Jan 26, 2024 Version 2

selSeq: A method for the enrichment of non-polyadenylated RNAs including enhancer and long non-coding RNAs for sequencing V.2

PLOS One

Peer-reviewed method

DOI
dx.doi.org/10.17504/protocols.io.j8nlkwpk6l5r/v2

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OPEN ACCESS

DOI: https://dx.doi.org/10.17504/protocols.io.j8nlkwpk6l5r/v2

External link: https://doi.org/10.1371/journal.pone.0289442

Protocol Citation: Jason D Limberis, Joel Ernst, John Metcalfe, Alina Nalyvayko 2024. selSeq: A method for the enrichment of non-polyadenylated RNAs including enhancer and long non-coding RNAs for sequencing. **protocols.io** **https://dx.doi.org/10.17504/protocols.io.j8nlkwpk6l5r/v2**Version created by **Jason D Limberis**

Manuscript citation:
Limberis JD, Nalyvayko A, Ernst JD, Metcalfe JZ (2023) *sel*Seq: A method for the enrichment of non-polyadenylated RNAs including enhancer and long non-coding RNAs for sequencing. PLOS ONE 18(11): e0289442. https://doi.org/10.1371/journal.pone.0289442

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

Created: January 26, 2024

Last Modified: January 26, 2024

Protocol Integer ID: 94220

Keywords: enhancer rna, polyadenylated rna, selective sequencing of crucial regulatory element, rna expression, rna, polyadenylated rnassuch, including enhancer, enhancer, selective sequencing, sequencing non, mrna, essential roles in gene regulation, gene regulation, crucial regulatory element, selseq, sequencing, method for the enrichment, large subset of crucial regulator

Funders Acknowledgements:
Program for Breakthrough Biomedical Research (PBBR)
Grant ID: NA

Abstract

Non-polyadenylated RNA includes a large subset of crucial regulators of RNA expression and constitutes a substantial portion of the transcriptome, playing essential roles in gene regulation. For example, enhancer RNAs are long non-coding RNAs that perform enhancer-like functions, are bi-directionally transcribed, and usually lack polyA tails. This paper presents a novel method, *sel*Seq, that selectively removes mRNA and pre-mRNA from samples to enable the selective sequencing of crucial regulatory elements, including non-polyadenylated RNAssuch as long non-coding RNA, enhancer RNA, and non-canonical mRNA.

Materials

Required

SuperScript® III First-Strand Synthesis System **Thermo Scientific** **Catalog #**18080-051

RNase H - 1,250 units **New England Biolabs** **Catalog #**M0297L

TURBO DNase 2 U/uL **Fisher Scientific** **Catalog #**AM2239

Agencourt RNAClean XP Magnetic Beads **Beckman Coulter** **Catalog #**A63987

Ethanol

A thermocycler and a qPCR machine

A magnetic rack

Optional

Luna Universal Probe One-Step RT-qPCR Kit - 200 rxns **New England Biolabs** **Catalog #**E3006S

Eukaryotic 18S rRNA Endogenous Control (FAM™/MGB probe, non-primer limited) **Thermo Fisher** **Catalog #**4333760F

TaqMan™ GAPDH Control Reagents (human) **Thermo Fisher** **Catalog #**402869

rRNA depletion oligos

Troubleshooting

Before start

Prewarm SuperScript III 10X Buffer to Room temperature

poly-A tailed cDNA synthesis

1 Mix the following in a 0.2ml tube

	A	B
	Component	Volume (µl)
	Total RNA (1-4ug total)	1
	Oligo dTs	1.5
	10 mM dNTP mix	1.5
	Nuclease-free H2O	10

poly-A tailed cDNA reaction synthesis components

2 Denature sample RNA/primer mixture for 00:05:00 at 65 °C then cool to 4 °C for ≥ 00:02:00 7m

3 Spin tube briefly and add the following and mix by pipetting 55m

	A	B
	Component	Volume (µl)
	10X SuperScript III Buffer	2
	25mM MgCl2	4
	0.1M DTT	2
	Superscript III Reverse Transcriptase	2

poly-A tailed cDNA reaction synthesis components

Incubate 50 °C for 00:50:00 followed by 00:05:00 at 85 °C to deactivate the enzyme, then cool to 4 °C and proceed to the next step

Optional: rRNA depletion

4 Add in the appropriate rRNA depletion oligos for you sample 2m

Incubate 90 °C for 00:02:00 and ramp down to Room temperature at 0.1 °C per second then proceed to the next step

poly-A tailed (and ribosomal) RNA depletion

5 Add 2 µL of RNase H

6 Incubate 37 °C for 00:20:00 followed by 00:05:00 at 65 °C to deactivate the enzyme, then cool it to 4 °C and proceed to the next step 25m

poly-A tailed (and ribosomal) DNA depletion

7 Add in the following components and mix gently by pipetting

	A	B
	Component	Volume (µl)
	10X Turbo DNase Buffer	4
	Turbo DNase	4
	Nuclease-free H2O	10

DNase treatment components

8 Incubate at 37 °C for 00:30:00 30m

Bead cleanup

9 Add 90 µl (1.8X) of resuspended RNAClean XP Beads to the sample
Mix by pipetting 10x

10 Incubate 00:15:00 at On ice 15m

11 Place on the magnet, allow the beads to aggregate, and remove and discard the supernatant

12 Add 200 µL [M] 80 % (v/v) ethanol and incubate (still on the magnet) for 00:00:30 30s

12.1 Remove the supernatant

12.2 Repeat for a total of 2 washes

13 Air dry for 00:00:30 , don't allow the beads to become cracked 30s

14 Remove the tubes from the magnetic rack
Add 50 µL H20 (optionally add-in 1 µL RNase inhibitor) and resuspend the beads by pipetting ≥10x

15 Incubate 00:05:00 at Room temperature 5m

16 Place on the magnet, aspirate 50 µL of the eluant into a new tube

Optional: One-step RT-qPCR quantification

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	A	B
	Component	Volume (µl)
	Luna Universal Probe One-Step Reaction Mix (2X)	5
	Luna WarmStart RT Enzyme Mix (20X)	0.5
	TaqMan GAPDH Control Reagents (human; 20x)	0.5
	TaqMan 18S rRNA Control Reagents (eukaryotic; 20x)	0.5
	RNA	2
	Nuclease-free H2O	1.5

Luna RT-qPCR one-step quantification

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	A	B	C	D	E
	Step	*Temp (C)*	*Time (s)*	*Cycles*	*Ramp Rate (C/s)*
	Reverse transcription	55	600	1	2.73
	Denaturation	95	60	45	2.73
	Denaturation	95	10		2.73
	Amplification	60	30		2.11
	Capture	60	0		–

Cycle parameters for QuantStudio 3