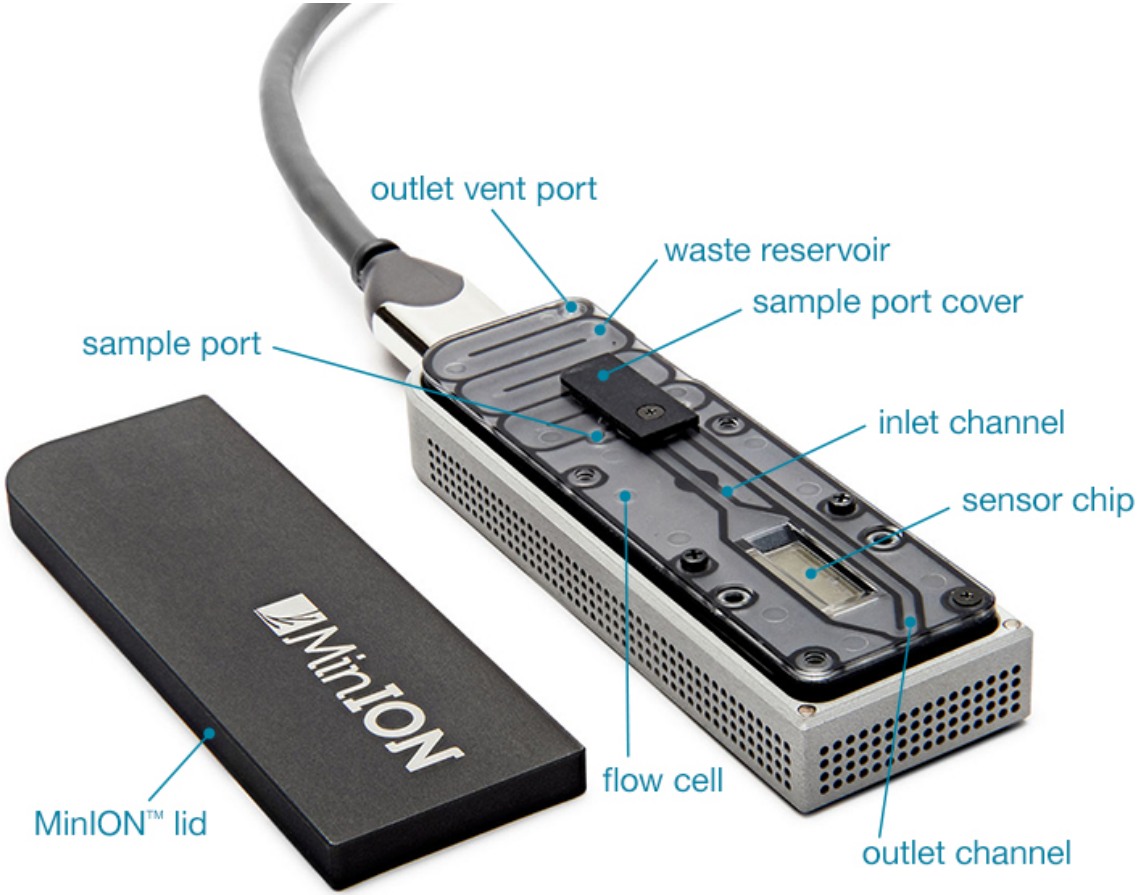
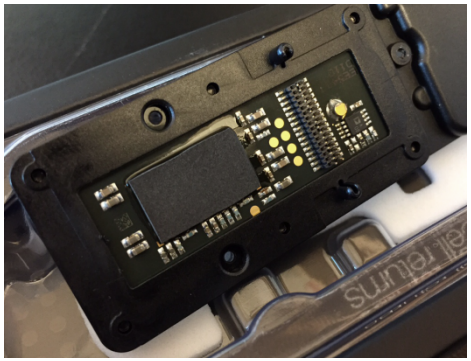
MinION Anatomy



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.



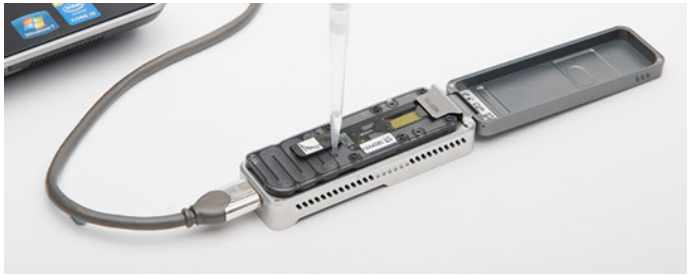
Preparing & Loading MinION Device

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)

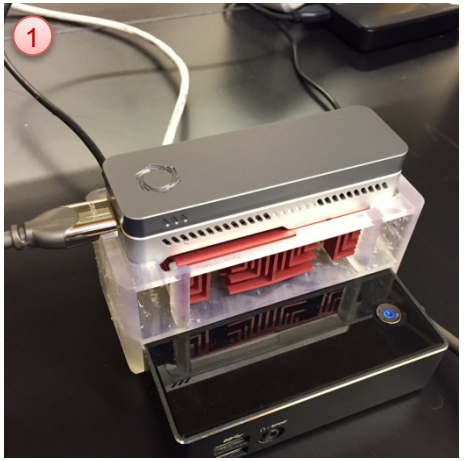
4. Prime flow-cell with 2x 500µl RB+Fuel for 10 mins each
   (500µl 2xRB + 473.4µl H_2O + 26.6µl Fuel)



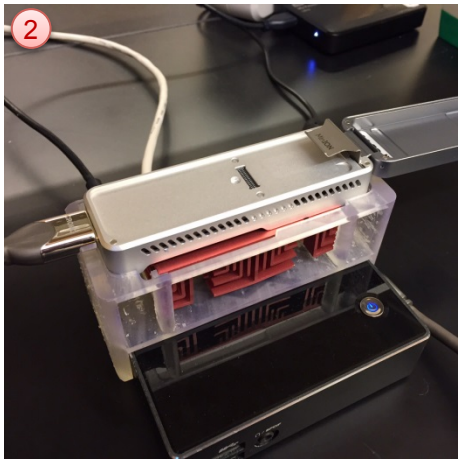
5. Load 150µl of Library in RB+Fuel
   (75µl 2xRB + 65µl H_2O + 4µl Fuel + 6µl Pre-sequencing Mix)

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

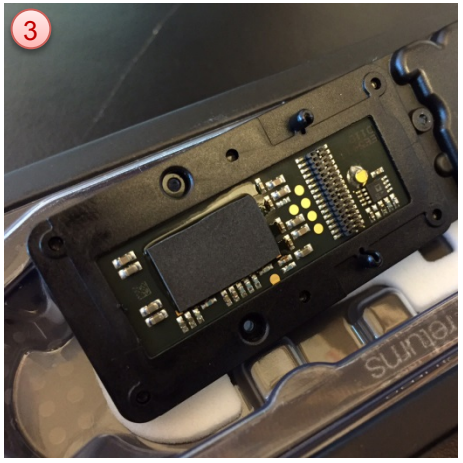
Inserting Flow-cell into MinION Device



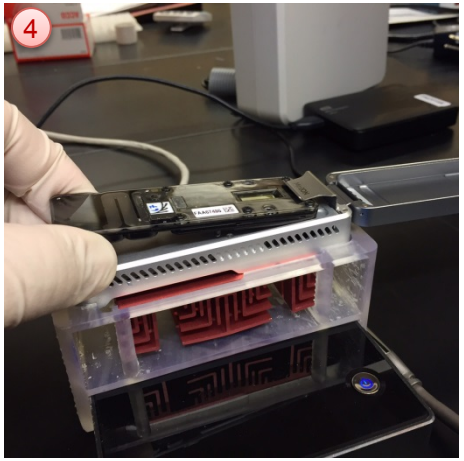
Ready MinION



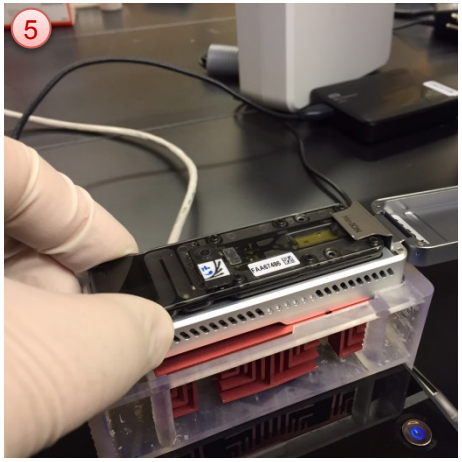
Open lid



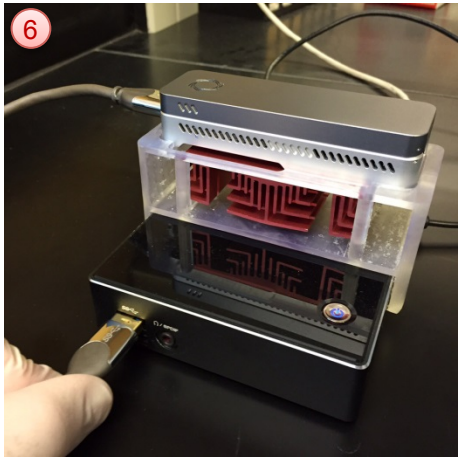
Unpack flow-cell, check heat pad intact & ASIC bubble free



Slide flow-cell into place



Make sure flow-cell is seated properly



Plug MinION into USB3 port

Flow-cell Quality Assessment

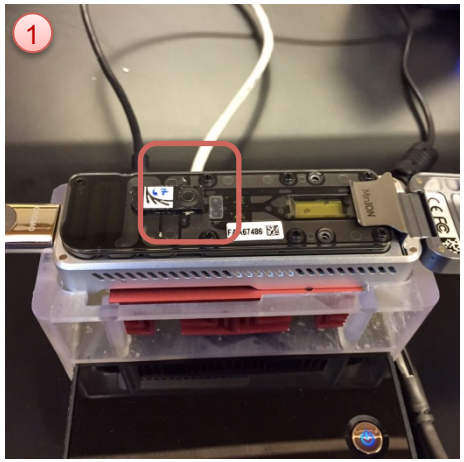
Platform-QC scans the 2048 channels as 4 Mux groups @ -180mV & reports back single good channel assignment to each of g1 to g4 groups with up to 512 wells each. These are best case numbers and you will have lower numbers as sequencing script start @ -140mV.

Good

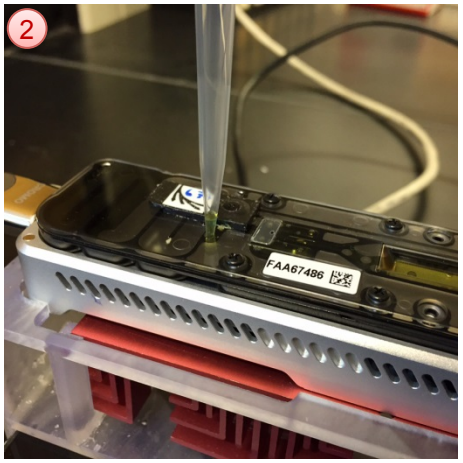
Bad

>650 Pore guarantee for purchased flowcells

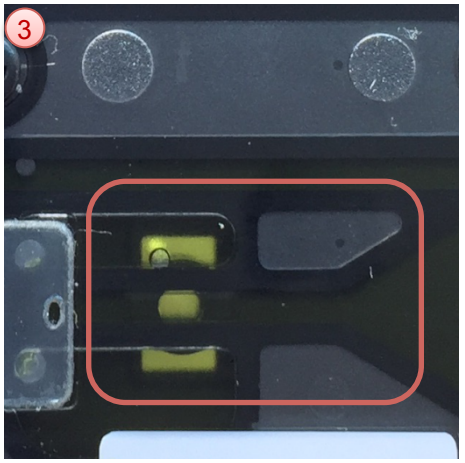
Priming and Loading Flow-cell



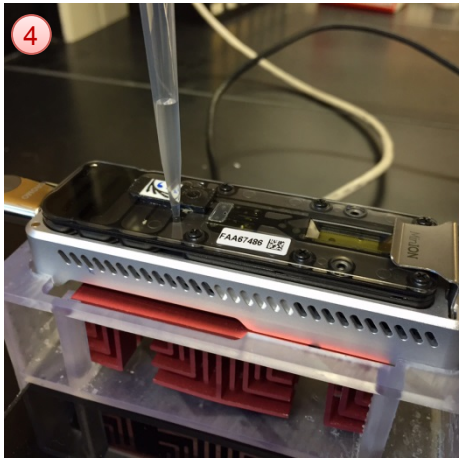
Open sample port



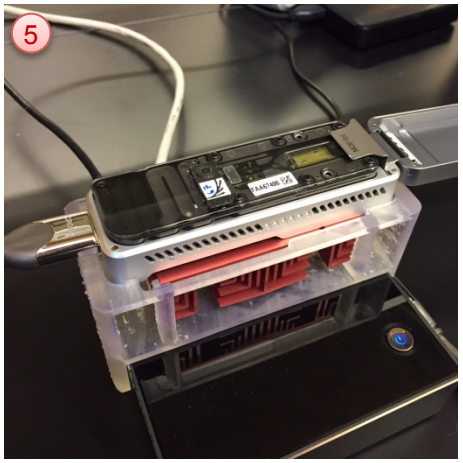
Slowly remove any air from sample port with pipette



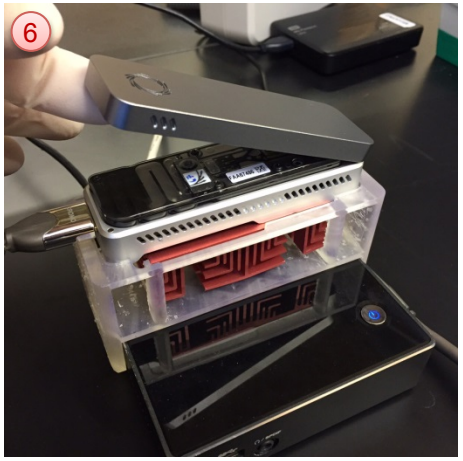
Confirm fluidic channel is free of bubbles



2 x 10 min 500µl RB+Fuel followed by 150µl Library



Close sample port



Close 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

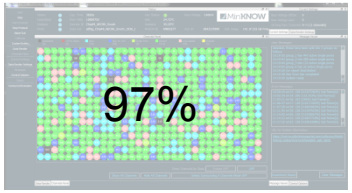
Select desired workflow:- 2D basecall or WIMP

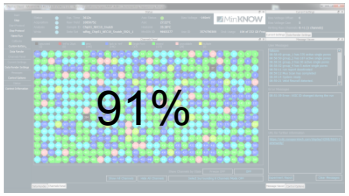
4 Start MinUp & Open MinoTour Browser Window

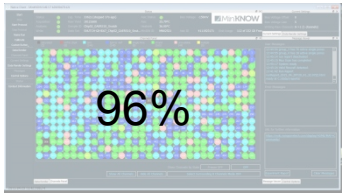
Issue command line parameters for read mapping and device control as below or use MinUP-GUI:

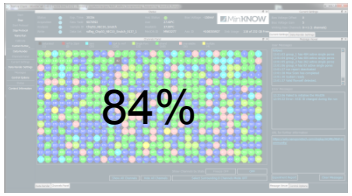
minUP.exe -dbh minotour.nottingham.ac.uk -dbu USER -pw PWD -w d:\data -f c:\reference\REF.txt -u USER -c -bwa -d -ip xxx.xxx.xxx.xxx -pin XXX -s minion

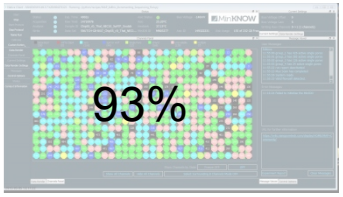
Good or Bad Library/Flowcell ?

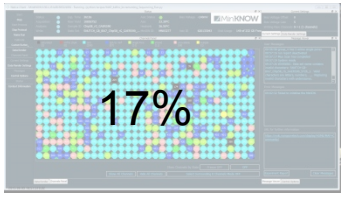


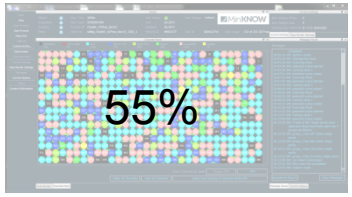


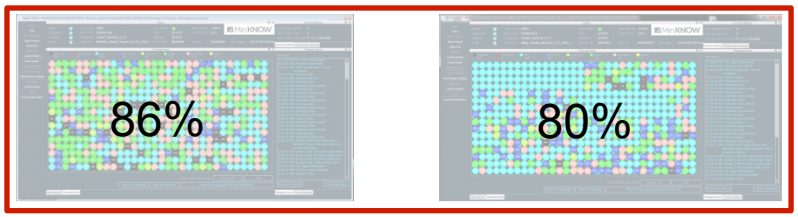












% of Single Good pores in Strand 1hr post addition

Red boxes indicate same library addition

Single Good Pore % in Strand as Measure of Library Quality

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

You would like >90% ~30mins after addition

$$\frac{292}{10 + 292} = 97\%$$

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

48hr Genomic DNA Sequencing Script

Mux @-140mV to assign g1- g4 pores

(-140mV to -165mV, -5mV every 4 hrs)

g1

24hrs

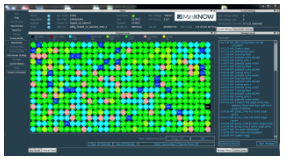
(-155mV to -180mV, -5mV every 4 hrs)

g2

24hrs

End Run

Does not use g3 or g4 pores



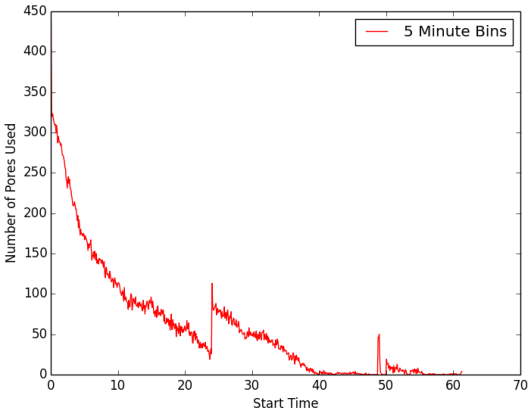
24hrs

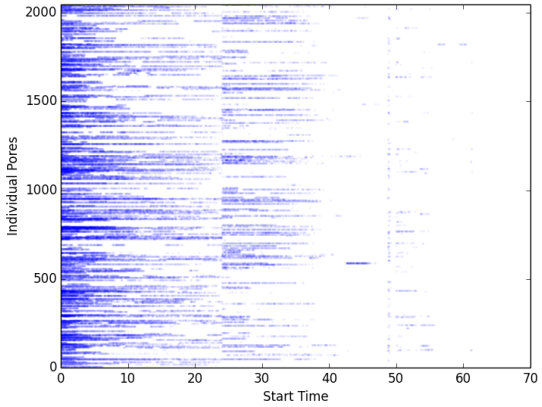
g1 to g2 switch

24hrs

Option to start other Scripts or have a play!

Example Pore Usage





Modified Scripts to Maximise Flowcell Yields

Yield-Monitoring_Voltage-Tuning Scripts

(If new flowcell use -140mV to start)

Multi-Voltage Re-mux scan selecting one with greatest "single good pore" count

30 min

Mux / Re-Mux Scan

(Voltage tuned across -140mV to -190mV range)

Switch when hourly event yield drops below 60% of first hour of this segment

Yield Dependent Time

(1 – 24 hours)

x5

-140mV

Start with fresh flow-cells

Yield dependent time

After remux voltage scan

Yield dependent time

After remux voltage scan

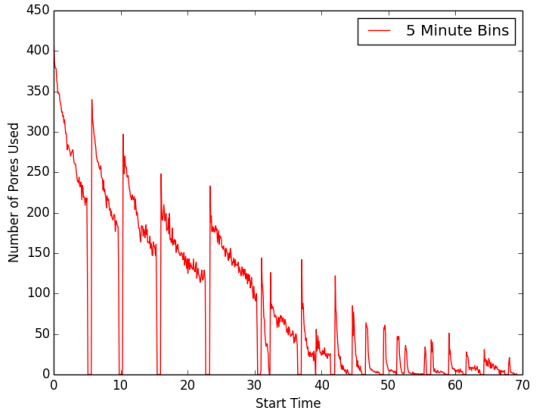
Yield dependent time

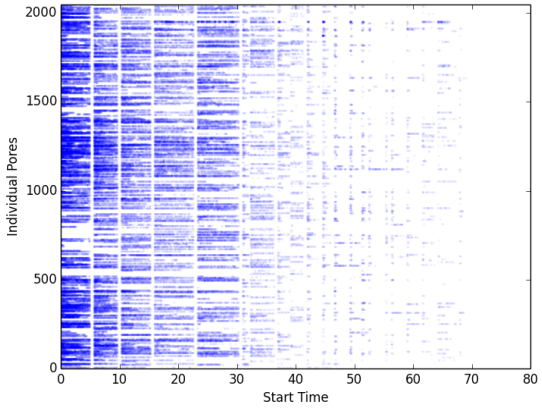
After remux voltage scan

Yield dependent time

.....

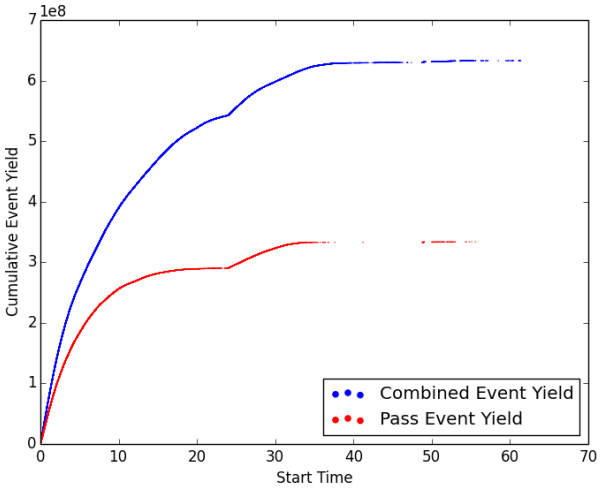
Example Pore Usage



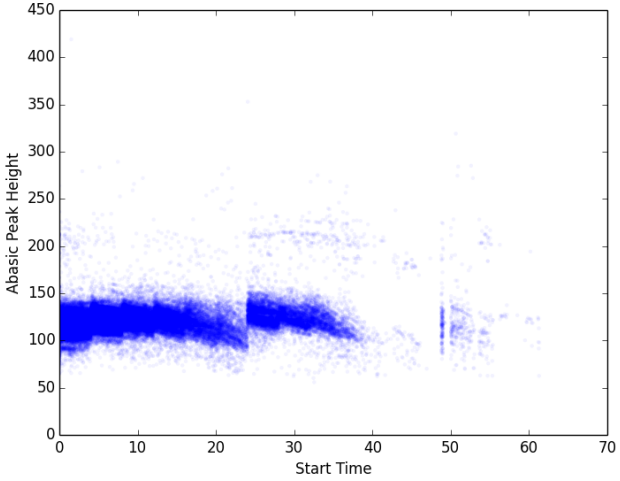


Run Dynamics:- SQK006 Standard Recipe Script

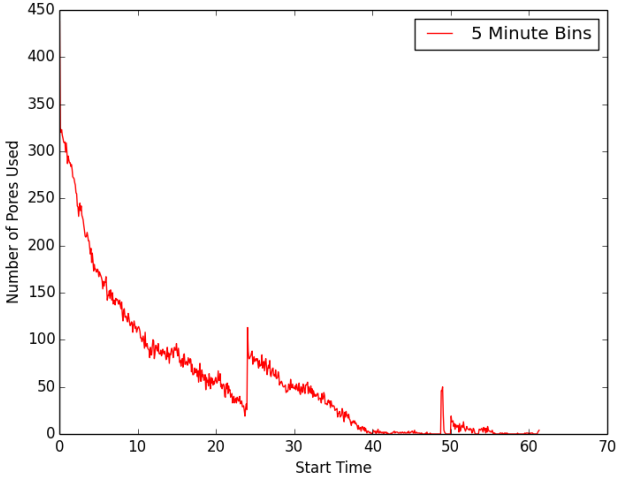
Event Accumulation

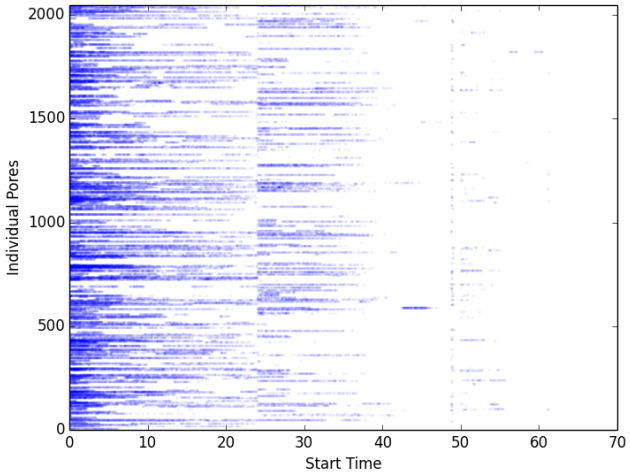


Abasic Current



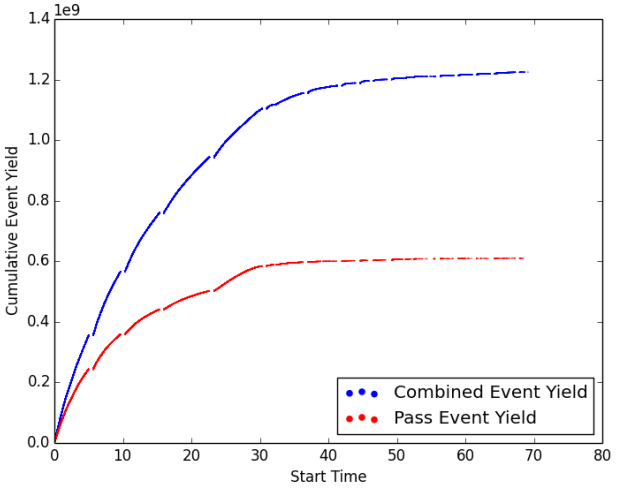
Pore Usage



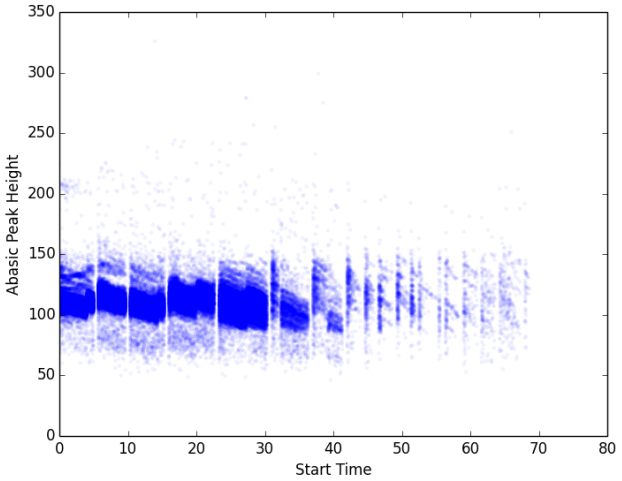


Run Dynamics:- SQK006 Tuning Recipe Script

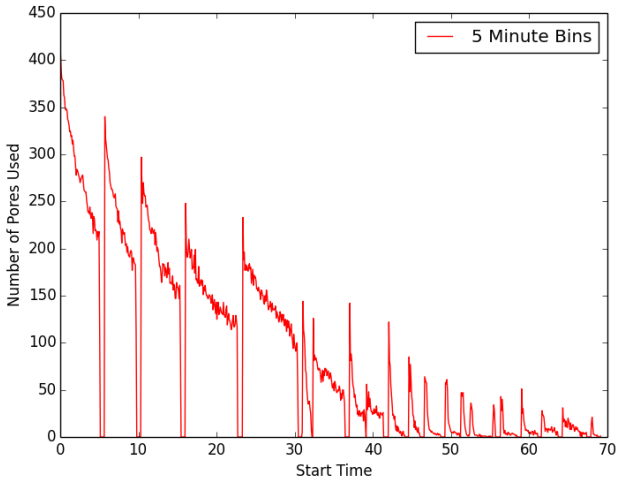
Event Accumulation

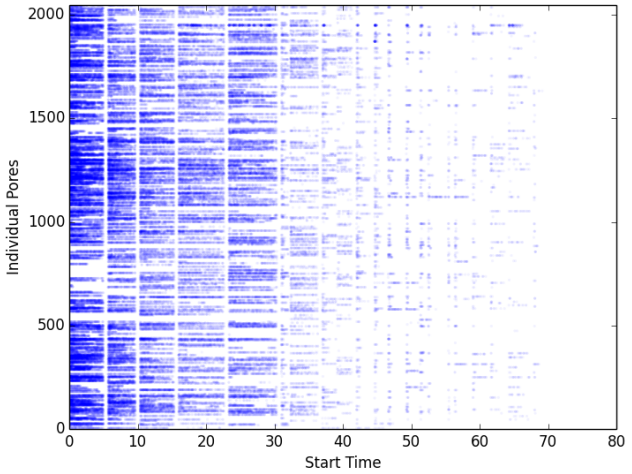


Abasic Current



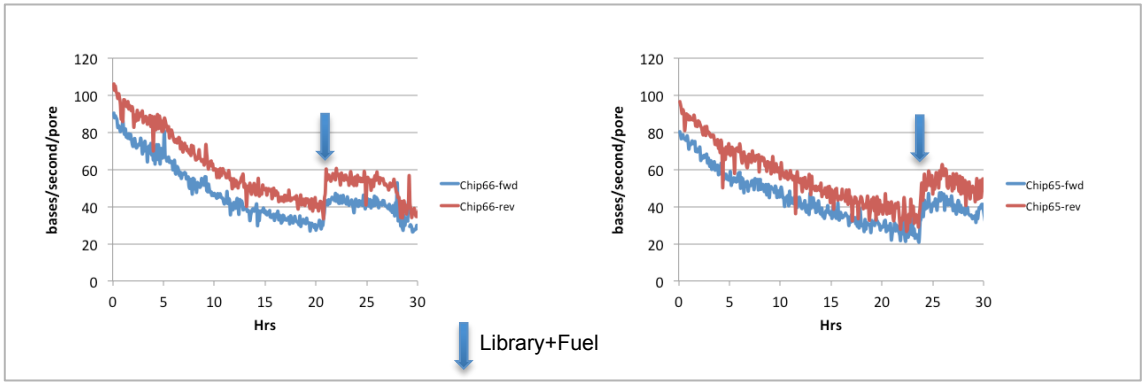
Pore Usage





Pore Speed Comparison

SQK6 Pore Speed



SQK5 Pore Speed