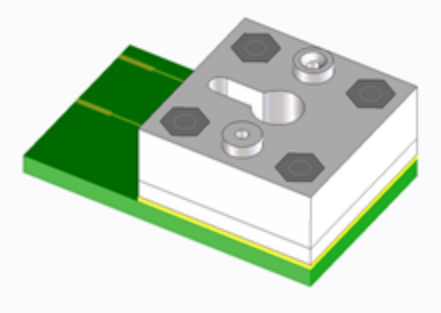
elements
enabling technologies for Life Science

eNPR - Flow cell for Nanopore Chips: Preparation, experimental setup and troubleshooting



This short user guide will help you with how to get started with a Nanopore Experiment and how to deal with possible problems.

ELEMENTS srl - ITALY - C.F/P.IVA/VAT 04113900403 - tel: +39 0547 482983 - www.elements-ic.com

commercial info: info@elements-ic.com - technical support: support@elements-ic.com

Nanopore treatment

For the best results, the 5x5 mm silicone nitride chip should be treated with oxygen plasma.

Oxygen Plasma treatment [1]:

Expose both sides of the 5x5 mm silicone nitride Nanopore Chip to oxygen plasma.

The following parameters were established using a Gatan Model 950 Advanced Plasma System:

Time per side	2-3 min
Oxygen flow rate	40 sccm
Forward RF power	30 W

Alternative Method: Piranha Cleaning [2; 3]:

Place the nanopore containing silicone nitride Chip into a 10 ml Pyrex beaker. Be careful not to break the nitride window, as it is very delicate. Place the beaker on a hotplate in a fume hood and set it to 80 °C.

Clean the Nanopore Chip with Piranha solution using great care. First, add 3 ml sulfuric acid to the container using a glass pipette. Next, carefully add 1 ml hydrogen peroxide to the sulfuric acid to make Piranha solution 14. Please take all appropriate precautions.

Allow the Nanopore Chip to soak in Piranha solution for 5 minutes.

Remove the Piranha solution from the beaker by using a glass pipette. Afterwards, place it into a proper storage receptacle.

Fill the beaker with degassed and deionized water using a clean glass pipette. Empty the beaker of water and repeat this step at least 5 times.

Remove the Nanopore Chip with a clean pair of tweezers and dry it by using light suction.

Testing of the Chip

- Use the Elements Data Reader (EDR) 3 software.

- Set the current range to 20 nA and the sampling rate to 20 kHz in the EDR control window.

- Click on the “Start” button in the RC estimation tab in the Analysis window.

- For **dry** Nanopore Chip: Check that the capacitance of the Chip is >10 pF. If it is below, see the troubleshooting section below.
  For **wet** Nanopore Chip: If you don’t see any current, check the capacitance of the chip by using the amplifier. If solution is in contact with the membrane window, the capacitance should be >20 pF (silicone Chips). If it is <20 pF, the solution has not yet made contact with the Chip itself. If this is the case, see the troubleshooting section below.

Troubleshooting

Dry Nanopore Chip

A capacitance value below 10 pF indicates that the buffer solution is not in contact with the nitride membrane.

- Inspect the top side of the Chip for any air bubbles and remove if necessary.
- If the value is still <10 pF, thoroughly flush and replace the top and bottom solutions with ddH_2O.
- Aggressively flush the bottom well with Isopropanol or Ethanol (this helps the wetting of the bottom chamber)
- Flush out the Isopropanol thoroughly with water several times. Be careful not to add any air bubbles.
- Replace the salt solution in the bottom well. Be careful not to add any air bubbles.
- Re-measure capacitance.

- An additional method that helps avoiding air bubbles is to turn the chamber upside down while filling up the bottom solution. This way the air bubbles will escape upwards from the O-Ring.

Wet Nanopore Chip

- Carefully check the top side of the chamber for air bubbles and remove them with a pipette if necessary.
- Flush the bottom chamber with water.
- Aggressively flush the bottom well with Isopropanol or Ethanol (this helps the wetting of the bottom chamber)
- Flush out the Isopropanol thoroughly with water several times. Be careful not to add any air bubbles.
- Replace the salt solution in the bottom well. Be careful not to add any air bubbles.

References

[1] **Smeets R. M. M., Keyser U. F., Dekker N. H., Dekker C.** (2008) Noise in Solid-State Nanopores. Proc. Natl. Acad. Sci. U.S.A., 105(2):417-421

[2] **Wanunu M., Meller A. Selvin, P. R., Ha, T.** (2008) Single-molecule techniques: a laboratory manual. Cold Spring Harbor Laboratory Press, New York. 395-420

[3] **Niedzwiecki D. J., Movileanu L.** (2011) Monitoring Protein Adsorption with Solid-state Nanopores. J. Vis. Exp. 58:P. e3560