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Protocol for Solid State Nanopore wetting

Materials:
Teflon chamber
Nanopore TEM grid
Hot plate
Large glass beaker for liquid waste
Large glass beaker for chamber washing
Small (20mL) glass beaker for TEM grid cleaning
Vacuum tubing and vacuum source
Glass Vacuum Flask
Glass Pasteur Pipette
Bath sonicator
Bunsen burner

Chemicals:
! Nitric Acid (HNO3)
! Concentrated Hydrogen Peroxide (H2O2)
! Sulfuric Acid (H2SO4)
Ethanol
Degassed water
Degassed KCl solution

1) Wash the Teflon chamber in 20% nitric acid

Warning*: working with nitric acid is dangerous. Do work under a fume hood, wear protective gear and read the MSDS.*

Rinse the Teflon chamber thoroughly with distilled water
Place the Teflon chamber in a glass beaker.
In a fume hood, add 4 parts distilled water and then 1 part Nitric Acid (HNO3) to the beaker so that the solution covers the Teflon chamber.
Place beaker on a hotplate and boil solution for 30 min (it will take a while to for the solution to boil.)
After boiling, allow the acid to cool for at least 20 min.
Set aside a large glass beaker partially filled with water for acid waste.
After the acid is cool, empty it into the large beaker.
Rinse the Teflon chamber with copious amounts of distilled water.
Rinse the Teflon chamber with ethanol
Dry the Teflon chamber under nitrogen and put it aside

2) Use microscope to confirm that TEM grid window is intact

3) Prepare piranha solution and wash nanopore TEM grid

Warning: *Sulfuric acid and Hydrogen peroxide are dangerous. Read MSDS. All work with should be done under a fume hood and with a lab coat, safety goggles and gloves. Piranha solution is extremely dangerous and can be explosive!* ***Read piranha MSDS.***

On a hotplate, add 3mL Sulfuric acid to a small (20mL) glass beaker.
Carefully add 1mL Hydrogen peroxide to this beaker with a Pasteur pipette.
Stir solution. It should boil.
Set hotplate to 140 degrees Celsius.

> If the solution does not boil then increase heat. If it still fails to boil, the peroxide may no longer be fresh.

(If solution is not clear it means that the glass beaker is not clean enough, boil piranha for 5 min. to clean the glass, remove piranha and then add fresh solution.)
Using tweezers carefully place the TEM grid into the piranha solution.
Make sure that the grid is submerged under the solution, if the grid rises, gently hold it under the piranha with tweezers for a few seconds or very gently use the glass pipette to cover it with piranha.
Once submerged, keep TEM grid in boiling piranha for at least 30 min.
(Prepare degassed water and KCl solution using steps 4 and 5)
Remove piranha with the same glass pipette and put in a large beaker filled with water.
Carefully fill the small beaker with degassed distilled water.

4) Degas water

Add distilled water to a vacuum flask.
Heat flask with Bunsen burner until the water just starts to boil.
Remove from heat and plug the top of the flask with a stopper.
Secure vacuum tubing to the side hole of the flask and put under vacuum.
Place beaker in a partially filled bath sonicator and Sonicate for at least 15 min. while solution is under vacuum.

5) Degassing KCl solution

Follow the same steps as above substituting KCl solution for water.

6) Clean o-rings

Rinse o-rings with ethanol and let dry

7) Load grid into o-rings

Carefully remove the TEM grid from water and dry quickly under suction.
Place the grid face down on top of one of the o-rings and make sure there are no gaps between the o-ring and grid.
Carefully bring the second o-ring on top of the grid so that the grid is sandwiched between the two o-rings.
Add 5uL of the degassed KCl solution to the interior of the o-ring onto each side of the TEM grid.

8) Load o-rings into the Teflon chamber

Place the o-rings into the groves of the chamber and seal the chamber using screws.
Add the desired amount of KCl to each bath of the chamber. Pipette the solution through the top flow channels of the chamber to make sure solution reaches the o-rings.
Let the chamber sit for at least 36 hours—note that some of the solution will evaporate. Placing the chamber next to a water bath minimizes evaporation.

9) Check current signature of chamber

The current across the nanopore should be symmetric with voltage, non-fluctuating, and match the expected conductance. Use IV curve protocol to measure conductance (protocol saved as *IVcurvetest.pro* in Clampex protocol folder)
A broken grid window will have a very large conductance (μS).
If current is asymmetric, too small, or fluctuating, let the chamber sit an additional 12 hours. If current signal is still unacceptable, put 5V DC across the pore for 2 seconds and re-measure.