Flexible Method for Targeted Transcript Depletion from RNA-Seq Libraries — Mouse, Rat, *Drosophila*, and *Arabidopsis*

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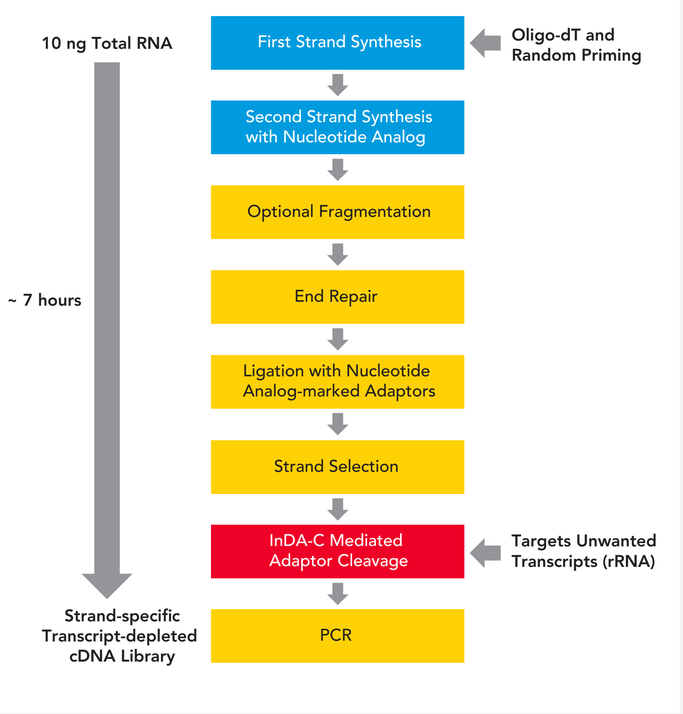
ABSTRACT

This poster describes a novel method, Insert Dependent Adapter Cleavage (InDA-C), for effective removal of specific transcripts from RNA-Seq libraries without impacting non-targeted transcripts. InDA-C employs specific and robust enzymatic steps to eliminate undesirable transcripts such as rRNA during library construction without perturbing the original total RNA population as with hybridization capture methods. The specificity of transcript depletion relies on InDA-C primers which can be designed to target virtually any class of unwanted transcripts from any species. The library construction workflow uses as little as 10 ng of input total RNA, produces a strand-specific library, and is highly adaptable for depletion of any unwanted transcript(s). Here we report the unbiased removal of rRNA and chloroplast RNA from RNA-Seq libraries across a variety of important model organisms including Mouse, Rat, *Drosophila* and *Arabidopsis*. Use of InDA-C primers designed against cytoplasmic and mitochondrial rRNAs from these species resulted in the reduction of sequencing reads derived from these transcripts to 2.5 – 8.7% of the total mapped reads. In the case of *Arabidopsis*, the combined use of InDA-C primers targeting rRNA and chloroplast rRNA resulted in an increase of informative reads (non-rRNA and non-chloroplast rRNA) from 8.5% in absence of InDA-C depletion to 57% following targeted depletion with InDA-C. Therefore, through the use of InDA-C technology a greater percentage of RNA-Seq sequencing reads can be directed towards desired coding and non-coding transcripts.

Fig. 1

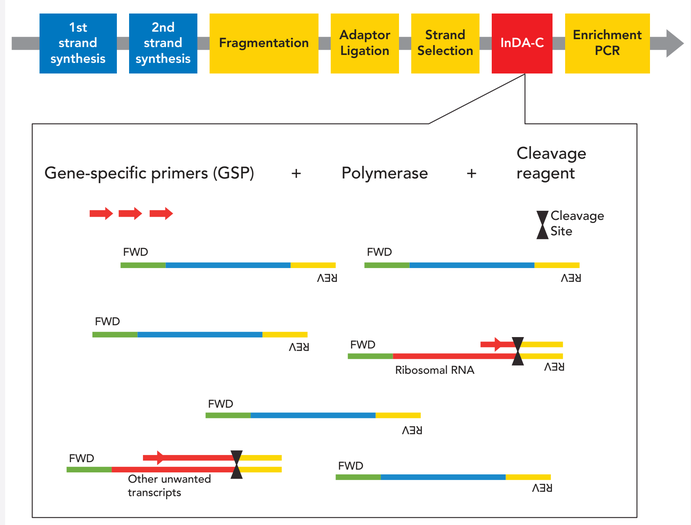


RNA-Seq library construction workflow. Library adaptors are specific for the Illumina sequencing platforms, and enable barcoding up to 96-plex.

FOR MORE INFORMATION

To receive information on the design of custom workflows using InDA-C, or for any other questions, please contact Steve Kain at skain@nugen.com

Fig. 2



Primer Extension to Form Cleavage Site

Assay workflow illustrating targeted depletion of unwanted transcripts using InDA-C. After adaptor ligation and strand selection the library is incubated with gene-specific primers (GSP) which target inserts containing unwanted transcripts such as rRNA. Primer extension into the reverse adaptor (REV) creates a cleavage site in the double-stranded adaptor. Addition of the cleavage reagent specifically cuts these reverse adaptors, making them non-amplifiable during enrichment PCR and cluster formation.

Table 1

Organism	Transcripts Targeted for Deletion
Human	Cardiac actin and myosin
Mouse	rRNA, globin
Goat	rRNA, casein protein RNAs
Yeast	rRNA, housekeeping genes
Grape	rRNA, chloroplast RNA
Drosophila	rRNA
Plant fungus	Top 30 expressed transcripts in host plant
Tuberculosis	Human host transcripts, TB rRNA

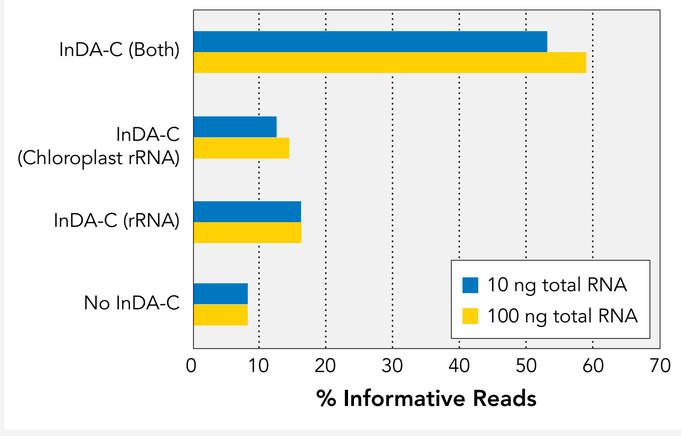
Examples of RNA-Seq projects. Projects used custom InDA-C primers to target the indicated unwanted transcripts for depletion from the RNA-Seq library.

Table 2

	InDA-C	% Aligned Reads	% rRNA Reads	% Strand Retention (Coding)	Transcripts Detected
Mouse	+	90	8.7	99.4	18,439
	–	95	80	99.3	15,395
Rat	+	94	2.5	98.9	12,098
	–	97	53.1	98.8	7,202
Drosophila	+	86	7.6	99.1	14,684
	–	96	85.7	98.8	12,003

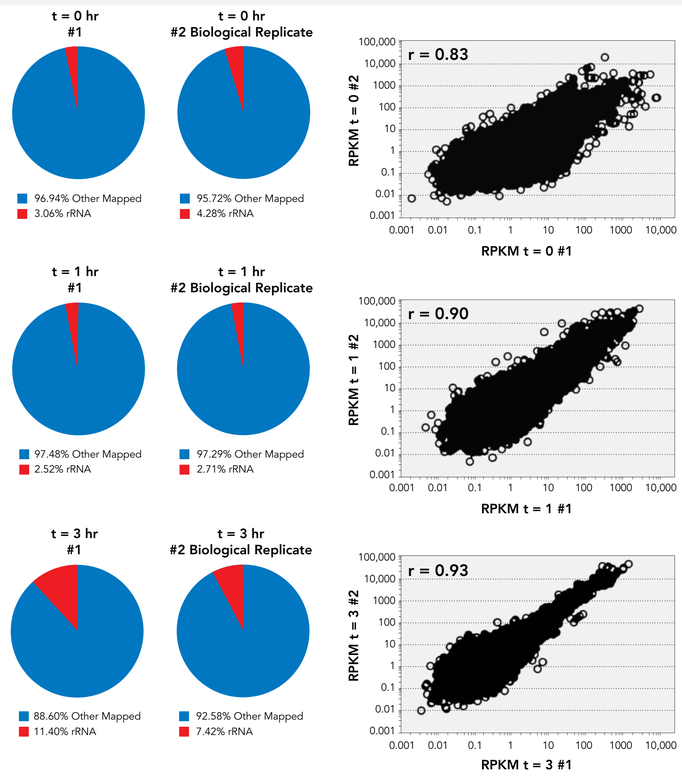
Sequencing performance metrics. Sequencing performance metrics with InDA-C primers targeting rRNA from different Model Organisms. Input is 10 ng total RNA from Mouse Universal Reference, Rat Brain, and *Drosophila* S2 RNAs; The number of detected transcripts is calculated using an FPKM threshold = 0.1.

Fig. 3



The percentage of informative reads was increased from ~8.5% to ~57% by targeted depletion using InDA-C primers; InDA-C primers designed to cytoplasmic 25S, 18S, 5.8S rRNA + chloroplast rRNA

Fig. 4



Measurement of genome-wide mRNA decay in whole fly embryos by pulse-chase labeling of nascent transcripts with 4-thiouridine. The results show a reduction in %rRNA reads to ~3–4% at t=0 hours using InDA-C, which increases to ~7–11% following the 3-hour chase to due to mRNA degradation and a relatively higher %rRNA

CONCLUSIONS

NuGEN has developed a strand-specific RNA-Seq library construction method that allows for researchers to customize depletion of unwanted transcripts:

- **Customizable:** NuGEN will assist researchers to design and source custom InDA-C primers for targeted depletion of unwanted transcripts
- **Integrated method for transcript reduction:** Uses targeted depletion during library construction to minimize undesired sequences in the final library
- **A complete solution for strand-specific RNA-Seq:** Includes all required components for preparation of strand-specific RNA-Seq libraries for use on all Illumina NGS platforms

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