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Protocol status: In development
While we have not tested this protocol, it is theoretically sound.

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Rapid Ribosome (Polysome) Profiling

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ABSTRACT

Ribosome profiling is a powerful technique used to study translation at a genome-wide level. It involves the sequencing of ribosome-protected mRNA fragments to determine the positions of ribosomes on transcripts. This information can be used to infer translation rates and identify translated open reading frames. While traditional ribosome profiling methods can be time-consuming and expensive, our method is rapid and cost-effective, and the inclusion of UMIs allows for precise quantification.

ATTACHMENTS

riboSeek.png

MATERIALS

	A	B
	Name	Sequence
	RiboS_linker	rCAAGCAGAAGACGGCATACGAGAT
	RiboS_linker_primer	ATCTCGTATGCCGTCTTCTGCTTG
	RiboS_IlluminaAdapt_TSO_UMI_RNA_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNGATrGrGrG
	RiboS_blocking_oligo	CTACCCCAAGCAG
	Index 1 Read	GATCGGAAGAGCACACGTCTGAACTCCAGTCAC[i7]ATCTCGTATGCCGTCTTCTGCTTG
	Index 2 Read	AATGATACGGCGACCACCGAGATCTACAC[i5]ACACTCTTTCCCTACACGACGCTCTTCCGATCT

Oligonucleotides. "r" indicated RNA base.

Monarch RNA Cleanup Kit (10 µg) - 100 preps **New England Biolabs Catalog #T2030L**

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

RtcB Ligase - 25 rxns **New England Biolabs Catalog #M0458S**

Superase-In RNase Inhibitor **Thermofisher Catalog #AM2694**

Template Switching RT Enzyme Mix – 100 rxns **New England Biolabs Catalog #M0466L**

Optional
Urea page
TBE
Agarose

BEFORE START INSTRUCTIONS

Read through the protocol and ensure you have the correct reagents depending on your chosen approach

RNA isolation and nuclease footprinting

1 Perform RNA isolation and nuclease footprinting as appropriate for your cell type (see steps 1-17 here for cultured mammalian cells)

Optional: preselection of small RNA fragments

5m

2 Optional but recommended preselection of small RNA fragments using either a urea PAGE gel or bead and membrane selection.
Step 2 includes a Step case.
15% UREA-TEB PAGE
Beads and membranes

step case

15% UREA-TEB PAGE

Recipe for casting own gel:
Urea 19.2g
40% Acrylamide/Bis (19:1) 15ml
10X TBE 4ml
25% APS 200ul
TEMED 20ul
Adding nuclease free water to 40ml

3 Add the appropriate volume of 2X RNA loading buffer to each RNA sample

4 Denature the samples for 80 °C 00:01:00 followed by ≥ 00:02:00 on On ice 3m

5 Load the samples on the polyacrylamide gel with TBE running buffer

6 Pre-run the gel in 1X TBE buffer at 100V for 10min

Optional: preselection of small RNA fragments

5m

7 Separate by electrophoresis for 01:30:00 at 100V 1h 30m

8 Stain the gel for 00:03:00 with 1X SYBR Gold in 1X TBE running buffer on a shaker 3m

9 Visualize the gel and excise desired region (20-40nts)

10 Transfer the excised gel slice to a 1.5 mL microcentrifuge tube and weigh it.

10.1 Pro Tip: Smashing the gel in the tube increases RNA recovery.

11 Add 4 volumes of Monarch RNA Cleanup Binding Buffer to the tube with the slice

12 Incubate the sample between 55 °C, gently mixing periodically until the gel slice is completely dissolved (generally < 00:10:00) 10m

13 Add two volumes of absolute ethanol to the sample and mix well by pipetting up and down

14 Incubate the sample between 55 °C or an additional 00:05:00 5m

15 Insert an RNA cleanup column into a collection tube, load the sample onto the column and close

the cap

15.1 Spin for 00:01:00, then discard flow-through 1m

16 Re-insert the column into the collection tube

16.1 Add 500 µL RNA Cleanup Wash Buffer

16.2 Spin for 00:01:00, then discard flow-through 1m

16.3 15 for a total of two washes.

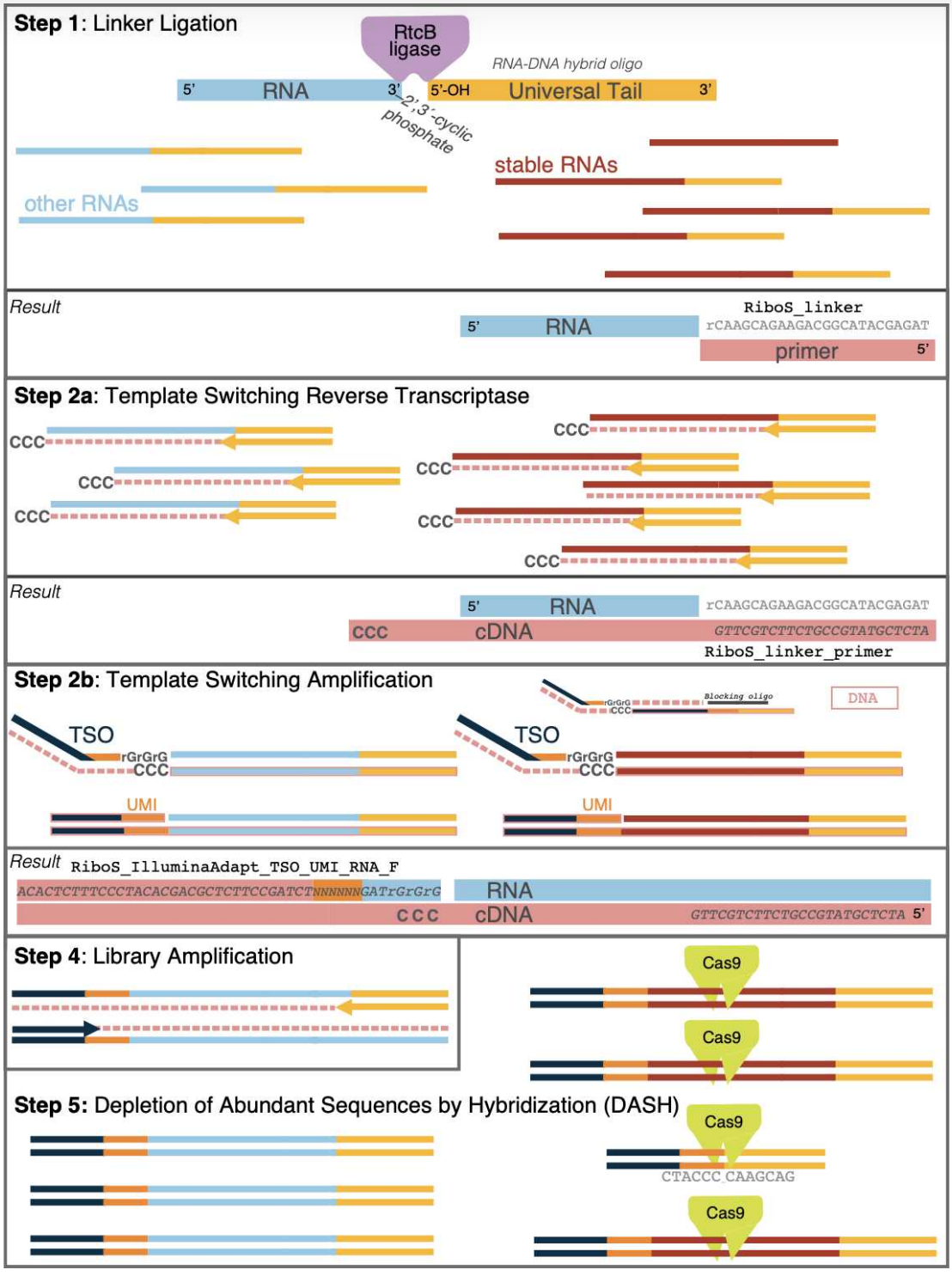
17 Transfer the column to a clean 1.5 ml microfuge tube

18 Add 10 µL nucleae-free water directly to the membrane and incubate at Room temperature for 00:05:00 5m

19 Spin for 00:01:00 1m

Overview

20



Linker ligation

21 Add 1 µL of [M] 100 micromolar (µM) RiboS_linker (linker RNA oligo) to 10 µL of RNA, denature it for 00:01:00 at 70 °C, and then cool it to Room temperature 1m

22 Set up the ligation reaction below and incubate for 01:30:00 at 37 °C 1h 30m

A	B
Component	Volume (µl)
RNA and linker	11
RtcB Reaction Buffer (10X)	2
SUPERase·In (20 U/µl)	1
1 mM GTP	2
10 mM MnCl2	2
RtcB RNA Ligase	1.5
H20	0

RNA cleanup

23 Add 100 µL RNA Cleanup Binding Buffer

24 Add 150 µL absolute ethanol and mix by pipetting

25 Insert an RNA cleanup column into a collection tube, load the sample onto the column and close the cap

25.1 Spin for 00:01:00, then discard flow-through

26 Re-insert the column into the collection tube

26.1 Add 500 µL RNA Cleanup Wash Buffer

26.2 Spin for 00:01:00, then discard flow-through

26.3 6 for a total of two washes.

27 Transfer the column to a clean 1.5 ml microfuge tube

28 Add 10 µL nucleae-free water directly to the membrane and incubate at Room temperature for 00:05:00

29 Spin for 00:01:00

RT (TSO) reaction

2h 37m 30s

30 6m

A	B

	A	B
	Component	Volume (µl)
	RNA	9
	RiboS_linker_primer (100uM; reverse transcription primer)	2
	dNTP mix (10 mM each)	2

Mix thoroughly by gently pipetting up and down at least 10 times, then centrifuge briefly to collect the solution to the bottom of the tube
Denature for 00:05:00 at 70 °C in a thermal cycler and then place on ice for ≥ 00:01:00

31 Vortex the Template Switching RT Buffer briefly followed by a quick spin
Combine the following components in a reaction tube

	A	B
	Component	Volume (µl)
	Template Switching RT Buffer	5
	RiboS_IlluminaAdapt_TSO_UMI_RNA_F (75uM)	1
	Template Switching RT Enzyme Mix	1

RT reaction mix

Mix thoroughly by gently pipetting up and down at least 10 times, then centrifuge briefly to collect the solution to the bottom of the tube

32 Combine 7 µL RT reaction mix (above) with 13 µL of the annealed mix, mix well by gently pipetting up and down at least 10 times, then centrifuge briefly to collect the solution to the bottom of the tube
Incubate as below

	A	B
	Temp (C)	Time (m)
	42	90
	85	5
	4	1

Index PCR

2h 37m 30s

33

	A	B
	Component	Volume (µl)
	10 µM Forward Index Primer	2.5
	10 µM Reverse Index Primer	2.5
	RiboS_blocking_oligo	2
	2X Phusion Master Mix	25
	TSO reaction	18

PCR components

	A	B	C
	Cycles	Temp (C)	Time (s)
	1	98	30
	10	98	5
		60	5
	1	72	10

PCR cycle parameters (for selection of <40bp inserts)

Fragment is 150bp excluding insert at this stage

AATGATACGGCGACCACCGAGATCTACAC[i5]ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNGATGGG[INSERT]CAAGCAGAAGACGGCATACGAGAT[i7]GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC

34 Add 90 µL AMPure XP beads to the PCR product 8m
Incubate at Room temperature for 00:05:00
Place on a magnetic rack
Aspirate supernatant
Add 200 µL [M] 70 % (v/v) ethanol
Wait for 00:00:30
Aspirate and discard the supernatant
Add 200 µL [M] 70 % (v/v) ethanol
Wait for 00:00:30
Aspirate and discard the supernatant
Resuspend beads in 10 µL of H20
Incubate for 00:02:00
Transfer to a clean PCR tube

Optional: Agarose gel size selection

35 Run a 1% agarose gel, excise the desired band, and perform a gel cleanup.

Optional: Depletion of Abundant Sequences by Hybridization

36 To perform DASH to deplete unwanted sequences (e.g., rRNA) follow the protocol here.

Quantification

37 Run a tapestation or bioanalyzer chip
Do qubit or qPCR using an Illumina library quant kit to quantify the library and pool the samples (see Illumina Sequencing Coverage Calculator for pooling information)

Analysis

38 Analysis can be done using common pipelines with the addition of umi_tools to count unique reads