

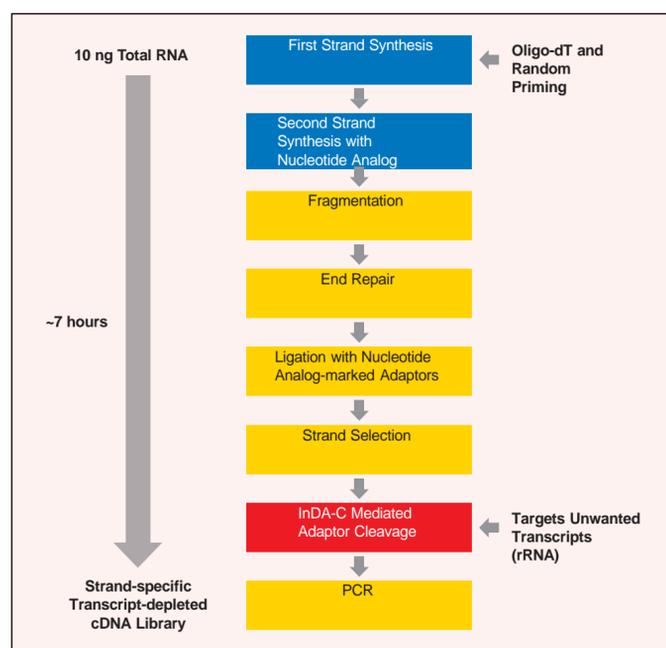
# Flexible Method for Targeted Transcript Depletion from RNA-Seq Libraries

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## ABSTRACT

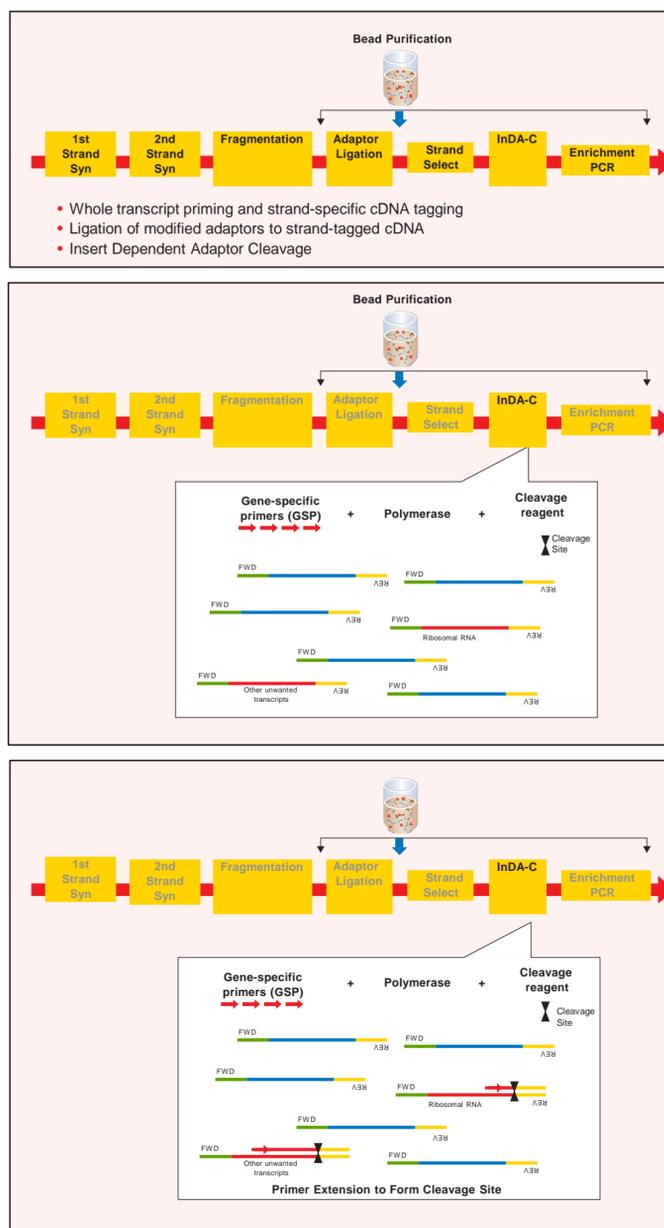
This poster describes a novel method, Insert Dependent Adapter Cleavage (InDA-C), for effective removal of specific transcripts from RNA-Seq libraries without impacting non-targeted transcripts. InDA-C employs specific and robust enzymatic steps to eliminate undesirable transcripts such as rRNA during 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 from RNA-Seq libraries across a variety of prokaryotic organisms and mixed species samples. Use of InDA-C primers designed against rRNAs from both bacterial and host species resulted in >98% reduction in rRNA transcripts compared to samples prepared without the InDA-C approach. As a result, 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

Fig. 2



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

100 ng input	Aligned to Human genome	Aligned to pathogen genome
Human:Pathogen 50:50 no InDA-C	36%	57%
Human:Pathogen 50:50 with InDA-C	21%	78%
Human:Pathogen 95:5 with InDA-C	77%	18%
Human:Pathogen 80:20 with InDA-C	45%	52%
Human:Pathogen 50:50 with InDA-C	21%	78%

Sequencing performance metrics with InDA-C primers targeting rRNA from both human and 'pathogen' (*S. aureus*) rRNAs. Input is 100 ng total RNA in varying mass ratios. This system was designed to simulate a host-pathogen mixed sample, with the goal of enriching the sample for pathogen transcripts by depleting human and pathogen rRNA reads.

Fig. 3

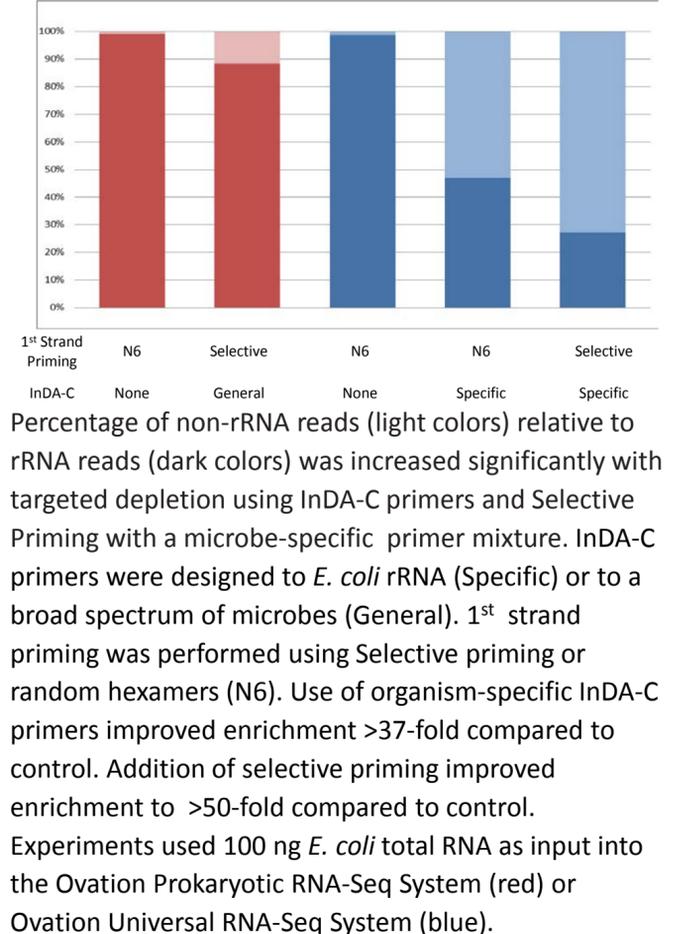
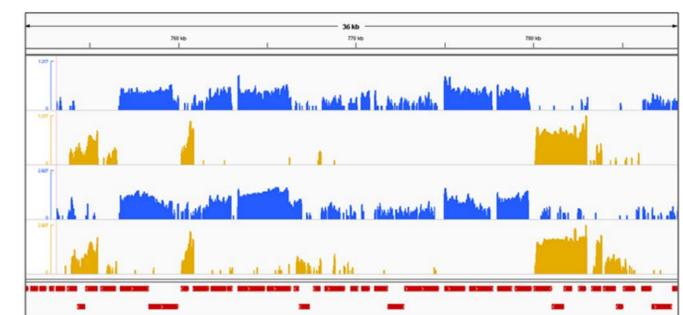


Fig. 4



Replicate plots of a representative region in the *S. aureus* genome strand-specific coverage. 100 ng *S. aureus* total RNA was used as input for the Ovation Prokaryotic RNA-Seq System.

## CONCLUSIONS

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

- **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 low input preparation of strand-specific RNA-Seq libraries for use on all Illumina NGS platforms
- **Affordable and scalable:** Features flexible barcoding capability for 2- to 96-plex sequencing to improve sample throughput and reduce sequencing costs
- **Customizable:** NuGEN will assist researchers to design and source custom InDA-C probes for targeted depletion of unwanted transcripts