

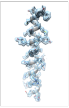
## Manual Blotting Protocol

This protocol is for individuals that have been certified to operate the Vitrobot. Please refer to the following paper Serbynovskyi et al 2025 (<https://pubmed.ncbi.nlm.nih.gov/38328036/>) for details on assembling the filter tips.

- i. Alert Cryo-EM staff if any assistance or supervision is needed.
  - ii. Reserve Vitrobot in iLab.
  - iii. Bring any supplies and/or tools you wish to use instead of what is already provided by the Core.
  - iv. Bring a facemask to protect samples from your breath (recommended).
  - v. Refer to the “Vitrobot Handout” or “Vitrobot Protocol” for basic Vitrobot operation.
  - vi. This protocol is for individuals that have been certified to operate the Vitrobot.
- Gather Tools and Supplies for Making Filter Tips
    - o 20 $\mu$ L pipet tips manual blot at least as many as the number of grids you plan to
    - o Filter paper at least one per 12 filter tips you intend to make
    - o 20 $\mu$ L pipet tip box(es) as many as needed to house the completed filter tips
    - o Scissors
    - o Box cutter or razor
    - o Ruler
    - o Cutting surface
    - o Clean disposable gloves



- Make the Filter Tips
  1. Wear clean disposable gloves.
  2. Cut a sheet of filter paper into quarters, and then those quarters into thirds, for a total of 12 pieces.
  3. Lay pipet tips on the cutting surface.
  4. Use the box cutter or razor to cut a pipet to the desired internal diameter (recommended: 3 mm).
  5. Insert an uncut pipet tip into a cut one.
  6. Cut the uncut pipet tip above the raw edge of the already cut pipet tip (recommended: 1 mm).
  7. Remove the long-cut pipet tip and cover it in a filter paper piece.
  8. Gently twist/shimmy a short-cut pipet tip over the filter-covered long-cut pipet tip.
  9. Hold the filter paper tip at 45° angle against a tabletop filter paper.
  10. Roll the filter tip to round out the edges.
  11. Flatten the filter tip against the tabletop filter paper.



12. Gently twist/shimmy the long-cut pipet tip out of the filter tip.
13. Store the filter tip in a designated pipet box.

- Set the blot total to zero on the Vitrobot.
- Dry Run Vitrobot to Test that It's Working Properly (see steps directly below).
- Manual Blot Vitrobot Operation
  - Operate Vitrobot as normal all the way through pipetting the sample onto the grid.
  - Remove a filter tip from its box and place it on a pipetman.
  - Use the pipetman to guide the filter tip into the Vitrobot chamber.
  - Gently blot the grid, manually counting the seconds.
  - Discard the filter tip.
  - Tap "Next" or footpedal for Vitrobot to plunge, skipping WT, BT<sub>i</sub>, BT<sub>o</sub>, and DT.
  - Resume normal Vitrobot operation.