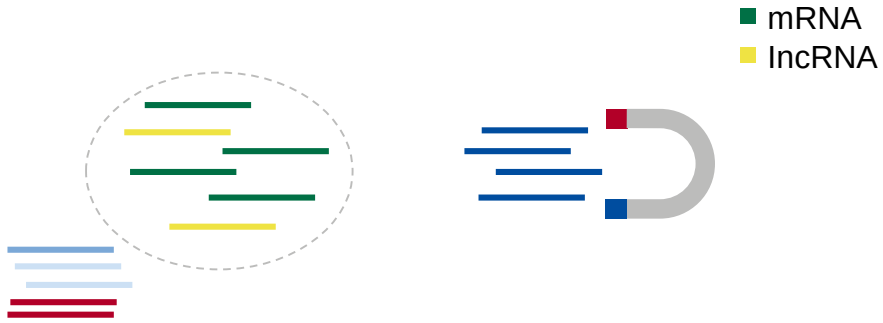
Enrichment/depletion strategies

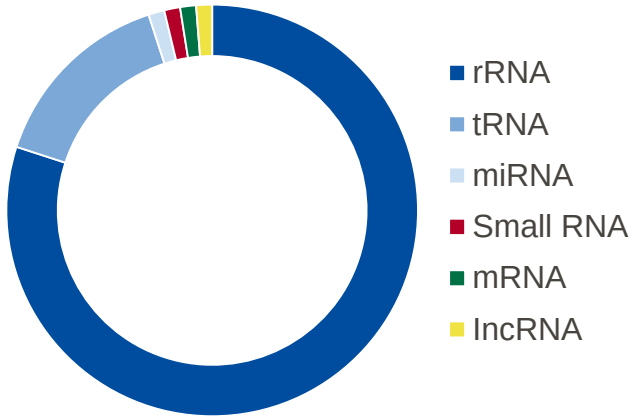
Poly(A) enrichment

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

rRNA depletion

- The “catch all” method





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

Agenda

Background

Mammalian rRNA depletion

Bacterial rRNA depletion

Summary

Mammalian rRNA depletion methods: Hybrid capture or RNase H

Pretreatment methodologies that take more than 2 hours and have ~30 steps

Hybrid capture method

Bead washing
(12 steps)

Probe hybridization
(5 steps)

Removal of rRNA
(6 steps)

RNA purification
(14 steps)

RNase H method

Bind ssDNA probes to rRNA
(6 steps)

Degrade rRNA using RNase H
(6 steps)

Degrade DNA probes
(6 steps)

RNA purification
(11 steps)

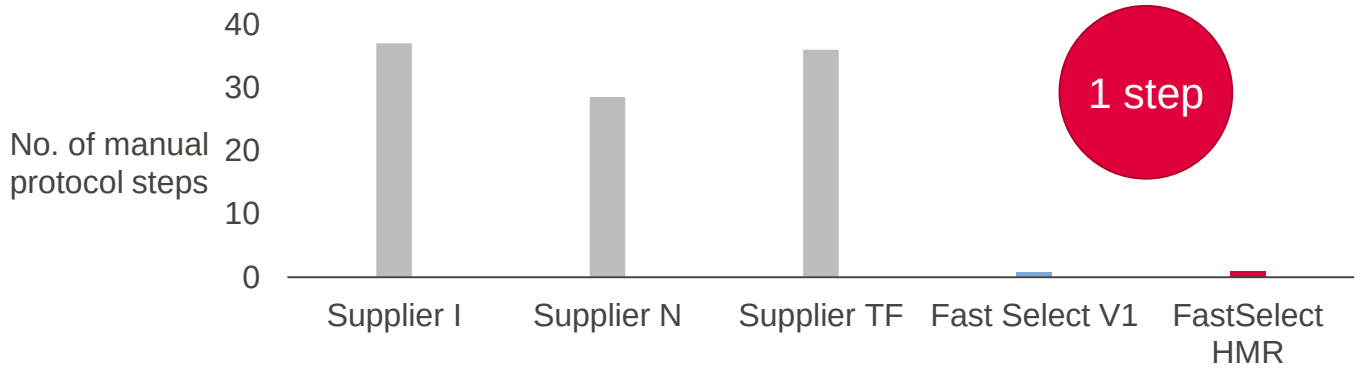
- Pretreatment methodologies are time-consuming and arduous with a higher chance for sample loss.

QIAseq® FastSelect –rRNA HMR Kit

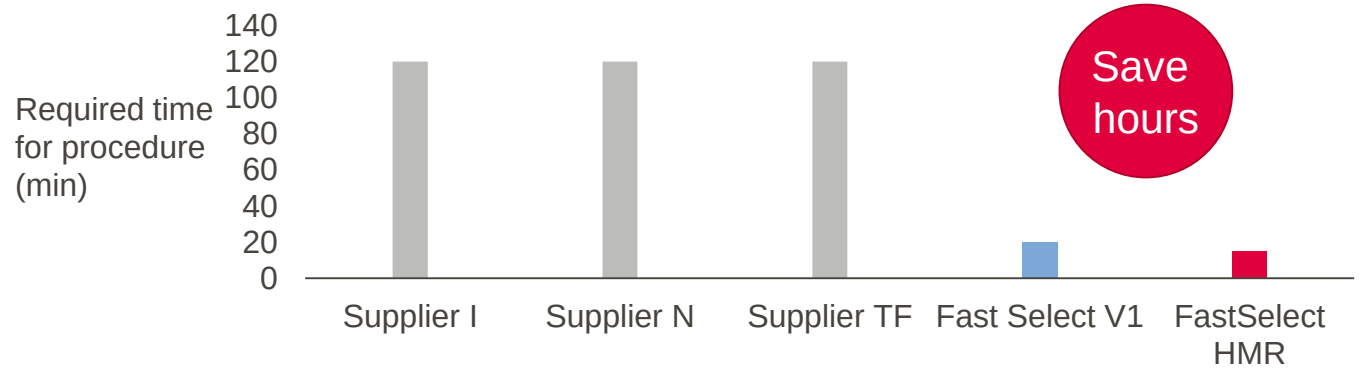
Removes rRNA in a single step requiring only 14 minutes

- 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

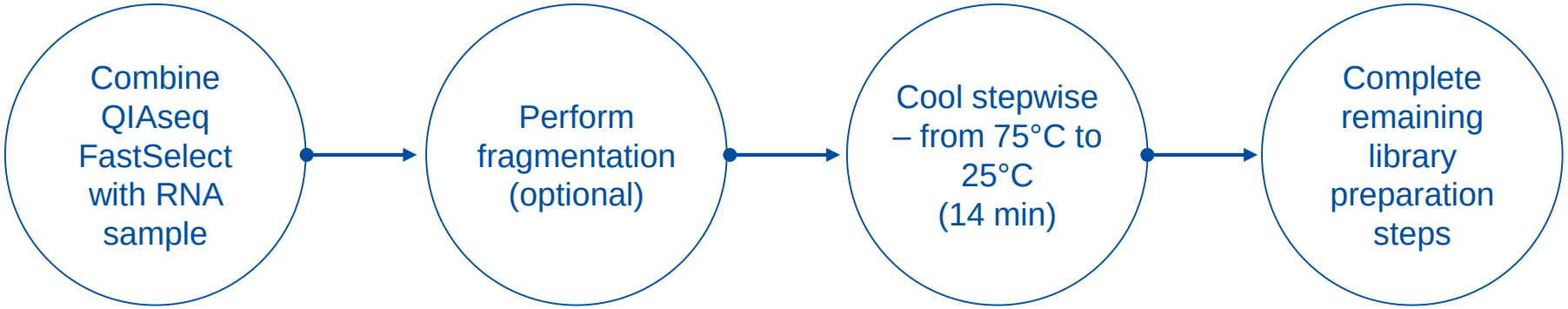


FastSelect –rRNA HMR: 90% less time



FastSelect –rRNA HMR and –Globin

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



NEW

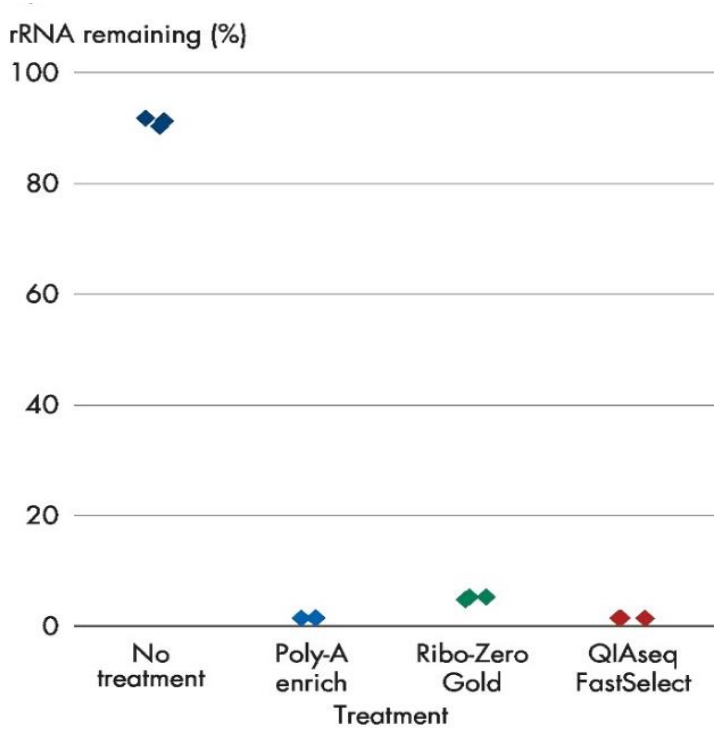
FastSelect –rRNA HMR and –Globin

- 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

QIAseq FastSelect vs. no treatment, Poly-A and Ribo-Zero Gold

Percentage of rRNA reads

Sample	rRNA remaining (%)	Protein coding (%)
No treatment, R1	90.61	87.42
No treatment, R2	91.83	88.55
No treatment, R3	91.72	88.79
Poly-A enrichment, R1	1.56	90.46
Poly-A enrichment, R2	1.68	90.64
Poly-A enrichment, R3	1.74	90.48
Ribo-Zero Gold, R1	5.34	88.39
Ribo-Zero Gold, R2	4.75	88.04
Ribo-Zero Gold, R3	5.24	88.55
QIAseq FastSelect, R1	1.68	88.90
QIAseq FastSelect, R2	1.46	89.02
QIAseq FastSelect, R3	1.52	89.20



Experimental overview

- Sample: 100 ng Universal Human Reference RNA (Promega)
- Enrichment: Poly-A (+mRNA), Ribo-Zero (–rRNA), FastSelect (–rRNA)
- Library prep: QIAseq Stranded Total RNA Lib Kit
- Sequencing: NextSeq® 550 (2 x 75 bp)
- Mapping: CLC Genomics Workbench

FastSelect efficiently removes rRNA to levels observed with Poly-A enrichment, without disturbing protein coding genes.

FastSelect –rRNA HMR, like FastSelect V1, robustly removes rRNA

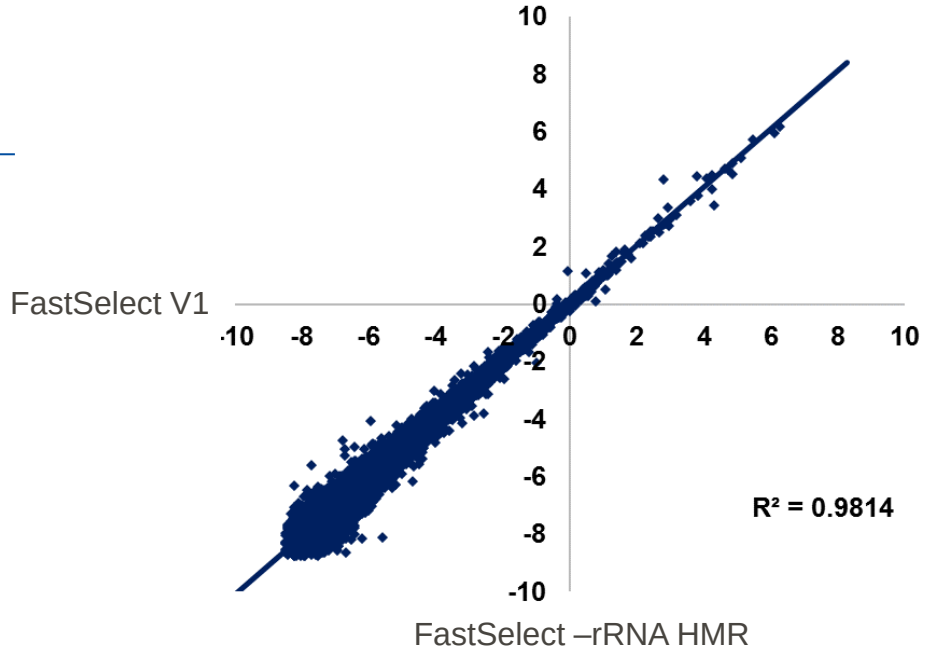
FastSelect substantially removes rRNA

Percentage of reads mapped to rRNA

Organism	FastSelect V1	FastSelect — rRNA HMR
Human, R1	2.60	1.15
Human, R2	2.15	1.27
Mouse, R1	7.18	2.43
Mouse, R2	7.74	2.38
Rat, R1	2.32	2.00
Rat, R2	3.01	2.07

FastSelect –RNA HMR correlates with FastSelect V1

Gene expression



Experimental overview

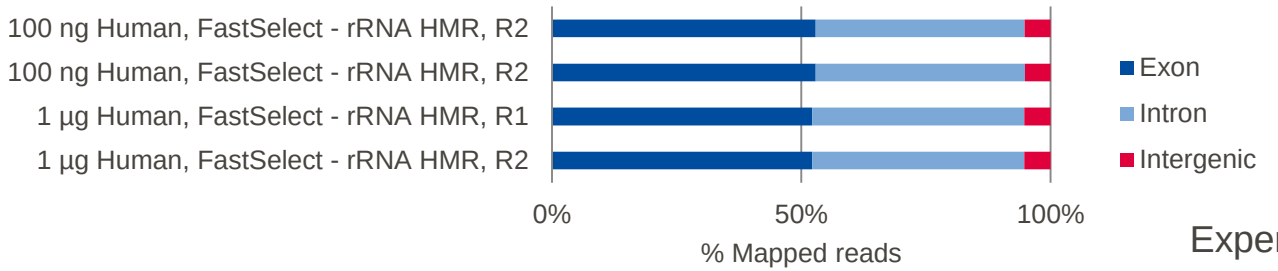
- Sample: 100 ng Universal Human, Mouse and Rat Reference RNAs
- Depletion: FastSelect V1, FastSelect –rRNA HMR
- Library prep: QIAseq Stranded Total RNA Lib Kit
- Sequencing: NextSeq 550 (2 x 75 bp)
- Mapping: CLC Genomics Workbench

Using a single-tube solution, FastSelect –rRNA HMR efficiently removes rRNA from human, mouse and rat samples. Highly similar results for human gene expression (not shown for mouse and rat) suggest FastSelect does not have spurious off-targets.

FastSelect –rRNA HMR: Consistent results at different inputs

Consistent exon mapping

Exon/intron/intergenic reads



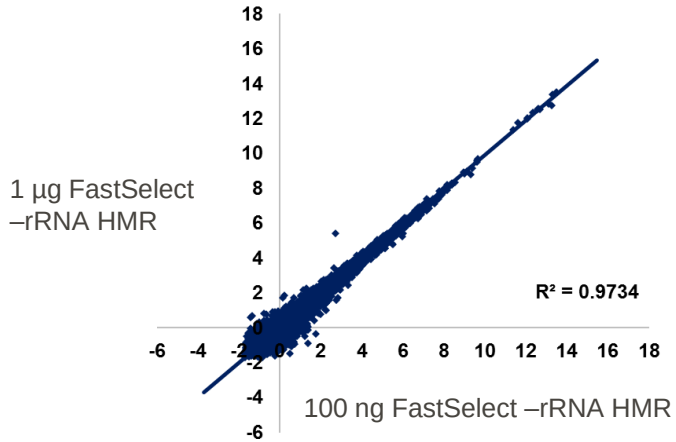
FastSelect substantially removes rRNA

Percentage of reads mapped to rRNA

Organism	RNA amount	FastSelect —rRNA HMR
Human, R1	1 µg	1.87
Human, R2	1 µg	2.17
Human, R1	100 ng	1.25
Human, R2	100 ng	1.41

Strong correlation between inputs

Gene expression analysis



Experimental overview

- Sample: 1 µg and 100 ng Universal Human Reference RNA
- Depletion: FastSelect –rRNA HMR
- Library prep: QIAseq Stranded Total RNA Lib Kit
- Sequencing: NextSeq 550 (2 x 75 bp)
- Mapping: CLC Genomics Workbench

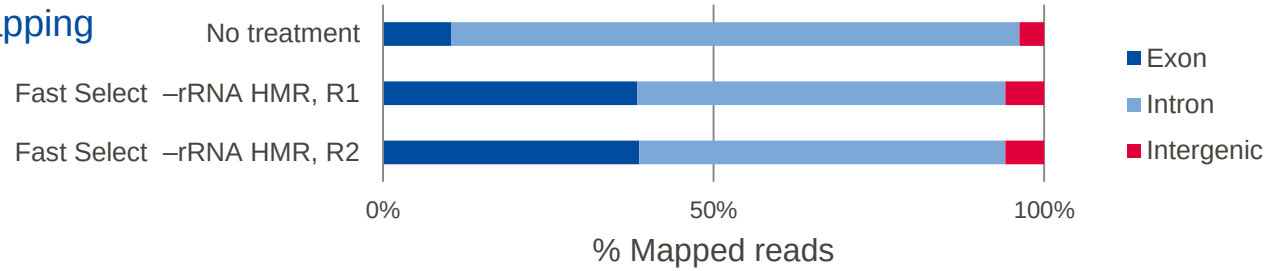
FastSelect efficiently removes rRNA, resulting in a consistent breakdown of mapped reads, regardless of RNA input. Gene expression values from FastSelect treatments are highly correlative, even between different RNA inputs (Log2 RPKM > 0.3).

FastSelect –rRNA HMR: Robust performance with FFPE samples

Increased/consistent exon mapping

Exon/intron/intergenic reads

Positive control



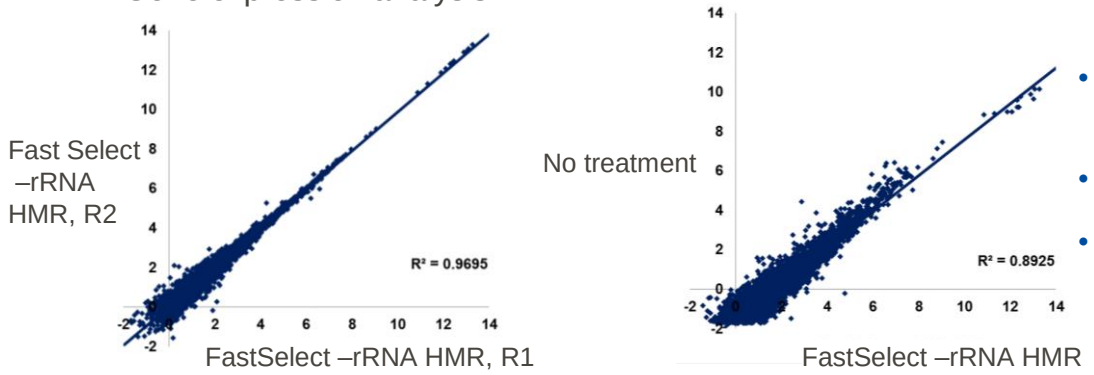
FastSelect substantially removes rRNA

Percentage of reads mapped to rRNA

Organism	Percentage of reads mapped to rRNA
No treatment	71.62
Fast Select –rRNA HMR,R1	2.62
Fast Select –rRNA HMR R2	2.83

Strong correlation: Replicates and treatments

Gene expression analysis



Experimental overview

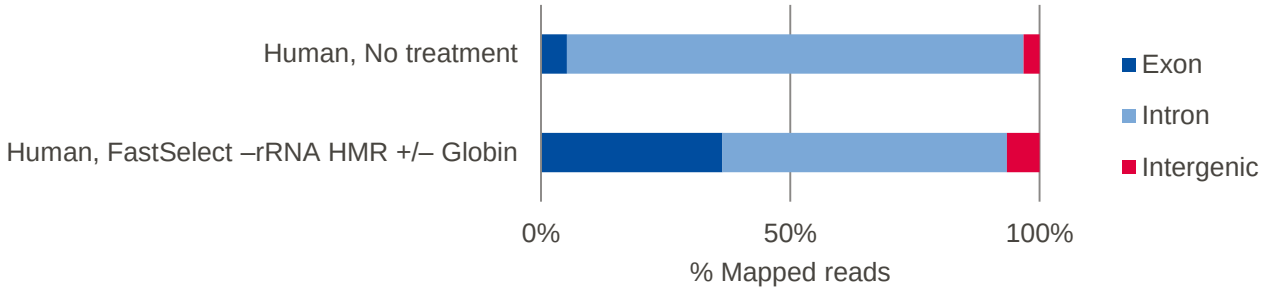
- Sample: 225 ng, Fusion RNA Positive Control (Horizon™)
- Depletion: No depletion, FastSelect –rRNA HMR
- Library prep: QIAseq Stranded Total RNA Lib Kit
- Sequencing: NextSeq 550 (2 x 75 bp)
- Mapping: CLC Genomics Workbench

FastSelect efficiently removes rRNA from FFPE samples, resulting in increased mapping to exons. Gene expression values from FastSelect treatments are highly correlative (Log2 RPKM > 0.3). Also, FastSelect does not have spurious off-target effects.

FastSelect –rRNA HMR +/– Globin: Robust removal of rRNA and Globin

Increased exon mapping

Exon/intron/intergenic reads



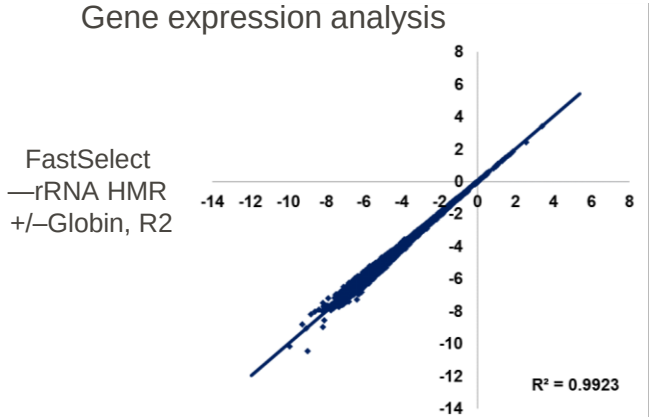
Substantial removal of rRNA and globin

Mapping metrics

Percentage of reads mapped to rRNA		Percentage of TPM mapped to Globin	
No treatment	**FastSelect —rRNA HMR +/–Globin**	**No treatment**	**FastSelect —rRNA HMR +/–Globin**
88.50	1.16	82.46	0.16

Strong correlation: Replicates

Gene expression analysis



Experimental overview

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

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).

Agenda

Background

Mammalian rRNA depletion

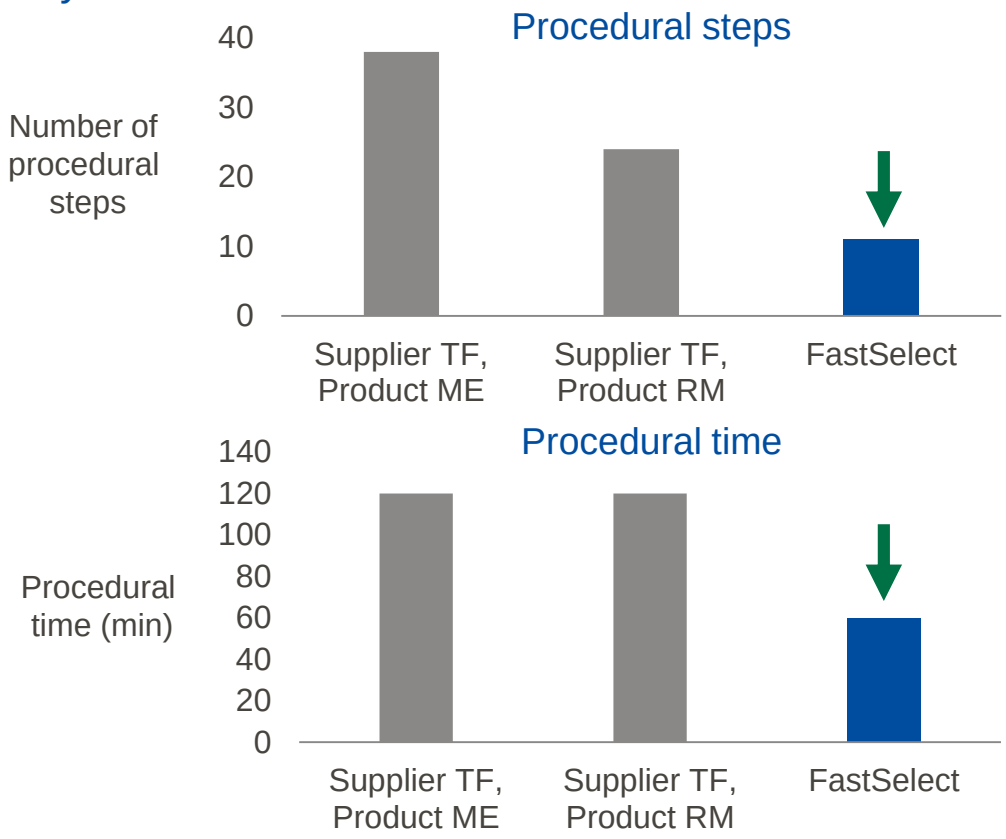
Bacterial rRNA depletion

Summary

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

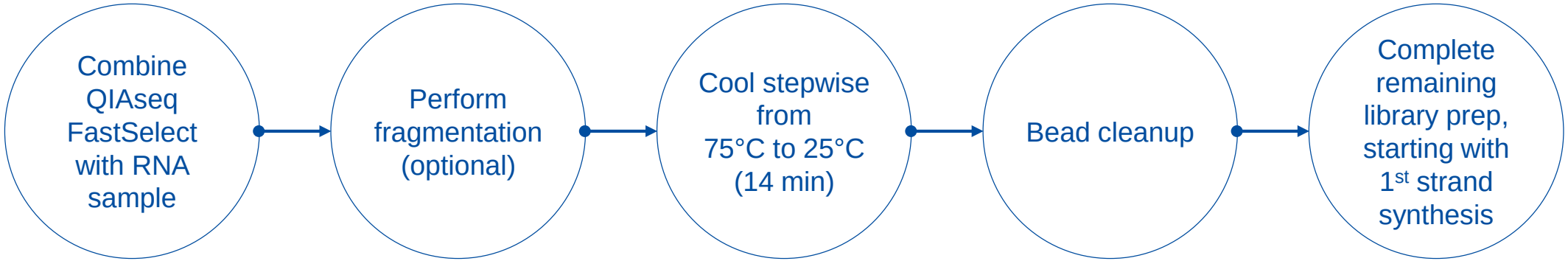
Add FastSelect reagent and ramp down temperature
(1 step)

Bead cleanup
(11 steps)

Library Prep

- FastSelect offers an attractive, streamlined workflow versus kits from other suppliers.

FastSelect –5S/16S/23S overview



NEW

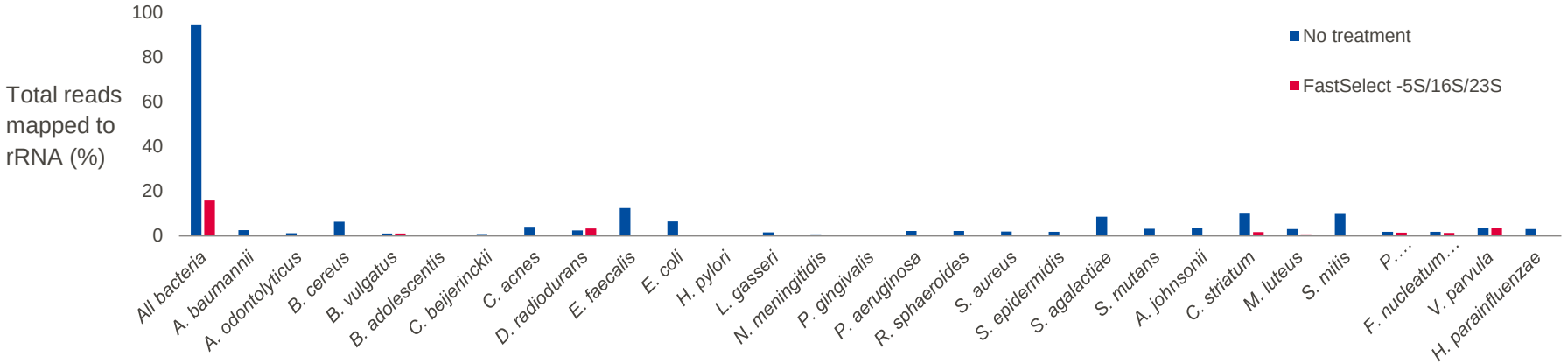
FastSelect –5S/16S/23S overview

- 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 23 rRNA sequences
- Total RNA input:
  - 5 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

Robust depletion of rRNA from individual species

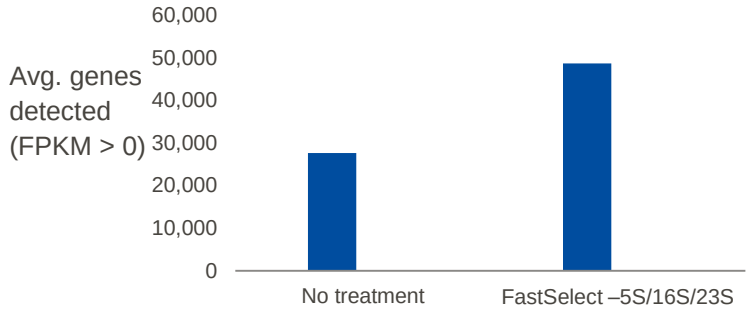
Percentage of reads mapped to rRNA (total)



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

Increased detected genes



Experimental overview

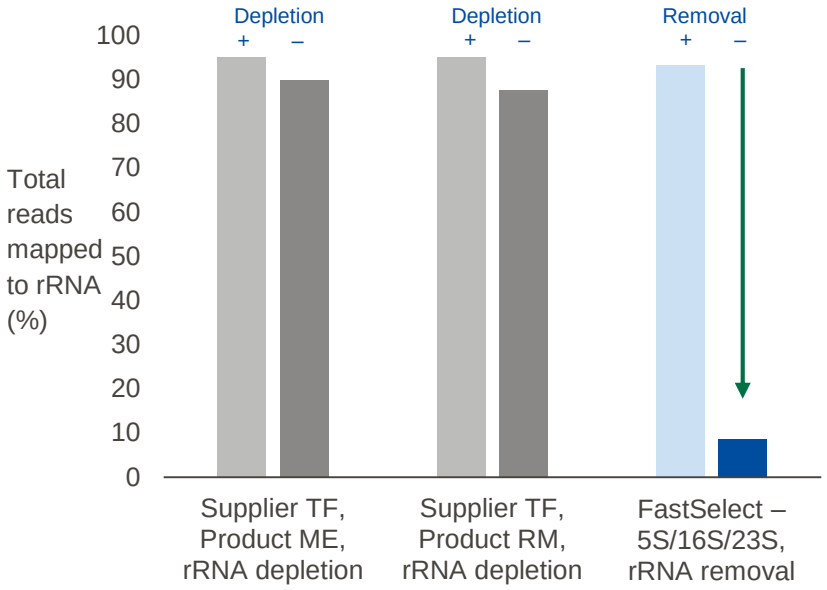
- Sample: 100 ng, 20 Strain Even Mix Whole Cell Material (ATCC) + Skin Microbiome Whole Cell Mix (ATCC) + Oral Microbiome Whole Cell Mix (ATCC)
- Depletion: No depletion, FastSelect –5S/16S/23S
- Library prep: QIAseq Stranded Total RNA Lib Kit
- Sequencing: NextSeq 550 (2 x 75 bp)
- Mapping: CLC Genomics Workbench

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

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

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

Percentage of reads mapped to rRNA



FastSelect substantially removes rRNA, freeing up reads for gene detection

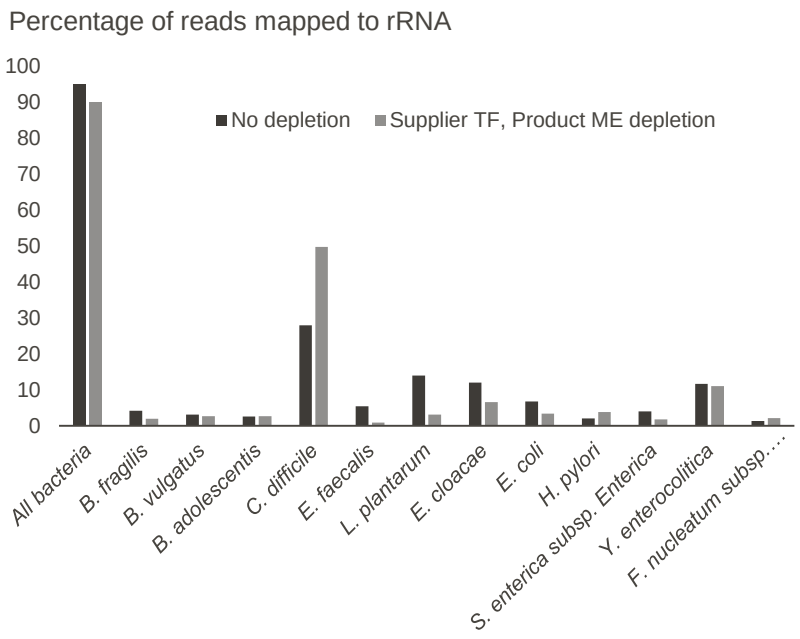
Experimental overview

- 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 Total RNA Lib Kit
- Sequencing: NextSeq 550 (2 x 75 bp)
- Mapping: CLC Genomics Workbench

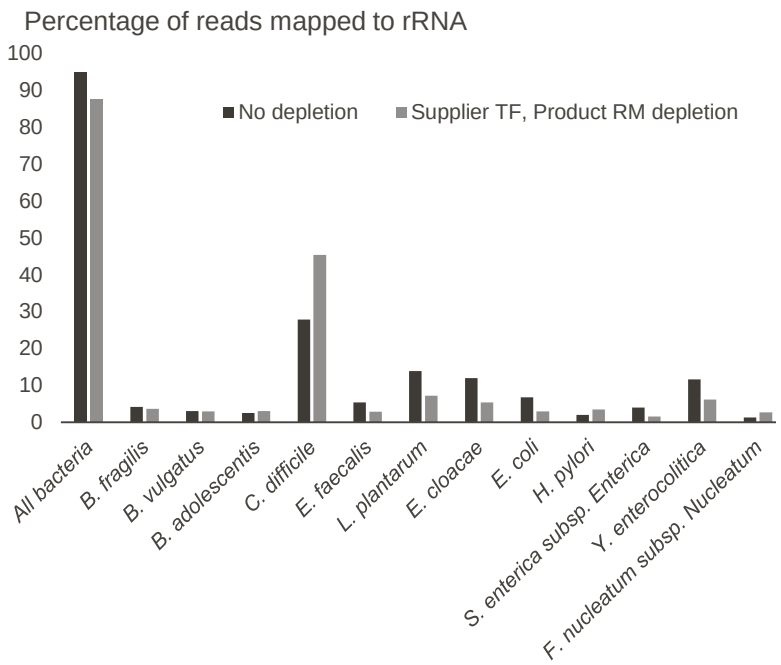
FastSelect efficiently removes rRNA, while Supplier TF's rRNA depletion products do not. The robust rRNA removal frees up a substantial amount of sequencing reads (9X compared to the other supplier's kits) for gene expression analysis.

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

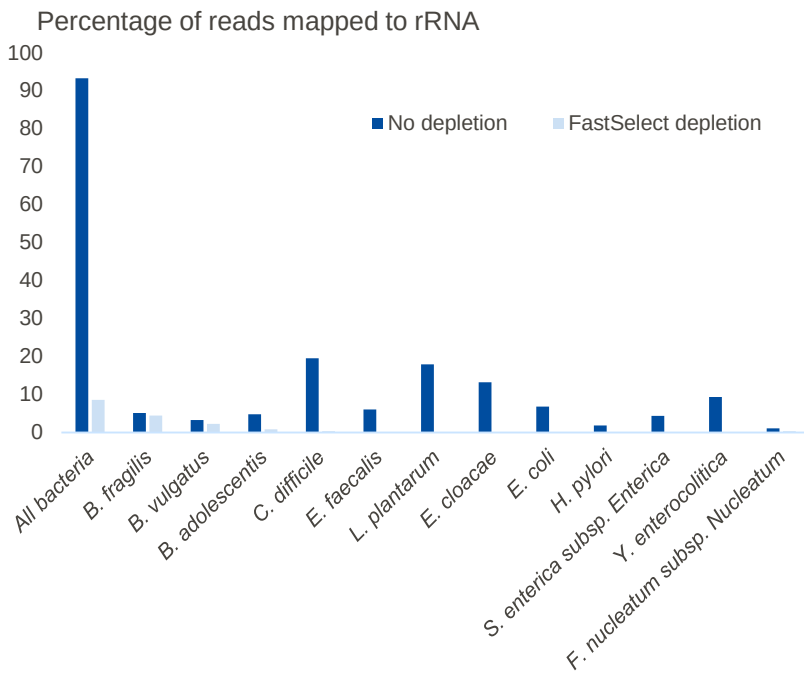
Supplier TF, Product ME



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 robustly removes rRNA from single-species samples

FastSelect substantially removes rRNA

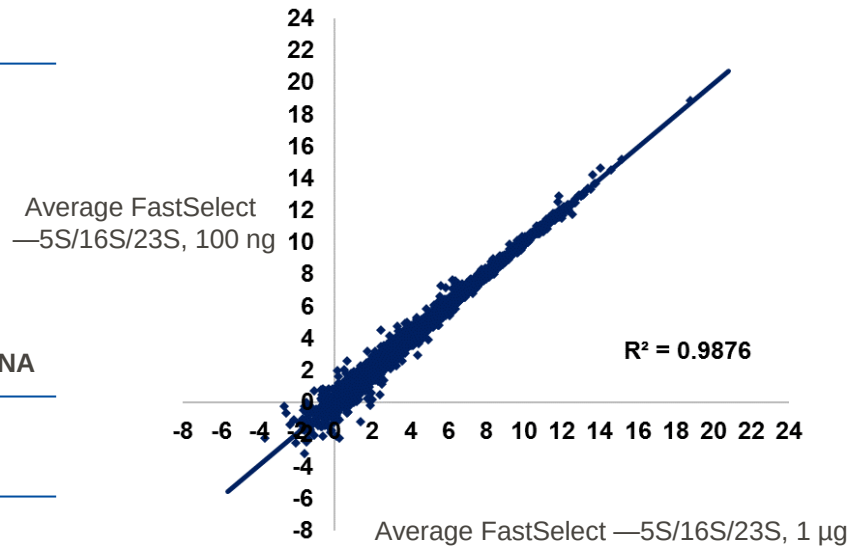
Percentage of reads mapped to rRNA

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 correlation between inputs

Gene expression analysis



Experimental overview

- Sample: 1 µg and 100 ng, DH5α *E. coli* total RNA (Thermo Fisher)
- 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 efficiently removes rRNA. Gene expression values from FastSelect-treated samples, even at different RNA input amounts, are highly correlative (Log2 RPKM > 0.3).

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Mammalian rRNA depletion

Bacterial rRNA depletion

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

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

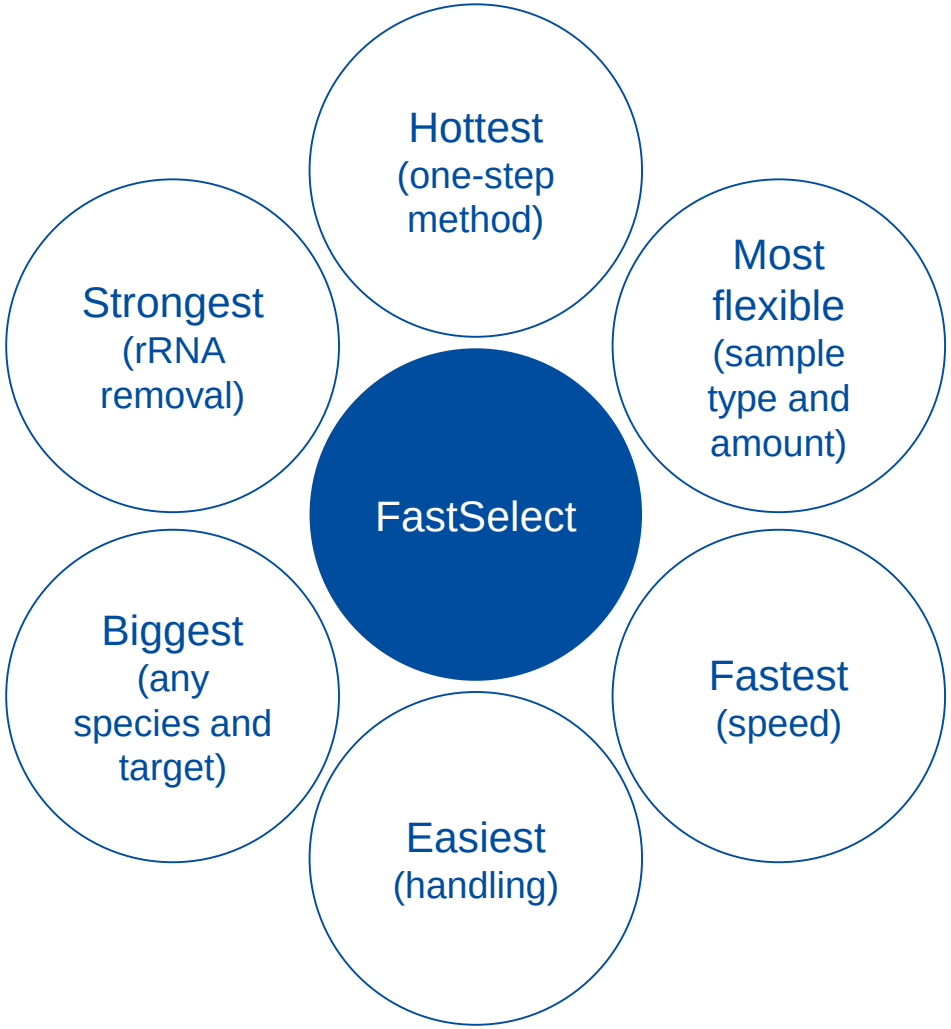
- Thirty percent faster than FastSelect V1, now in a single-tube
- Covers human, mouse, rat (HMR) and other mammalian species
- Compatible with several sample types, RNA types and input amounts and RNA library prep kits

FastSelect –5S/16S/23S

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

QIAseq FastSelect: An unparalleled unwanted RNA removal solution



Thank you for attending

Questions?

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