SEQ YOUR TRUE DIRECTION

REALLY®| rNONE™ a simplified workflow for ribo-depleted RNA-Seq library preparation **from severely damaged FFPE RNA**

WHY RETHINK THE RNA-SEQ WORKFLOW?

Current RNA-Seq protocols are riddled with superfluous steps

RNA-Seq is a next-generation sequencing (NGS) workflow routinely used for gene expression profiling and whole transcriptome analyses, key to characterizing biological states in all organisms.

ClaretBio's newest NGS offering is a directional RNA-Seq library preparation method that uses single-strand ligation to generate libraries directly from the first strand cDNA, **eliminating the need for second strand synthesis and DNA end-polishing.** Total RNA is immediately converted to cDNA and depletion of ribosomal reads is only performed after ligation, thus reducing the exposure of labile RNA to processing steps. This simplified workflow results in significant reductions in reagent requirements, protocol steps and time, while generating high quality, sequencing-ready RNA-Seq libraries.

REALLY™ is a robust RNA-Seq workflow that includes **rNONE**™ a ribosomal RNA depletion module that significantly reduces rRNA carryover

Workflow				Experiment		
Method	Ribo-depletion method	Adapter Ligation	Directionality	Input DNA	Input Amount	Index PCR Cycles
REALLY	Tiling probes, bead-pull-down based on ligated cDNA	Single-stranded	No 2nd Strand synthesis	Univeral Human Total RNA	10 ng - 250 ng	9 - 14
dsPrep	Tiling probes, RNaseH-based on total RNA	Double-stranded	Uracil-based 2nd strand removal			12 - 16

We compared the performance of ClaretBio's REALLY I rNONE workflow with a traditional double-stranded library preparation method that performs upstream ribodepletion on total RNA followed by library preparation. We used the Agilent® Technologies's Universal Human Total RNA Reference input to represent good quality RNA and assessed the performance with inputs as low as 10ng, upto 250ng. Both methods were performed following manufacturer's instructions.

HOW DO **REALLY** LIBRARIES COMPARE?

Generate libraries from the low amount of RNA with fewer PCR cycles

Condition	Input	PCR cycles	Yield (ng)	Adapter dimer %	Avg. bp
REALLY	10ng	11	26.16	1.01	479
	50ng	9	22.52	0.21	491
	100ng	9	46.8	0.1	463
	250ng	9	81.2	0.02	464
dsPrep	10ng	16	16.4	0	379
	50ng	14	17.16	0.08	383
	100ng	14	24.8	1.14	390
	250ng	14	66.4	0.03	414

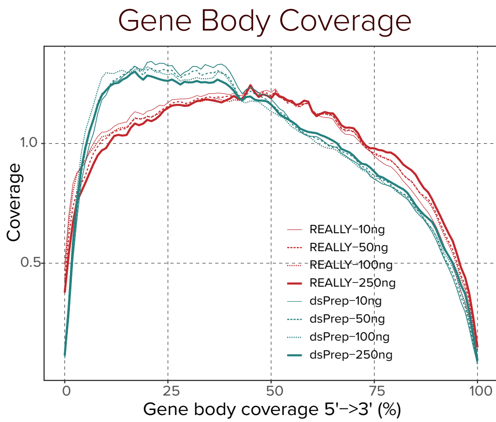
For high quality and RNA high input amounts, both REALLY protocol and the dsPrep protocol showed equivalent molecular metrics however, the double-stranded methods required additional 5 cycles of PCR. Fewer PCR cycles reduces PCR biases and formation of PCR duplicates. To further delinate PCR duplicates form true transcript copies, the workflow can be seamless integrated ClaretBio's with UMI Add-on module.

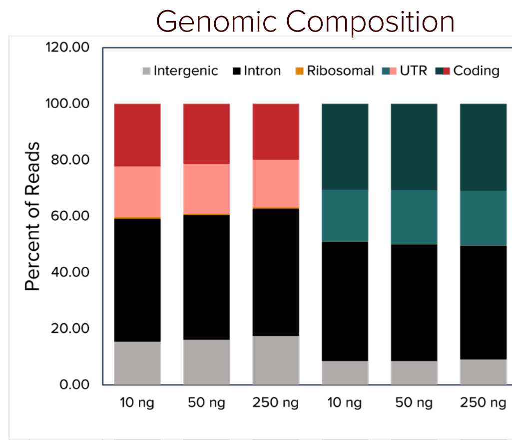
Libraries were sequenced on Illumina NextSeq 500 and analyzed using a comprehensive bioformatic pipeline called really-run that includes UMI deconvolution when required. **Available for download at www.claretbio.com/software/really-run**

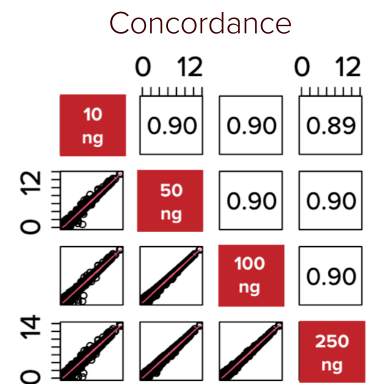
HOW DO **REALLY** LIBRARIES PERFORM?

Make libraries of high mapping quality and uniform gene body coverage

Condition	Input	Total Reads	Percent Kept	Percent Uniquely Mapped	Percent Unmapped	Percent duplicates (Picard)	Percent Correct Strand
REALLY	10ng	34161360	96.69	87.74	4.92	24.56	97.95
	50ng	41115391	97.98	89.62	4.11	11.66	98.28
	100ng	42018937	99.32	90.54	3.94	10.09	98.31
	250ng	38043713	99.44	90.76	4.06	8.18	98.28
dsPrep	10ng	37819288	99.77	92.21	3.49	85.74	97.86
	50ng	35110622	99.88	92.05	3.29	55.55	97.39
	100ng	22001510	99.83	90.62	4.51	30.62	97.38
	250ng	37867109	99.91	91.83	3.52	27.01	98.32





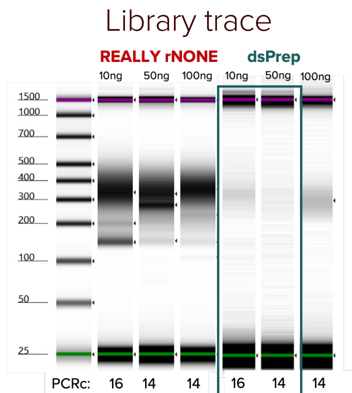


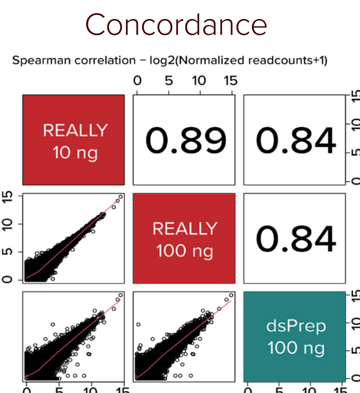
Both approaches generated libraries of high quality mapping metrics across the range tested. REALLY demonstrated more uniform coverage across the transcipts, whereas dsPreps showed 5' bias. The dsPreps captured slightly higher amount of coding region and lower percent of intergenic genes. REALLY libraries have high concordance of read counts between libraries across the input range tested.

WHAT ABOUT **REALLY** BAD SAMPLES?

Degradation during processing and storage make RNA poor inputs for RNA-Seq

Method	Input	pairs	Percent Kept	Percent Mapped	Percent Unmapped	Percent duplicates	Percent correct strand
REALLY	10ng FFPE	35416649	80.30	78.40	11.55	85.68	97.91
	100ng FFPE	10475270	98.46	88.36	6.05	69.06	92.26
dsPrep	100ng FFPE	33338471	95.79	83.32	8.25	45.70	98.51





Formalin and paraffin fixed can severely damage RNA in preserved tissue. REALLY outperformed double-stranded methods in generating high quality RNA-Seq libraries from degraded RNA. dsPreps were unable to generate libraries even after 16 cycles of PCR from 10 - 50 ng of FFPE RNA. Whereas REALLY generated sequence ready libraries for the range tested with higher mapping percent. High concordance was seen between REALLY libraries made from 10 ng and 100 ng of FFPE RNA.

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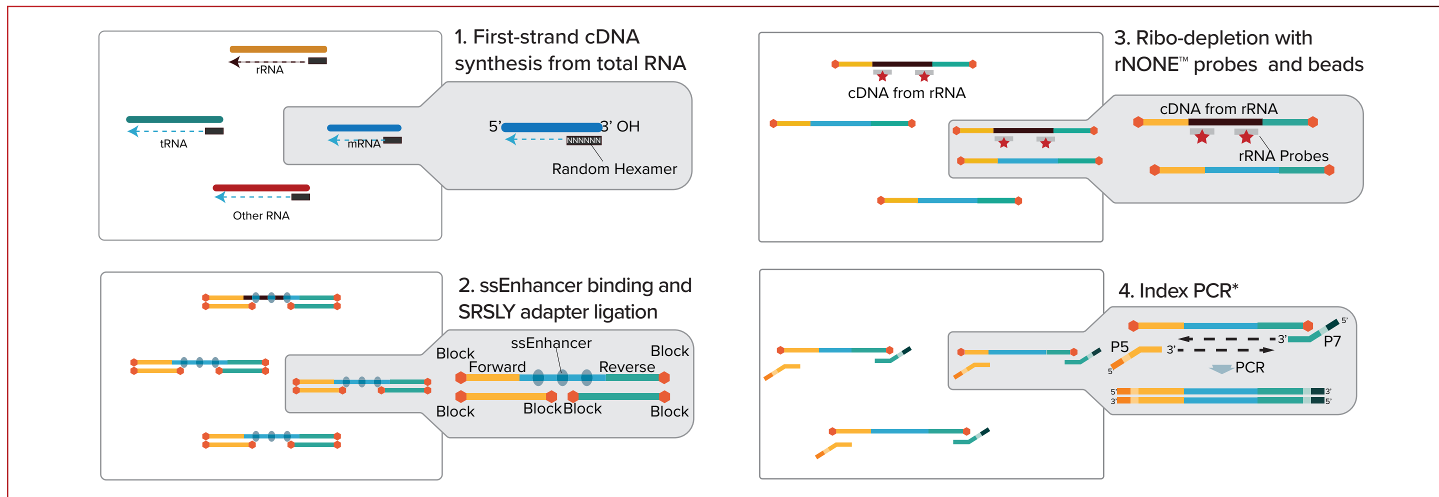
WORKFLOW



PRODUCT SPECIFICATIONS

- Generates final libraires from total RNA in ~7 hours
- Contains reagents for first strand synthesis, adapter ligation, ribo-depletion indexing primers and magnetic beads for purification.
- Depletes all human rRNA, mitochondrial rRNA and other over-abundant ribosomal transcripts
- Compatible with an optional step for Unique Molecule Indentifier (UMI) addition
- Enzymatic method; compatible with automation platforms

INPUT TYPES

Total RNA from

- Formalin-fixed paraffin embedded tissue
- Fresh-frozen tissue
- Cell-cuture
- Other degraded and low quantity RNA sources

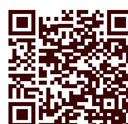
KIT SPECIFICATIONS

The REALLY® bundle contains the follow components (available in 24 or 96 reaction formats)

Modules	Components
rNONE™ kit	First Strand Synthesis reagents, Ribodepletion probes and Streptavidin Beads
SRSLY® Base Kit	ssEnhancer, Adapters, Ligation Master Mix, Index-PCR Master Mix
Clarefy™ Beads for REALLY™	DNA Purification beads (Recommended)
UDI Primers (Optional)	UDI primer mix
UMI Add-on (Optional)	UMI Extension Primer, UMI Extension Enzyme, Buffer, UMI/UDI Primers

REALLY. ORDER TODAY!

Early adapter and bulk discounts available



Scan the QR code to order a trial kit

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