qNano protocol

Please use the more detailed manufacturer protocol until you are not familiar with the machine. This is only a short checklist to help you to remember the most important steps.

Setting up the system

1. Connect the power cable to the power supply. The power indicator will glow.
2. Turn on the computer and check the USB cable connection
3. Start the program on the computer.
4. Clean the fluid cells.
5. Measure the stretch with a ruler and set it between 40-41.5 mm.
6. Load the Nanopore (serial number should be facing upwards).
7. Calibrate stretch at 45 mm approximately.
8. Stretch Nanopore to 47 mm.

Opening the Nanopore

9. Load the upper cell and pipette 40 µl 0.5% Triton X in PBS into the upper chamber.
10. Apply maximum pressure for at least 5 min. (1. rotate the plunger under the "PRE" scale is uppermost. 2. Vent valve OPEN. 3. Plug VPM nozzle, PRE scale is set on to ZERO. 4. Close vent valve. 5. Push in the plunger to apply a pressure)
11. Remove Triton X from the upper chamber. Clean it (upper and lower chamber as well) 2 times with HPLC-clean water and then 2 times with EV-free fresh buffer (electrolyte!).

Setting the baseline

12. Pipette 80 µL buffer to the lower chamber. Try to avoid bubbles. If the outside of the chamber gets wet, dry it before continue.
13. Put on the upper chamber and pipette 40 µL buffer to the upper chamber. Try to avoid bubbles. (0.01% Tryton X can be added to the buffer.)
14. Apply the fluid cell cap.
15. Apply pressure (see above) or vacuum to set the baseline.
16. Turn on the voltage (in the programme). The power should be between 70-150 nA
17. Wait till the baseline is nice flat and stable

Calibration

18. Make dilution of the appropriate calibration beads according to the table. Use the same buffer as for EV dilution (if needed apply 0.01% triton X)
19. Switch off the pressure.
20. Remove the puffer from the upper chamber.
21. Add 40 µl diluted calibration beads to the upper chamber.
22. Apply pressure (see above, order of steps is very important).
23. Find the best pressure, voltage and stretch. Red dots must be in the green zones (relative particle size and relative particle speed). Events should be far enough from the noise line to make reliable measurement.

24. Record calibration for at least 30 sec and 500 events.
25. Save calibration with the adequate parameters.
26. Remove beads from the upper chamber
27. Wash upper chamber with puffer

Measurement

28. Make at least two different dilution of your samples.
29. Pipette 40 µl sample to the upper chamber
30. Apply pressure similar as by calibration
31. Do not change voltage or stretch! Otherwise you won't be able to calibrate your results
32. Record and save your sample
33. Wash the upper chamber between every sample
34. It is advisable if you calibrate after at least every third sample.
35. If finished you should shut down the machine properly!

Cleaning

36. Remove buffer from the upper and lower chamber. Wash chambers at least 2 times with HPLC-clean water
37. Pipette 80 µl HPLC clean water to the lower and 40 µl water to the upper chamber and apply maximum pressure for at least 5 minutes.
38. Remove cell and dry it.
39. Remove nanopore and dry it. If you will use it next day or it blocked you may keep it in 0.5% triton X for maximum one day.
40. Do not forget to nplug the machine from the power supply!

Trouble shouting

- If your nanopore is blocked or you do not see any events you can try many thing to solve it
- Pipette up and down the sample in the upper chamber to remove any possible bubble
- Wash the upper chamber wit 0.01% triton X in buffer
- Apply maximum pressure if necessary
- Check the lower chamber if there is enough buffer in it or if there is any bubble in it.
- Replace the buffer in the lower chamber
- Dry the outside of the lower chamber if it is wet
- Try to set the baseline again (it can take longer)
- Start again from the beginning staring with the pore opening
- Restart the computer and the program
- Check the USB connection
- If nothing works change the nanopore and start it again from the beginning ☹