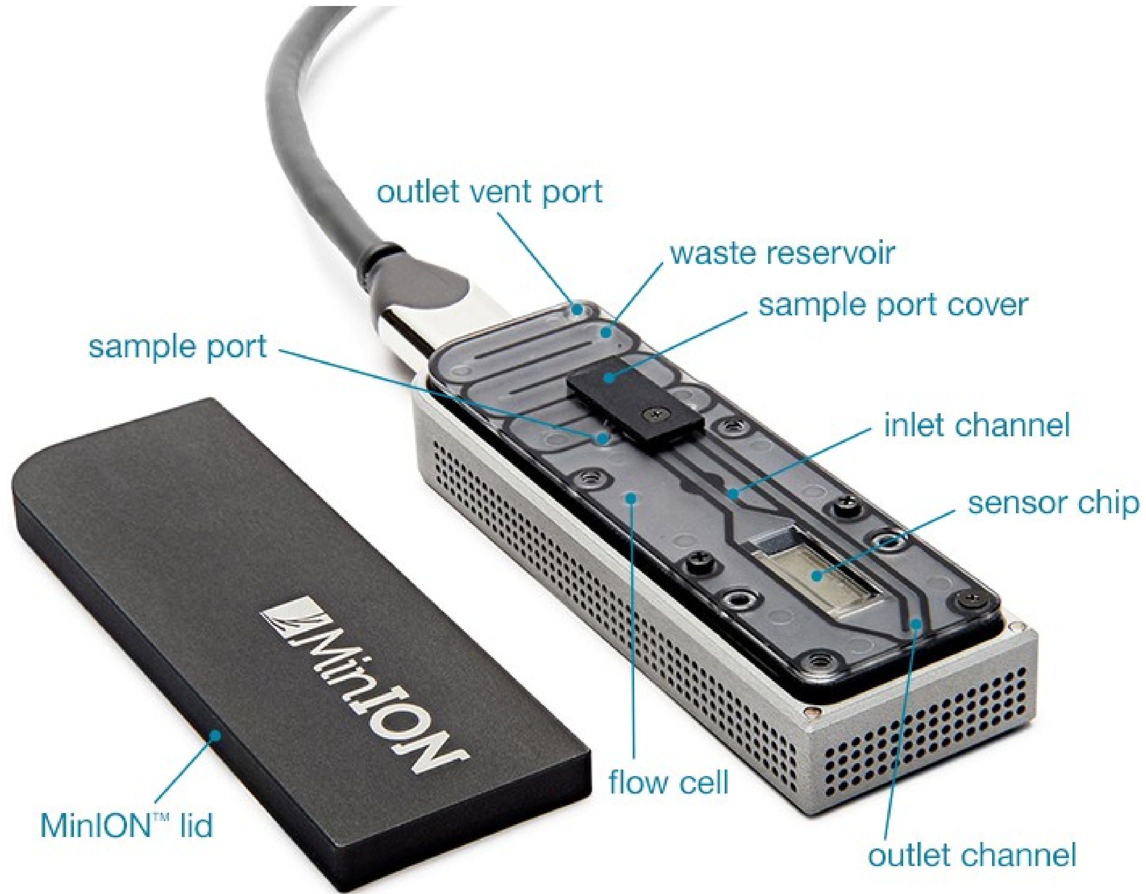
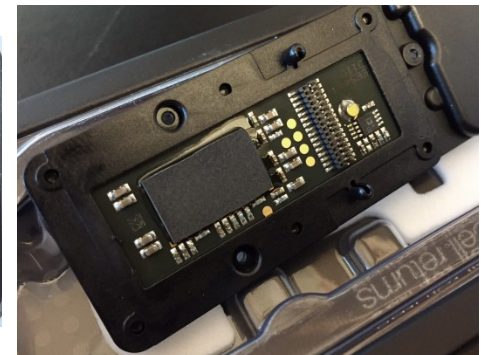
MinION Anatomy



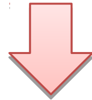
Pre-Run Check List

1. Make sure required Software is installed:-
   - MinKNOW Control of MinION device & run parameters
   - Metrichor Cloud basecalling of event data
   - MinoTour Live monitoring / control of run while sequencing
   - Chronolapse Screen image grabber for record keeping
   - Poretools, poRe Sequence extraction and data summaries
   - TeamViewer Remote control of MinION computer
2. Confirm automatic software updates and sleep modes are disabled
3. Check computer SSD for available storage space >150Gb
4. Flowcell inspection Remove bubbles in fluid lines and on surface of flowcell where possible if present, & confirm conductive heat pad is installed on bottom surface of ASIC chip.

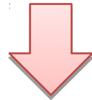


Preparing & Loading MinION Device

1. Attach Flowcell to MinION device and plug into USB3 port

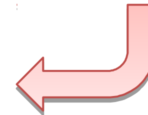


2. Start MinKNOW software and start device

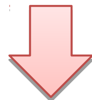


3. Name Run and start platform-QC protocol

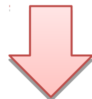
If flow-cell good proceed
(Single Good pores >650)



4. Prime flow-cell with 2x 500µl 1xRBF1 for 10 mins each
(500µl 2xRBF1 + 500µl H_2O)

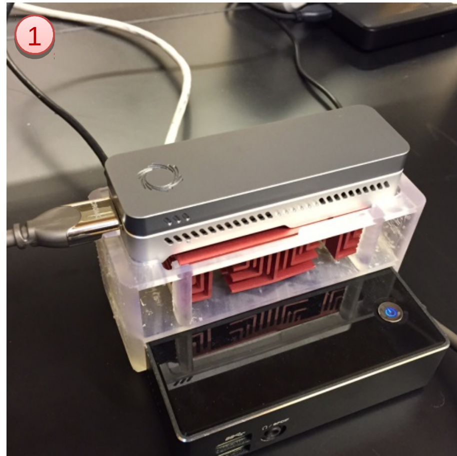


5. Load 150µl of Library in 1xRBF1
(75µl 2xRBF1 + xµl H_2O + 4µl Fuel + 6-11µl Pre-sequencing Mix)

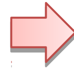


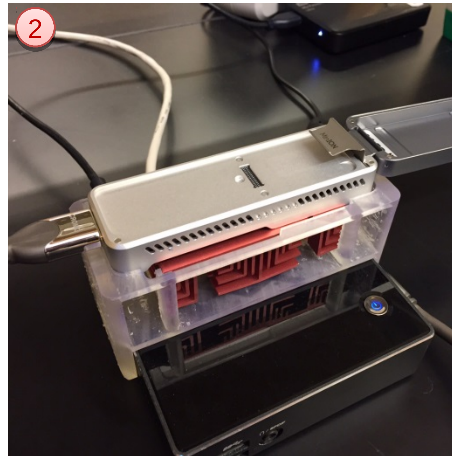
6. Name run and start Sequencing Protocol – Standard / Modified :o)
(Start Metrichor & required workflow plus screen capture software)

Inserting Flow-cell into MinION Device

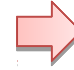


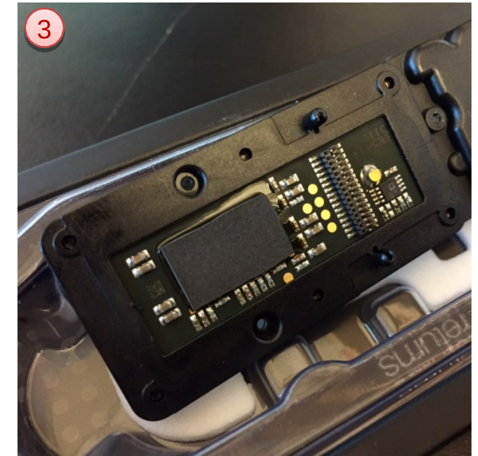
Ready MinION



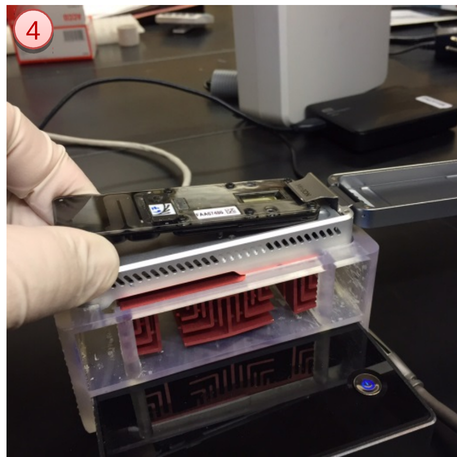


Open lid

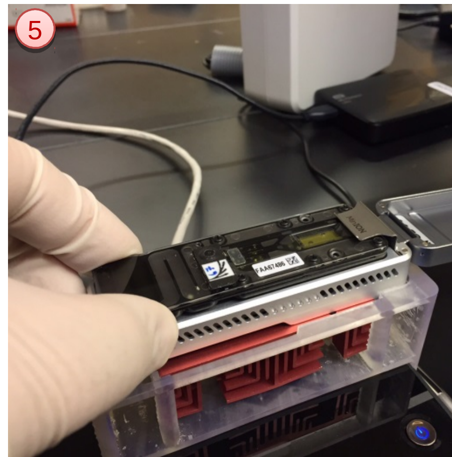




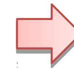
Unpack flow-cell, check heat pad intact & ASIC bubble free

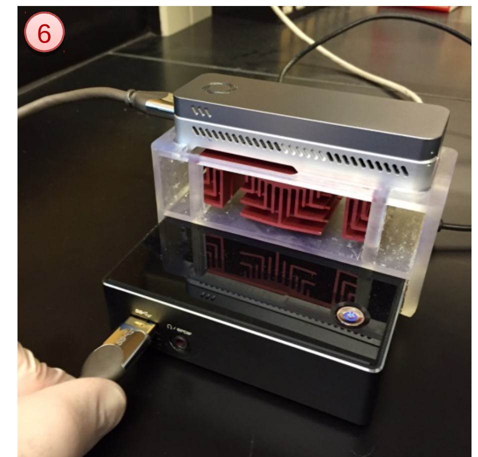


Slide flow-cell into place



Make sure flow-cell is seated properly





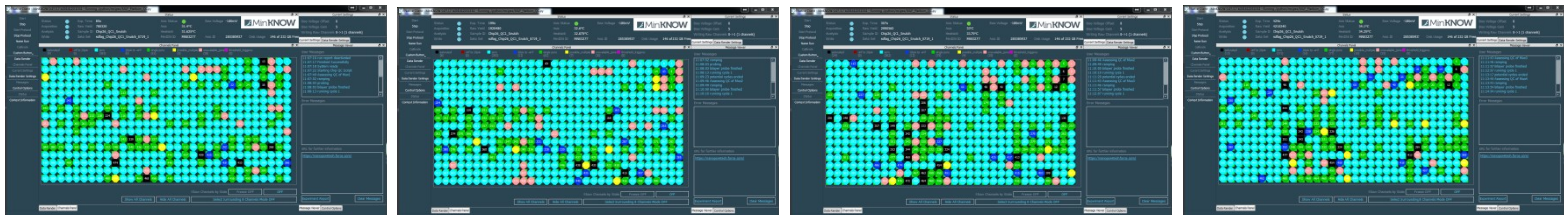
Plug MinION into USB3 port

Flow-cell Quality Assessment

Platform-QC scans the 2048 channels as 4 Mux groups @ -180mV with R9 & reports back single good channel assignment to each of g1 to g4 groups with up to 512 wells each. Expect Good correlation using R9 flowcells between Platform QC pore numbers and those obtained at the start of a run.

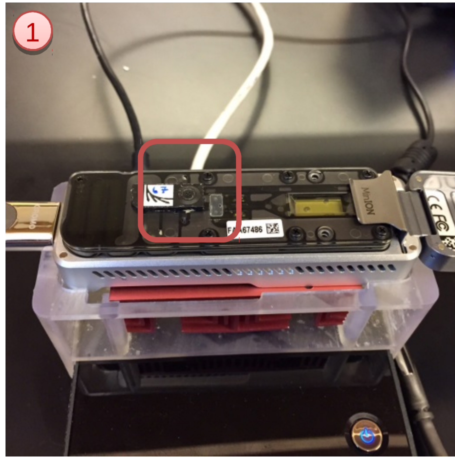
Good

Bad

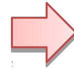


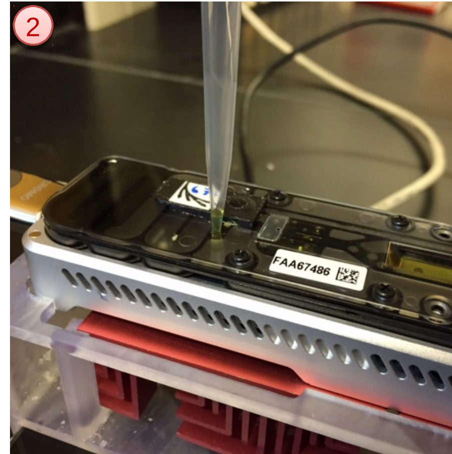
>650 Pore Guarantee

Priming and Loading Flow-cell

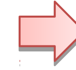


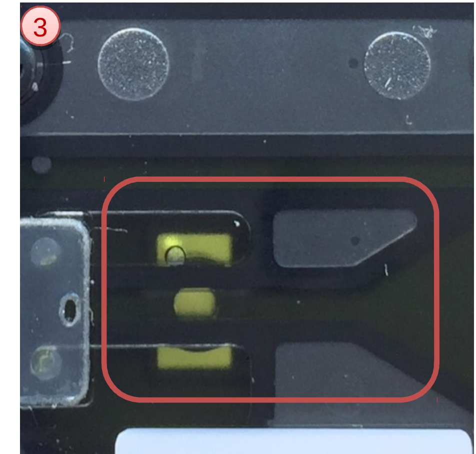
Open sample port



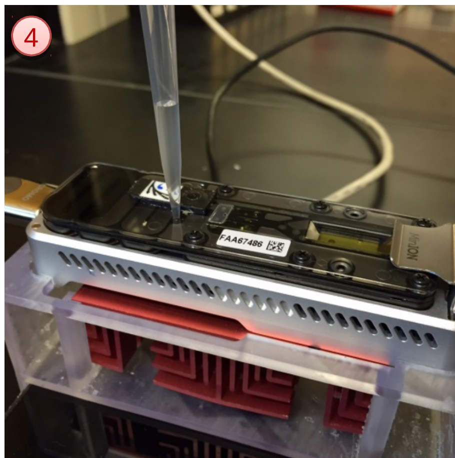


Slowly remove any air from sample port with pipette

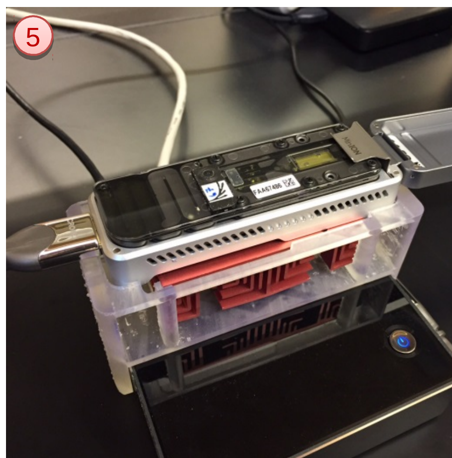




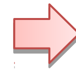
Confirm fluidic channel is free of bubbles

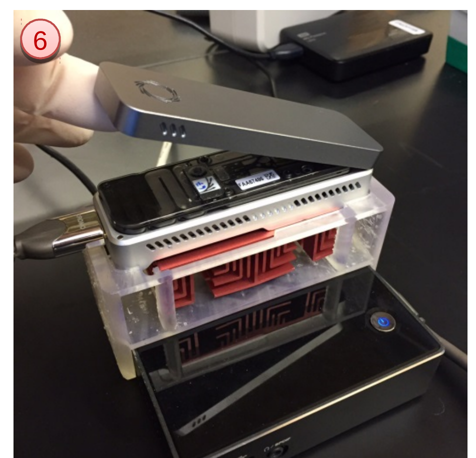


2 x 10 min 500µl 1xRBF1 followed by 150µl Library



Close sample port

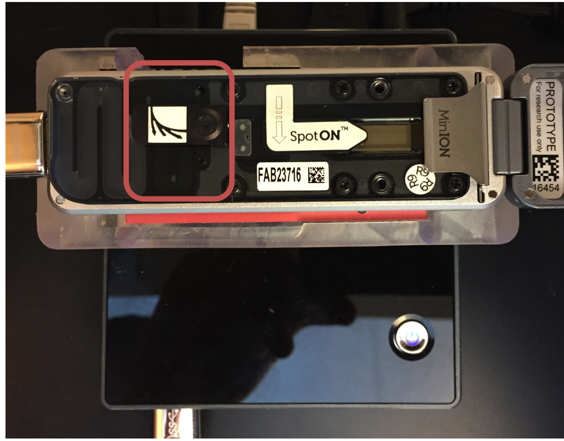




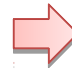
Close lid and GO!

Priming and a Loading SpotON Flow-cell

1



Open sample port



2

Flowcell Priming

Slowly remove any air from sample port with pipette

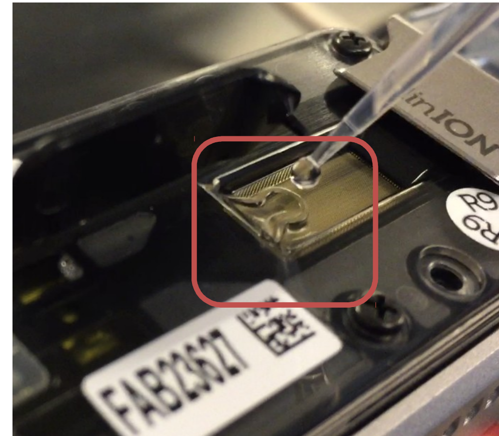
1x 10 min 500µl RBF1 through sample port

1x 10 min 300µl RBF1 through sample port

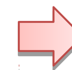
3

Open SpotON port and then add 200µl RBF1 through sample port, NOT SpotON port.

4



Add 75ul of Sequencing Mix to SpotON well dropwise



Close ports, lid and GO!

Start Run

1. ## Start Sequencing Recipe Script

   Name run and select required Recipe Script

2. ## Start Chronolapse screen capture

   Image grab every 30 seconds and as required at start

3. ## Start Metrichor workflow

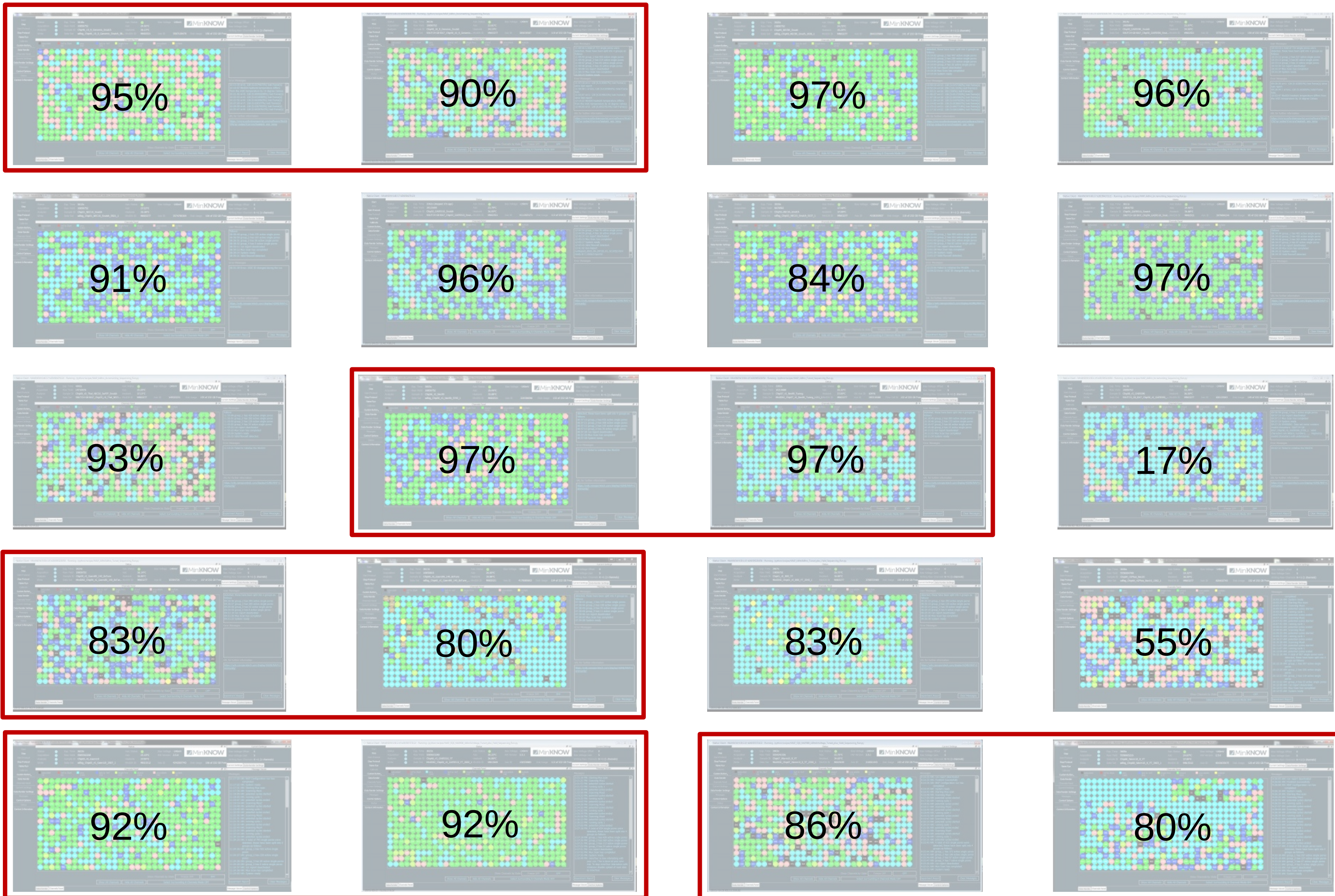
   Select desired workflow:- 2D basecall or WIMP

4. ## Start MinUp & Open MinoTour Browser Window

   Issue command line parameters for read mapping and device control as below or use MinUP-GUI:

   minUP.exe -dbh minotour.nottingham.ac.uk -dbu USER -pw PWD -w d:\data -f c:\reference\REF.txt -u USER -c -bwa -d -ip xxx.xxx.xxx.xxx -pin XXX -s minion

Good or Bad Library/Flowcell ?



% of Single Good pores in Strand

Single Good Pore % in Strand as Library QC

Use the relative quantity of in Strand reads as a measure of library quality independent of pore number

292 / 302 = 97%

Script Tinkering

1 Bias-Voltage Setting & Remux

Bias voltage directly controls induced current flow across the membrane, and current flow is used to assign pores to different categories. With time greater bias-voltage is required to produce current that is in the "single good" pore range. Bias-voltage is the master control. Selection or "re-mux" of pores at a particular targeted voltage must be carried out for maximum efficiency.

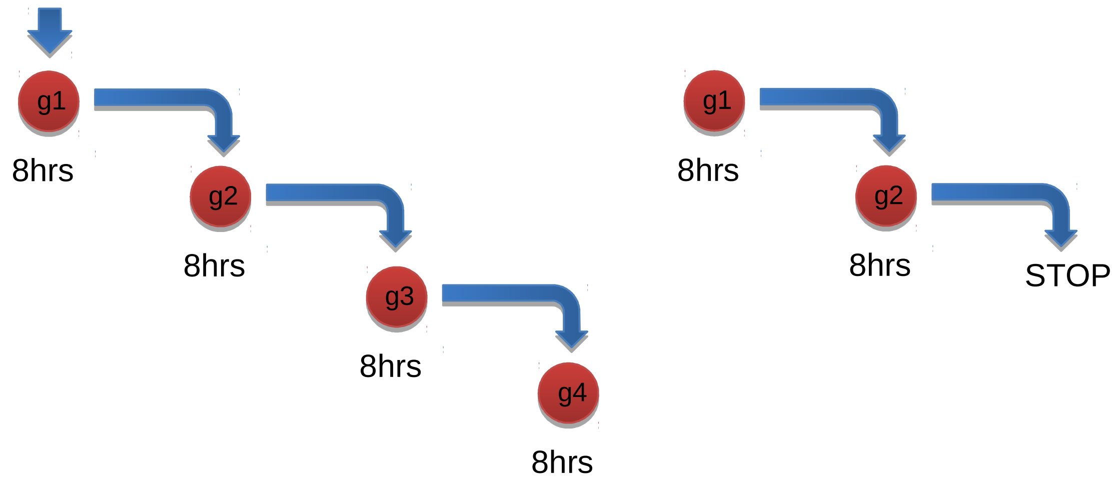
2 Yield Monitoring

As pore numbers fall and/or reagent is depleted you will see a drop in the event yield over time. Gaining access to still functional, but unselected, pores once event accumulation rate drops below a set value makes much better use of a flow-cell. This lessens unproductive electrochemical gradient deterioration observed with standard recipe scripts.

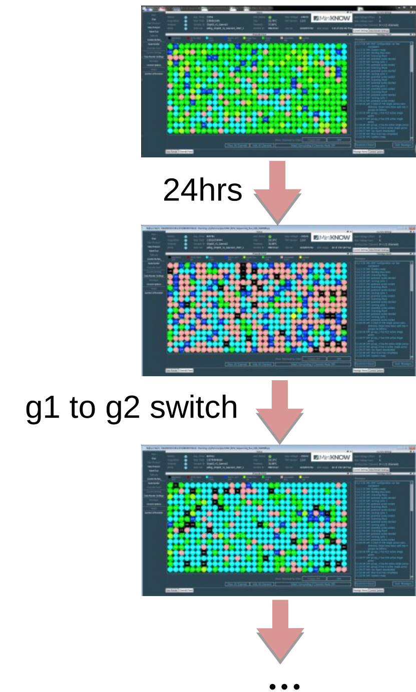
3 Pore Shepherding

Wells actively sequencing experience an electrochemical gradient deterioration that is twice that of inactive pores, but even inactive pores show a deterioration that requires bias-voltage increases for optimal functioning. Because of the 4-1 (wells - electrical channel) nature of current flow-cells this results in a spreading of the optimal bias-voltage required for a particular well depending on its on/off history. To mitigate this, sub-peak bias-voltage selection can "shepherd" well population more tightly by attempting to deplete least used wells.

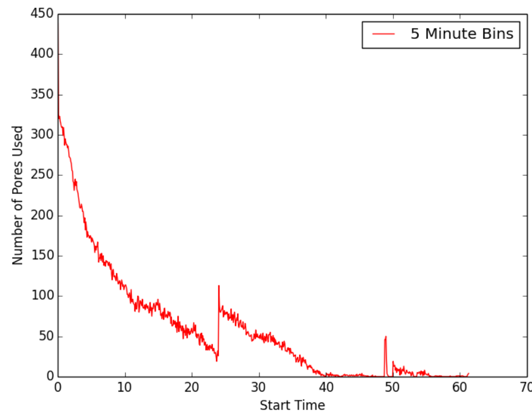
Standard 48hr Sequencing Recipe Script

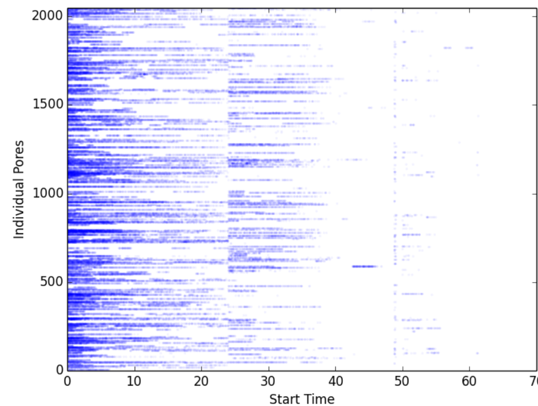


(example from previous R7.3 Version)



Example Pore Usage from retired R7.3





Modified Scripts to Maximise Flowcell Yields

Yield-Monitoring_Voltage-Tuning Scripts

(If new flowcell use -180mV to start)

Multi-Voltage Re-mux scan selecting one within a defined range of the greatest “single good pore” count voltage

15 min

Mux / Re-Mux Scan

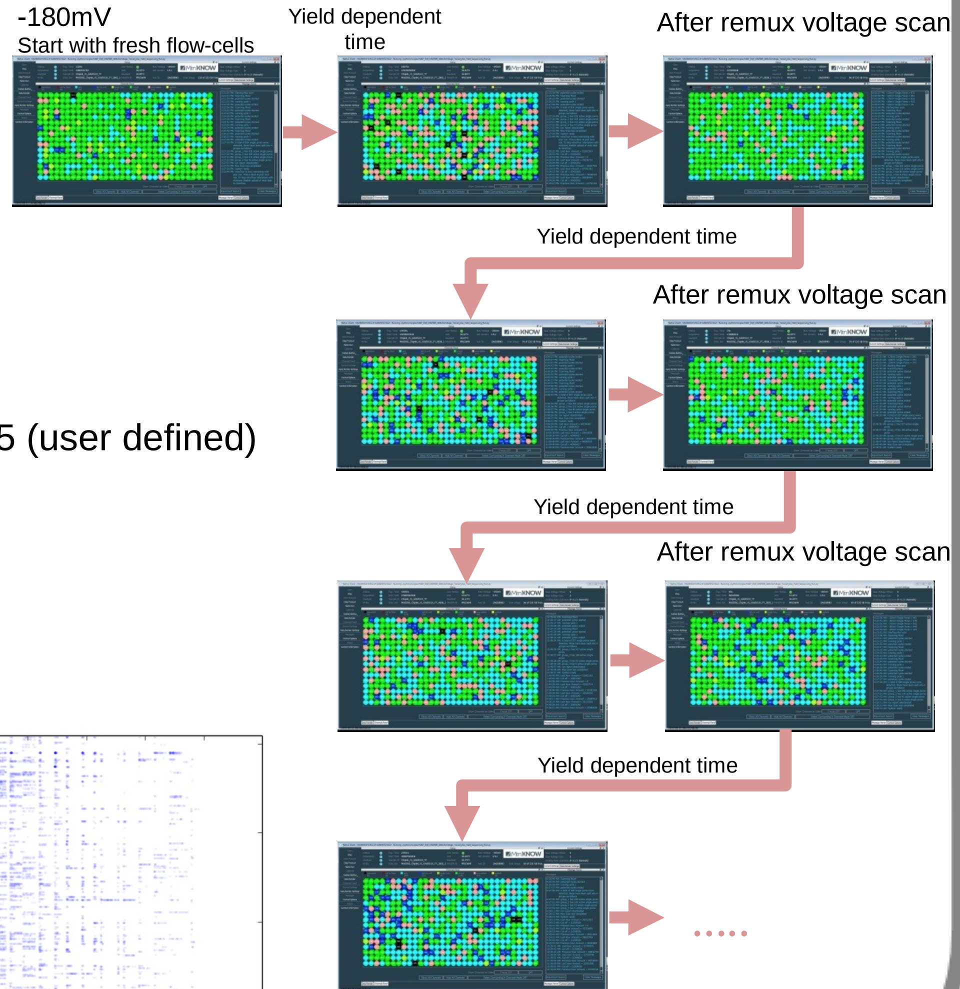
(Voltage tuned across 30mV range)

Switch when hourly event yield drops below 67% of first hour of this segment

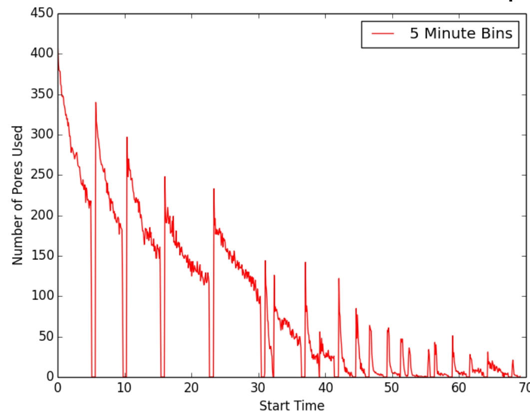
Yield Dependent Time

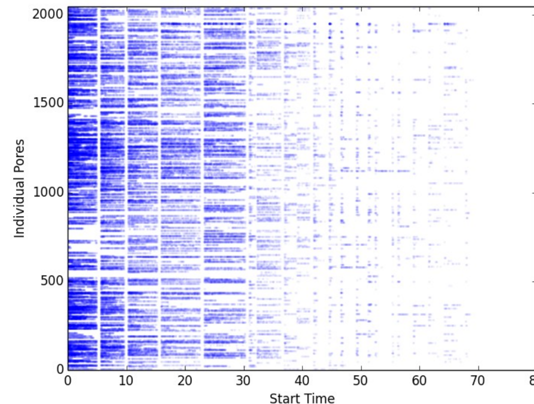
(~1-5 hrs)

x5 (user defined)



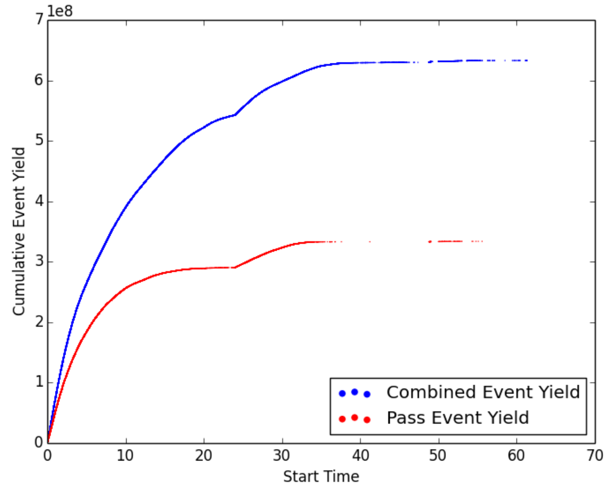
Example Pore Usage



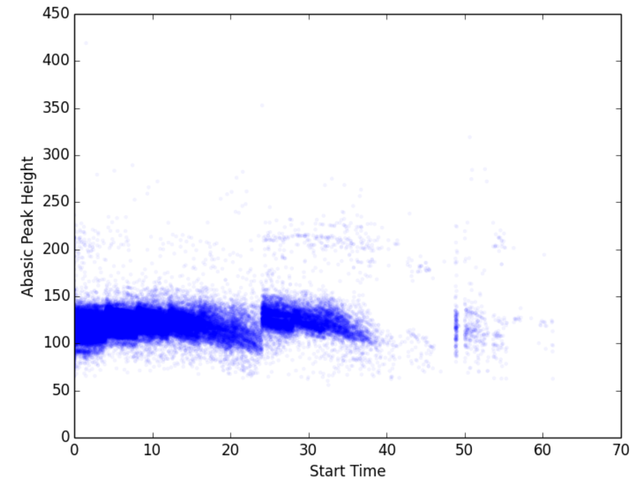


Run Dynamics:- SQK006 Standard Recipe Script

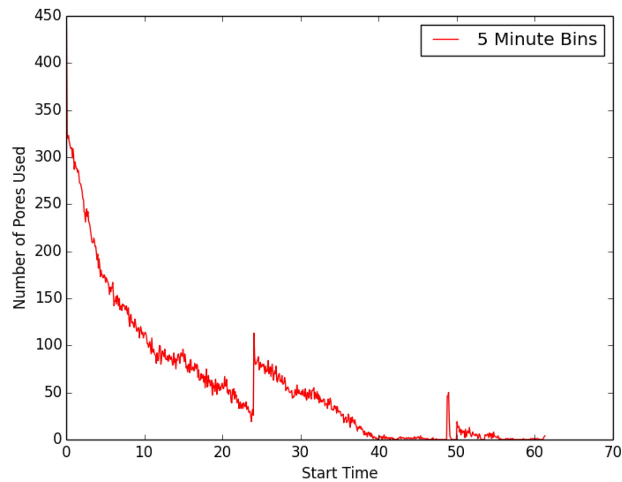
Event Accumulation

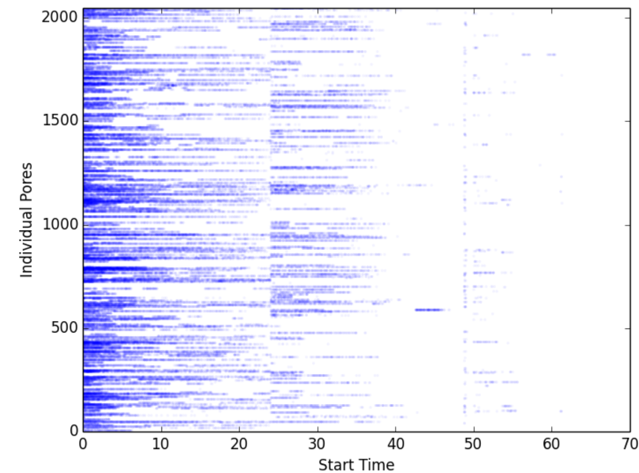


Abasic Current



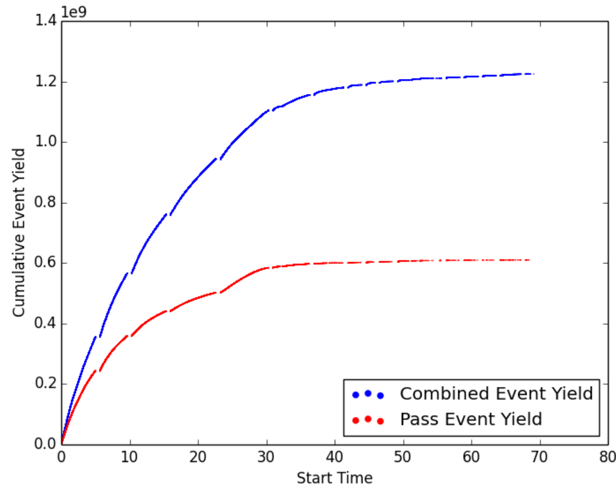
Pore Usage



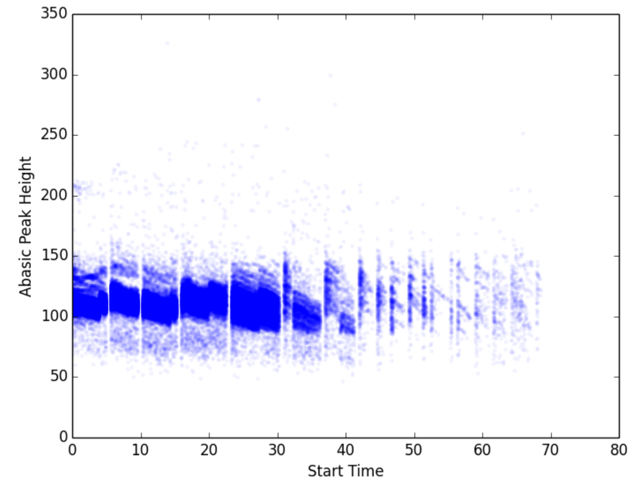


Run Dynamics:- SQK006 Tuning Recipe Script

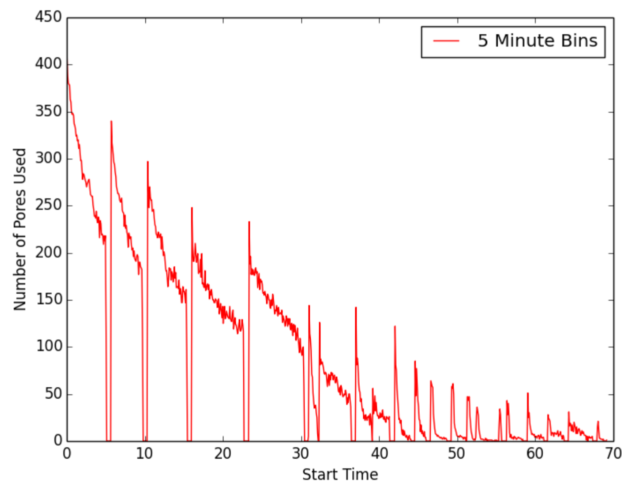
Event Accumulation

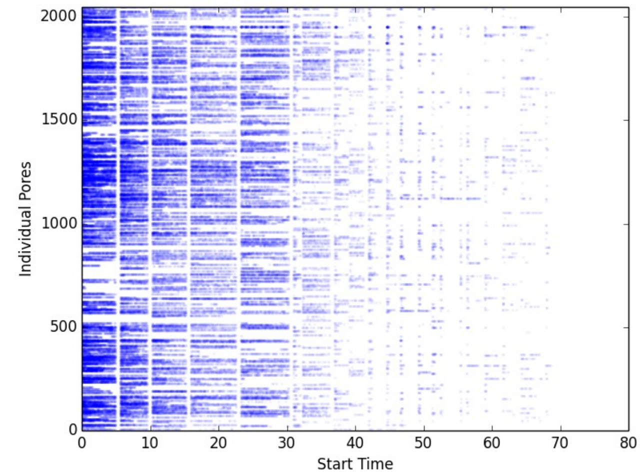


Abasic Current

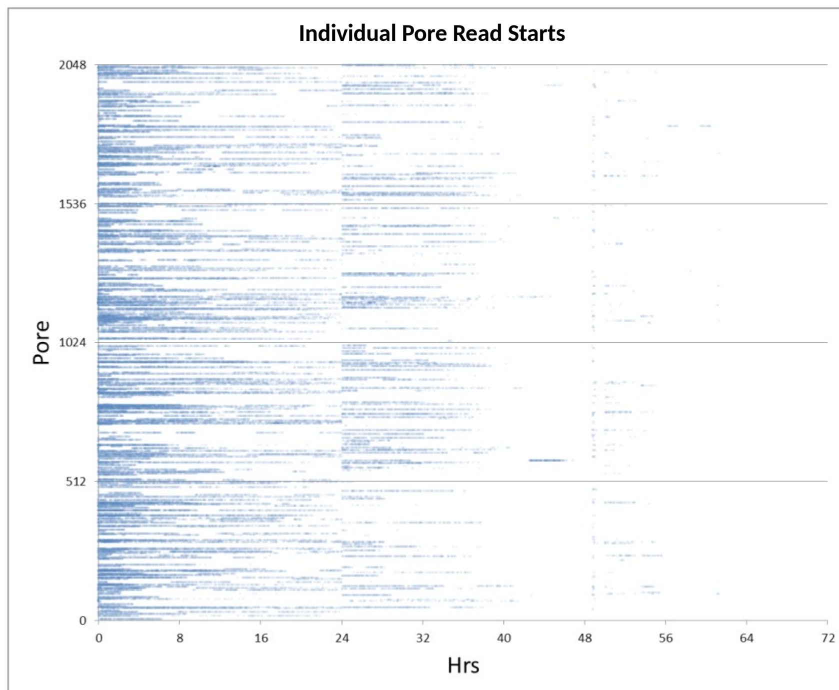


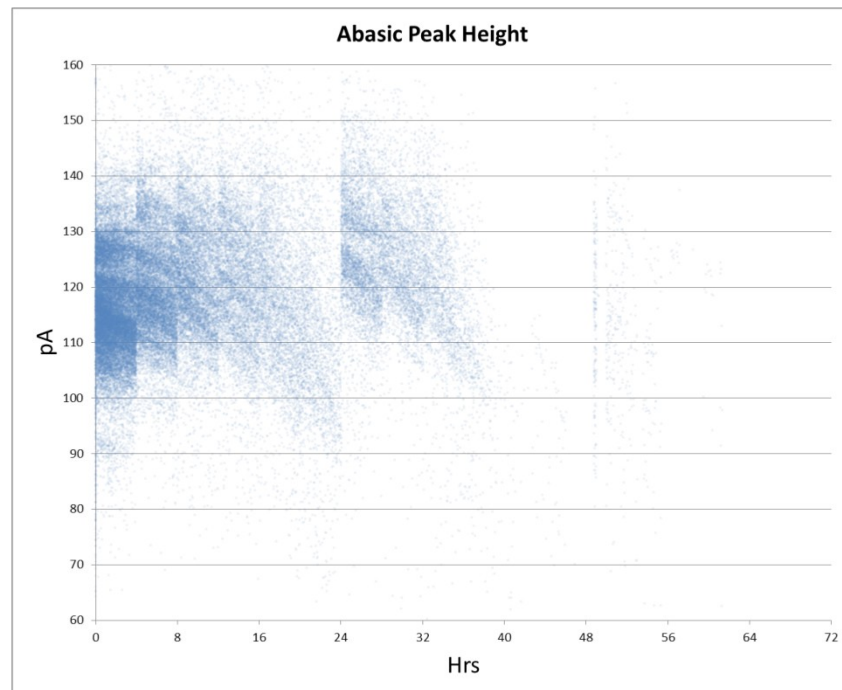
Pore Usage





Standard Script
(Chip65)





Tuning Script
(Chip66)