

NANOPORE UNBLOCKING GUIDE

To troubleshoot partial or complete nanopore blocking when running a sample, complete the following steps until a stable baseline has been re-established. It is not necessary to continue on to subsequent troubleshooting steps once the baseline is stabilised, if the nanopore blocks again later then start this unblocking guide from the beginning.

Apply a Negative Pressure

- ✔ Making sure the pressure nozzle is inserted, apply a negative pressure of -2500 Pa for 10–30 seconds.

Tap the Pressure Nozzle

- ✔ Without changing the pressure, take the back of the Pressure Application Device (PAD) and tap the top of the pressure nozzle firmly.

Apply the PAD

- ✔ Set the pressure to 0 Pa and remove the pressure nozzle.
- ✔ Using the plunging end of the PAD, apply pulses of pressure to the upper fluid cell.

Pipette Mix Contents

- ✔ Using a further $35\ \mu\text{L}$ of sample, mix the fluid in the upper fluid cell.
- ✔ Make sure to remove $35\ \mu\text{L}$ once this is done.

Stretch and Positive Pressure

- ✔ Stretch the nanopore up to 50 mm and apply a full positive pressure of 2500 Pa for 10–30 seconds. You may need to reduce the voltage to avoid current saturation.

Stretch and Negative Pressure

- ✔ Stretch the nanopore up to 50 mm and apply a full negative pressure of -2500 Pa for 10–30 seconds.

Re-setup the System

- ✔ Set the pressure to 0 Pa and remove the pressure nozzle.
- ✔ Reduce the stretch and remove the nanopore.
- ✔ Rinse with DI and dry the nanopore, upper fluid cell and pressure nozzle.

Re-assemble the System

- ✔ Re-assemble the system with measurement electrolyte in the upper and lower fluid cells and stretch to 47 mm, or the stretch previously being used.

Flush the System

- ✔ Replace all the fluids with DI water and apply maximum pressure of 2500 Pa until the current is <5 nA. This may need repeating to meet the criteria.

