# Ribo-lite Protocol

**Day1,** from cell lysis to ribosome and ribosome protected fragment (RPF) purification.

1, Prepare buffers:

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| 1xpolysome buffer (w/o water) | Stock | 3ml(3samples) | 4.5ml(4samples) |
| 20mM Tris7.4, 150mM NaCl, 5mM MgCl2 (5x polysome buffer) | 5x | 600ul | 900ul |
| 1mM DTT | 100mM  | 30ul | 45ul |
| 100ug/ml CHX | 50mg/ml | 6ul | 9ul |

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| 1xlysis buffer | Stock | 1ml(3samples) | 1.5ml(4samples) |
| 1xpolysome buffer+DTT+CHX |  | 212ul | 318ul |
| 1% Triton X-100 | 10%  | 100ul | 150ul |
| 25U/ml Turbo Dnase | 2U/ul  | 12.5ul | 18.75ul |
| NF water |  | 675.5ul | 1013.25ul |

2, Put the sample into 1.5ml tube, leaving less than 2ul liquid. Add 20ul iced Ribo-seq lysis buffer. Quickly vortex the tube, then spin down the solution and immediately place it on ice. Put it on ice for 10 mins. Then get out 2ul of the lysis for Smart-seq (If you want to perform Ribo-RNA-lite to co-profile translatome and transcriptome).

3, Then supplement 300ul to the tube, slightly flick the tube and incubate it on ice for 10 mins.

4, Centrifuge at 4 ℃, 20000g for 10 mins, then move the supernatant to another tube.

5, Add 1ul RNase I (100U/ul), mix thoroughly, then incubate on thermomixer at 22℃, 1000rpm for 45 mins.

6, Add 3ul SUPERase•In™ RNase Inhibitor to stop nuclease digestion, mix it thoroughly.

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| 1M Sucrose cushion | Stock | 2.56ml(3samples) | 3.84ml(5samples) |
| 1xpolysome buffer+DTT+CHX | 5x | 212ulx2 | 212x3ul |
| NF water |  | 788ulx2 | 788ulx3 |
| 34% sucrose |  | 0.87g | 1.305g |
| Super Rnase Inhibitor | 20U/ul | 2.56ul | 3.84ul |

7, Add 700ul of sucrose cushion to Beckman tube (with a mark on the outside edge), then carefully drip 300ul lysate at the top, leaving an interface between layers.

8, Pellet ribosomes by centrifugation in MLA150 rotor in Beckman Optima MAX-XP ultracentrifuge, at 4℃, 76400rpm (260,000g) for 4hrs.

9, Gently pipette the supernatant out of the tube, then resuspend the ribosomal pellet by slowly pipetting 50ul pellet buffer (10 mM Tris pH7.5, 1% SDS) at the bottom for several times into phase-lock tube.

10, Purify RNA by Trizol:

1. Add 1ml Trizol to phase-lock tube, vortex it thoroughly, then leave it at RT for 5 mins.
2. Add 0.2ml chloroform, vortex it thoroughly, then leave it at RT for 2 mins.
3. Centrifuge at 4℃, 12000g for 15min. Transfer the aqueous phase to a new tube.
4. Add 1ul glycogen and 750ul isopropanol, then leave at -20 ℃ overnight.

**Day2**, from ribosome purification to RPF purification.

11, Centrifuge the tube at 4℃, 12000g for 40min.

12, Wash by 75% ethonal for once then centrifuge at 4℃, 7500g for 5min.

13, Open the tube to let it dry for few minutes until it become slightly transparent.

14, Dissolve the pellet in 6ul NF water. Add 6ul 2x gel loading+6ul RNA.

15, Prepare gel (6.5~7ml for each gel):

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| --- | --- |
|  | 15% denatured RNA PAGE gel |
| 4.8g Urea+1ml 10xTBE+3.4ml Acrylamide (19:1)+NF water | 10ml |
| 10% APS | 50ul  |
| TEMED  | 10ul |

16, Prepare 1xTBE. Rinse the wells of gel. Pre-run gel at 200V, 30min.

17, Denature the samples at 80℃ 90s, then place it on ice immediately.

18, Rinse the wells of gel. Run electrophoresis at 200V, 65min, until the blue marker run out of the gel.

To minimize contamination, each sample was run in a separate gel and the electrophoresis was run without RNA/DNA markers. The region from 26-to-34nt nucleotides for the RPFs was excised as a ~1 cm square according to the light blue loading dye (Xylene Cyanol), and further determined on the basis of the relative position of RNA oligo markers to the loading dye on a separate and parallel gel.

19, Excise the gel down the light blue marker and place it in a new tube. Add 400ul RNA extraction buffer (300mM sodium acetate, pH5.5; 1mM EDTA; 0.25% SDS).

20, Freeze the sample at -80℃ for 30min.

21, Leave the samples overnight or 6~8hrs at 22℃, 1000rpm.

**Day3,** RPF library construction using Dplex-Small-RNA-seq Kit (diagenode, C05030001)

22, Briefly centrifuge the tube then transfer the supernatant into another tube. Precipitate the RNA with 1.5ul glycogen and 500ul isoproponal.

23, incubate for at least 30min at -80℃ or 30~40min at -20℃.

24, Centrifuge the tube at 4℃, 12000g for 40min.

25, Wash by 75% ethonal for once then centrifuge at 4℃, 7500g for 5min.

26, Open the tube to let it dry for few minutes.

27, Dissolve the pellet in 2ul NF water.

28, Add 1.25ul Crowding Buffer (CB) to the sample. And add 0.5ul Dephosphorylation buffer (DB)+0.125ul Dephosphorylation Reagent (DR).

Incubate at 37℃, 15min then place on ice.

29, Add 0.25ul Small Tailing Buffer (STB)+0.125ul Small Tailing Reagent (STR).

Incubate at 37℃, 40min then place on ice.

30, Add 0.25ul RPTM.

Incubate at 70℃, 10min+slowly decrease to 25°C for 2 minutes by ramping down at 0.5°C/second.

31, Add 1.25ul Reverse Transcription Buffer (RTB) and 0.25μl of Reverse Transcription Reagent (RTR).

Incubate at 25℃, 15min

32, Add 0.5ul 1/5 Small Template Switch Oligo (STSO).

Incubate at 42℃, 120min+70℃, 10min

33, Add 2.5ul Forward primer+2.5ul Reverse primer+12.5ul PCR master mix

PCR for 20 cycles.

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| Cycles | Temperature | Time |
| 1 | 98℃ | 30s |
| 20 | 98℃ | 15s |
| 20 | 62℃ | 30s |
| 20 | 72℃ | 30s |
| 1 | 72℃ | 10min |
| 1 | 4℃ | hold |

34, Add 37.5ul beads (1.5x into 25ul reaction mix) to purify the PCR product. Then dissolved in 50ul water.

35, Barcoded transcriptome and translatome libraries were pooled and sequenced on the Illumina platforms with 150 bp paired-end reads. Read 1 (sense strand) of Ribo-lite data were used for analysis, as most RPFs were short and were already covered by Read 1 alone.

Information of some reagents are shown below:

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| Reagent | Item No. |
| 1 mL, Open-Top Thickwall Polycarbonate Tube | Beckman, 343778 |
| Invitrogen Phasemaker Tubes | Invitrogen™ Phasemaker™ Tubes A33248 |
| low binding，0.2 mL tube | PCR-02-L-C，LOT0819035 |
| Isopropanol | Sigma 2-propyl alcohol 59304-500ML |
| Ethyl alcohol | Sigma Ethyl alcohol, Pure, E7023 |
| Chloroform | Amresco 0757-500ml |
| Nuclease-free water | Invitrogen AM9932 |
| [DTT (1 M)](http://g.labscout.cn/115/%21orders/order/index.757) | Sigma-Aldrich cat. no. 43816-10ML |
| 50mg/ml CHX | Sigma #C4859-1ml  |
| Smart-seq ERCC spike-in  | Invitrogen; 4456740 |
| 2×Gel loading buffer II | Gel loading buffer II, Thermo Fisher，AM8546G |
| Urea | Amresco #0568-1KG |
| Acrylamide/Bis 19:1, 40% (w/v) solution | Invitrogen #AM9022 |
| 2U/ul turbo Dnase | Ambion AM2239 |
| SUPERase•In™ RNase Inhibitor | life technologies AM2696 |
| RNase I  | Invitrogen AM2294, Ambion™ RNase I |
| RNA-grade glycogen | Fermentas #r0551  |
| Trizol | life tech #15596026 |
| Sucrose | AMRESCO - M117-500G/Aladdin S112226-500g |
| BORIC ACID | AMRESCO, M139-500G/Aladdin B111601-500g |
| sucrose | AMRESCO, M117-1KG |
| 1M Tris-cl(PH7.5) | Invitrogen, 15567027 |
| 5M NaCl | Invitrogen, AM9760G |
| 1M MgCl2 | Invitrogen, AM9530G |
| 3M sodium acetate,ph5.5 | Invitrogen, AM9740 |
| APS | Amresco, 0486-5 |
| 20% SDS | Invitrogen, AM9820 |
| 0.5M EDTA | Invitrogen, AM9260G |
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