

Materials List for:

# Dissection of *Drosophila melanogaster* Flight Muscles for Omics Approaches

Shao-Yen Kao<sup>\*1</sup>, Elena Nikonova<sup>\*1</sup>, Keshika Ravichandran<sup>\*1</sup>, Maria L. Spletter<sup>1,2</sup>

<sup>1</sup>Biomedical Center, Department of Physiological Chemistry, Ludwig-Maximilians-University Munich

<sup>2</sup>Center for Integrated Protein Science Munich (CIPSM), Department of Chemistry, Ludwig-Maximilians-University Munich

\*These authors contributed equally

Correspondence to: Maria L. Spletter at [maria.spletter@bmc.med.lmu.de](mailto:maria.spletter@bmc.med.lmu.de)

URL: <https://www.jove.com/video/60309>

DOI: [doi:10.3791/60309](https://doi.org/10.3791/60309)

## Materials

Name	Company	Catalog Number	Comments
5x High Fidelity (HF) buffer	Thermo Fisher	F518L	
60 mm culture dishes	Sigma-Aldrich	Z643084-600EA	Greiner dishes, 60 mm x 15 mM, vented
Black dissecting dish (glass)	Augusta Laborbedarf	42021010	Lymphbecken, black glass, 4 cm x 4 cm
Black silicon dissecting dishes: activated charcoal powder	Sigma-Aldrich	C9157	Also available from most pharmacies
Black silicon dissecting dishes: Sylgard 184	Sigma-Aldrich	761036	Dishes are made by mixing the Sylgard (~50g) with activated charcoal powder (200 mg) and curing it in Petri dishes (~4 60 mm dishes).
Blue pestle	Sigma-Aldrich	Z359947-100EA	Any pellet pestle that can be sterilized, also can be used with a motor-driven grinder
Cell phone camera, Samsung Galaxy S9	Samsung	SM-G960F/DS	used for photos not taken under a microscope
Chloroform	PanReac AppliChem	A3691,0500	
Confocal microscope, Leica SP8X upright confocal	Leica		<a href="http://www.leica-microsystems.com">www.leica-microsystems.com</a>
Confocal microscope, Zeiss LSM 780 inverted confocal	Zeiss		<a href="http://www.zeiss.com">www.zeiss.com</a>
Double stick tape	Scotch/3M	3M ID 70005108587	Double-sided tape, available at most office supply handlers
Dumont #5 Forceps	Fine Science Tools	11252-20	Inox straight tip 11 cm forceps, Biology grade with 0.05 mm x 0.02 mm tip
EtOH (100%, RNase free)	Sigma-Aldrich	32205-M	
fluorescent dissecting microscope camera, Leica DFC310 FX camera	Leica		<a href="http://www.leica-microsystems.com">www.leica-microsystems.com</a>
Fluorescent dissecting microscope software, Leica Application Suite (LAS) version 4.0.0	Leica		<a href="http://www.leica-microsystems.com">www.leica-microsystems.com</a>
Fluorescent dissecting microscope, Leica M165 FC	Leica		<a href="http://www.leica-microsystems.com">www.leica-microsystems.com</a>
Fly: Bru1[M2]			Fly stock; This paper
Fly: Bru1[M3]			Fly stock; This paper
Fly: Mef2-GAL4	Bloomington Stock Center	BDSC:27390	Fly stock
Fly: salm[1]	Bloomington Stock Center	3274	Fly stock

Fly: salm[FRT]			Fly stock; see Spletter et al., Elife, 2018
Fly: UAS-Bru1IR	Vienna Drosophila Research Center	GD41568	Fly stock, RNAi hairpin
Fly: UAS-GFP::Gma	Bloomington Stock Center	BDSC:31776	Fly stock
Fly: UAS-mCD8a::GFP	Bloomington Stock Center	BDSC:5130	Fly stock
Fly: w[1118]	Bloomington Stock Center	3605	Fly stock
Fly: weeP26			Fly stock; see Clyne et al., Genetics, 2003
GFP detection reagent, GFP-Booster	ChromoTek	gba488-100	
Glycogen	Invitrogen	10814-010	
Image processing software, Photoshop CS6	Adobe		www.adobe.com
Isopropanol	Sigma-Aldrich	I9516-25ML	2-propanol
Method 1 (RNA isolation): TRIzol	Life Technologies	15596018	Guanidinium isothiocyanate and phenol monophasic solution
Method 2 (RNA isolation): Method 1 + TURBO DNA-free Kit	Invitrogen	AM1907	TRIzol isolation followed by treatment with a kit to remove DNA
Method 3 (RNA isolation): Direct-zol RNA Miniprep Plus Kit	Zymo Research	R2070S	RNA isolation in TRIzol, but over commercial columns instead of using phase separation. Recommended DNase treatment performed with Monarch DNase I in Monarch DNase I Reaction buffer.
Method 4 (RNA isolation): RNeasy Plus Mini Kit	Qiagen	74134	We used the provided DNase treatment. IFM pellets were homogenized in RTL buffer as suggested for animal tissues.
Method 5 (RNA isolation): ReliaPrep RNA Tissue Miniprep System	Promega	Z6110	We applied the protocol for 'Purification of RNA from Fibrous Tissues'.
Method 6 (RNA isolation): Monarch Total RNA Miniprep Kit	New England Biolabs	T2010G	We applied the protocol for tissues/leukocytes and lysed in 300 $\mu$ L of RNA Protection Reagent.
Microcentrifuge tubes	Thermo Fisher	AM12400	RNase-free Microfuge Tubes, 1.5 mL
Microscope slides	Thermo Fisher	12342108	Standard slides, uncharged, 1 mm
Microtome blades	PFM Medical	207500003	C35 feather 80 mm
Monarch DNase I	New England Biolabs	T2004-21	
Monarch DNase I Reaction Buffer	New England Biolabs	T2005-21	
Normal goat serum	Thermo Fisher	16210072	
OneTaq Polymerase	New England Biolabs	M0480X	
Paintbrush	Marabu	1910000000	Marabu Fino Round No. 0, or similar brush from any art supply
Paraformaldehyde	Sigma-Aldrich	158127	
PBS buffer (1x)	Sigma-Aldrich	P4417	Phosphate buffered saline tablets for 1 L solutions, pH 7.4
PFA PureTip Pipette Tips	Elemental Scientific	ES-7000-0101	Optional substitute for standard pipette tips to reduce sample loss; 100 mL, 0.8 mm orifice
Phusion High Fidelity Polymerase	Thermo Fisher	F-530XL	
Pipette tips	Sigma-Aldrich	P5161	Universal 200 mL pipette tips

Preomics iST 8x Kit	Preomics	P.O.00001	peptide preparation kit for mass spectrometry
Q Exactive mass spectrometer	Thermo Fisher	725500	mass spectrometry was performed at the Protein Analysis Unit of the LMU Biomedical Center
Qubit RNA Assay Kit	Life Technologies	Q32855	
Rhodamine-phalloidin	Invitrogen, Molecular Probes	10063052	
RNA concentration Approach 1 & RNA integrity traces, Bioanalyzer	Agilent Technologies	G2939BA	
RNA concentration Approach 2, Nanodrop	Thermo Fisher	ND-2000	
RNA concentration Approach 3, Qubit 4 Fluorometer	Invitrogen	Q33238	
RNA Pico Chips	Agilent Technologies	5067-1513	
RNase A	Promega	A7937	
RNase-free water, Diethyl pyrocarbonate (DEPC)	Sigma-Aldrich	D5758	DEPC treat water overnight and then autoclave, to remove all RNase.
RT Kit #1: Super Script III Reverse Transcriptase Kit	Invitrogen	18080-044	reverse transcription kit
RT Kit #2: LunaScript	New England Biolabs	E3010S	reverse transcription kit
RT Kit #3: QuantiNova Reverse Transcription Kit	Qiagen	205410	reverse transcription kit
Slide mounting buffer, Vectashield	Vector Laboratories	H-1200	containing DAPI
Statistical software: GraphPad Prism	GraphPad Prism		www.graphpad.com
Statistical software: Microsoft Excel	Microsoft		Purchased as part of the bundle: Office Home & Student 2019
Table-top centrifuge	Eppendorf	5405000760	Eppendorf Centrifuge 5425 or equivalent
Tissue/ Kimwipes	Sigma-Aldrich	Z188956	Standard tissue wipes
Transfer pipette	Sigma-Aldrich	Z350796	Plastic pipette
Triton-X100	Sigma-Aldrich	T9284-500ML	
Vannas spring scissors	Fine Science Tools	15000-00	3 mm cutting edge, tip diameter 0.05 mm, length 8 cm
Whatman paper	Sigma-Aldrich	1004-070	Filter paper circles, Grade 4, 70 mm