

Materials List for:

Low-Cost Cryo-Light Microscopy Stage Fabrication for Correlated Light/ Electron Microscopy

David B. Carlson¹, James E. Evans¹

¹Department of Molecular and Cellular Biology, University of California Davis

Correspondence to: James E. Evans at JEEvans@ucdavis.edu

URL: <https://www.jove.com/video/2909>

DOI: [doi:10.3791/2909](https://doi.org/10.3791/2909)

Materials

| Name | Company | Catalog Number | Comments |
|--|--------------|----------------|--|
| 1 – 22.86 cm (9") pie pan with sloped edge | Good Cook | | Local grocery store |
| 1 – 22.86 cm (9") cake pan | Good Cook | | Local grocery store |
| 4 pairs of chopsticks | | | Local grocery store |
| 1 - Great Stuff spray foam | Great Stuff | | Local hardware store |
| 1 - 4cm x8cm x1cm block of aluminum | | | Local hardware store or metal scrap yard |
| 5 - #10 1.91 cm (3/4") flat head slotted bolts w/ nuts | | | Local hardware store |
| 3 - #4 0.95 cm (3/8") round slotted bolts w/ nuts | | | Local hardware store |
| 15 - #10 washers | | | Local hardware store |
| 2 - transparent plastic clipboards | | | Local office store |
| 1 - Cryo-EM grid box holder | Ted Pella | 160-41 | |
| 1 - Cryo-EM grid box handling rod | Ted Pella | 160-46 | |
| 1 - R2/1 holey carbon film, 400-mesh copper cryo-EM support grid | SPI Supplies | 4340C-XA | |

Experimental materials

1. *Yeast strain*: Hta1-CFP::Kan (KSC-3382: MATa, ura3-52, lys2-801, ade2-101, trp1-Δ63, his3-Δ200, leu2-Δ1, Hta1-CFP::KanMX6 from the Kaplan lab at UC Davis).
2. *Cryo-Light Microscopy*: Cryo-light microscopy images were taken on a Leica DM4000M reflecting microscope with a Leica DFC310 FX CCD camera. Bright Field, Dark Field and Fluorescence images were acquired using either a HCX PL FLUOTAR 5x 0.15 N.A. air objective, a HCX PL FLUOTAR 10x 0.30 N.A. air objective, a PL FLUOTAR 50x 0.55 N.A. air objective or a NPLAN 100x 0.75 N.A. air objective. Bright Field and Dark Field images used a 12V 100W tungsten lamp as the light source. For Fluorescence, an EL6000 UV lamp source was used in conjunction with a Leica JC1 (Ex.F.: BP 480/30, D.M.: LP 505, S.F. BP 535/40 & LP 580) Filter Cube.
3. *Cryo-Transmission Electron Microscopy*: Cryo-EM images were acquired on a JEM-2100-FEG Transmission Electron Microscope (JEOL, Japan) operating at 200 KeV utilizing a Gatan 626 cryo-transfer holder (Gatan, USA). Micrographs were recorded at nominal magnifications of 50, 200, 1,200 and 4,000X on a 4,096 x 4,096 pixel Tietz CCD camera (TVIPS, Gauting, Germany).