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RNAseH-based ribodepletion

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Nicole Schonrock[1], Helaine Graziele Santos Vieira[1], Oguzhan Begik[2], Eva Maria Novoa[3]
[1]Garvan Institute for Medical Research; [2]Centre for Genomic Regulation; [3]Center for Genomic Regulation (CRG)

Eva Maria Novoa
Center for Genomic Regulation (CRG)

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Protocol status: Working
We use this protocol and it's working

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Keywords: using rnaseh nuclease, rnaseh enzyme, rnaseh nuclease, based ribodepletion goal, rnaseh, ribodepletion goal, rna sample, rna molecule, dna oligonucleotide, complementary to human rrna, rna, approaches as nuclease, dna oligo, samples from rrna, rrna molecule, ribodepletion, human rrna, many rounds of ribodepletion, ribodepleted material, nuclease, rrna, 28s ribodepletion, dna, enzyme, dna duplex

Disclaimer

Do not use for long read approaches.
This protocol is adapted from the protocol used in Adiconis et al., Nature Methods 2013 (https://doi.org/10.1038/nmeth.2483)

Abstract

Goal: Method to ribodeplete samples from rRNAs using RNAseH nuclease.
Summary: DNA oligos are complementary to human rRNAs. They will anneal to the rRNA molecules. RNAseH will degrade RNA-DNA duplexes, leaving the RNA samples depleted from rRNAs
Tested on :Human, Mouse and Yeast (for 18s and 28s ribodepletion).
Cost: DNA oligonucleotides will be expensive the first time ordered, but will last for many rounds of ribodepletion. Once the DNA oligonucleotides have been purchased, the major cost comes from the RNAseH enzyme. In our hands, the cost is much lower than commercial kits, thus being scalable for high input samples where the ribodepleted material needs to be high.
Recommended for: NGS-based approaches. Do not use for Nanopore-based approaches as nuclease-based methods degrade RNA molecules.

Troubleshooting