

Materials List for:

Methods to Investigate the Regulatory Role of Small RNAs and Ribosomal Occupancy of *Plasmodium falciparum*

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Materials

Name	Company	Catalog Number	Comments
REAGENTS-All reagents must be RNase-free.			
Diethyl pyrocarbonate	Sigma	D5758	DEPC
DEPC-treated water (see REAGENT SETUP)			
Yoyo-1 DNA fluorescent dye	Thermo Fisher		
Gene Pulser II electroporator	Bio-Rad		
0.2 cm Electroporation cuvettes			
4 M potassium acetate	Mallinckrodt	6700	
2 M potassium HEPES	Sigma	H0527	pH 7.2
1 M magnesium acetate	Sigma	M-2545	
1 M dithiothreitol	Research Products International	D11000	DTT
100 mM phenylmethylsulfonate fluoride	Sigma	P7626	PMSF, dissolved in isopropanol
10% (v/v) Igepal CA-630	Sigma-Aldrich	I3021	
10% (w/v) sodium deoxycholate	Sigma	D-6750	DOC
Cycloheximide	Sigma	C-7698	CHX
Sucrose	Mallinckrodt	8360-04	
RNAseOUT (Invitrogen, cat. no. 100000840)	Invitrogen	100000840	
RPMI-1640 w/ L-glutamine	Cellgro	10-040-CV	
10% Albumax I	Invitrogen	11020-039	w/v in sterile water
Gentamicin	Gibco	15750-060	
HT supplement (100x) (Invitrogen, cat. no. 11067030)			
45% (w/v) sucrose (Sigma, cat. no. G8769)			
1 M HEPES buffer (Gibco, cat. no. 15630-080)			
1x phosphate buffered saline (PBS; Cellgro, cat. no. 2109310CV)			
Corning 500 ml vacuum filter flask (VWR, cat. no. 430769)			
Glass slides (VWR, cat. no. 48311-702)			

Giemsa stain (50X) (VWR, cat no. m708-01)			
mirVana miRNA isolation kit (Ambion, ThermoFisher Scientific).			
5' Biotin conjugated mature miRNA (sequence varies by miRNA of interest) (Dharmacon)			
Biotin powder (Sigma-Aldrich)			
1 M Potassium Chloride			
Streptavidin Sepharose High Performance Beads (GE Healthcare)			
Ribosome resuspension buffer (see REAGENT SETUP)			
Lysis buffer (see REAGENT SETUP)			
15% (w/v) sucrose gradient solution (see REAGENT SETUP)			
50% (w/v) sucrose gradient solution (see REAGENT SETUP)			
0.5 M sucrose cushion (see REAGENT SETUP)			
60% (w/v) sucrose chase solution (see REAGENT SETUP)			
Plasmodium cell culture media (see REAGENT SETUP)			
RNA capture Wash Buffer (see REAGENT SETUP)			
RNA Elution Buffer (see REAGENT SETUP)			
REAGENT SETUP			
Solutions			
Plasmodium cell culture media			
500 ml RPMI-1640 w/ L-glutamine			
0.5 ml gentamicin			
5 ml HT supplement			
2.5 ml 45% glucose			
25 ml 10% AlbuMAX I			
12.5 ml 1 M HEPES			
Sterile filter with 0.2 mm vacuum filter flask.			
Plasmodium cell culture media containing 10x CHX			
Same recipe as Plasmodium cell culture media above, with the addition of:			
2 mM cycloheximide			
Media is prepared as normal, then add CHX to a final concentration of 2 mM and filter.			
1x PBS containing cycloheximide			

Cycloheximide is added fresh to 1x PBS to a final concentration of 200 mM, and kept at 4 °C until use.			
Ribosome resuspension buffer			
400 mM potassium acetate			
25 mM potassium HEPES, pH 7.2			
15 mM magnesium acetate			
200 mM cycloheximide (add fresh)			
1 mM DTT (add fresh)			
1 mM PMSF (add fresh)			
40 U/ml RNaseOUT (add fresh)			
Lysis buffer			
Same recipe as ribosome resuspension buffer above, with the addition of:			
1% (v/v) Igepal CA-630			
0.5% (w/v) DOC			
Sucrose solutions			
Same recipe as ribosome resuspension buffer above, with the addition of varying amounts of sucrose:			
15% (w/v) sucrose to make 15% sucrose gradient solution			
50% (w/v) sucrose to make 50% sucrose gradient solution			
0.5 M sucrose to make 0.5 M sucrose cushion			
As above, cycloheximide, DTT, PMSF, and RNaseOUT must be added fresh.			
The 60% sucrose chase solution requires only sucrose and water, no other components			
60% (w/v) sucrose in DEPC-treated water to make 60% sucrose chase solution			
RNA Capture Wash Buffer			
20 mM KCl			
5 unit/ml Rnase Out (Invitrogen)			
RNA Capture Elution Buffer			
Same recipe as RNA Capture Wash Buffer, with the addition of:			
2 mM Biotin			
EQUIPMENT			
SW55 Ti ultracentrifuge rotor (Beckman, cat. no. 342194)			
SW41 Ti ultracentrifuge rotor (Beckman, cat. no. 331362)			

Ultra-Clear™ ½ x 2 in (13 x 51 mm) ultracentrifuge tubes for the SW55 (Beckman, cat. no. 344057)			
Polyallomer 9/16 x 3 ½ in (14 x 89 mm) ultracentrifuge tubes for the SW41 (Beckman, cat. no. 331372)			
L8-80M ultracentrifuge (Beckman)			
Steel blunt syringe needle, 4 inch, 16 G (Aldrich, cat. no. Z261378)			
1 ml syringe with 27 G½ needle (Becton Dickinson, cat no. 309623)			
5 ml syringe (Becton Dickinson, cat. no. 309646)			
Parafilm			
Tube rotator, end-over-end			
Microcentrifuge, refrigerated			
Density gradient fractionation system (Teledyne Isco, cat. no. 69-3873-179)			
TracerDAQ (Measurement Computing)			
Eppendorf 5810R centrifuge			
Eppendorf A-4-81 rotor (Eppendorf, cat. no. 022638807)			