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2020

ZYMO RESEARCH
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Accurate probe-free ribosomal depletion

RNA-seq made simple

By Casey Cruz, Ken Vittayarukskul, Justin J. Lin

INTRODUCTION:
RNA analysis is rapidly becoming a standard assay in the molecular biologist's toolkit. Applications range from gene expression analysis, novel transcript discovery, and alternative splicing analysis to the detection of gene-fusion events and SNPs in expressed RNA transcripts[1].

A major challenge in RNA-Seq analysis is the abundance of ribosomal RNA (rRNA), which can comprise up to 95% of library reads. Poly(A)-enrichment has been a common strategy used to select for the 3′ poly-adenylated tail found on mRNA in order to produce protein-coding RNA libraries. However, poly(A)-enrichment is not effective for prokaryotic RNA (which lack 3′ poly-adenylation), degraded RNA samples, and does not measure nascent, pre-processed RNA.

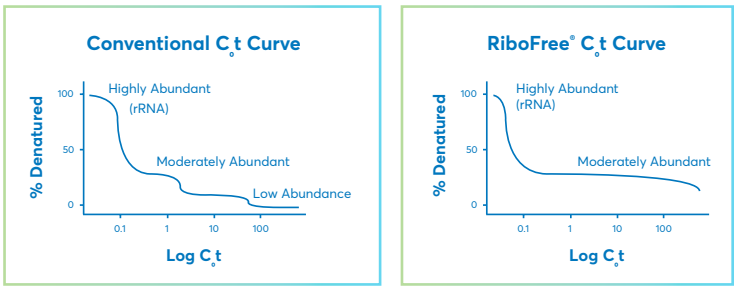
A more recent strategy, ribosomal removal, depletes the sample of rRNA sequences using complementary oligonucleotide probes to target and remove ribosomal sequences by magnetic bead-based separation, or enzymatic digestion. However, the off-target effects of probe-based ribosomal depletion (compared to the untreated, native transcriptome) are rarely addressed[2].

We demonstrate that off-target effects from probe-based ribosomal depletion are significant but can be avoided using a probe-free method based on the principle of DNA reassociation kinetics, available as RiboFree® Universal Depletion.

The RiboFree® method integrates seamlessly into library preparation after cDNA synthesis, allowing researchers to prepare ribo-depleted total RNA-seq libraries in as few as 3.5 hours. Because RiboFree® Universal Depletion is probe-free, it is universally applicable to any sample type or species, from human whole blood to plant and prokaryote RNA.

RIBOFREE® UNIVERSAL DEPLETION:
Complementary sequences in a denatured, heterogeneous pool re-hybridize at a speed proportional to their initial concentrations[3]. Nucleases specific to double-stranded DNA can be added to the re-association reaction to remove sequences from the pool as they anneal. Sequences present at specific abundances (such as highly abundant repetitive elements) can be selectively removed by timing the dsDNA digestion to specific stages of the reassociation curve[4].

Zymo Research greatly simplifies this process with RiboFree® Universal Depletion. Re-hybridization is performed after cDNA synthesis, allowing the researcher to capture transcript abundances directly from the native, untreated sample. Because the enzyme used in RiboFree® Universal Depletion removes only the cDNA strand in a cDNA/RNA hybrid, highly repetitive RNA



FIGURES 1 & 2: Representative plots demonstrating nucleic acid sequence rehybridization kinetics in either conventional or RiboFree® reaction conditions.

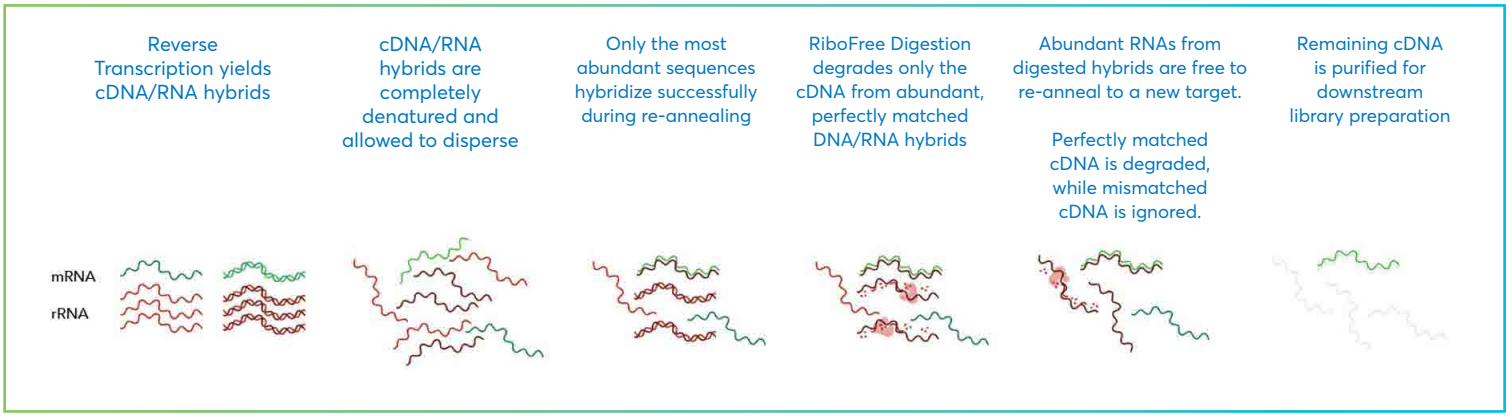


FIGURE 3: Schematic of RiboFree® Universal Depletion Reaction

transcripts (e.g., Globin, rRNA) are free to hybridize to another target for highly efficient removal of ribosomal cDNAs under multi-turnover kinetics (abundant transcripts can initiate multiple rounds of target hybridization and cDNA depletion). Meanwhile, the enzyme and buffers in the RiboFree® reaction are optimized to be mismatch intolerant – only perfectly matched sequences are digested, while imprecisely hybridized sequences are ignored, resulting in accurate representation of the untreated transcriptome without off-target removal of mis-hybridized cDNAs.

MATERIALS AND METHODS:

RNA samples

To evaluate each technology on a set of controlled samples that can be easily obtained for reproducibility, we utilized the Universal Human Reference RNA (UHR).

To evaluate the performance of the RiboFree® Universal Depletion of ribosomal RNA across diverse species, we purchased Universal Human Reference RNA, Human Leukocyte Reference RNA, Mus musculus reference RNA, and Rattus norvegicus reference RNA. RNA representing plant (Arabidopsis thaliana) and prokaryote (Escherichia coli) were extracted using the Zymo Research Quick-RNA Kit (VWR Cat. No. 76020-388).

Library Preparation and Sequencing

RNA-Seq libraries were prepared in duplicate from 500ng RNA samples, using either Illumina's probe-based pulldown to deplete rRNAs, or Zymo Research's probe-free enzymatic depletion. Control samples were similarly prepared in duplicate, without any treatment, for each library kit workflow. Libraries were indexed and analyzed using a TapeStation 2200 (Agilent) instrument for quality control and normalized by ZymoTaq Library Quantification. Normalized libraries were then pooled and sequenced on an Illumina HiSeq 2500 at an average sequencing depth of ≥35 million reads per library.

Sequencing and Analysis

Reads were adapter-trimmed according to library kit manufacturer recommendations and aligned against hg38/ensembl92 annotation using STAR v2.7.0f with the following flags:

args = c("--outReadsUnmapped", "Fastx",
"--outSAMmultNmax", "1",
"--outSAMtype", "BAM Unsorted",
"--limitOutSJcollapsed", "2000000"),

Count tables were created using featureCounts from the R (3.6.2) Rsubread (2.0.1) package using the following flags:

args = c("--outReadsUnmapped", "Fastx",
"--outSAMmultNmax", "1",
"--outSAMtype", "BAM Unsorted",
"--limitOutSJcollapsed", "2000000"),

RiboZero, poly(A)-plus mRNA, and probe-free RiboFree® libraries were compared to their respective Untreated control libraries using standard differential expression analysis using DESeq2 (version)

For absolute transcript abundance, the NIST ERCC RNA Spike-in Standards from Ambion™ were added at a concentration of 3% total RNA abundance to Universal Human Reference RNA.

RNA-Seq libraries were prepared from 500ng total RNA, and either treated with the probe-free, RiboFree® Universal Depletion, or left untreated. ERCC transcript read counts were calculated using the featureCounts function from Rsubread, normalized using the RPKM function from Rsubread, and plotted against the known molar concentration of each ERCC Spike-in Standard transcript.

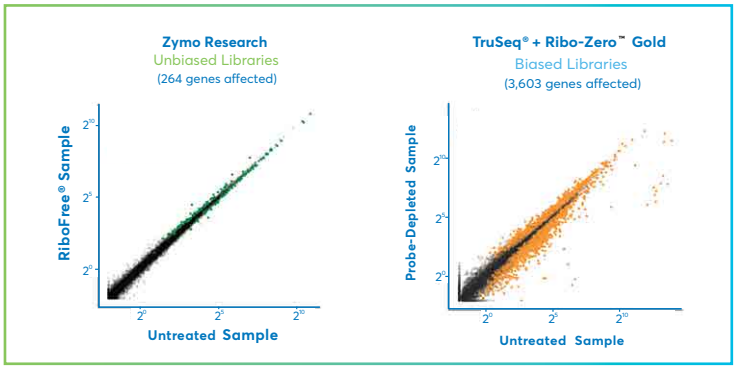


FIGURE 4: Scatterplot representation of transcriptome profile differences between untreated samples (X-axes) and depleted samples (Y-axes) using either the RiboFree® Total RNA Library Kit (left), or the TruSeq® Total RNA Kit with Ribo-Zero™ Gold (right). Genes statistically biased (p.adj ≤ 0.05) are colored in green (Zymo) or orange (Illumina), respectively.

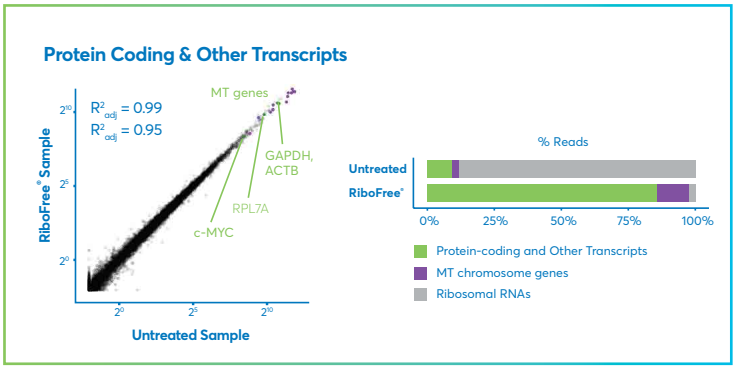


FIGURE 5: Measurement of specific, high-abundance transcripts within the context of transcriptome profile comparison between untreated samples and RiboFree® samples using either scatterplot representation (left) or stacked barplot representation (right). Highly abundant genes are not affected by RiboFree® Universal Depletion.

RESULTS:

RiboFree® Universal Depletion avoids bias found in probe-based depletion:

We compared a leading total RNA library kit, TruSeq with Ribo-Zero Gold, to the Zymo-Seq RiboFree® Total RNA Library Kit. Both ribo-depleted and untreated libraries were prepared in replicate from each library kit.

Since differential gene expression analysis is commonplace in RNA-Seq experiments, we used DESeq2 (a commonly used open-source software package for differential gene expression analysis) to measure the number of protein-coding genes for which differential expression between depleted and untreated samples were flagged as statistically significant[6]. We find that probe-based ribosomal depletion results in as many as 3,603 genes (18%) passing the $p < 0.05$ filter for statistically significant differential expression, while the enzymatic probe-free depletion (RiboFree®) results in only 264 genes (1.3%) passing the same filter (Fig. 4).

Significantly differential genes as determined by DESeq2 are shown as colored dots in the scatterplots above. Average protein-coding gene abundances from ribo-depleted libraries are plotted on the Y-axis, while protein-coding gene abundances from untreated libraries are plotted on the X-axis.

Highly expressed mRNAs are not affected by RiboFree® Universal Depletion

As RiboFree® Universal depletion is based on transcript abundance, we examined a suite of highly expressed housekeeping genes (Fig. 5) to verify that RiboFree® depletion preserves the absolute abundance of highly expressed mRNAs. Genes such as GAPDH and β-actin are not significantly affected by RiboFree® Universal Depletion. Indeed, even mitochondrial genes, which are expressed more highly by an order of magnitude, are not affected by RiboFree® depletion (R2 = 0.98).

ERCC Spike-in Control Transcripts are accurately represented by RiboFree® Total RNA libraries

To further validate the accuracy of RiboFree® depletion, we used the ERCC Spike-in controls as a source of absolute truth. The ERCC RNA Spike-In Control Mixes are a pre-formulated set of 92 poly-adenylated transcripts from the ERCC plasmid reference library (NIST). These controls span a 106-fold concentration range with varying transcript size and GC content.

ERCC Spike-in control RNA was added to Universal Human Reference RNA at 3% total RNA abundance. Shown are scatterplots where ERCC Mix 1 (top) abundances from Untreated (left) and RiboFree® libraries (right) are plotted on the Y-axis, while the absolute abundances of the respective ERCC Spike-in Controls are plotted on the X-axis.

We find that the detection of ERCC Spike-in control RNA is improved in RiboFree (R2 = 0.93) compared to native samples (R2 =0.91), and that the high-abundance tier of ERCC Spike-in control transcripts is unaffected by RiboFree® depletion.

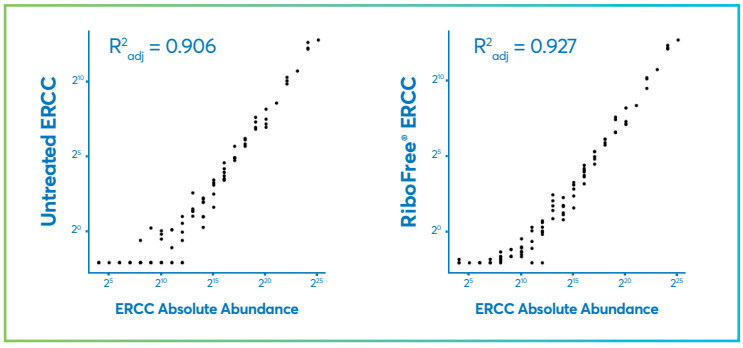


FIGURE 6: Measurement of ERCC Mix 1 spike-in transcripts (Y-axes) in untreated samples (left) and RiboFree® samples (right), against known ERCC Mix 1 abundances (plotted on the X-axes).

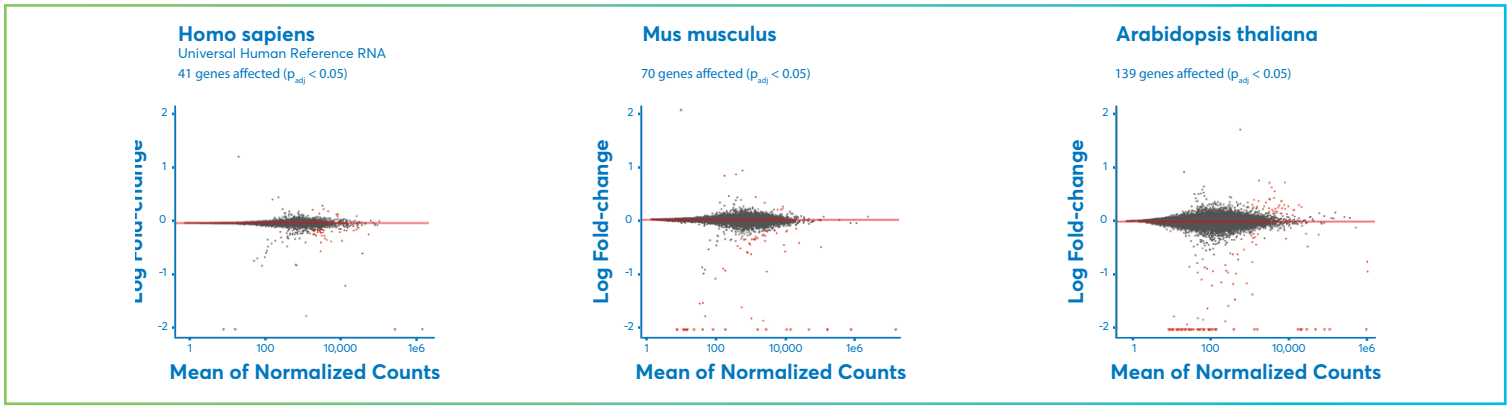


FIGURE 7: Mean-abundance (MA) plots of "differential expression" of genes affected by RiboFree depletion in human (left), mouse (middle), and plant (right) samples. Genes statistically significant (p.adj ≤ 0.05) are plotted in red.

RiboFree® Universal Depletion is compatible with diverse sample types

Since probe-free depletion is based on transcript abundance, and is not sequence-specific, we prepared RiboFree® Total RNA libraries from Universal Human Reference RNA (Ambion), Human Clinical Leukocyte Sample, Mouse Reference RNA (Ambion), Rat Reference RNA (Ambion), Arabidopsis RNA, and *E. coli* RNA, to compare transcriptomes with and without ribo-depletion.

Stacked bar charts represent Untreated and RiboFree® libraries. Reads corresponding to 18S and 28S ribosomal RNAs are grey, while reads corresponding to protein-coding and other transcripts are green.

Libraries were sequenced at a depth of >10 million reads/sample to test the compatibility of the protocol with a range of diverse organisms. Removal of cDNAs complementary to ribosomal RNA was efficient across all species and sample types tested. We also examined the effect of RiboFree® depletion on gene expression analysis and found that genes significantly biased by RiboFree® removal were minimal in human (41 genes), mouse (70 genes), and plant (139 genes) RNA. RiboFree® depletion is both efficient and specific for RNA from species spanning diverse branches of the tree of life.

SUMMARY

The RiboFree® Total RNA Library Kit integrates universal, probe-free depletion into total RNA library preparation and avoids off-target biases found in other probe-based protocols. Because the probe-free depletion targets only highly abundant sequences that were present to begin with, the RiboFree® Total RNA Library Kit is compatible with any sample type or species.

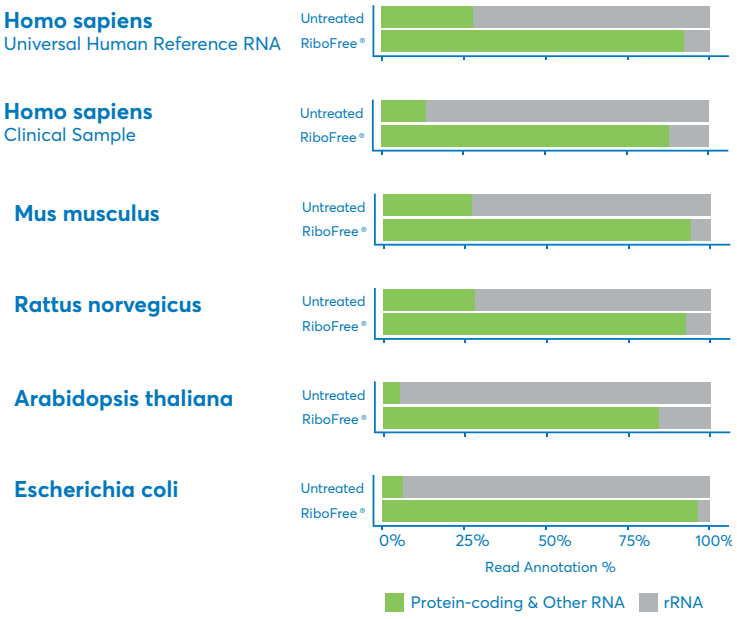


FIGURE 8: Stacked barplot representation of reads corresponding to either rRNA or protein-coding and other RNA transcripts from untreated or RiboFree samples in a wide range of species and sample types spanning the tree of life. Samples were prepared simultaneously using the sample protocol.

REFERENCES

1. Wang Z, Gerstein M, Snyder M. 2009. RNA-Seq: a revolutionary tool for transcriptomics. Nat Rev Genet. 10(1):57–63. doi:10.1038/nrg2484
2. Zhao S, Zhang Y, Gamini R, Zhang B, von Schack D. 2018. Evaluation of two main RNA-seq approaches for gene quantification in clinical RNA sequencing: polyA+ selection versus rRNA depletion. Sci Rep. 8(1):4781. doi:10.1038/s41598-018-23226-4
3. Britten RJ, Graham DE, Neufeld BR. 1974. Analysis of repeating DNA sequences by reassociation. Methods Enzymol. 29:363–418. doi:10.1016/0076-6879(74)29033-5
4. Yi H, Cho YJ, Won S, et al. 2011. Duplex-specific nuclease efficiently removes rRNA for prokaryotic RNA-seq. Nucleic Acids Res. 39(20):e140. doi:10.1093/nar/gkr617
5. Anders S, Huber W. 2010. Differential expression analysis for sequence count data. Genome Biol. 11(10):R106. doi:10.1186/gb-2010-11-10-r106

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Direct-zol™ RNA Microprep Kit	Spin Column	50 Preps	≤1×10⁶ Cells	≥6 µl	**76020-456**
Direct-zol™ RNA Microprep Kit	Spin Column	200 Preps	≤1×10⁶ Cells	≥6 µl	**76211-324**
Direct-zol™ RNA Miniprep Plus Kit	Spin Column	50 Preps	≤1×10⁷ Cells	≥50 µl	**76020-110**
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AccuBlue® High Sensitivity dsDNA quantitation Kit with 8 DNA standards	1000 assays	**89139-008**
AccuBlue® High Sensitivity dsDNA quantitation solution	1000 assays	**89139-012**
AccuBlue® High Sensitivity dsDNA standards, set of 8	0.5 ml each	**89493-588**
AccuBlue® NextGen dsDNA Quantitation Kit		
AccuBlue® NextGen dsDNA quantitation Kit	500 assays	**76204-714**
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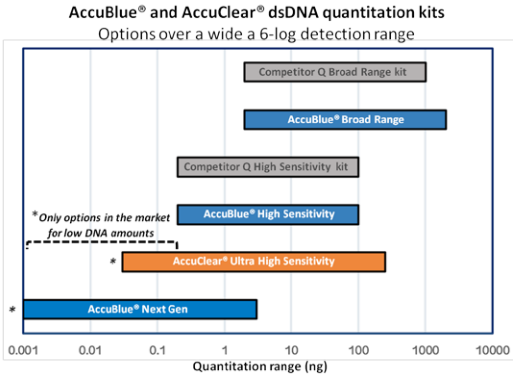
AccuClear® Ultra High Sensitivity dsDNA quantitation solution	1000 assays	**89427-076**
AccuClear™ Ultra High Sensitivity dsDNA Quantitation Kit with 7 DNA Standards	1000 assays	**89427-078**



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Selective depletion of abundant RNAs to enable transcriptome analysis of low-input and highly-degraded RNA from FFPE breast cancer samples

By Daniela B Munafó[1], Bradley W Langhorst[1] Christine L Chater[1], Christine J Sumner[1] Deyra N Rodríguez[1], Salvatore Russello[1], Fiona J Stewart[1] Dominick Sinicropi[2], John Morlan[2], Kunbin Qu[2], Eileen T Dimalanta[1] and Theodore B Davis[1] , [1] New England Biolabs, Ipswich, MA; [2] Genomic Health, Inc., Redwood City, CA.

INTRODUCTION

Deep sequencing of cDNA prepared from total RNA (RNA-seq) has become the method of choice for transcript profiling and discovery. However, the standard whole-transcriptome approach faces a significant challenge, as the vast majority of reads map to ribosomal RNA (rRNA). One solution is to enrich the sample RNA for polyadenylated transcripts using oligo (dT)-based affinity matrices; although, this also eliminates other biologically relevant RNA species, such as microRNAs and noncoding RNAs, and relies on having a high-quality and high-quantity RNA sample.

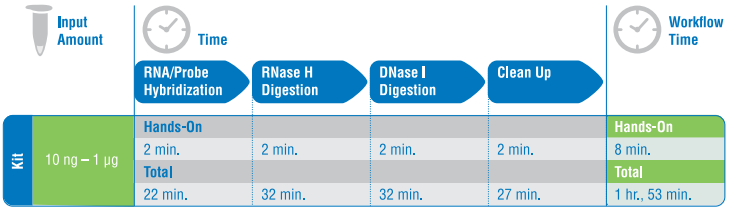


Figure 1. NEBNext® rRNA Depletion Kit workflow input amounts and times

Here, we present a method to eliminate abundant RNAs from total RNA with different degradation levels, from intact RNA to highly degraded formalin-fixed paraffin-embedded (FFPE) samples. This method is based on hybridization of probes to the targeted abundant RNA, followed by subsequent enzymatic degradation (Figures 1 & 2).

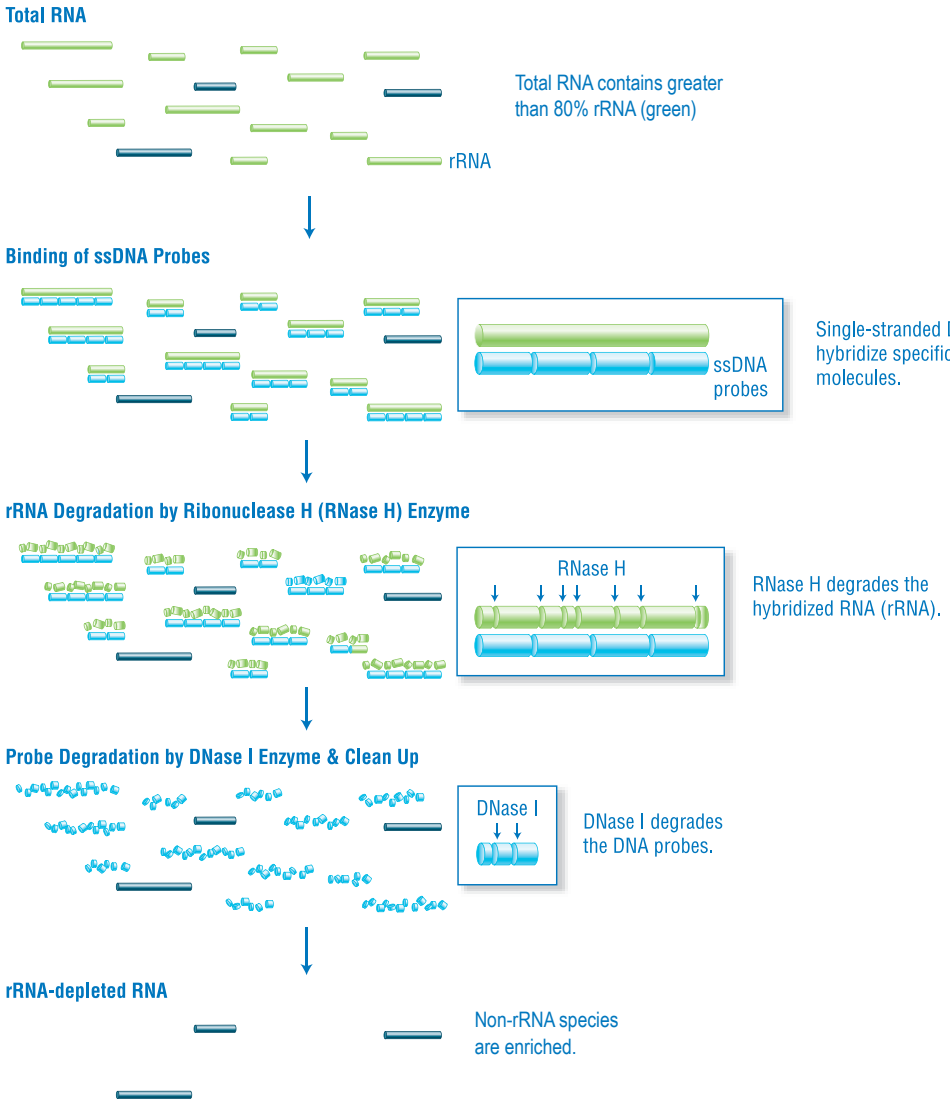


Figure 2. NEBNext rRNA Depletion Kit workflow. Total RNA (0.1-1 µg) is hybridized with single stranded DNA probes targeting cytoplasmic (5S, 18S, 28S, 5.8S rRNAs) and mitochondrial (12S and 16S rRNAs) ribosomal RNA, followed by RNase H digestion to degrade targeted RNA. Finally, DNA probes are digested with DNase I. The ribosomal-depleted RNA is purified using Agencourt RNAClean XP beads. Ribosomal RNA depletion can be immediately followed by RNA-seq library preparation.

We applied this method to remove cytoplasmic and mitochondrial rRNA from different eukaryotic total RNA samples (human, mouse, and rat), as well as degraded (archive age of 1 year) and highly-degraded (archive age of 10 years) human FFPE breast tumor biopsy RNA samples.

MATERIALS AND METHODS

For the full protocol, see the full-length technical article on **vwr.com/neb.**

RESULTS

The NEBNext® rRNA Depletion Kit is effective on RNAs of varying quality, from low quality (FFPE 1–10 years) to high quality.

Multiple RNA samples were processed using the NEBNext® rRNA Depletion Kit in order to examine effectiveness with different RNA degradation levels: "10-year FFPE RNA" was a pool of equal portions of RNA extracted from eight FFPE breast cancer samples with an approximate archive age of 10 years. "1-year FFPE RNA" was a pool of equal portions of RNA extracted from >100 breast tumor biopsy samples with an archive age of one year. "UHR", or Universal Human Reference DNA, is a commercially available non-degraded RNA (Agilent). RNA profiles before and after rRNA depletion were analyzed by an Agilent® Bioanalyzer® on an RNA Pico chip (Figure 3).

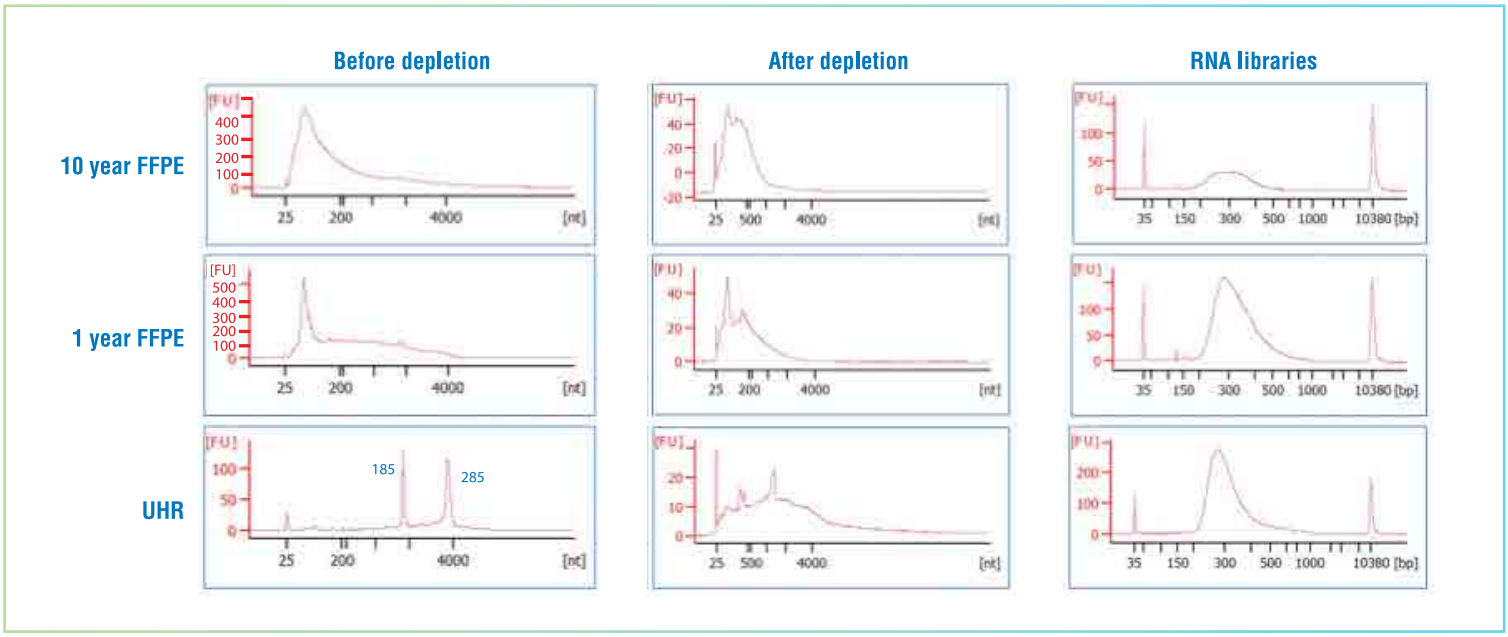


Figure 3. Comparison of rRNA depletion on RNA samples of varying quality. RNA samples of varying quality were analyzed on a Bioanalyzer® using an RNA Pico chip before and after rRNA depletion, and depleted libraries were analyzed on a Bioanalyzer® using a High-Sensitivity DNA chip.

Directional RNA-Seq libraries were then made from rRNA-depleted RNA and from non-depleted total RNA (not shown) using the NEBNext® Ultra™ Directional RNA Library Prep Kit for Illumina® (VWR Cat. No. 102715-920). Libraries were analyzed on a Bioanalyze® using a DNA High sensitivity chip (Figure 3).

As can be seen in the intact UHR sample, two distinct peaks, corresponding to the ribosomal subunits 18S and 28S, are visible in the sample that was not depleted ("Before Depletion"). These peaks are not visible in the 10-year or 1-year FFPE samples, because the RNA is degraded. After depletion, the 18S and 28S peaks are no longer present, indicating that rRNA depletion was successful. All rRNA-depleted samples were used to make high-quality RNA libraries, with optimal size distribution for subsequent RNA-seq experiments.

The NEBNext® rRNA Depletion Kit offers superior performance with fragmented and FFPE RNA

To further examine rRNA depletion efficiency, performance was assessed on different input amounts of FFPE RNA, and on intact and fragmented UHR RNA. Total RNA was treated with the NEBNext® rRNA Depletion Kit, Ribo-Zero® Gold (Epicentre®) or the Ribo-Zero Gold rRNA depletion reagents provided within the TruSeq® Stranded Total RNA Kit with Ribo-Zero Gold (Illumina®).

Libraries were made from samples depleted of rRNA and sequenced on the Illumina® platform. The percentage of sequence reads that aligned to rRNA was then determined.

NEBNext® rRNA-depleted libraries contained a minimal percentage of total reads that aligned to rRNA subunits, regardless of the quality of the input RNA (FFPE, Fragmented, or intact UHR). In contrast, Ribo-Zero rRNA-depleted libraries

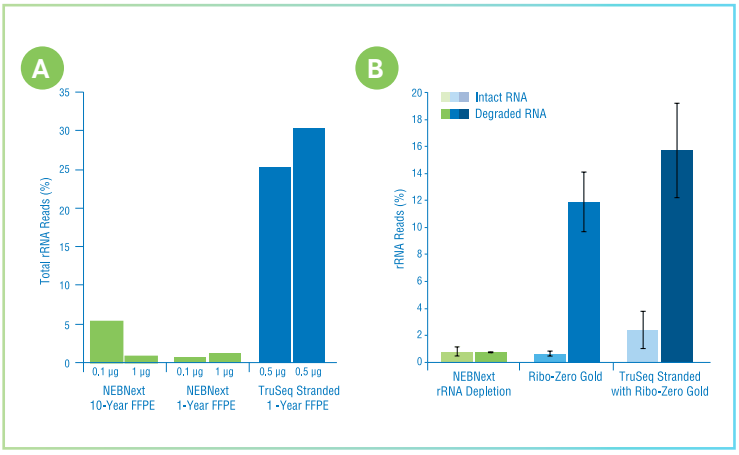


Figure 4. rRNA depletion efficiency. rRNA was depleted from human breast cancer FFPE Total RNA (Panel A) or from intact and fragmented Universal Human Reference Total RNA (UHR, Panel B) using the NEBNext® rRNA Depletion Kit, Ribo-Zero® Gold or the Ribo-Zero Gold reagents provided within the TruSeq™ Stranded Total RNA Kit. rRNA-depleted RNA libraries were made using either the NEBNext® Ultra™ Directional RNA Library prep or the TruSeq Stranded Total RNA Kit. Total reads were aligned to the cytoplasmic (5S, 18S, 5.8S and 28S) and mitochondrial (12S and 16S) ribosomal RNA subunits using Bowtie 2.0 (local, sensitive).

resulted in a higher percentage of reads (>10% total reads) mapping to rRNA for the degraded samples (FFPE RNA or fragmented RNA) (Figure 4).

Transcript Composition

The composition of transcripts after rRNA depletion was assessed by determination of the proportion of reads mapping to annotated exons, introns, and intergenic regions, and this was compared to the composition of non-depleted total RNA and of poly(A) mRNA-enriched RNA.

Libraries generated from rRNA-depleted RNA resulted in low rRNA reads, comparable to poly(A) mRNA-enriched RNA, while also retaining more noncoding reads. Effective rRNA depletion efficiency was achieved even with FFPE RNA. The exonic ratio was constant between total RNA input amounts of 100ng (shown) and 1µg (not shown). FFPE RNA contained a higher percentage of intronic reads than fresh RNA, as previously reported[1] (Figure 5).

The NEBNext rRNA Depletion Kit also effectively depletes rRNA from mouse and rat samples

To assess rRNA depletion efficiency for other eukaryotic total RNA, rRNA was depleted from mouse and rat kidney total RNA using the NEBNext rRNA depletion method. Libraries were made and sequenced on the Illumina platform, and the corresponding percentage of rRNA-aligned reads was determined (Figure 6).

Transcript expression correlation with non-depleted sample libraries

To investigate any effects on non-ribosomal RNA, correlation of transcript expression was determined between rRNA-depleted and non-depleted samples, for UHR and FFPE samples. FPKM (Fragments Per Kilobase of transcript per Million mapped reads) correlation analysis of sequencing reads from libraries generated with these samples indicated very good transcript expression correlation (R>0.93) between rRNA-depleted and non-depleted libraries. Moreover, NEBNext® rRNA depletion did not alter transcript expression levels (Figure 7).

CONCLUSION

Regardless of the quality or amount of input RNA, this method efficiently removes rRNA, while retaining non-coding and other non-poly(A) RNAs that are lost with oligo d(T) poly(A) mRNA enrichment methods, offering a more complete picture of the transcript repertoire.

REFERENCES:

1. Morlan, JD, et al. 2012. Selective depletion of rRNA enables whole transcriptome profiling of archival fixed tissue. PLOS One (7):e42882.

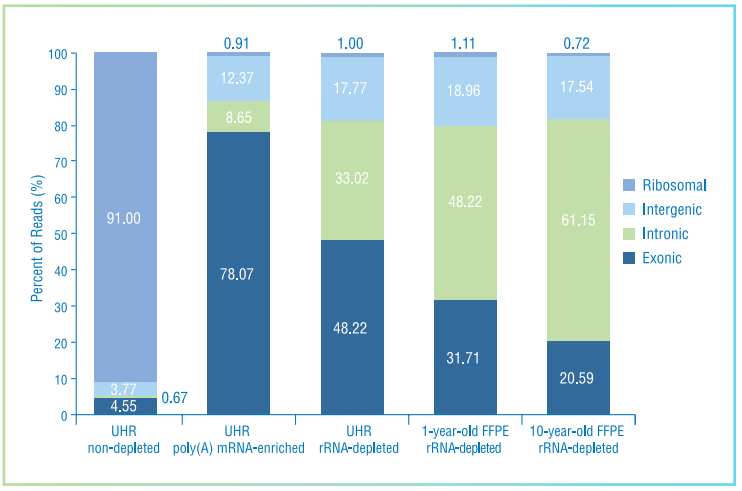


Figure 5. Read distribution across transcripts. RNA-seq libraries were generated from Universal Human Reference Total RNA (UHR, Agilent) or Breast Cancer FFPE RNA (with an archive age of 1 year and 10 years). RNA was either untreated or treated with the NEBNext® poly(A) mRNA Magnetic Isolation Module (VWR Cat. No. 102715-962) or the NEBNext® rRNA Depletion Kit. RNA-seq libraries were made using the NEBNext® Ultra™ Directional RNA Library Prep Kit for Illumina®. Reads were mapped to the hg19 genome and read distributions were determined using Picard RNA-Seq Metrics.

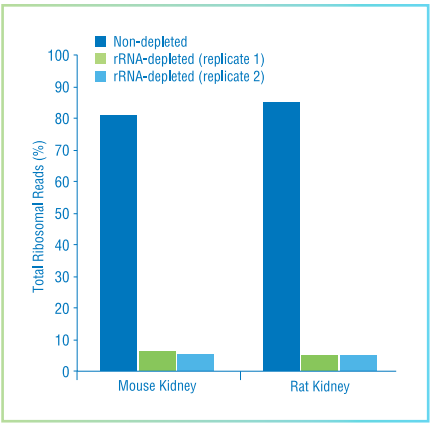


Figure 6. rRNA depletion in mouse and rat. Ribosomal RNA was depleted from mouse and rat kidney total RNA (two technical replicates) using the NEBNext® rRNA Depletion Kit. RNA-seq libraries were made from non-depleted total RNA or rRNA-depleted RNA, using the NEBNext® Ultra™ Directional RNA Library Prep Kit for Illumina®. Total rRNA-aligned reads were determined using Bowtie 2.0 (local, sensitive). NEBNext® rRNA-depleted mouse and rat libraries contain a minimal percentage of reads mapping to rRNA.

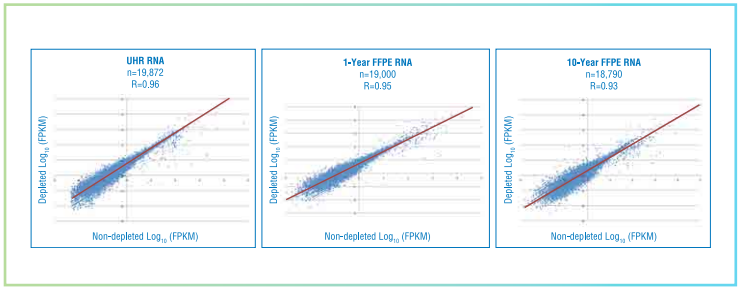


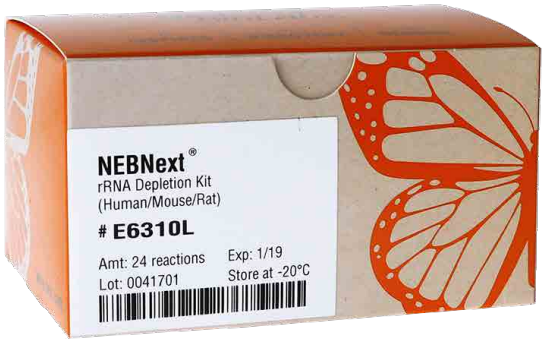
Figure 7. Transcript expression correlation between depleted and non-depleted libraries. Libraries were made from UHR RNA (Agilent) and Breast Cancer FFPE RNA (with archive age of 1 year and 10 years), both non-depleted RNA and RNA depleted using the NEBNext® rRNA Depletion Kit. All libraries were made using the NEBNext® Ultra™ Directional RNA Library Prep Kit for Illumina®. TopHat2 and Cufflinks were used for read mapping and transcript assembly and quantification.

Lose the haystack — find the needle

NEBNEXT® rRNA DEPLETION KIT (HUMAN/MOUSE/RAT), NEW ENGLAND BIOLABS

EMPLOYS AN RNASEQ-BASED METHOD TO DEPLETE BOTH CYTOPLASMIC AND MITOCHONDRIAL RIBOSOMAL RNA FROM TOTAL RNA PREPARATIONS

This product is suitable for both intact and degraded RNA (e.g. FFPE RNA). The resulting rRNA-depleted RNA is suitable for RNA-Seq, random-primed cDNA synthesis, or other downstream RNA analysis applications.



Description	Size	Cat. No.
NEBNext rRNA Depletion Kit (Human/Mouse/Rat)	24 Reactions	**102877-566**
NEBNext rRNA Depletion Kit (Human/Mouse/Rat)	6 Reactions	**102877-568**
NEBNext rRNA Depletion Kit (Human/Mouse/Rat)	96 Reactions	**102877-570**

Isolate viral DNA and RNA at the same time from the same sample

innuPREP VIRUS DNA/RNA KIT

SUITABLE FOR AN EXCEPTIONALLY WIDE VARIETY OF STARTING MATERIALS WHEN USED IN CONJUNCTION WITH INNUPURE C16 touch

- Preparation of up to 200 µL, 400 µL and even 600 µL serum, plasma, and other cell-free body fluids
- Automation for ideal reproducibility
- Improvement of qPCR results: win up to 1.7 Ct values
- Sensitive protocols for optimized yields
- Reduce false negative findings in COVID-19 testing
- Extraction time is approximately 75 minutes

Description	Size	Cat. No.
Strip Tubes		
innuPREP Virus DNA/RNA Kit	16 Reactions	**76411-362**
innuPREP Virus DNA/RNA Kit	96 Reactions	**76411-364**
Plates		
innuPREP Virus DNA/RNA Kit	16 Reactions	**76411-366**
innuPREP Virus DNA/RNA Kit	96 Reactions	**76411-368**
innuPREP Virus DNA/RNA Kit	480 Reactions	**76411-370**
innuPURE C16 touch Nucleic Acid Extractor	—	**76414-004**

Fast, reliable DNA purification & size selection

SPARQ PUREMAG BEADS, QUANTABIO

NUCLEIC ACID PURIFICATION SYSTEM FOR REACTION CLEANUP AND SIZE SELECTION IN NEXT GENERATION SEQUENCING (NGS) WORKFLOWS

- High recovery of DNA fragments greater than 100 bp
- Efficient removal of unwanted components from adapter ligation and PCR reactions
- Consistent single or double-sided size selection
- Seamless integration into existing NGS workflows with little or no protocol change
- Easy-to-use and compatible with manual processing or automated liquid handling robots
- Cost effective alternative to AMPure® XP with equivalent performance

Size	Cat. No.
450 mL	**76302-830**
60 mL	**76302-832**
5 mL	**76302-834**

Fast & efficient DNA end repair for library prep

END-IT™ DNA END-REPAIR KIT, SHEARED DNA REPAIR, PHOSPHORYLATED BLUNT DNA ENDS, BIOSEARCH TECHNOLOGIES

CONVERT SHEARED, DAMAGED, OR PROTRUDING DNA ENDS TO PHOSPHORYLATED, BLUNT-ENDED DNA

- Completely convert DNA into 5′ blunt-ended, phosphorylated DNA
- Repair sheared, nebulized, or restriction enzyme-digested genomic DNA
- Prepare templates for NGS (next-generation sequencing) or shotgun library preparation

The End-It™ DNA End-Repair Kit converts DNA containing damaged or incompatible 5´- and/or 3´-protruding ends to 5´-phosphorylated, blunt-ended DNA. The end-repaired DNA can be rapidly ligated into a cloning vector or to blunt next-gen sequencing adaptors, using the Fast-Link™ DNA Ligation Kit.

Description	No. of Reactions	Cat. No.
End-It™ DNA End-Repair Kit	20	**75927-936**
End-It™ DNA End-Repair Kit	50	**75927-938**

US ONLY

*These products are not yet available in Canada. Please contact your VWR Life Science Specialist on information on similar products currently available in your region.

Library prep with unrivaled speed & performance

SPARQ DNA FRAG AND LIBRARY PREP KIT, QUANTABIO

ROBUST ENZYMATIC FRAGMENTATION AND CONSISTENT RESULTS

- High quality libraries in 2.5 hours from 1 ng to 1 µg of input DNA
- Tunable and reproducible fragmentation size range
- Simple, convenient 2-step workflow with minimal hands-on time
- Novel chemistry and high-fidelity amplification minimizing bias
- Superior sequence coverage uniformity and low duplication rate

This kit is optimized for enzymatic fragmentation of DNA and streamlined construction of high-quality libraries for sequencing on Illumina® NGS platforms. Quantabio's engineered DNA frag and polishing enzymes work in concert to generate fragment sizes that are tunable and reproducible based on reaction time.

Description	Cat. No.
Fragment and Library Prep Kits	
sparQ DNA Fragment and Library Prep Kit	**76183-242**
sparQ DNA Fragment and Library Prep Kit	**76183-244**

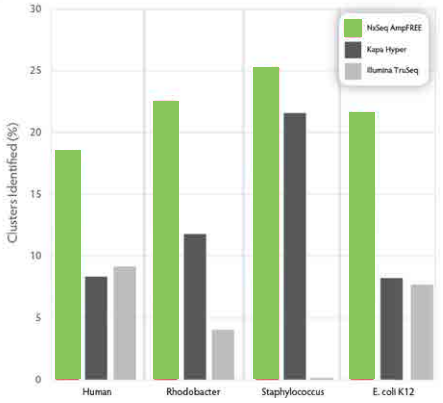
High-efficient library construction, improved sequencing results

NXSEQ® AMPFREE LOW DNA LIBRARY KIT, NEXT GEN SEQUENCING (NGS) LIBRARY PREP KITS, PCR-FREE, ILLUMINA-COMPATIBLE, BIOSEARCH TECHNOLOGIES

NEXT GENERATION SEQUENCING (NGS) DNA FRAGMENT LIBRARIES FOR ILLUMINA SEQUENCERS,PCR-FREE DNA LIBRARY PREPARATION KIT

- High-efficiency next generation sequencing (NGS)
- Optimized to ensure peak performance
- Prevents the introduction of PCR bias in the sequencing results
- Compatible with a larger number of starting samples
- Produces more sequenceable DNA fragments

Greater Percentage of Identifiable Clusters
More fragments sequenced per run - More data



Libraries generated with this kit are suitable for de novo whole genome sequencing, resequencing, mutation/SNP detection, and more. Each step of the NxSeq AmpFREE Low DNA Library Kit protocol is optimized to ensure peak performance on Illumina sequencers and provide the most sequencing data possible from each library.

Description	Cat. No.
NxSeq AmpFREE Library Kit	**10837-142**
NxSeq AmpFREE Library Kit	**10837-144**
NxSeq Adaptors, Box 2	**10837-148**
NxSeq Adaptors, Box 1	**10837-146**

High-fidelity, high-efficiency library amplification while maintaining even coverage

SPARQ HIFI PCR MASTER MIX, QUANTABIO

INCREASING EFFICIENCY AND YIELD WHILE LOWERING BIAS

- Increased amplification efficiency results in higher yields for NGS library amplification even from low input DNA
- Unbiased amplification of DNA fragments provides improved coverage across AT- and GC-rich regions
- Improved overall sequencing economics

A high-efficiency, high-fidelity, and low bias PCR master mix for NGS workflows requiring DNA library amplification prior to sequencing.

Description	Reactions per Kit	Cat. No.
sparQ HiFi PCR Master Mix	50 Reactions	**76121-366**
sparQ HiFi PCR Master Mix	250 Reactions	**76121-368**

analytikjena
An Endress+Hauser Company

Biometra tAdvanced

BIOMETRA tADVANCED AND BIOMETRA tADVANCED TWIN THERMAL CYCLERS, ANALYTIK JENA

THE BIOMETRA tADVANCED IS THE HIGH-PERFORMANCE THERMAL CYCLER FOR DNA AMPLIFICATION BY PCR

- Integrated 7" touchscreen control
- Whisper quiet, with a low noise emission of 45 dB (max.)
- Linear gradient tool allows easy gradient programming using the primer annealing temperature
- Units include a two-year warranty

Description	For Use With	Cat.No.
Biometra tAdvanced		
Biometra tAdvanced 96	Standard 96-Well Plates, 0.2 ml Tubes, 8-Well Strips, or 12-Well Strips	**76102-486**
Biometra tAdvanced 96G	Standard 96-Well Plates, 0.2 ml Tubes, 8-Well Strips, or 12-Well Strips	**76102-488**
Biometra tAdvanced Twin		
Biometra tAdvanced Twin 48G	2×48-Well Block (0.2 ml) and Gradient Function	**76102-438**
BiometratAdvanced Modules		
Biometra tAdvanced Block Module 96	96-Well, 0.2 ml Tubes, Plates, Strips	**76102-420**
Biometra tAdvanced Twin Modules		
Biometra tAdvanced Twin Block Module 48G	2×48-Well Block (0.2 ml) and Gradient Function	**76102-446**

A faster, smaller, better way to qPCR

Q qPCR INSTRUMENT, QUANTABIO

A FASTER, SMALLER, BETTER WAY TO qPCR

- **Ultra-Fast Data Acquisition -** 35 cycles in 25 minutes
- **Unrivaled Performance -** Detect 2-fold expression level differences
- **Portable & Compact -** 4.5 lbs - transport without ever calibrating
- **Scalable & Wireless -** Connect up to 10 instruments (48 samples/instrument)
- **Magnetic Induction Technology -** Eliminate variability vs block-based cyclers

Q uses a patented magnetic induction technology to rapidly heat samples coupled with fan-forced air for cooling to acquire data in only 25 minutes. Available in 2- or 4-channel models, the robust optical system acquires all channels simultaneously and allows for running the fastest multiplexed assays.

Description	Size	Cat. No.
Q 2-channel qPCR Instrument	1 instrument	**76175-392**
Q 4-channel qPCR Instrument	1 instrument	**76175-394**
20 racks/ box	1 box	**76202-252**

Fastest qPCR-based library quant in 40 minutes

sparQ FAST LIBRARY QUANTIFICATION KITS (FOR Q), QUANTABIO

ACCURATE qPCR-BASED LIBRARY QUANTIFICATION IN 40 MINUTES

- Faster time to results – 50% shorter run time than traditional cycling protocols
- Accurate, reliable quantification of NGS libraries of various sizes and GC-content
- High amplification efficiency across a wide linear dynamic range
- Stabilized, ready-to-use sparQ Fast Mastermix to reduce pipetting steps
- Superior run-to-run uniformity ensuring highly precise measurements

This kit provides rapid and accurate quantification of libraries prepared for sequencing on Illumina NGS platforms. Accurate quantification of the number of amplifiable library molecules prior to loading onto a flow cell is a critical step in the NGS workflow and it ensures optimal cluster generation and cost-effective use of sequencing capacity. The sparQ Fast Library Quant Kit uses real-time qPCR (qPCR) to specifically quantify the number of library molecules that possess the appropriate adapter tag at each end.

40 min

US ONLY

No. of Reactions	Cat. No.
50	**76323-384**
500	**76323-386**

*These products are not yet available in Canada. Please contact your VWR Life Science Specialist for information on similar products currently available in your region.

Agilent Technologies

Easy NGS QC from FFPE samples that are qPCR-based

NGS FFPE QC KITS, AGILENT

qPCR-BASED ASSAY ENABLING FUNCTIONAL DNA QUALITY ASSESSMENT OF DNA PRIOR TO PREPARATION OF NGS LIBRARIES

- Efficiently assess both DNA integrity and accurate quantitation of amplifiable templates prior to library preparation
- Accurately qualify and quantify amplifiable DNA in challenging formalin-fixed, paraffin-embedded tissue samples
- Includes an optimized low input library prep workflow for improved complexity and target coverage
- Provides complete cancer research solutions from sample to data

Description	Cat. No.
NGS FFPE QC kit 16 reaction	76335-750
NGS FFPE QC kit 96 reaction	76335-752

* These products are not yet available in Canada. Please contact your VWR Life Science Specialist for information about similar products currently available in your region.

US ONLY

Agilent Technologies

NGS library quantification for Illumina® sequencers

sizes, and

qPCR NGS LIBRARY QUANTIFICATION KIT, AGILENT TECHNOLOGIES

THE AGILENT qPCR NGS LIBRARY QUANTIFICATION KIT PROVIDES RESEARCHERS WITH AN ACCURATE AND SENSITIVE METHOD FOR QUANTIFYING NGS LIBRARIES

- Accurate library quantification leading to optimal cluster densities for improved sequencing efficiency and data quality
- Consistent library quantification across a broad range of samples, varying library insert sizes, and GC content
- Sensitivity of detection to quantify low concentration libraries, particularly those that have been prepared with protocols that reduce or eliminate PCR amplification steps
- Ideal for use in barcode sequencing when need to quantify a large number of libraries in a single quantification run
- Adaptable to high-throughput library quantification for larger barcoding applications
- Quantify 84 libraries with each kit

Description	Cat. No.
qPCR NGS Library Quant Kit, Illumina GA	97066-988

* These products are not yet available in Canada. Please contact your VWR Life Science Specialist for information about similar products currently available in your region.

US ONLY

FOCUS: NEXT-GENERATION SEQUENCING

2020

BECKMAN COULTER Life Sciences

Automated Next-Gen Sequencing (NGS) library construction

BIOMEK® 4000 AUTOMATED WORKSTATION, BECKMAN COULTER

IDEAL FOR NGS SAMPLE PREPARTION OF UP TO 24 SAMPLES PER RUN

- Increasing throughput while minimizing hands-on time
- Reducing errors and accelerating implementation with pre-programmed methods
- Providing flexibility to maximize scheduling during your day
- Tracking sample progress and manipulation with DART software
- Reducing downtime with remote technical support, Beckman Connect

The Biomek 4000 has been demonstrated to run multiple pre-programmed methods, including Illumina's TruSeq DNA PCR-Free kit and TruSeq Nano DNA kit, TruSight Cancer kit and TruSight Tumor 15, Nextera® Rapid Capture kit from Illumina and NEBNext® Small RNA Kit from NEB.

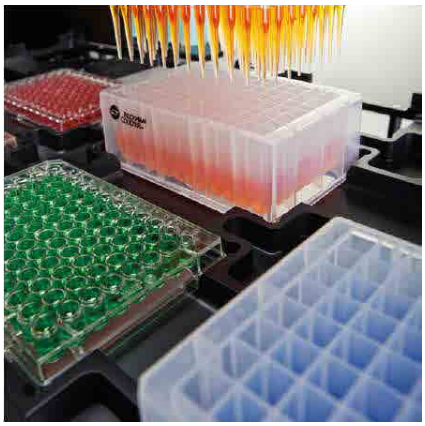
Furthermore, Biomek systems are versatile and can run different methods on the same instrument, including nucleic acid extraction from cells, fresh or frozen blood, fresh/frozen/FFPE-fixed tissues, etc.

Description	Cat. No.
Biomek® 4000 Automated Liquid Handler	**75816-488**
Biomek® 4000 Automated Liquid Handler for Genomics	**75816-486**
Biomek® 4000 Automated Liquid Handler for PCR Set-up	**75816-480**
Biomek® 4000 Automated Liquid Handler for PCR Clean-up	**75816-482**

BIOMEK® PIPETTE TIPS FOR BIOMEK LIQUID HANDLERS, BECKMAN COULTER®

Biomek disposable tips from Beckman Coulter are the only tips designed, validated, and approved for Biomek Liquid Handlers. Manufactured under stringent manufacturing processes, we guarantee the tips to be free of DNAse, RNAse, human & mouse DNA, pyrogens, trace metals, and PCR inhibitors.

Description	Volume, µL	Sterility	Cat. No.
Biomek® P50 Tips	90	Non-sterile	**BKA21578**
Biomek® AP96 P20 Tips	100	Non-sterile	**BK717254**
Biomek® AP96 P250 Tips	220	Non-sterile	**BK717251**
Biomek® Span-8 P1000 Tips	1050	Non-sterile	**BKB01122**



To learn more about the Biomek® 4000 Automated Workstation or additional accessories that are available, please contact your VWR Life Science Specialist.

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