Actual observations:
Without MB
With MB
Both

I. **HARVEST** (Gandre, S., & Bliek, A. M., 2016)

Materials

- ~~5 worm plates per harvest~~ [see next bullet]
- 1 (large) plate per treatment group
- 0.1M NaCl
- M9 buffer
- ~~Mitochondrial IB~~
    - ~~210 mM mannitol~~
    - ~~70 mM sucrose~~
    - ~~0.1 mM EDTA, pH 8.0~~
    - ~~5 mM Tris-HCl, pH 7.4~~
    - ~~1 mM PMSF~~
- Centrifuge

1. Harvest worms from 5 plates (either MB treated or untreated) by washing off the plate with 10 mL M9 buffer per plate
    a. x3 10mL/plate + 3mL to catch stragglers
2. Pellet the worms (all 5 plates together) by centrifugation for 5 min @ ~~1000g~~ 2500g
    a. Loose pellet formed; sent in for +10 min @ 2500g
    b. Pellet still loose (especially blue); + 10 min @ 5000g
    c. + 10 min @ 5000g
3. Resuspend the pellet in 10 volumes ice-cold 0.1M NaCl
    a. Add an equal volume of ice-cold 60% sucrose solution; mix by inversion
    b. Split into x2 1 mL tubes/group
4. Centrifuge for 2 min @ 500g
5. Transfer the uppermost layer (floating worms) to a new tube using a Pasteur pipet
    a. Pool top 250μL from each tube (each group) → 1 tube per group
6. ~~Dilute the worm suspension with 10 volumes ice-cold 0.1M NaCl~~
    a. Centrifuge for 2.5 min @ ~~500g~~ 5000g
    b. +10 mins @ 10000g
7. ~~Discard the supernatant and resuspend the pellet in 50 volumes ice-cold 0.1M NaCl~~

II. **ISOLATE** (Gandre, S., & Bliek, A. M., 2016)

Materials (volumes were adjusted to reduced sample quantity)

- 5 plates worth of worms, pelleted
- Mitochondrial Isolation Buffer (IB) (for 25 mL)
    - 210 mM mannitol -- 0.956 g
    - 70 mM sucrose -- 0.599 g

    - 0.1 mM EDTA, pH 8.0 -- 0.00093 g (0.93 mg)
    - 5 mM Tris-HCl, pH 7.4 -- 0.0151 g (15.1 mg)
    - 1 mM PMSF -- 0.00435 g (4.35 mg) -- fume hood?
- Potter-Elvehjem homogenizer/Dounce/honestly, I will use a rock at this point
- Centrifuge

1. Pellet worms by centrifuging for 5 min @ 2000g
2. Resuspend 5 g worms (this was originally done for twice the amount of sample, so it's probably going to be *way* less than 5 g) in 5 mL ice-cold IB
3. Homogenize with 15 strokes of a chilled Potter-Elvehjem homogenizer
4. Collect the homogenate in a 25 mL Falcon tube and increase the volume to 13 mL with IB
    a. Centrifuge for 10 min @ 750g
5. Transfer the supernatant to a fresh tube and resuspend the pellet in 5 mL IB
6. Homogenize the remaining pellet with 15 more strokes of the homogenizer
    a. Repeat Steps 4 & 5
7. Combine the supernatants from Steps 5 & 6a; ~~save an aliquot as total worm lysate~~
8. Centrifuge the supernatants for 10 min @ 12,000g; ~~save an aliquot of the resulting supernatant as postmitochondrial supernatant~~
9. Resuspend the mitochondrial pellet in 6 mL IB
    a. Centrifuge for 10 min @ 750g
10. Transfer the supernatant to a new tube without disrupting the pellet
    a. Centrifuge for 10 min @ 12,000g and discard the supernatant
11. Combine and resuspend the mitochondrial pellets from Steps 9a & 10a in IB

III. **ASSAY** (Sigma-Aldrich, 2019)

<u>Materials</u>

- Isolated C. elegans mitochondria (MB treated or untreated)
- Equine heart cytochrome c
- Reaction buffer, on ice
    - 50 mM Tris-HCl, pH 7.4 -- 6.057 g
    - 250 mM sucrose -- 85.575 g
    - 10 mM KCl -- 0.746 g
    - 1 mM EDTA -- 0.372 g
    - 10 mM KH_2PO_4 -- 1.361 g
    - Final volume 1L w/distilled water
    - Filter-sterilize through a 0.22 µm filter (optional)
- Spectrophotometer

1. Add ~~50 µL 50 µM~~ 50 µL 4.4 mM oxidized cytochrome c to 950 µL of buffer in a cuvette (blank)
    a.
2. Add ~~50 µM~~ 50 µL 4.4 mM oxidized cytochrome c to 950 µL of buffer to a new cuvette
    a. Add 50 µL mitochondria
3. Allow the reactions to proceed at 25°C for 5 minutes; monitor absorbance

4. Add dithiothreitol (DTT) to a cuvette containing 950 µL of buffer and 50 µL 4.4 mM oxidized cytochrome c to observe the full reduction of cytochrome c.
5. Calculate reduction rate
    a. $\frac{\Delta A}{min} = (A/\min)_{sample} - (A/\min)_{blank}$
    b. $Enzyme\ Activity\ (Units/mL) = \frac{\Delta A/\min * dil * V_{reaction}}{V_{enzyme} * 21.84}$

    where:
    21.84 = extinction coefficient of reduced cytochrome c ($mM^{-1}cm^{-1}$)
    $V_{reaction}$ = total reaction volume
    V_{enzyme} = volume of mitochondrial sample
    dil = dilution of mitochondrial sample
6. Compare treatment groups
    a. $Difference\ (\%) = \frac{Activity_{MB} - Activity_{untreated}}{Activity_{untreated}} * 100$