Ribosomal Depletion (Ribominus™) for Whole Transcriptome RNA-Seq Studies

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Introduction

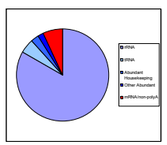
As analysis of gene expression moves from microarrays to massively parallel sequencing technologies, abundant ribosomal RNA transcripts are significant barriers to efficient use of valuable instrument capacity. These are not the target of gene expression studies, but are highly abundant in total RNA preparations. Current strategies include specific selection of only mRNA with oligo dT, an approach that ignores all non-polyadenylated transcripts with potentially interesting function. An alternative approach that depletes abundant, unwanted ribosomal RNA is essential for optimal applications of next generation expression studies. Invitrogen's RiboMinus™ platform technology utilizes specific locked nucleic acid capture probes to bind unwanted ribosomal RNA, which are subsequently removed from the sample via binding to streptavidin coated Dynabeads®. The remaining whole transcriptome RiboMinus™ RNA is suitable for direct sequencing using any of the next generation sequencing platforms or microarray analysis.

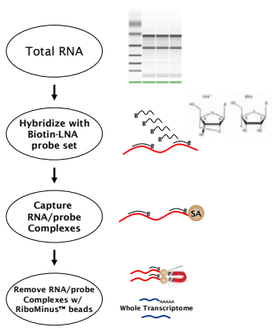
Here we demonstrate the performance of the RiboMinus™ Eukaryote Kit for RNA-Seq and RiboMinus™ Plant Kit for RNA-Seq to deplete ribosomal RNAs (28s, 18s, 16s, 5.5s and 5s) from several mammals and plants and results from RiboMinus™ and oligo-dT selected RNA samples.

Figure 1 – RiboMinus™ Parameters

RiboMinus™ Overview

- Abundant RNA depletion system
- Biotin-LNA probe sets
- Streptavidin magnetic beads
- Whole transcriptome RNA





Specificity of probes for Ribosomal large and small subunits

Model Organisms		
Turf	Surf	Plants
Human	Sea squirt	Arabidopsis thaliana
Mouse	Coelacanth	Brassica napus
Rat	Lancelet	Oryza sativa
Frog	Eel	Lycopersicon esculentum
Rabbit	Salmon	Humulus lupulus
Cow	Sturgeon	Zea mays
Pig	Ratfish	Wheat
Chicken	Lamprey	Soybean
Drosophila	Brown Plankton	Pine
C.elegans	Zooplankton	Aspen
Midge		
Mosquito		
Yeast - S. cerevisiae		
Yeast - S. pombe		

5S - zero to 1 mismatches	
Model Organisms	Plants
Human	Arabidopsis thaliana
Mouse	Zea mays
Rat	
Frog	
Chicken	
Drosophila	
C.elegans	

5.8S - zero mismatches	
Model Organisms	
Human	
Mouse	
Rat	

Figure 2 Whole transcriptome enrichment by ribosome depletion; Optimized depletion of 28s and 18s rRNA

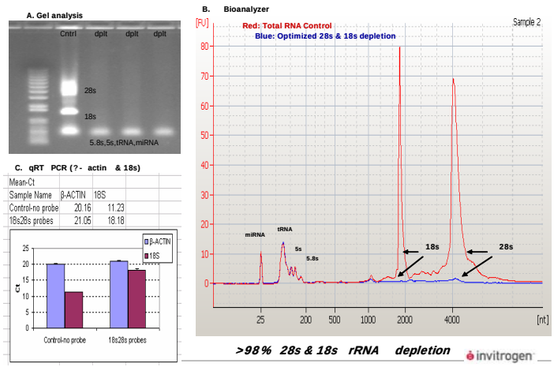


Figure-2 Optimized 28s and 18s rRNA depletion. Panel A shows the control (undepleted) total RNA and triplicate lanes of the new optimized 28s/18s rRNA depletion. Panel B. shows the Bioanalyzer sketch (Agilent) of the total RNA control (red line) overlaid with the optimized 28s and 18s rRNA depletion protocol. Panel C shows the depletion of 18s rRNA by a shift upwards in qRTPCR cycle threshold; while the levels of β-actin mRNA (normalized by volume) remain constant. Greater than 98% of 28s and 18s is depleted.

Figure 3 – New depletion of 5.8s and 5s rRNA

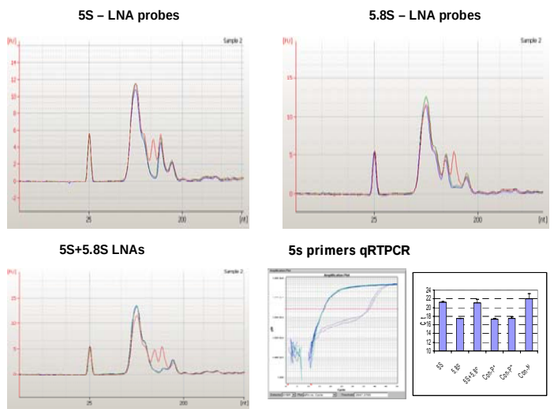
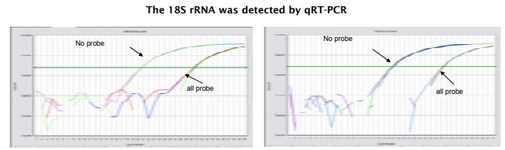


Figure-3 New depletion of 5s and 5.8s rRNA. Upper panels show bioanalyzer overlays, the depletion of the 5s and 5.8s ribosomal subunits with sequence specific biotinylated LNA probes. Lower panels demonstrate combined depletion of both 5s and 5.8s rRNA depletion on the Bioanalyzer overlays and confirm specific depletion of the 5s rRNA by qRTPCR with 5s specific primers that show increased cycle threshold number following the addition of 5s specific LNA probes.

Figure 4 – Up to 99% depletion of ribosomal RNA

Evaluation of new kit by – qRT-PCR



	Mouse RNA	Human RNA
*ΔCt	9.84	9.72
% depletion	99.99	99.99

* ΔCt = Ct number of no probe-Ct number of all probes

Figure 5 – Evaluation of steady state genes by qRT-PCR

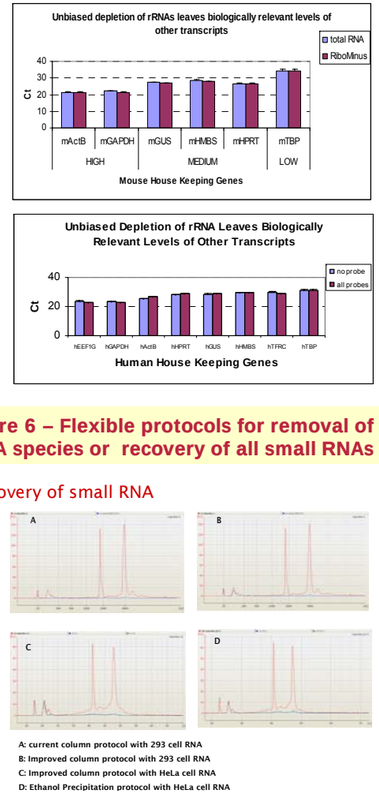
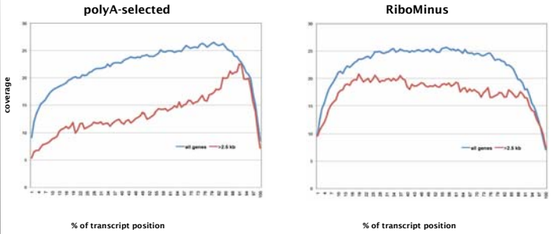


Figure 6 – Flexible protocols for removal of tRNA species or recovery of all small RNAs

Recovery of small RNA

A: current column protocol with 293 cell RNA
B: Improved column protocol with 293 cell RNA
C: Improved column protocol with HeLa cell RNA
D: Ethanol Precipitation protocol with HeLa cell RNA

Figure 7. mRNA-Seq shotgun profiling; Ribominus enhances transcriptome sequence coverage



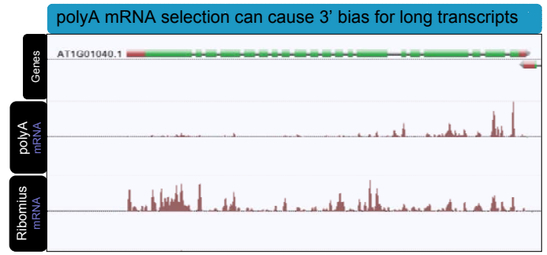
Resource

Cell

Highly Integrated Single-Base Resolution Maps of the Epigenome in *Arabidopsis*

Cell 133, 1–14, May 2, 2008 ©2008 Elsevier Inc.

polyA mRNA selection can cause 3' bias for long transcripts



Discussion

Understanding of the transcriptome is under going a revolution in which its annotation and regulated expression are drastically more complex under the new paradigm. Observations that at least ten times more transcription is occurring in mammalian species, that a large fraction is cell type specific, that nearly half is non-polyadenylated, - that many transcripts are non-protein coding (NC) yet possess regulatory functions, and that low copy units of extra small and large transcripts are located in unannotated regions - indicates that an enormous number of transcriptional elements are yet to be discovered, annotated and functionally defined. Enrichment of the whole transcriptome elements – specifically the nonpolyadenylated elements through depletion of ribosomal elements will enhance gene discovery and expression profiling through RNA-Sequencing and related methods which will facilitate the elucidation of normal physiologic and pathologic processes.