Roche Sample Prep Solutions:

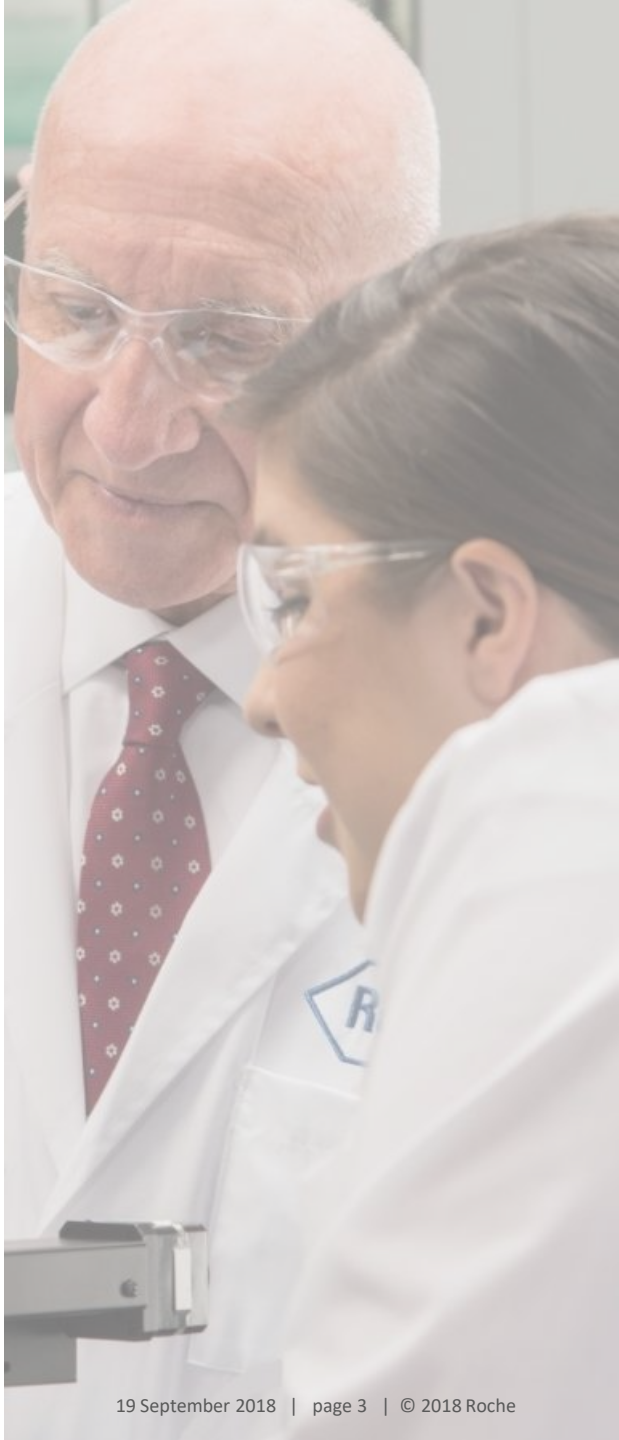
Unlock the Potential of Every RNA-Seq Sample

July 2018

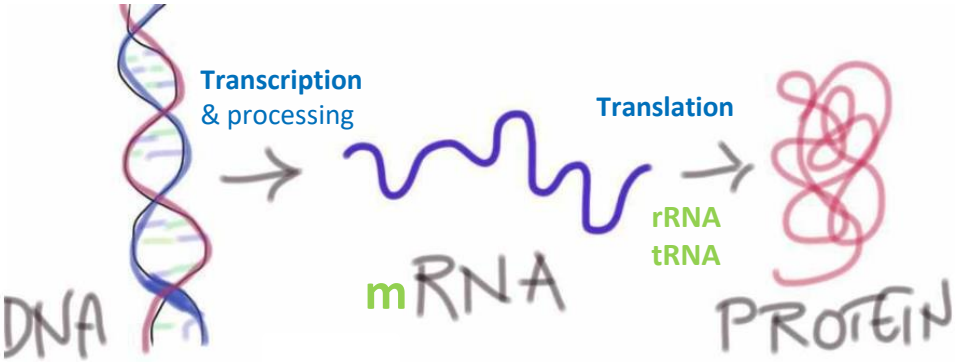
Overview

- RNA and RNA-Seq
- Roche Sample Prep Solutions for RNA-Seq: Features & Benefits
- RNA-Seq Application Data

RNA and RNA-Seq



Biological Roles of RNA



The Central Dogma of Molecular Biology: genetic information flows from **DNA** to **RNA** to **proteins:**

- The process of **transcription** generates RNA (the **transcriptome**) from DNA
- After processing, mature coding or messenger RNAs (**mRNA**) are **translated** into proteins
- Translation is mediated by ribosomes, which contain ribosomal RNA (**rRNA**). Transfer RNAs (**tRNA**) are also an important component of the translation process.
- Together, rRNAs and tRNAs (non-coding RNA) make up ~95% of the RNA in the cell.[1] These RNA species are relatively "uninteresting" to study.

mRNA and **other non-coding RNAs** (e.g. **lncRNA**, **siRNA**, **miRNA**, **shRNA** and **piRNA**) comprise the **RNA species most researchers want to study**. We still have a lot to learn about these non-coding RNAs, but know that they play an important role in the regulation of gene expression.

1. Paralkar, V, Weiss M. Long noncoding RNAs in blood and hematopoiesis. *Blood* 2013 ; 121(24):4842-4846
Image adapted from: https://steemit.com/biology/@pjheinz/biology-the-study-of-life-part-9-central-dogma. Accessed May 2018.

Next-Generation RNA Sequencing

RNA-Seq is the set of experimental procedures that generates cDNA sequences from all or some of the RNA molecules in a sample, followed by library construction and massively parallel deep sequencing, to base-pair resolution[1]

RNA-Seq allows us to:

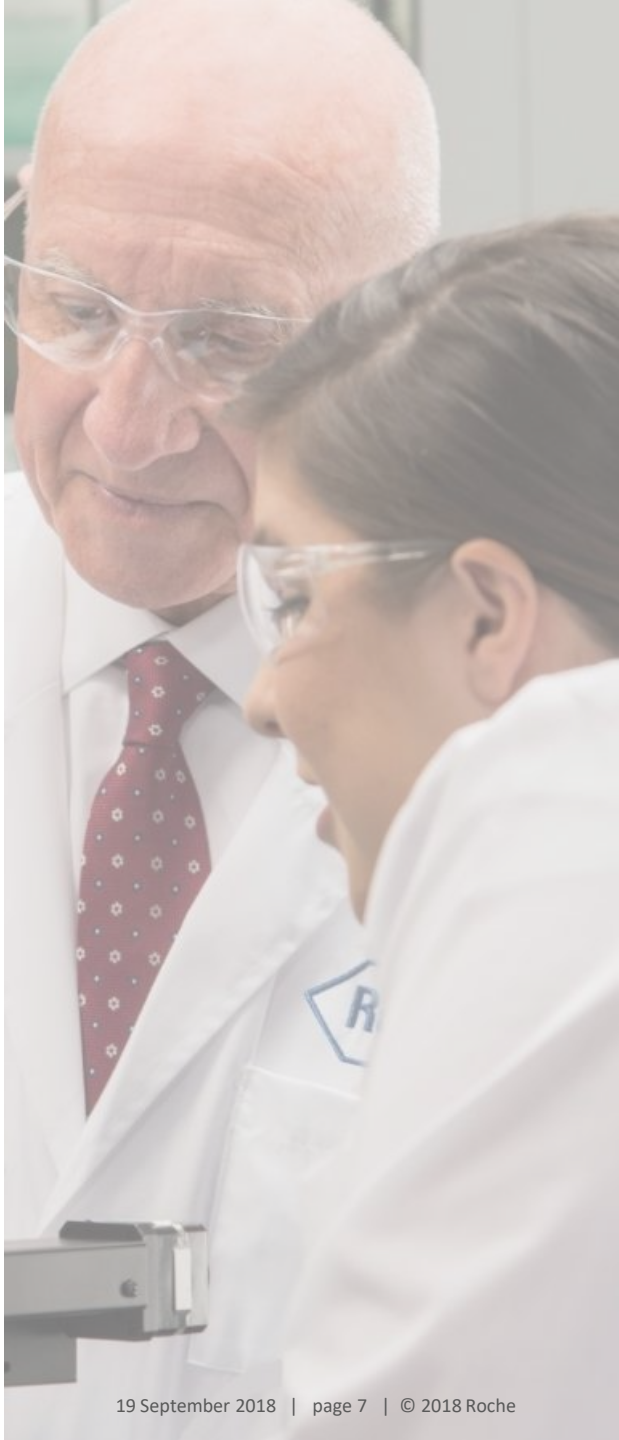
- Assess both **known** and **novel** features in a single assay
  - Discover and annotate transcripts
- Measure (quantify) the **abundance** (expression level) or **relative change** (differential expression) of genes (**coding RNA**) and other transcripts (**non-coding RNA**), for different biological or experimental conditions
- … and study gene/transcript **structure**:
  - 5'- and 3'-boundaries, transcript **isoforms** (alternative splicing), post-transcriptional modifications
  - **gene fusions** and other **genetic variants** (including SNVs)

[1]Han et al. Advanced Applications of RNARNARNA Sequencing and Challenges. Bioinformatics and Biology Insights 2015:9(S1) 29–46 . doi: 10.4137/BBI.S28991

Challenges associated with RNA-Seq

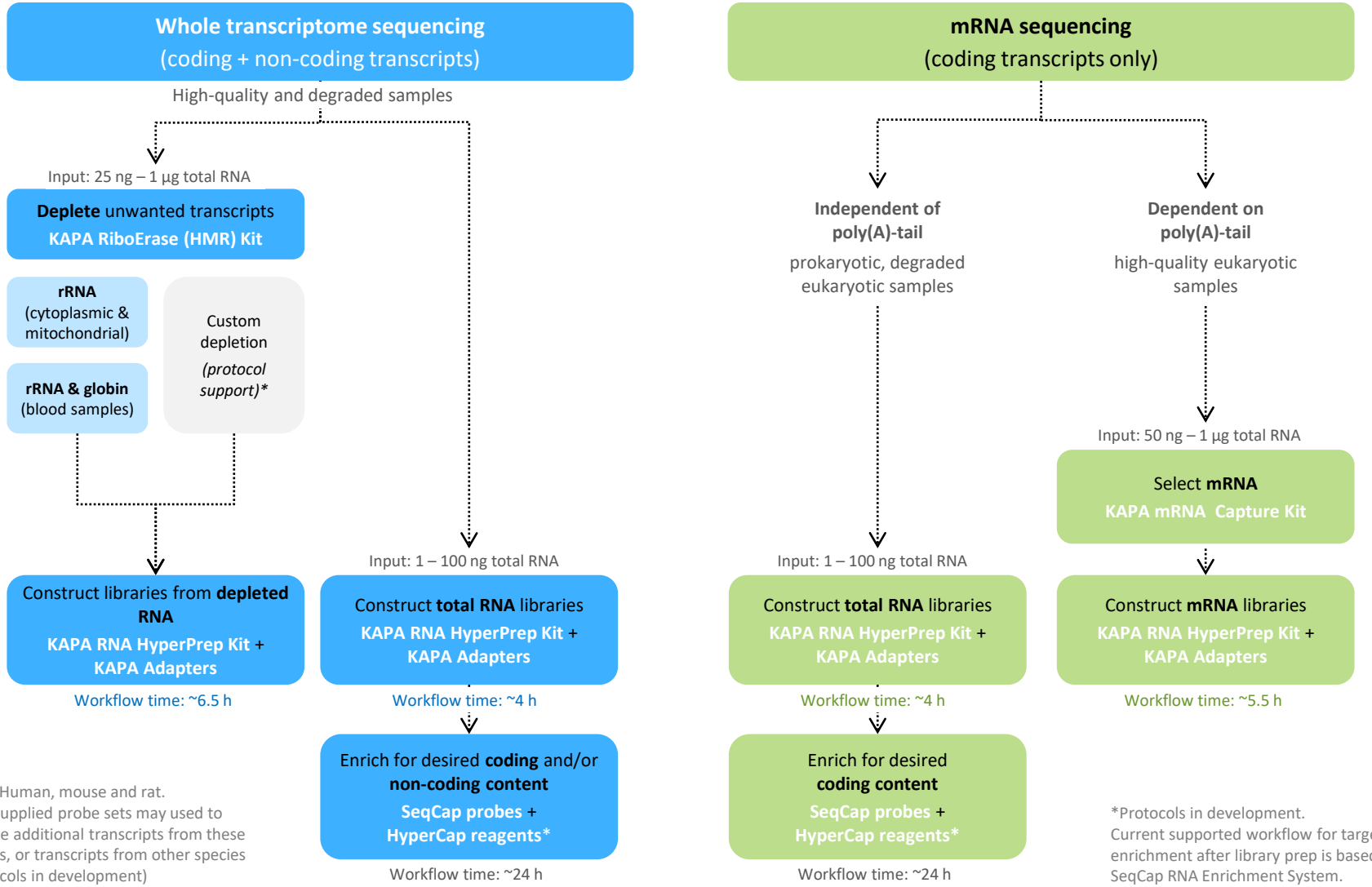
- **Library prep challenges:**
  - **Long, multi-step protocols**
  - **RNA quantity and quality**
  - **Bias** associated with library amplification (always needed when making RNA-Seq libraries)
- Ability to detect and quantify **all** of the RNA of interest, especially:
  - **GC-rich** regions
  - **Low-abundance transcripts**
- Accurately assess **transcript orientation** (stranded RNA-Seq)
- Data analysis

Roche Sample Prep Solutions for RNA-Seq: Features & Benefits



Roche Sample Prep Solutions for RNA-Seq

Flexible workflow options for a wide range of applications



HMR: Human, mouse and rat.
User-supplied probe sets may used to deplete additional transcripts from these species, or transcripts from other species (protocols in development)

*Protocols in development.
Current supported workflow for target enrichment after library prep is based on the SeqCap RNA Enrichment System.

KAPA RNA HyperPrep Portfolio

Kit specifications

	KAPA RNA HyperPrep	KAPA mRNA HyperPrep	KAPA RNA HyperPrep with RiboErase (HMR)	KAPA RNA HyperPrep with RiboErase (HMR) Globin
Applications	• **Whole transcriptome profiling*** • Isoforms, gene fusions*	• **mRNA profiling** • Annotation • Isoforms, gene fusions	• **Whole transcriptome profiling** • Long non-coding RNA • Annotation • Isoforms, gene fusions	• **Whole transcriptome profiling (blood samples)** • Long non-coding RNA • Annotation • Isoforms, gene fusions
Input	1 ng – 100 ng	50 ng – 1 µg	25 ng - 1 µg	25 ng - 1 µg
Sample type	High and low quality total or enriched RNA	High-quality poly(A)-tailed mRNA	High and low quality (e.g. FFPE) total RNA	High and low quality total RNA from blood
Species	Eukaryotic, prokaryotic	Eukaryotic	Human, mouse, rat (protocol support for other species)	
RNA Fragmentation	Tunable, based on input quality			
cDNA synthesis	Random priming, KAPA Script Reverse Transcriptase (all reagents included)			
Adapters	KAPA Dual-Indexed Adapters (available separately)			
Cleanup beads	KAPA Pure Beads (included)			
Library amplification	KAPA HiFi + amplification primers			
Stranded libraries	Yes			
Workflow time	4 hr*	5.5 hr	6.5 hr	6.5 hr
Automation-friendly	Yes			

*With downstream target enrichment (TE). TE reagents are not included in kit and can be purchased separately from Roche. Workflow time does not include TE.

KAPA RNA HyperPrep Kits

Key Features

- KAPA RNA HyperPrep Kits utilize novel chemistry that enables the combination of enzymatic steps and fewer reaction purifications, resulting in a truly **streamlined** solution for the preparation of high-quality libraries for RNA-Seq on the Illumina platform

- The **strand-specific** workflow is **flexible**; supporting library construction from a range of applications – either with upfront RNA enrichment (mRNA capture or depletion of unwanted transcripts), or downstream target enrichment (hybridization capture)

- Kits include **all reagents** required for RNA enrichment (if performed) and library preparation, with the exception of KAPA Adapters (available separately)

KAPA RNA HyperPrep Kits

Benefits

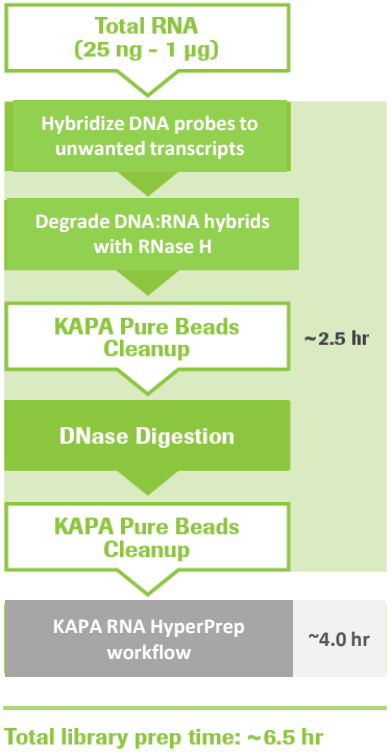
- **Single-day** library construction, inclusive of RNA enrichment
- **Automation-friendly** RNA enrichment and library prep workflows
- **Highly efficient RNA enrichment and library prep** reduces the number of sequencing reads associated with unwanted transcripts
- More content is preserved through **even coverage across transcript lengths**, and efficient detection of **GC-rich transcripts**
- **Robust and reliable performance** across different sample types and input amounts
- **Higher success rates** with lower input and degraded samples
- **Integrated service and support** for the entire workflow from RNA to sequencing-ready library

Streamlined, Single-tube Library Construction

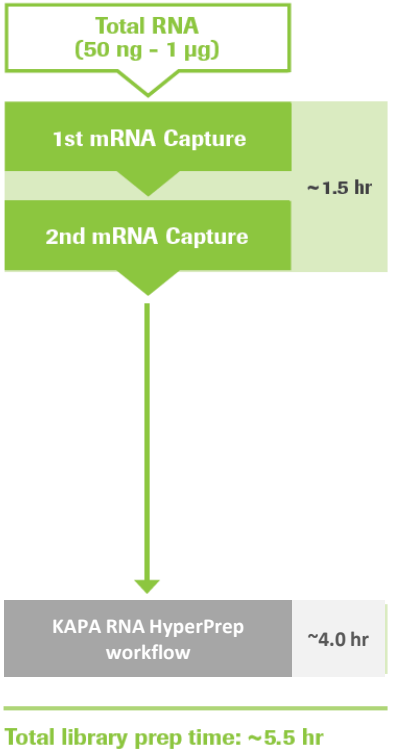
- Reduce hands-on and overall turnaround time with fewer enzymatic and cleanup steps
- Produce strand-specific libraries from input RNA in ~4 hours with an automation-friendly workflow
- Complete the entire workflow, inclusive of upfront RNA enrichment, in a standard work day

Automation-friendly RNA Enrichment Options

KAPA RNA HyperPrep Kits with KAPA RiboErase (HMR)
Globin & custom depletion available



KAPA mRNA HyperPrep Kits

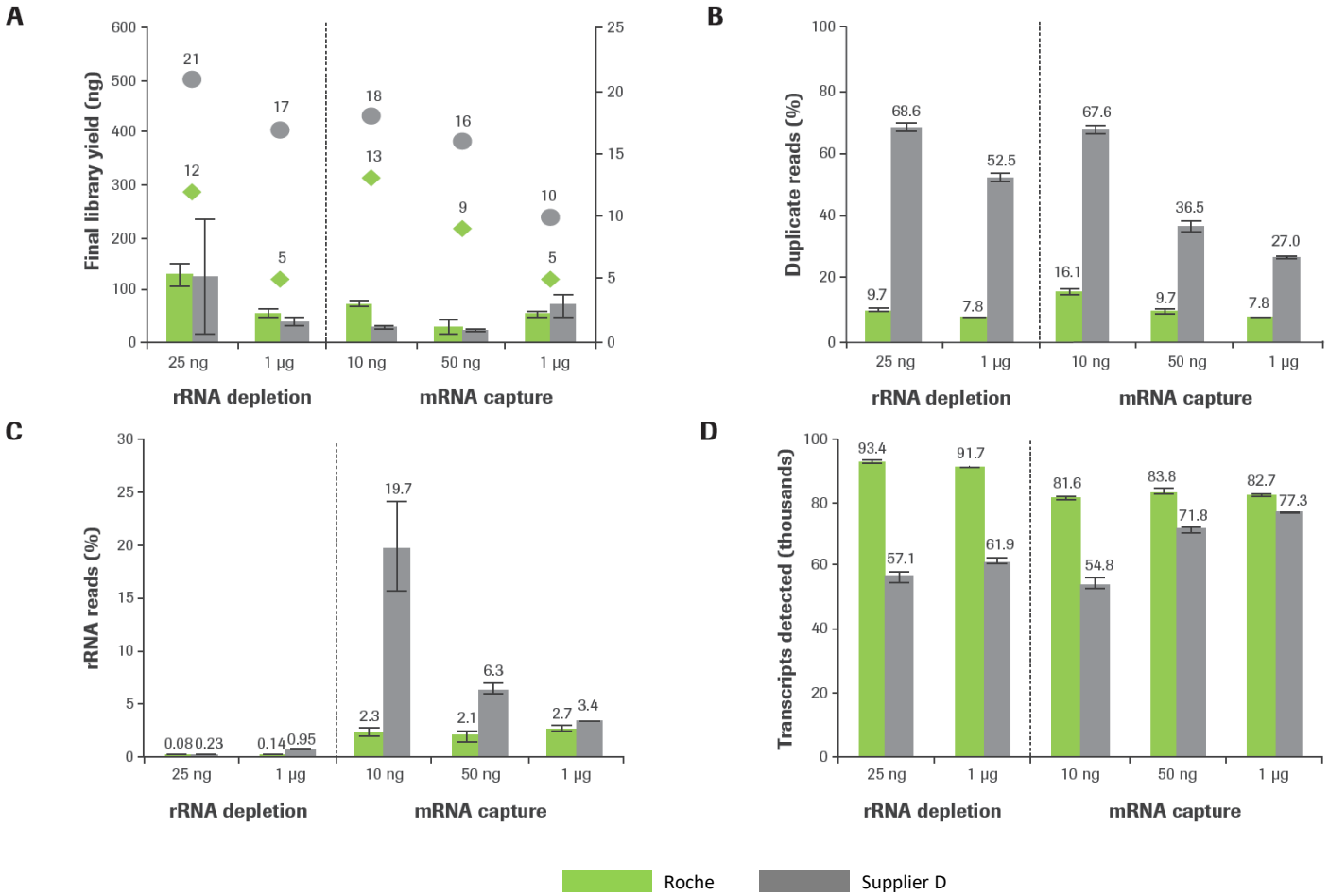


KAPA RiboErase (HMR) workflow. Sequencing of total RNA samples depleted of unwanted rRNA (cytoplasmic and mitochondrial), globin (for blood samples) or other transcripts provides a more comprehensive representation of the whole transcriptome. Unwanted transcripts are targeted and depleted enzymatically using complementary DNA probes and RNase H. This results in improved coverage of transcripts of interest, including precursor mRNAs and important regulatory noncoding RNAs.

KAPA mRNA Capture workflow. Sequencing of mRNA-enriched samples provides a focused view of the protein-coding regions in the transcriptome. KAPA mRNA capture beads are used prior to library preparation with the KAPA RNA HyperPrep workflow, which enriches for mRNA over non-polyadenylated species, such as ribosomal, precursor, and noncoding RNAs.

Highly Efficient RNA Enrichment & Library Construction

- Effective RNA enrichment (rRNA depletion or mRNA capture), combined with highly efficient library construction, reduces the number of reads associated with unwanted content and PCR duplicates
- More unique transcripts and genes are detected from a fixed amount of sequencing



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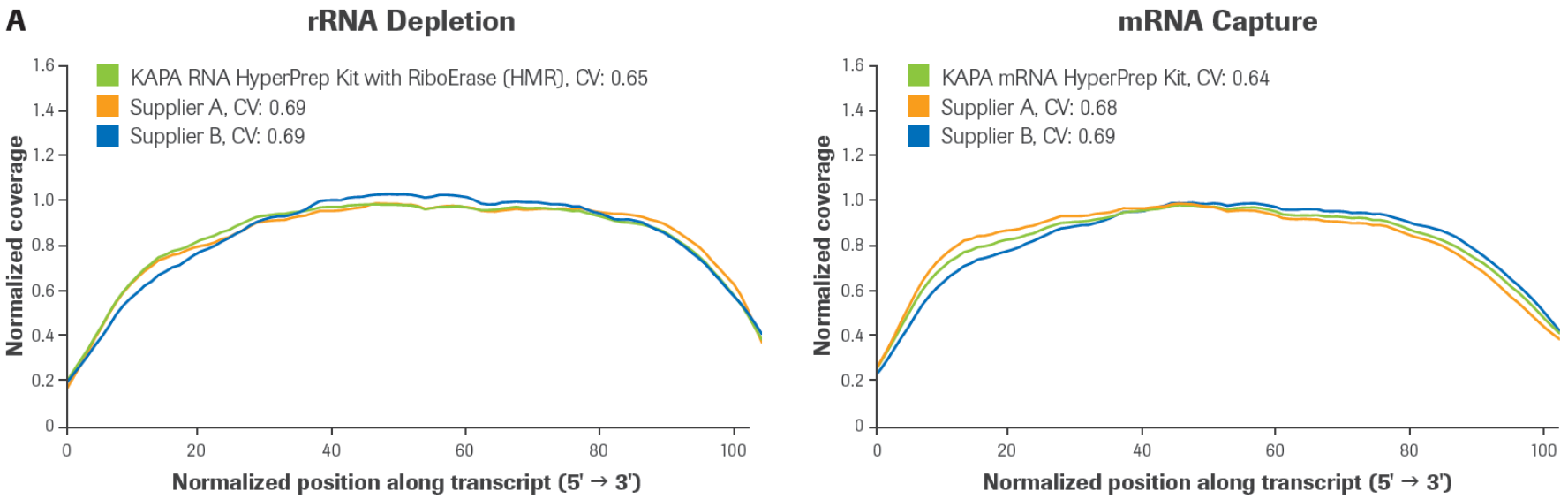
Higher Coverage Uniformity Across Transcript Length

- Efficient RNA enrichment and library construction processes results in more even coverage along transcript lengths



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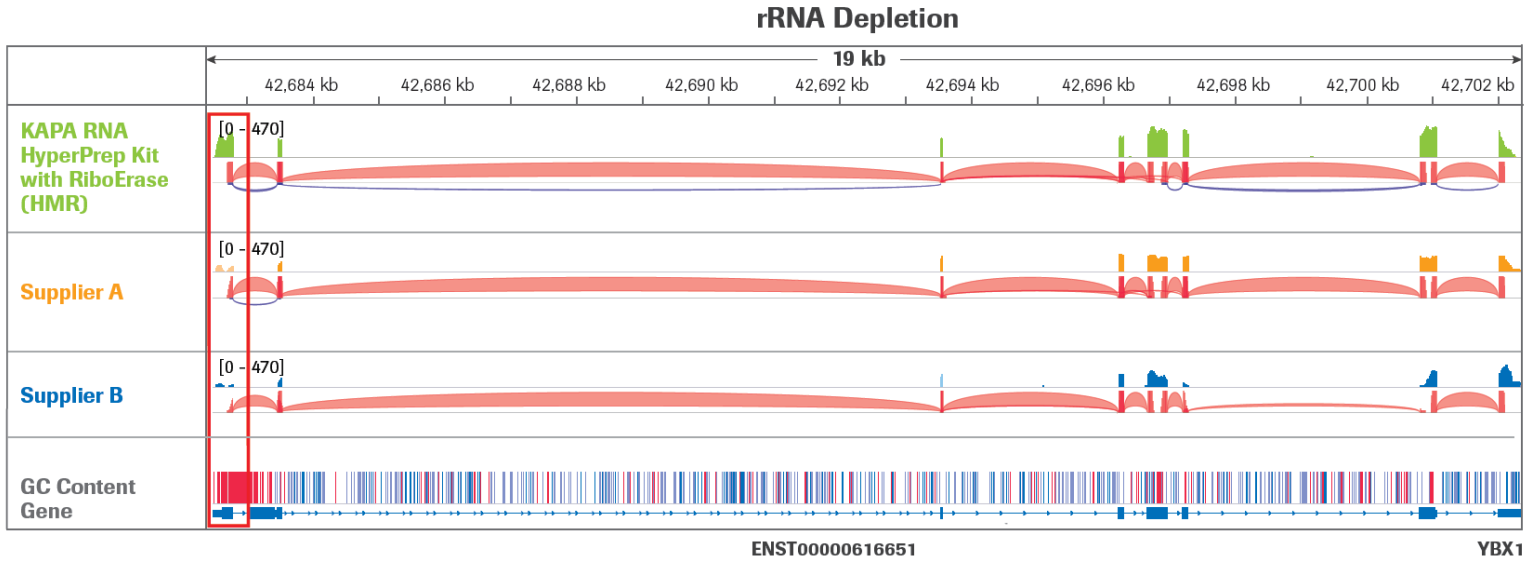
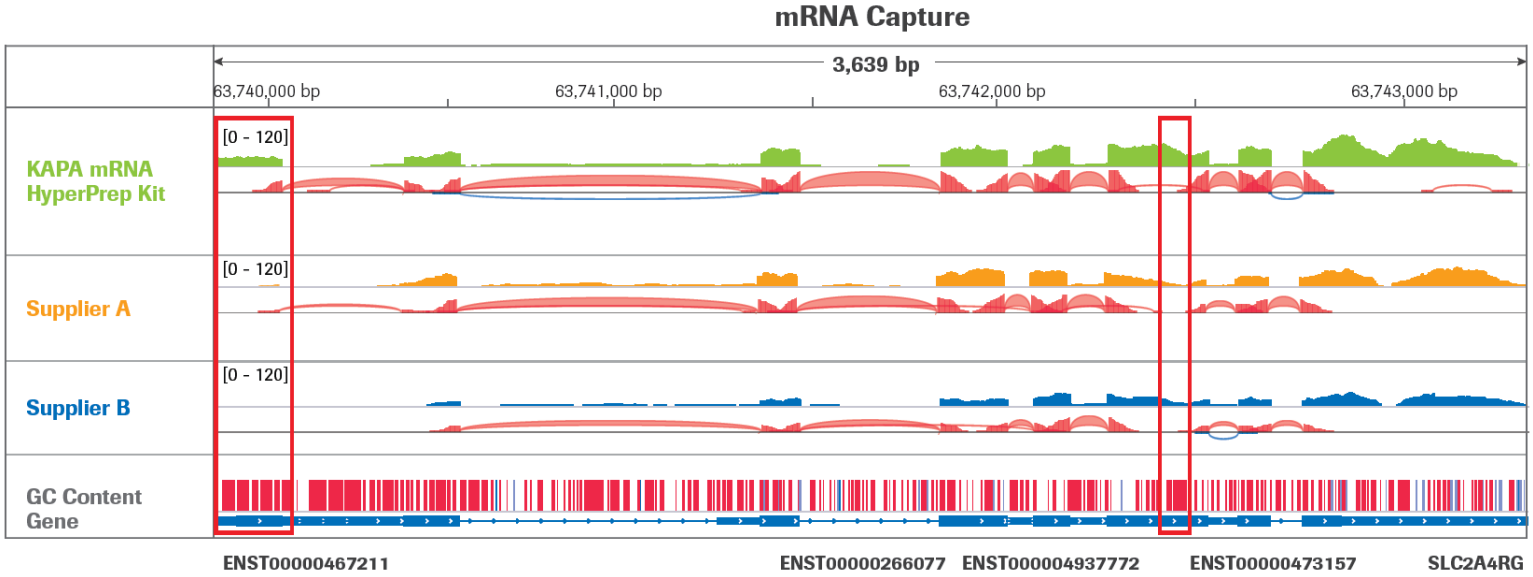
KAPA HiFi Enables Better Coverage of GC-rich regions





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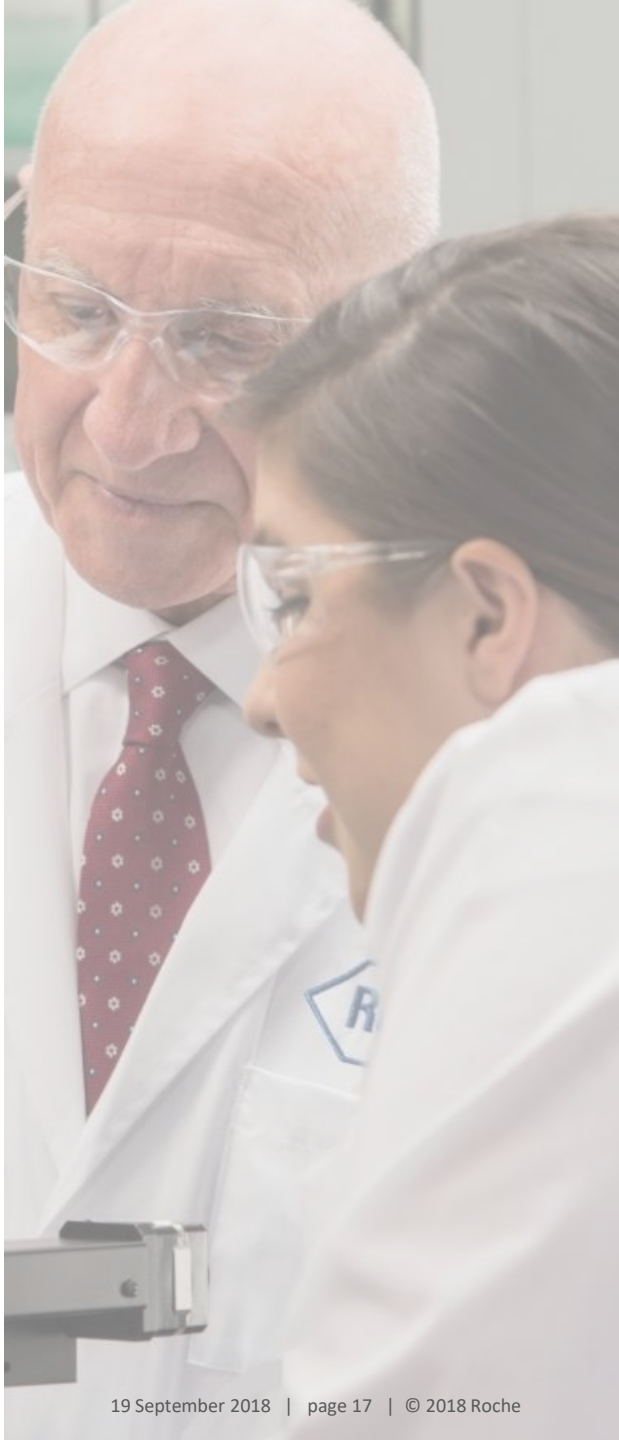
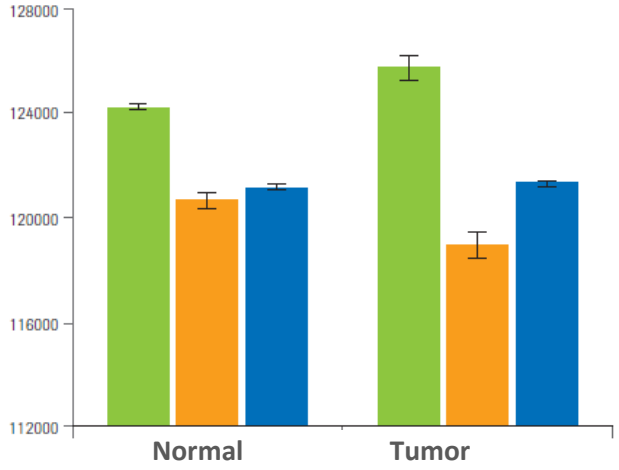
RNA-Seq Application Data



Gene Expression Analysis

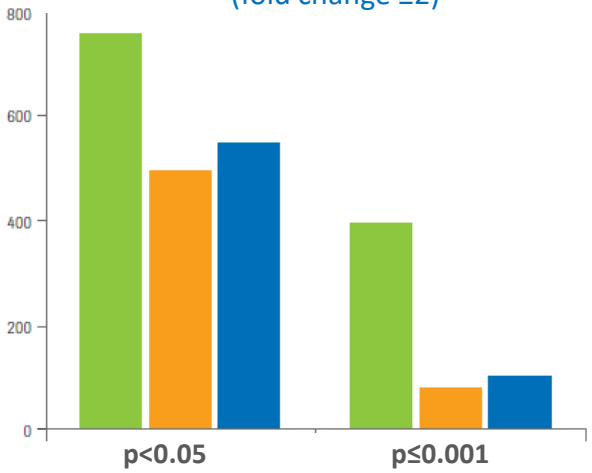
Discover more differentially expressed transcripts

Unique transcripts identified



Differentially expressed transcripts

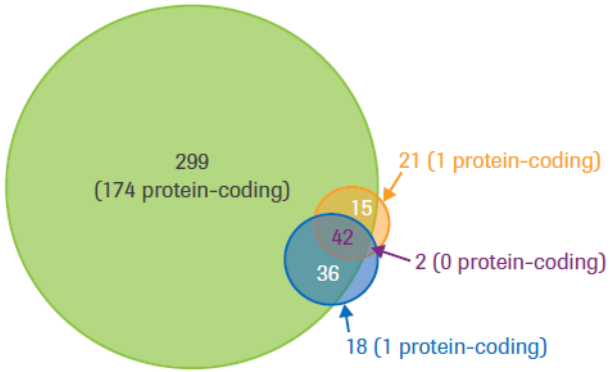
(fold change ≥2)



KAPA RNA HyperPrep Kit with KAPA RiboErase (HMR)
Supplier A (bead-based rRNA depletion)
Supplier B (enzymatic rRNA depletion)

Differentially expressed transcripts

(fold change ≥2; p≤0.001)

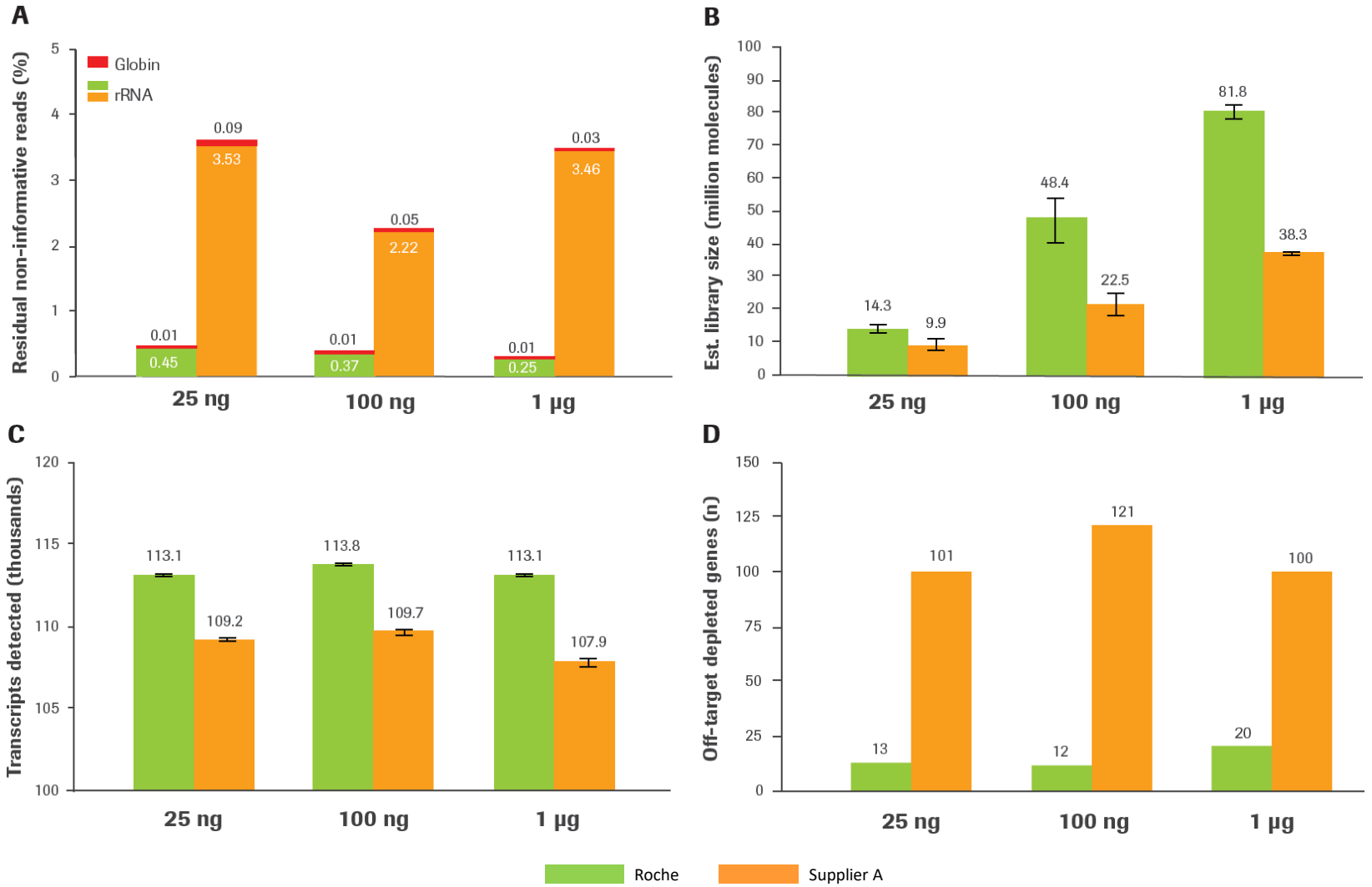


Comparative gene expression analysis by RNA-Seq. Libraries were prepared using an rRNA depletion workflow from 100 ng of RNA isolated from matched fresh-frozen primary breast tumor and adjacent normal breast tissue (AMSBIO). Paired-end sequencing (2 x 100 bp) was performed on an Illumina HiSeq 2500 instrument, and data were down-sampled to 14 million reads per replicate prior to analysis. Differentially expressed genes were validated using a custom-designed RealTime ready qPCR array (Roche).

Data on file.

Whole Transcriptome Profiling from Blood Samples

Effective depletion of globin transcripts



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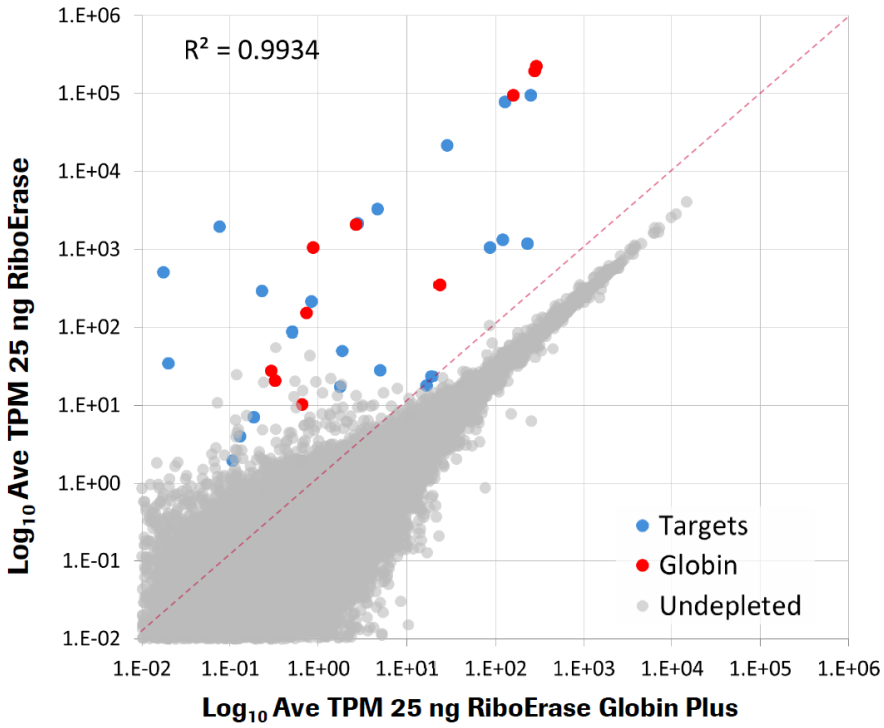
Custom Depletion*

KAPA RiboErase workflow provides for use of custom probes



Transcripts targeted for depletion	
RiboErase (HMR)	Human, mouse and rat cytoplasmic & mitochondrial rRNA transcripts
RiboErase (HMR) Globin	Above + globin transcripts
RiboErase Globin Plus* (this study)	• 12 highly expressed targets in human blood • 8 transcripts present at high concentrations in the ERCC Spike-In Controls Mix 1 (Ambion).

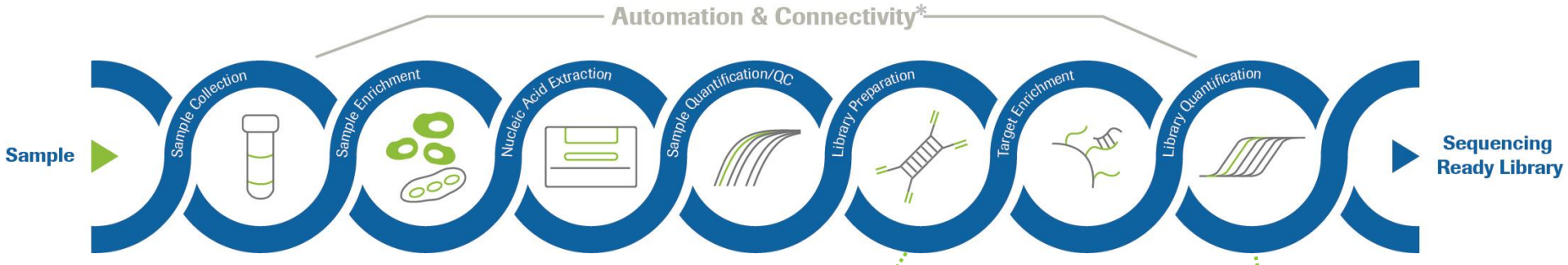
*Not a product; custom depletion protocol in development

Expression correlation plots of human blood total RNA showing efficient removal of high expressed RNA transcripts. TPM values for transcripts mapping to the human reference sequence for the KAPA RiboErase (HMR) Kit and "RiboErase Globin Plus" workflows (25 ng input into depletion) shows that the majority of the globin and additional targeted RNA transcripts were effectively depleted from the sample

Data on file.

Whole Transcriptome Sequencing from FFPE samples

Reliable results from degraded samples



- **KAPA RNA HyperPrep Kit** with **KAPA RiboErase (HMR)**
- **KAPA DI or SI Adapters**
- **KAPA Pure Beads**

KAPA Library Quant Kits

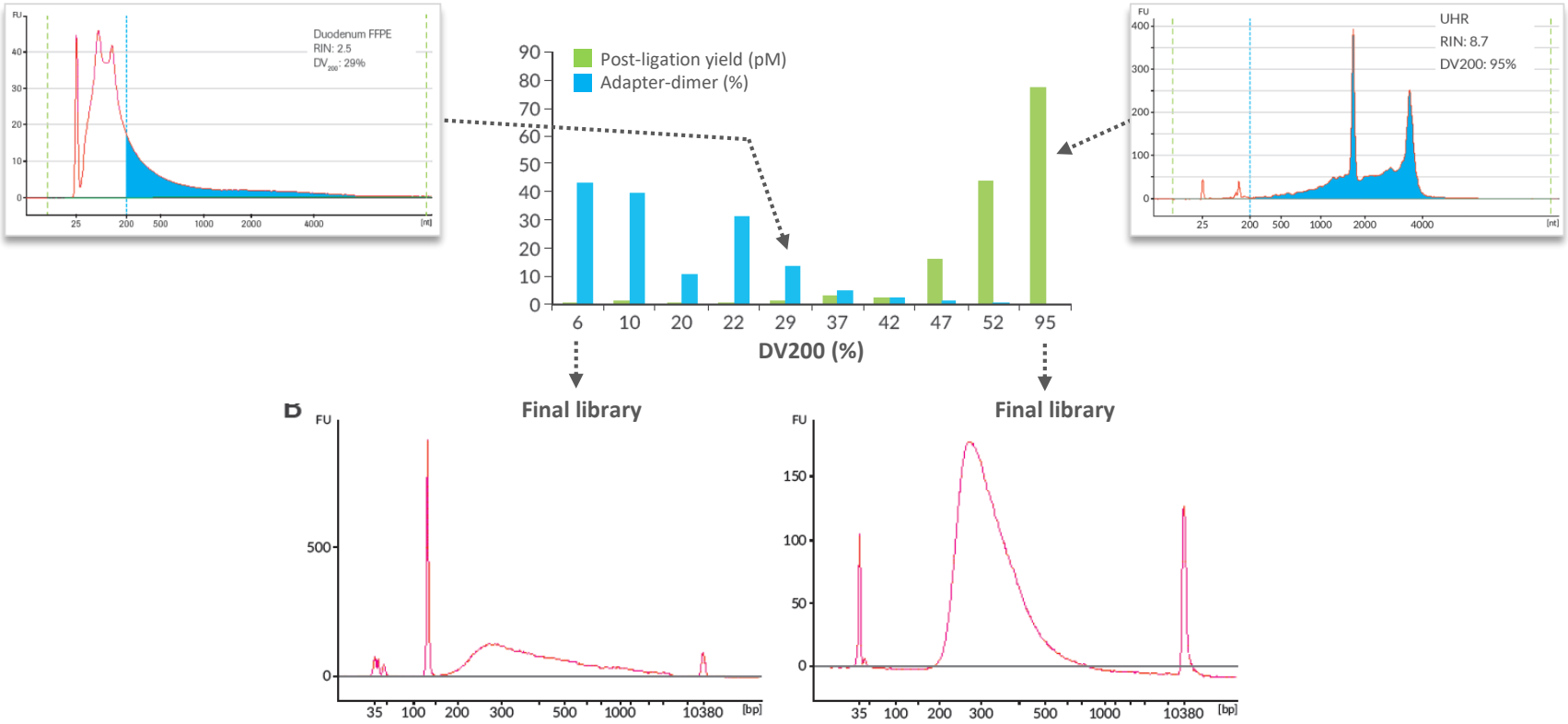
Valuable for protocol optimization

High library yields,
higher success rates from low-quality samples
flexible, single-day protocol

Unlock the Potential of Every Sample

Whole Transcriptome Sequencing from FFPE samples

Understanding the impact of input sample quality



Sample quality affects RNA-seq library construction. Libraries were constructed with the KAPA RNA HyperPrep Kit with KAPA RiboErase (HMR), from 100 ng of RNA from FFPE tissues (DV_{200} range: 6 – 52%) and a high-quality UHR control (DV_{200} = 95%). Electropherograms were generated using an Agilent RNA 6000 Pico Kit. Post-ligation yield was measured by qPCR, and adapter-dimer rates were calculated from electrophoretic assessment of final libraries. Representative traces of final libraries from low-quality (left) and high quality (right) samples were obtained using an Agilent High Sensitivity DNA Kit.

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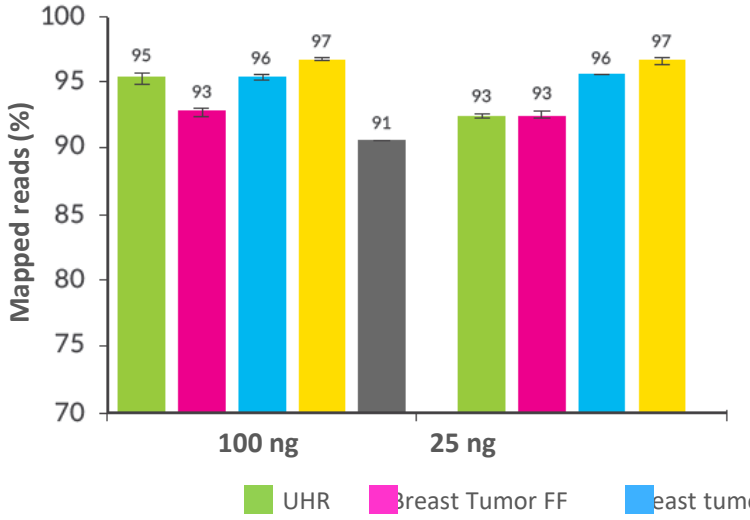
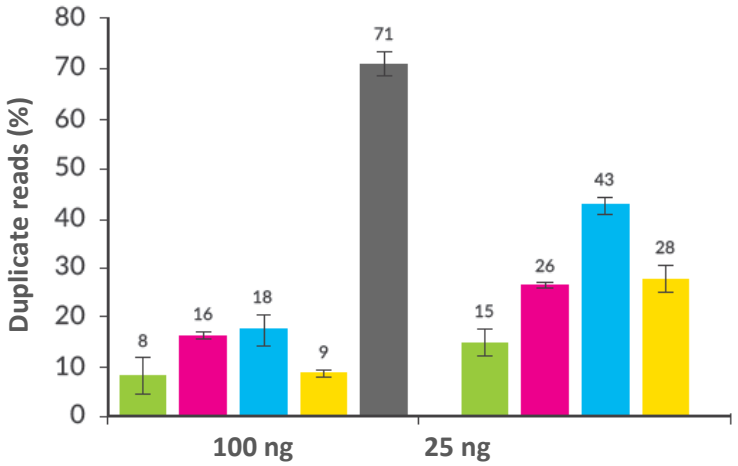
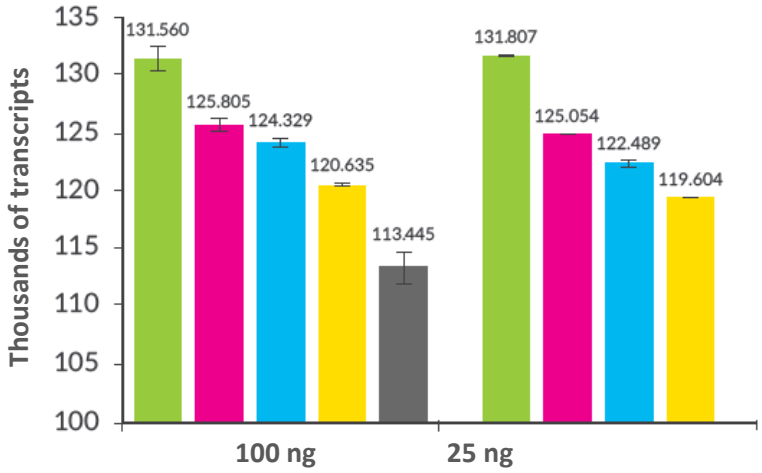
Whole Transcriptome Sequencing from FFPE samples

Understanding the impact of input sample quality





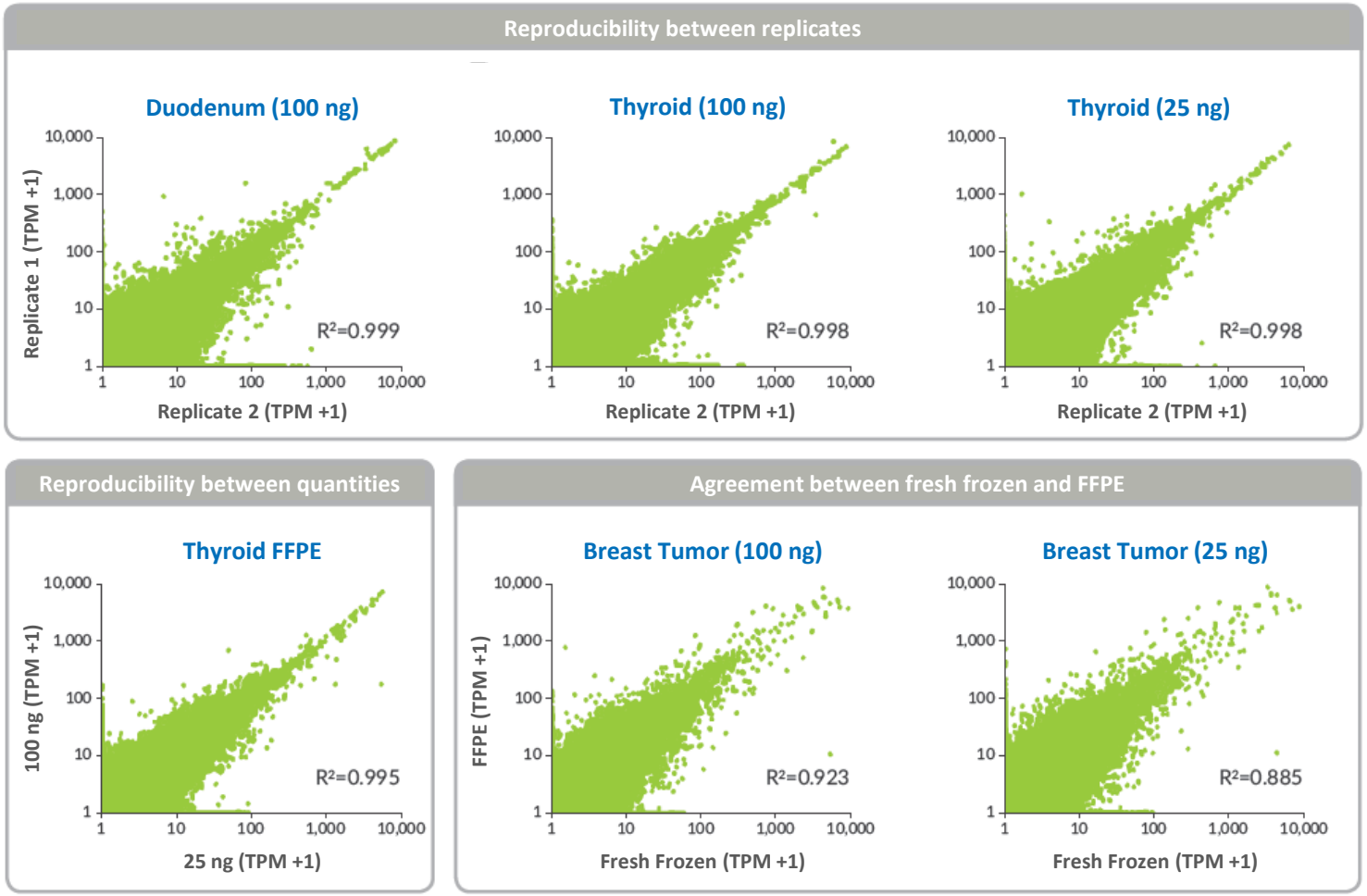


Impact of sample input and quality on sequencing metrics. DV_{200} values ranged from 95% (UHR) to 29% (duodenum sample). Sequencing (2 x 100 bp) was performed on an Illumina HiSeq 2500 instrument. Reads were aligned to a hard-masked version of human reference GRCh38, filtered to remove rRNA reads, and sub-sampled to 14M paired reads per sample. Gene expression was TMM-normalized and quantified using Kallisto.

Data on file.

Whole Transcriptome Sequencing from FFPE samples

Reliable results from degraded samples



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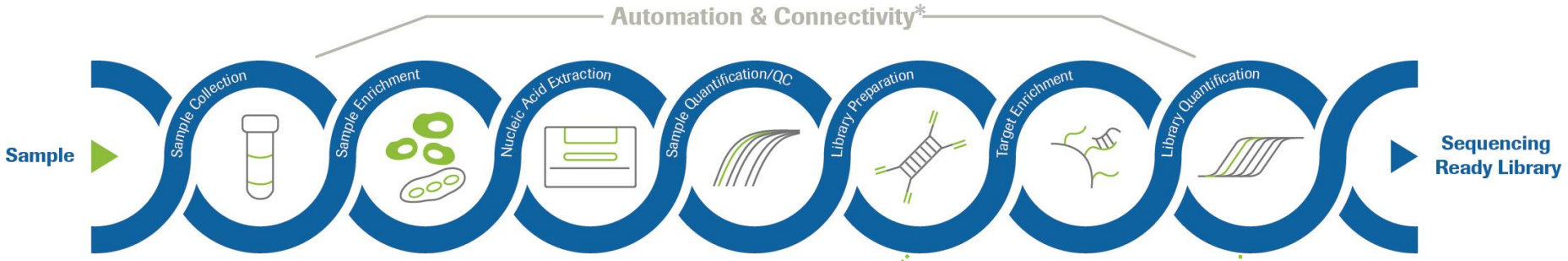
Transcriptome Profiling of Single Cells

KAPA HiFi improves efficiency of Smart-Seq[1]



- **KAPA HiFi Kit for cDNA pre-amplification**
- KAPA HyperPlus Kit
- KAPA DI or SI Adapters
- KAPA Pure Beads

Protocol in development

KAPA Library Quant Kits

Valuable for protocol optimization

Efficient, low-bias pre-amplification, streamlined library construction

1. Picelli S, Björklund ÅK, Faridani OR, et al. Smart-seq2 for sensitive full-length transcriptome profiling in single cells. Nature Methods. 2013; 10:1096

BRIEF COMMUNICATIONS

Smart-seq2 for sensitive full-length transcriptome profiling in single cells

Simone Picelli[1], Åsa K Björklund[1,2], Omid R Faridani[1], Sven Sagasser[1,2], Gösta Winberg[1,2] & Rickard Sandberg[1,2]

Single-cell gene expression analyses hold promise for characterizing cellular heterogeneity, but current methods compromise on either the coverage, the sensitivity or the throughput. Here, we introduce Smart-seq2 with improved reverse transcription, template switching and preamplification to increase both yield and length of cDNA libraries generated from individual cells. Smart-seq2 transcriptome libraries have improved detection, coverage, bias and accuracy compared to Smart-seq libraries and are generated with off-the-shelf reagents at lower cost.

Several methods exist for constructing full-length cDNAs from large amounts of RNA, including cap-enrichment procedures[1–3], but it is still challenging to obtain full-length transcriptome coverage from single cells. Existing methods either use 3′-end poly(A) tailing of cDNA[4,5] or template switching[6,7], or they sacrifice full-length coverage altogether for multiplexing before cDNA amplification[8,9]. We recently showed that Smart-seq, which relies on template switching, provides mo
transcripts than poly(A)-tailing
common use of template switchi
capture RNA 5′ ends[8,10]. Despite
transcriptome profiling methods,
made to improve cDNA library
single-cell amounts.

We systematically evaluated a
reverse transcription, template-sw
and PCR preamplification (for a
compared the results to those fron
after called SMARTer) in terms of
from 1 ng of starting total RNA
particular, exchanging only a singl
acid (LNA)[11] guanylate at the TSO
fold increase in cDNA yield rela
SMARTer IIA oligo ($P = 7.2 \times 10^{-}$
Supplementary Table 2 and **Supp**
a consequence of the increased t
base pairs (1–8 °C per LNA mon
that the presence of the methyl gr
nation with higher $MgCl_2$ concen
yield (by two- to fourfold; $P \leq 1.$

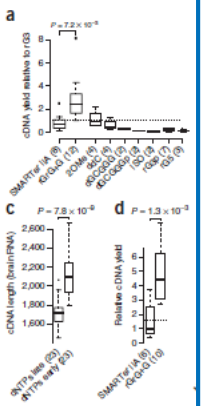


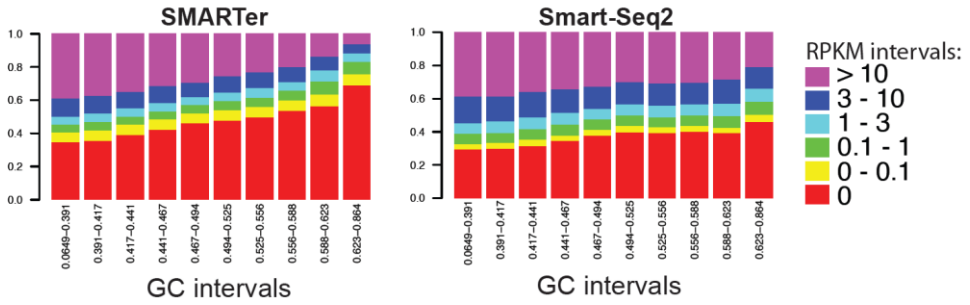
Figure 1 | Improvements in cDNA library yield and length. (**a**) Median yield of preamplified cDNA obtained using different TSOs, relative to those obtained using the rG3 oligo. All oligo sequences are found in **Supplementary Table 1**. (**b**) Median yield of preamplified cDNA in reactions with (black) or without betaine (gray) and as a function of increasing Mg^{2+} concentration, relative to cDNA yields obtained using SMARTer-like conditions. (**c**) Length of preamplified cDNA generated in reactions where dNTPs were added before RNA denaturation (early) or in the reverse transcription master mix (late). Experiments shown in **a**–**c** were based on 1 ng total RNA of mouse brain origin. (**d**) Median yield of preamplified cDNA from HEK293T cells using the LNA-G (rGrG+G) and SMARTer IIA template-switching oligos with the optimized protocol. Dotted horizontal line indicates median yield from commercial SMARTer reactions. (**e**) Median yield of preamplified cDNA from DG-75 cells in reactions with or without betaine. (**f**) Lengths of cDNA libraries generated from single HEK293T cells in reactions with or without bead extraction. Throughout figure, data are represented as box plots with numbers of replicates in parenthesis. Significant differences were determined using Student's *t*-test.

[1]Ludwig Institute for Cancer Research, Stockholm, Sweden. [2]Department of Cell and Molecular Biology, Karolinska Institutet, Stoc
should be addressed to R.S. (Rickard.Sandberg@ki.se).

RECEIVED 6 MAY; ACCEPTED 17 JULY; PUBLISHED ONLINE 22 SEPTEMBER 2013; DOI:10.1038/NMETH.2639

KAPA HiFi DNA Polymerase:

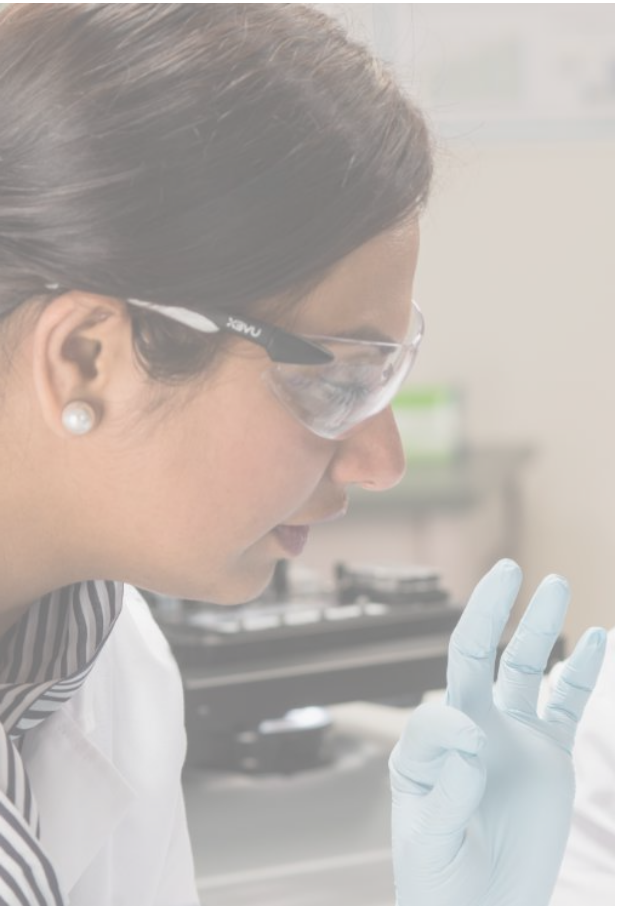
- **does not require bead purification** of the 1st strand synthesis reaction, yielded longer cDNAs
- allowed for the detection of **more genes at higher GC levels** (lower pre-amplification bias)
- provided **improved sensitivity and accuracy** of absolute expression levels
- enabled **more even coverage of both the 5′ and 3′ ends** of the transcripts



From: Picelli S, Björklund ÅK, Faridani OR, et al. Smart-seq2 for sensitive full-length transcriptome profiling in single cells. Nature Methods. 2013; 10:1096

Roche Sample Prep Solutions for Transcriptome Profiling

Summary



- **Flexible KAPA RNA HyperPrep protocol** supports sequencing of desired coding and/or non-coding transcripts
- **Variety of enrichment options**: mRNA capture; depletion of rRNA, globin and/or other transcripts; or capture of desired content after library construction
- **Efficient enrichment and library prep improves sequencing economy** (fewer reads wasted on unwanted content and PCR duplicates)
- **Robust, reliable performance** with **FFPE-derived RNA**
- **KAPA HiFi DNA Polymerase improves the efficiency of cDNA pre-amplification** for ultra-low input applications[1]

Unlock the Potential of Every Sample

1. Picelli S, Björklund ÅK, Faridani OR, et al. Smart-seq2 for sensitive full-length transcriptome profiling in single cells. *Nature Methods*. 2013; 10:1096

KAPA RNA HyperPrep Portfolio

Product Codes

Roche Mat. No.	KAPA Code	Description*	Pack Size
08098093702	KK8540	KAPA RNA HyperPrep	24 rxn
08098107702	KK8541	KAPA RNA HyperPrep	96 rxn
08098131702	KK8560	KAPA RNA HyperPrep with KAPA RiboErase (HMR)	24 rxn
08098140702	KK8561	KAPA RNA Hyper Prep with KAPA RiboErase (HMR)	96 rxn
08308314702	KK8562	KAPA RNA HyperPrep with KAPA RiboErase (HMR) Globin	24 rxn
08308241702	KK8563	KAPA RNA HyperPrep with KAPA RiboErase (HMR) Globin	96 rxn
08098115702	KK8580	KAPA mRNA HyperPrep	24 rxn
08098123702	KK8581	KAPA mRNA HyperPrep	96 rxn

*All KAPA RNA HyperPrep Kits contain KAPA Pure Beads for reaction cleanups

Roche Mat. No.	KAPA Code	Description	Kit Size
08278555702	KK8722	KAPA Dual-Indexed Adapters Kit (15 µM)*	96 adapters x 20 µL each
08278539001	KK8721	KAPA Adapter Dilution Buffer	25 mL

*Contains KAPA Adapter Dilution Buffer, as well as three additional sealing films to support multiple use.

Disclaimer

Roche Sample Prep Solutions comprise products that are For Research Use Only.
Contact your Roche sales representative for more information.

Automation and Connectivity products are in development.

KAPA, HYPERCAP and SEQCAP are for Research Use Only.
Not for use in diagnostic procedures.

Millsect and MagNA Pure 24 and 96 products are for use for *in vitro* diagnostics.

Cell-Free DNA Collection Tubes are for RUO* and IVD** use.
*For Research Use Only. Not for use in diagnostic procedures.
**Available for CE-IVD where the CE-mark is accepted.

Unless otherwise stated, all data generated by and on file with Roche.

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