Selective removal of abundant RNAs enhances the sensitivity of transcript detection across different Prokaryotic and Archaeabacterial species

Genomics

Amanda Hoppers (ahoppers@neb.com), NEB, **Keerthana Krishnan**, New England Biolabs, **Deyra Rodríguez**, NEB, **Bradley Langhorst**, New England Biolabs, **Gautam Naishadham**, New England Biolabs, **Jian Sun**, NEB, **Kaylinnette Pinet**, Neb, **Anetta Nowosielska-Vecchietti**, NEB, **Christine Sumner**, NEB, **Fiona Stewart**, NEB, **Lynne Apone**, NEB, **Eileen Dimalanta**, New England Biolabs, **Theodore Davis**, New England Biolabs

RNA-Seq is a widely used technology with a broad range of applications, including studying unique prokaryotic species. Tools to study these organisms can frequently lag behind the resources available for eukaryotic species, making it difficult to take an omics approach for their analysis. Moreover, the dynamic range of transcript expression within a sample presents a challenge in whole-transcriptome sequencing. Highly expressed transcripts with minimal biological interest can dominate readouts, masking detection of more informative lower abundant transcripts. Here, we present a robust method to enrich for RNAs of interest by eliminating rRNA in diverse bacterial species. We further introduce an approach to customize RNA depletion and eliminate specific RNAs in any organism not well covered by a pre-optimized kit.

Using strand-specific RNA sequencing we measured sequencing metrics before and after depletion in both bacterial monocultures and communities across a range of input amounts (10ng – 1ug). We achieved high depletion efficiencies (up to 99.9 %) in all samples with minimal off-target effects. We detect a high number of transcripts, with even coverage across the transcript length, while retaining transcript complexity even at the lowest inputs.

We introduce a user-friendly web tool (highlighted here) to enable custom probe design to supplement an existing kit or to target other species. We used this web tool and depletion method to remove rRNA from total RNA of various species, including archaea Thermococcus kodakarensis and Pyrococcus furiosus. Additionally, we used this approach to target highly abundant coding RNAs in more complex total RNA samples, and supplemented an existing anti-rRNA probe set to achieve depletion of both rRNA and the selected coding RNAs.

We conclude that the reduction of abundant transcripts for RNA-Seq studies significantly increases the ability to detect true biological variation that could not be efficiently detected in non-depleted samples. The method described here is a reliable and simple solution that greatly improves sensitivity in transcriptome studies and is amenable to high-throughput automation.