

# Introduction to the MinION sequencer



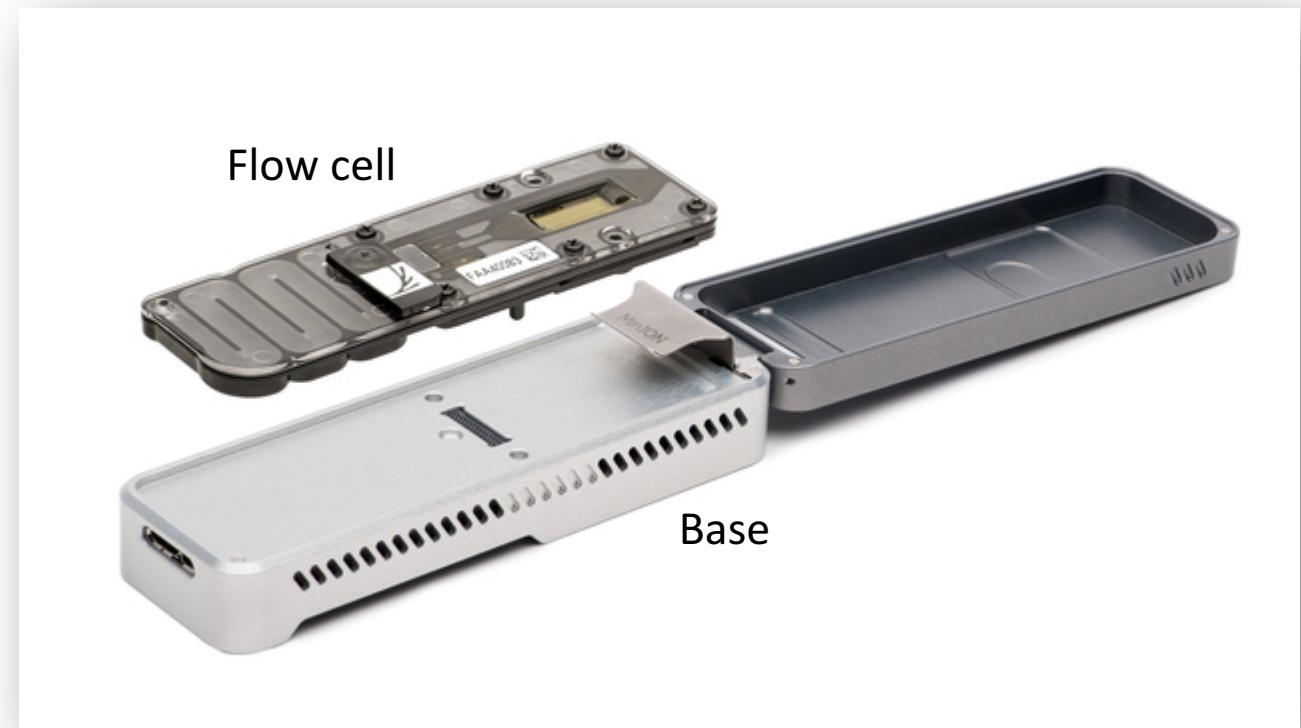
# MinION Sequencer

## Base

- Connects to Computer
- Fan / thermal regulation

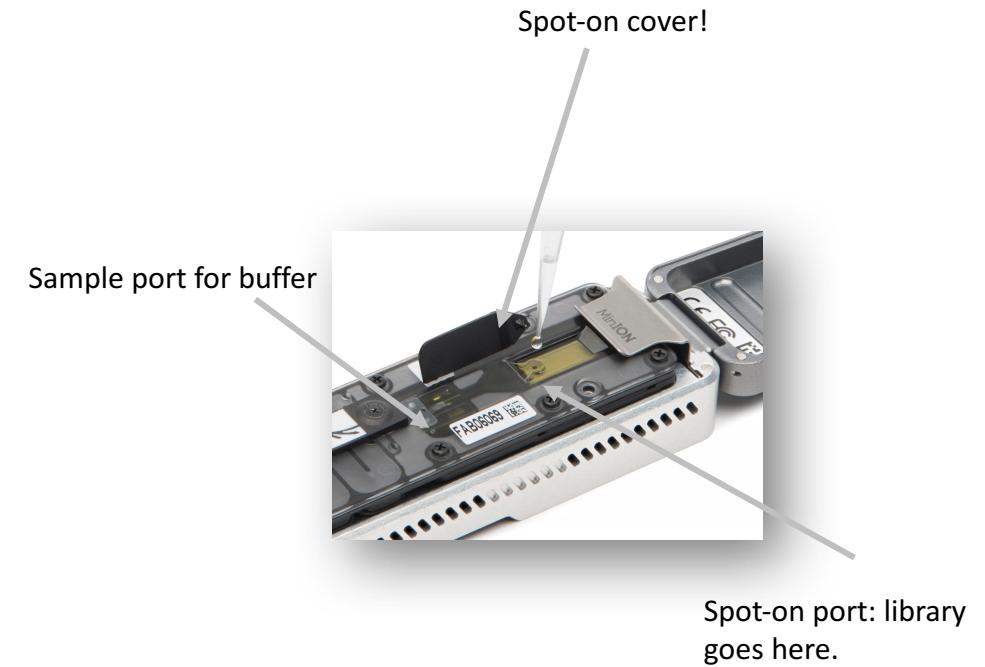
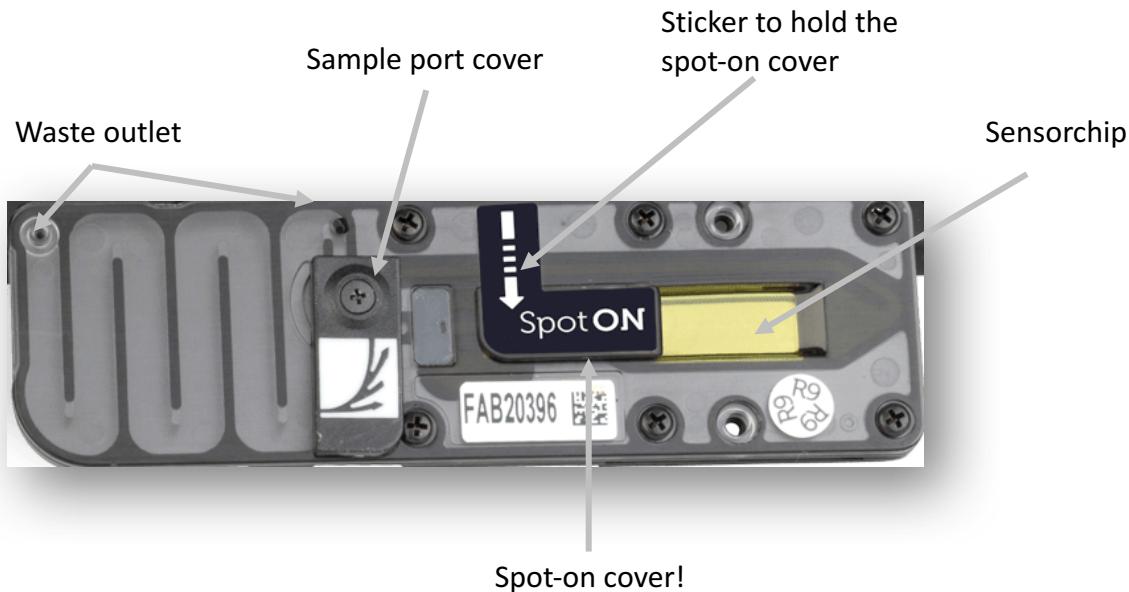
## Flow Cell

- Sample goes here
- Includes chip with nanopores
- Enables up to 48h of sequencing  
(Consumable)



Source: <http://www.the-scientist.com>

# Flow cell – Overview



# Flow cell

## Sample port

- Main inlet for buffers etc
- Confusing name: Don't put the sample here!
- Used to wash and prime the flow cell before / after use



# Flow cell

## Spot-On port & cover

- Library goes here
- Rigid cover connected to flow cell with a thin sticker.
- **Always keep closed unless you're loading library.**



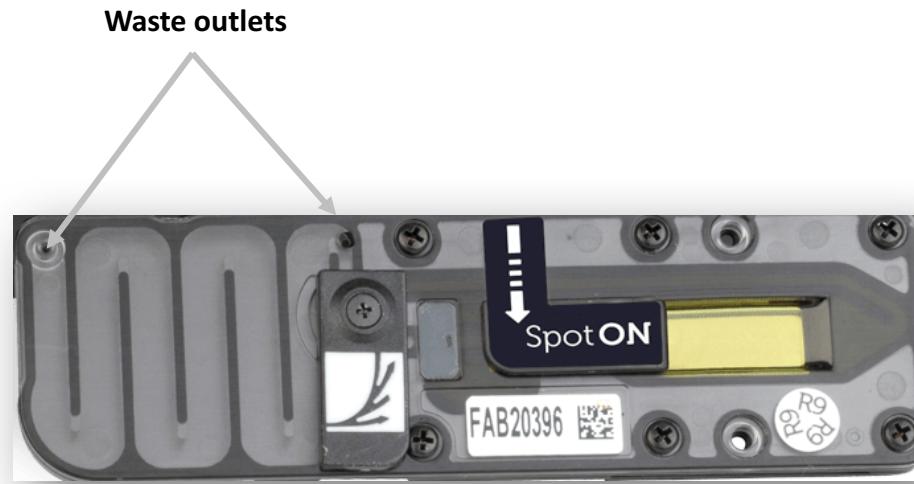
Spot-on port & cover



# Flow cell

## Waste outlets

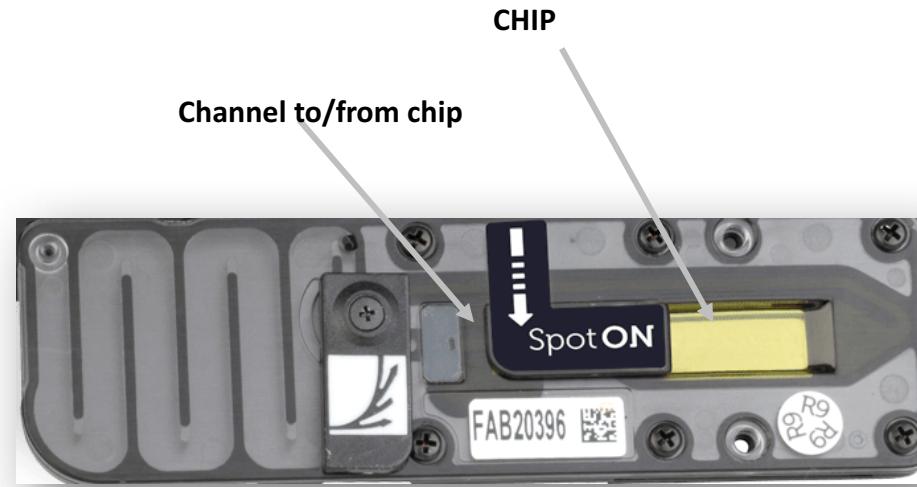
- To remove old primer, library etc using these
- **Never remove liquid from waste outlets unless Spot-on port and Sample port are closed!!**



# Flow cell

## Chip

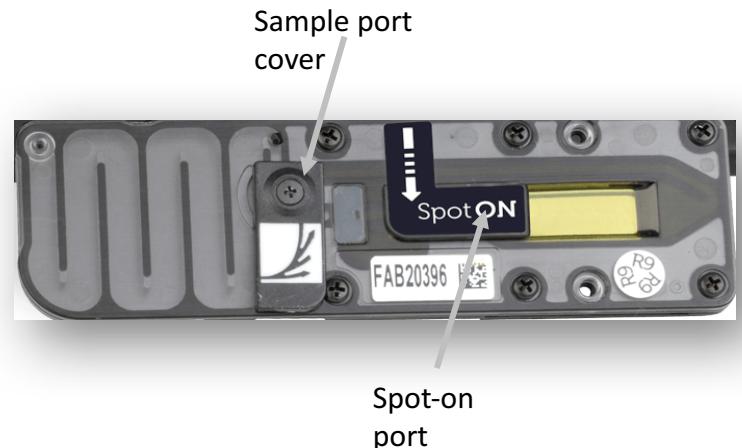
- **NO BUBBLES!!!**
- Always check for bubbles
- While loading sample port always check for air in channels in front of chip



# Sequencing workflow

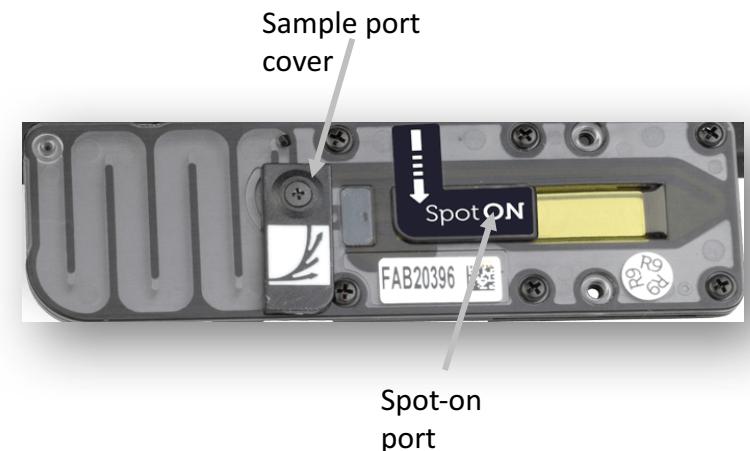
- Prepare library
- Add Flow Cell to MinION
- Quality check of Flow Cell with MinKNOW software
- Load priming Buffer into Flow cell
  - Open Sample port
  - Take a few ul from sample port to make sure there is no air in the port
  - Load buffer (NO BUBBLES)

Sample port open



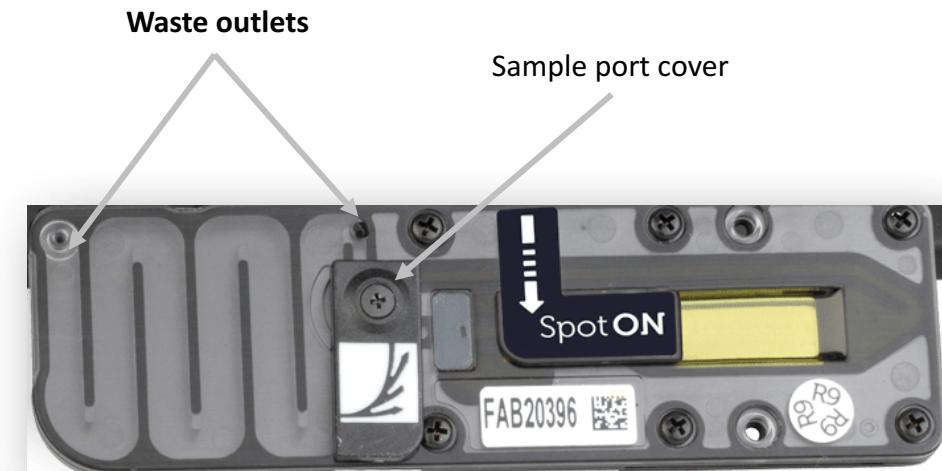
# Sequencing workflow

- Both ports open
- Prepare Library for loading
  - Add more priming buffer into sample port
  - Load library
    - Open Spot-on port cover
    - Drop library into spot-on port
  - Close Spot-on port & sample port
  - Start sequencing



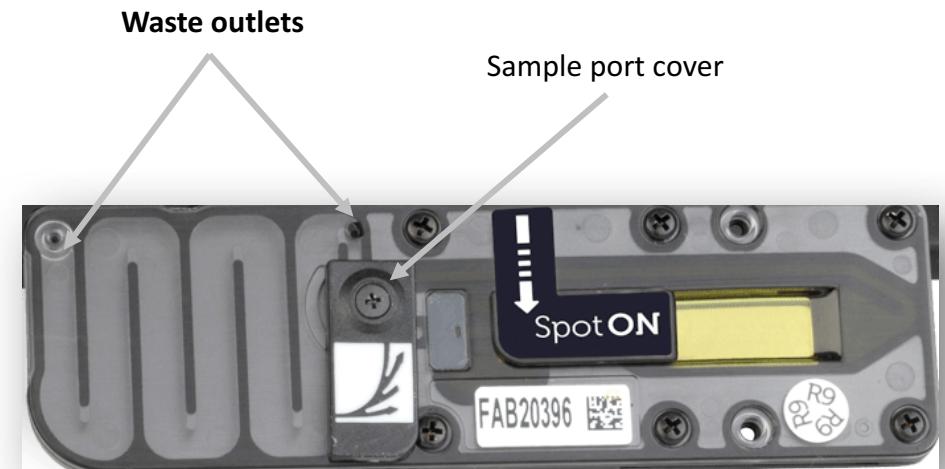
# Flow Cell washing (Storage)

- Sample port open
- 1. Stop sequencing
  - 2. Open sample port
  - 3. Add solution A in sample port
  - 4. Add storage buffer into sample port
  - 5. Close sample port
  - 6. Take old library from waste outlets



# What to keep in mind

1. No bubbles in the system!
2. Take up a few ul from sample-port to make sure there is no air in it
3. Remove all air from pipette tip
4. Close spot-on port before using sample port
5. Close all port before using waste outlets

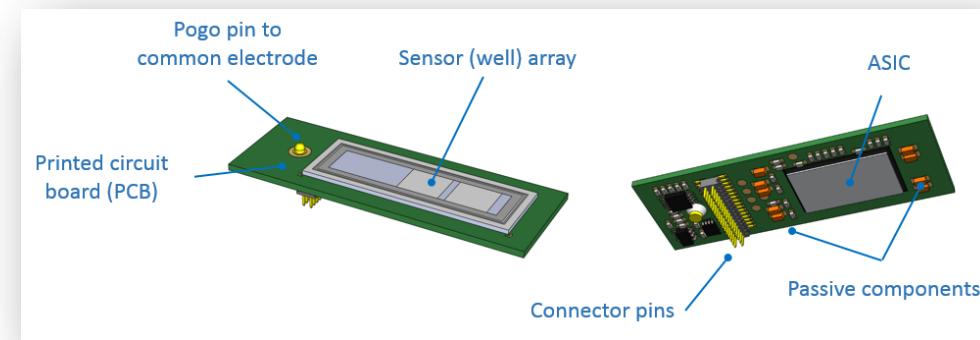


# How does it work ?

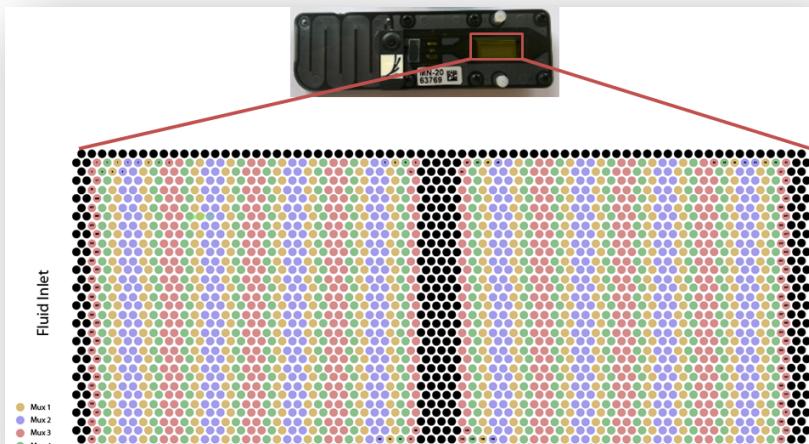
<http://nanoporetech.com>

# Flow Cell Chip

- High-density array of circuits
- Circuits measure and control current



Source: <https://community.nanoporetech.com>

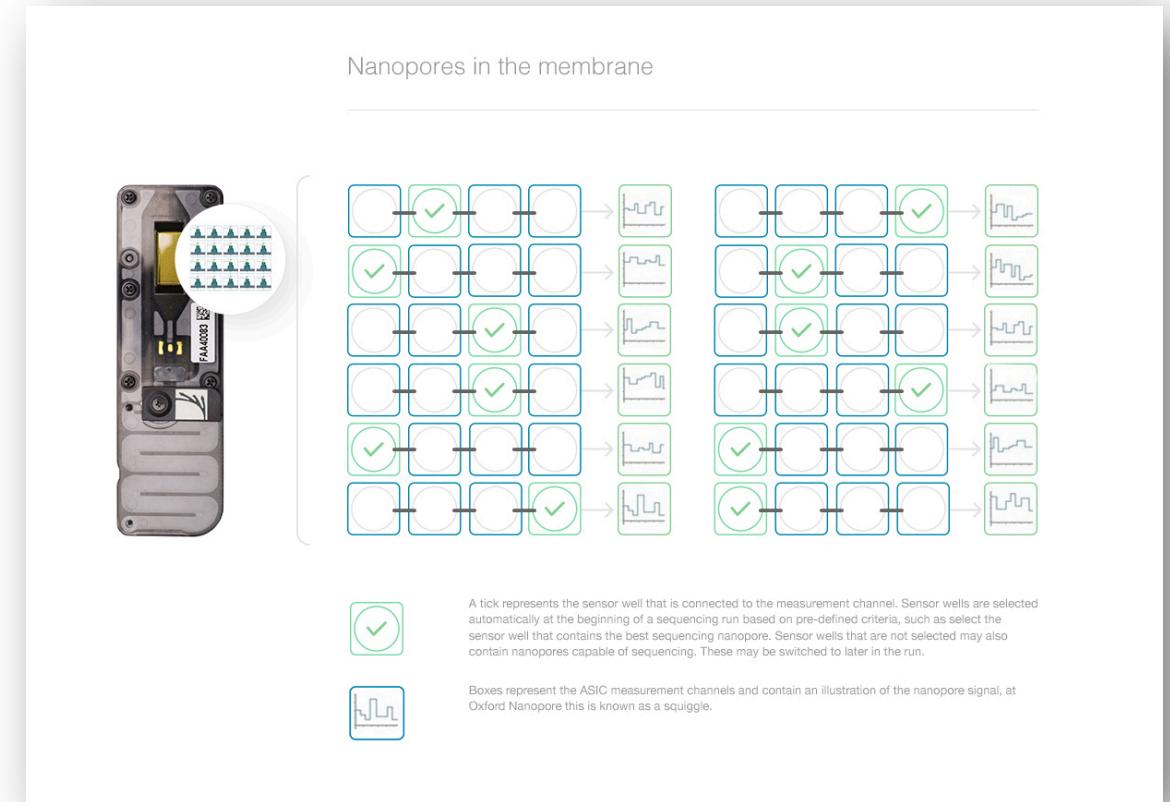


Source: <https://community.nanoporetech.com>

- 2048 (32 x 64) hexagonal wells
- 512 measurement channels, 4 times multiplexed  
=> per row always 4 wells are controlled by one channel

# Flow Cell - multiplexing

- During MUX scan the best 512 channels are chosen as group1, second best as group2 etc



# Questions?

# Sample Quality Considerations

## For Long Read Sequencing

Tonia Russell

March 2018



*“Delivering internationally competitive genomic services to the research community”*

# Ramaciotti Centre Overview

*Delivering  
internationally  
competitive  
genomic  
services to the  
research  
community*

## National Infrastructure Facility (NCRIS)



**GENOMICS**  
AUSTRALIA



**BIOPLATFORMS**  
AUSTRALIA

**15 ARC LIEF Grants**

>\$9M ARC

>\$15M Universities

**>500 research groups**  
**>1,000 individual researchers**

**Not for profit**



**Est. 1999**

**18 staff**

**Steering Committee:**  
UNSW, USyd, Macquarie,  
UTS, Newcastle  
Garvan, Kolling, VCCRI

**60% clients  
external**

**85% income  
is external**

# Team RAMAC



# Technology

## Sequencing

Illumina:

NovaSeq 6000

NextSeq 500

MiSeq

PacBio:

RSII

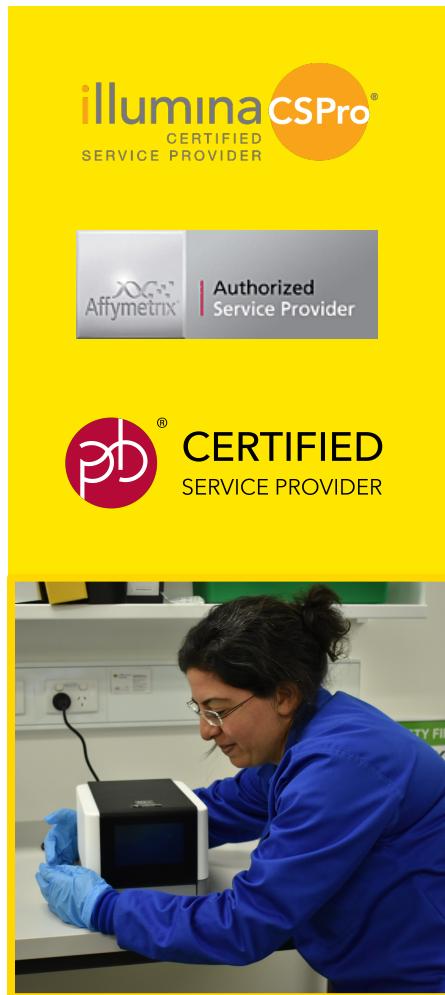
Sequel

Oxford Nanopore:

Minlon

Life Technologies:

AB3730 (Sanger)



## Gene Expression & Genotyping

Affymetrix

Agilent

Fluidigm BioMark

NanoString

## Single Cell

10X Genomics Chromium

Fluidigm C1 single-cell

# Research Supported

## Nationally Significant Projects:

- Melanoma genome
- Antibiotic resistance
- Stem cell characterisation
- Koala reference genome
- Oz mammals genomes
- Marine microbial diversity
- Great Barrier Reef
- Soil biodiversity
- Wheat pathogenomics
- Wine yeast biology





Ramaciotti Centre  
for Genomics

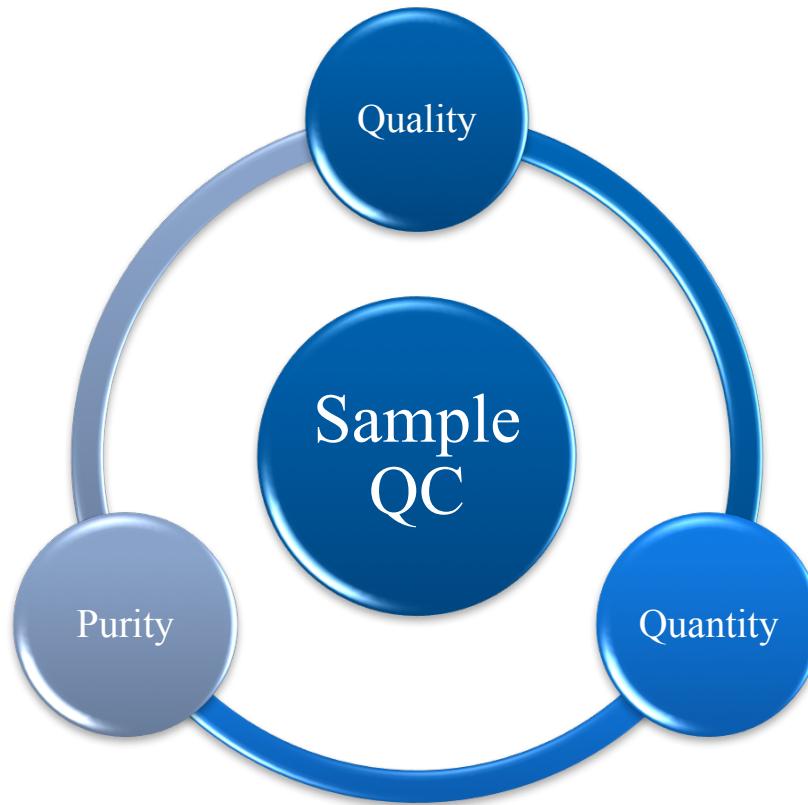
# The Importance of Sample Quality





Ramaciotti Centre  
for Genomics

# Three Aspects to Assessing Quality





# Genomic Sample Quality

It is essential that DNA:

- ❶ Is double stranded.
- ❷ Has not been exposed to high temperatures (e.g.: > 65° C for 1 hour) or pH extremes (< 6 or > 9).
- ❸ Has not been exposed to UV or intercalating fluorescent dyes.
- ❹ OD260/280 ratio between ~1.8 and a OD260/230 ratio between 2.0-2.2.
- ❺ Does not contain insoluble material or RNA contamination
- ❻ Does not contain divalent metal cations, denaturants (e.g. guanidinium salts or phenol) or detergents (e.g. EDTA).
- ❼ Does not contain carryover contamination from the original organism/tissue (e.g. polysaccharides.)



# RNA Sample Quality

It is essential that RNA:

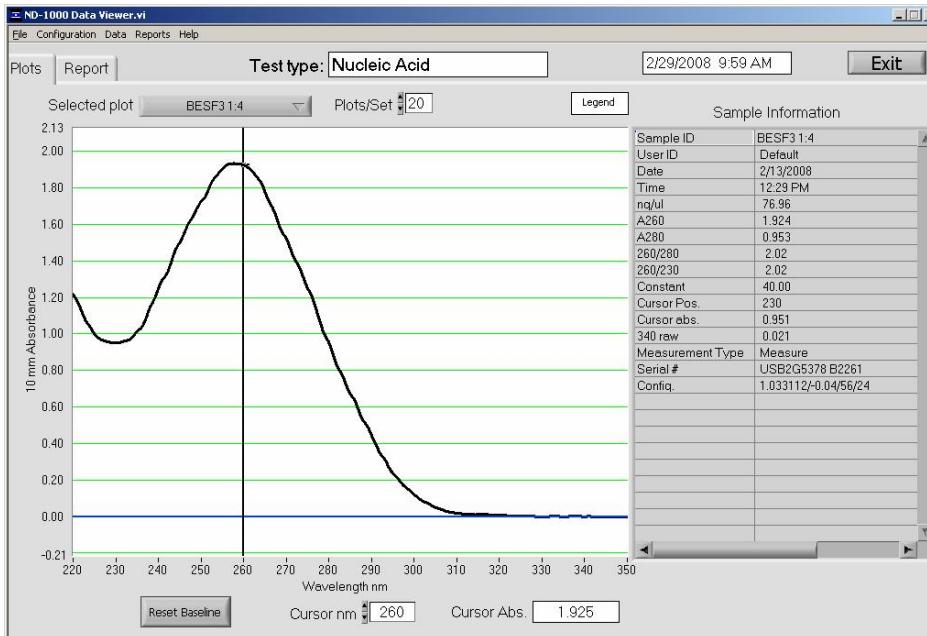
- ❶ Has not undergone multiple freeze-thaw cycles
- ❷ Has not been exposed to high temperatures (e.g.: > 65° C for 1 hour) or pH extremes (< 6 or > 9).
- ❸ OD260/280 ratio between 2.0-2.2 and a OD260/230 ratio between 1.8-2.2.
- ❹ Does not contain insoluble material.
- ❺ Does not contain DNA contamination.
- ❻ Does not contain denaturants (e.g. guanidinium salts or phenol) or detergents (e.g. SDS or Triton-X100).
- ❼ Does not contain carryover contamination from the original organism/tissue (e.g. heme, humic acid, polyphenols etc.)



Ramaciotti Centre  
for Genomics

# Quantification/Purity: Nanodrop

NanoDrop ND-1000



## Advantages

- Assess contamination
- No cuvette required
- Needs only 1-2ul of sample
- Broad spectral output
- DNA & RNA quantification

## Disadvantages

- Only reliable >30ng/μl
- Quantification errors



Ramaciotti Centre  
for Genomics

# The Good!

## Optimal Ratios

### DNA

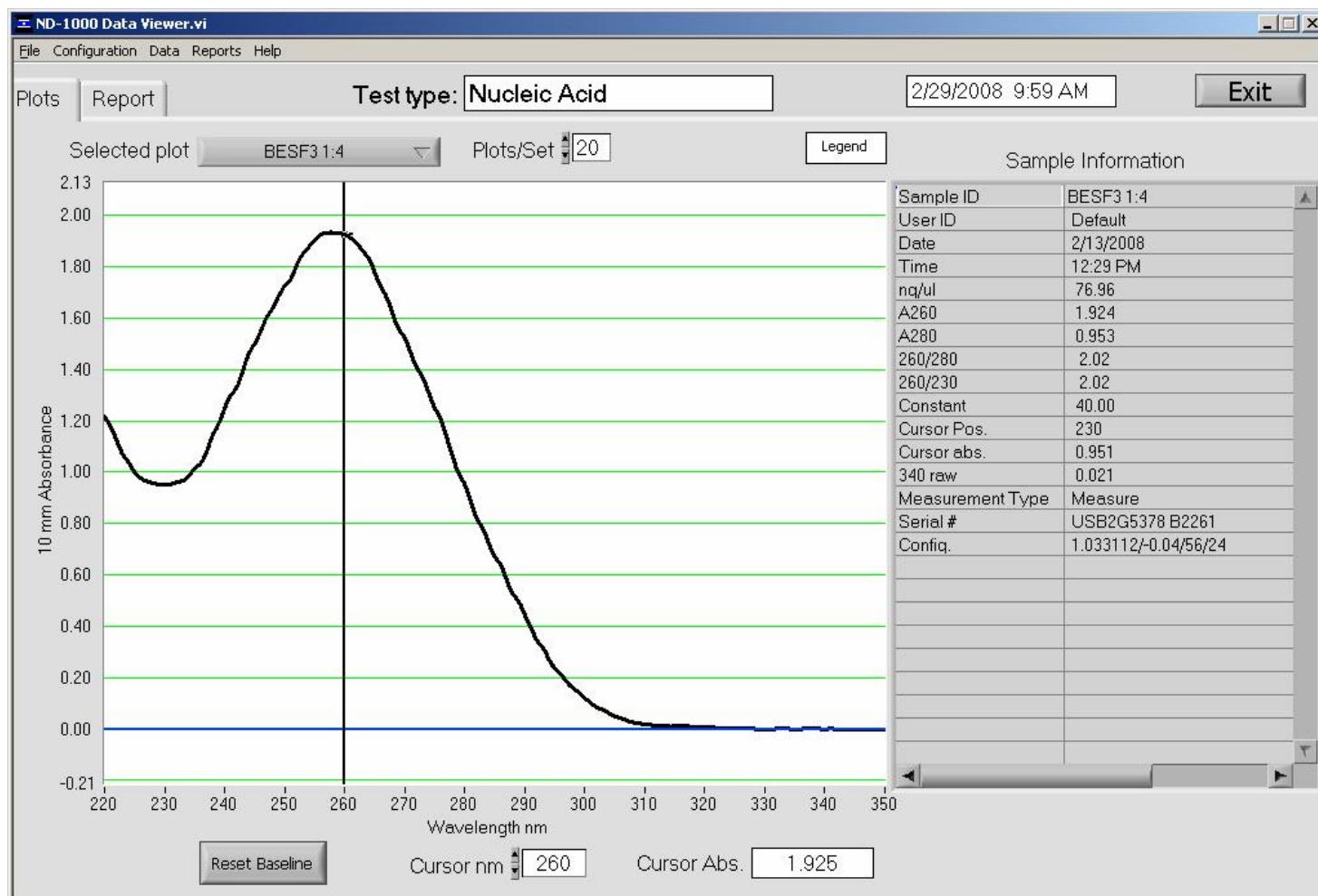
260/280 ~1.8- 2.0

260/230 ~ 2.0-2.2

### RNA

260/280 ~2.0

260/230 ~ 2.0-2.2





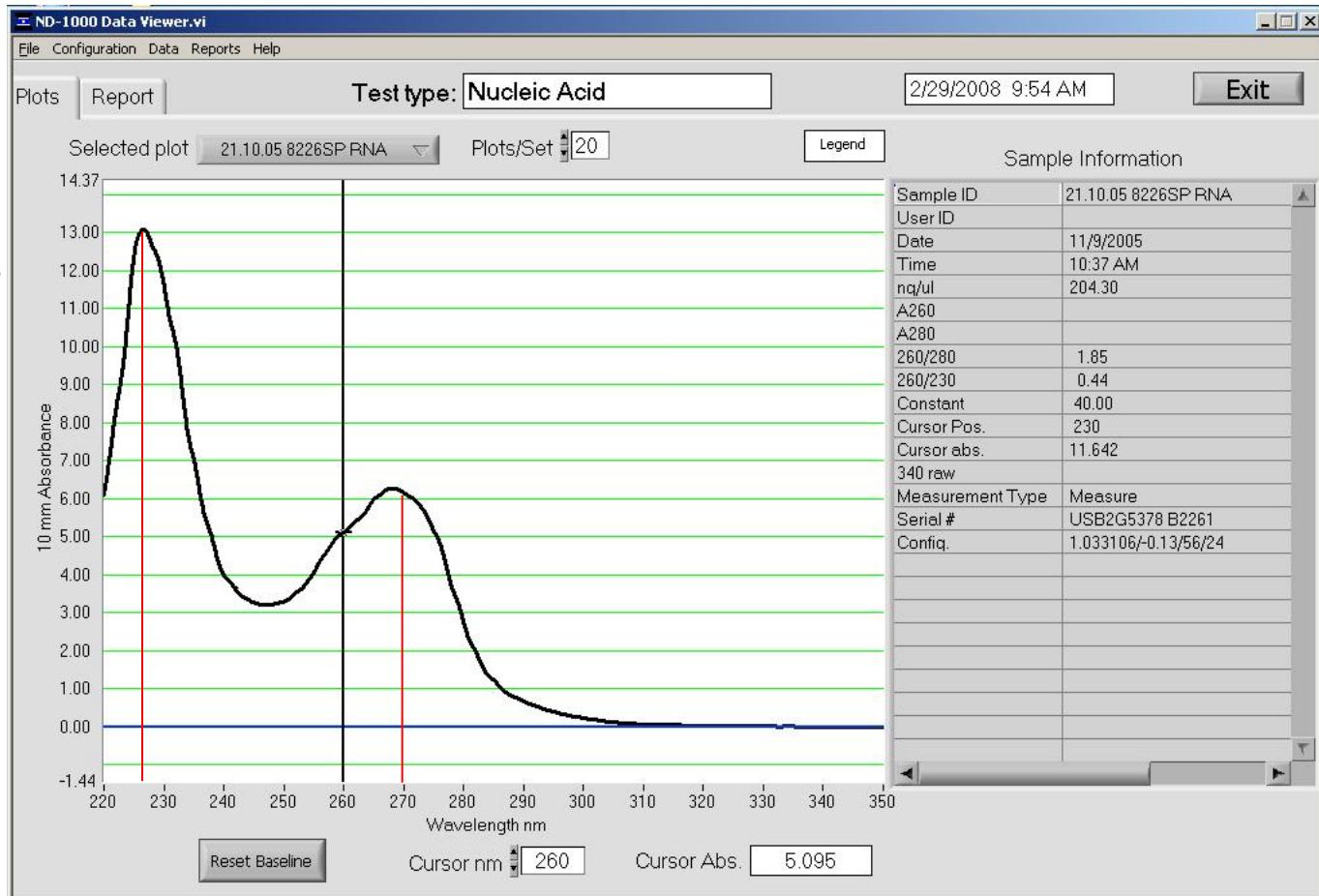
Ramaciotti Centre  
for Genomics

# The Ugly!

Low 260/230 ratios;  
Presence of organic compounds

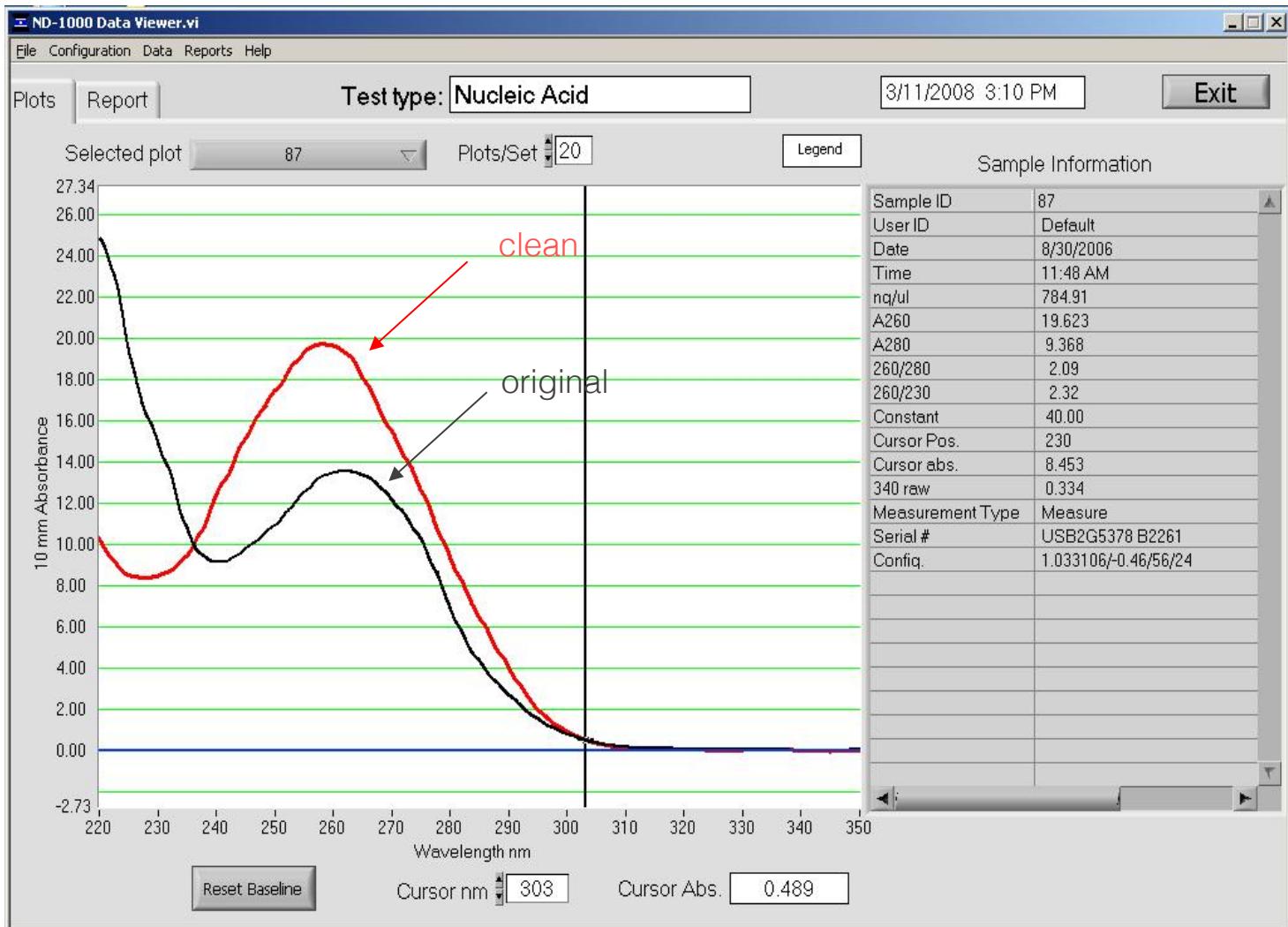
- Phenol
- Trizol
- Chaotropic salts

Low 260/280 ratios;  
-Protein  
-Guanidine  
-Phenol





# Sample Clean up





Ramaciotti Centre  
for Genomics

# Quantification/Purity:Trinean Xpose



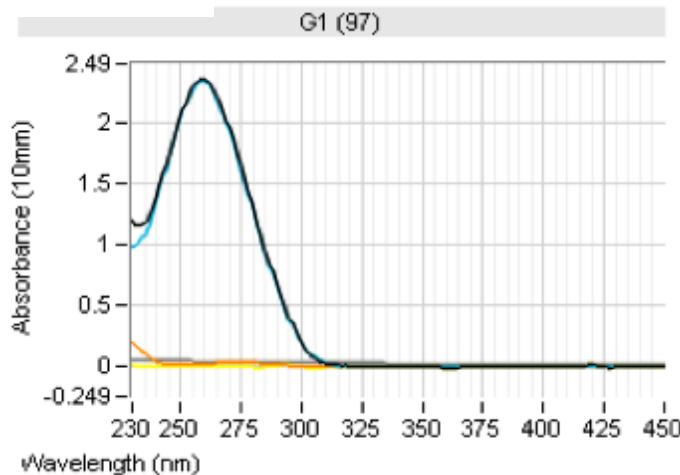
## Advantages

- Reads 16 samples in 1 minute
- Broad spectral output
- Spectral content profiling
- Specific quantification of DNA, RNA and protein fraction
- Subtraction of other impurities

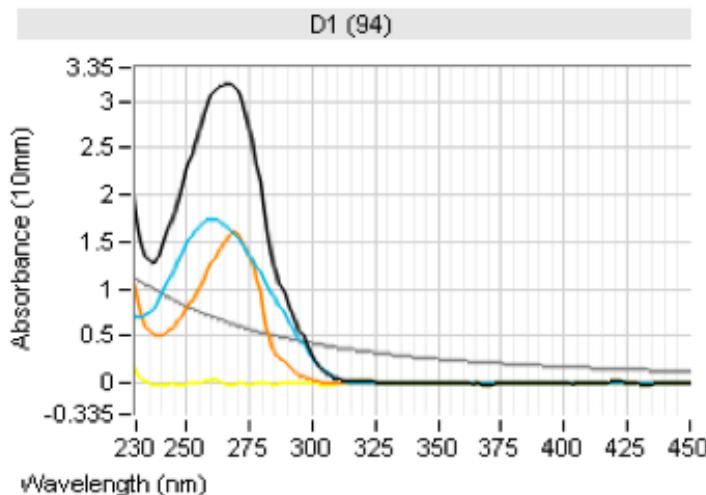


Ramaciotti Centre  
for Genomics

# Quantification/Purity: Trinean Xpose



RNA (ng/ul)      Nucleic acids (ng/ul)      A260/A280      A260/A230  
93.6                  93.6                  1.82                  1.98



RNA (ng/ul)      Nucleic acids (ng/ul)      A260/A280      A260/A230  
69.5                  94.7                  1.56                  1.74



Ramaciotti Centre  
for Genomics

# Quantification – Fluorescence



## Methods for Quantifying Nucleic Acid

- Qubit assays for RNA and DNA
- RiboGreen for RNA
- PicoGreen for DNA



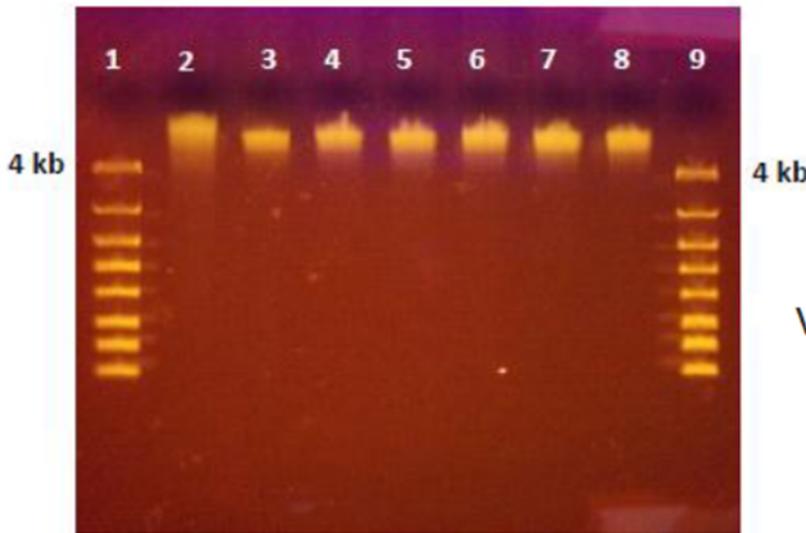


Ramaciotti Centre  
for Genomics

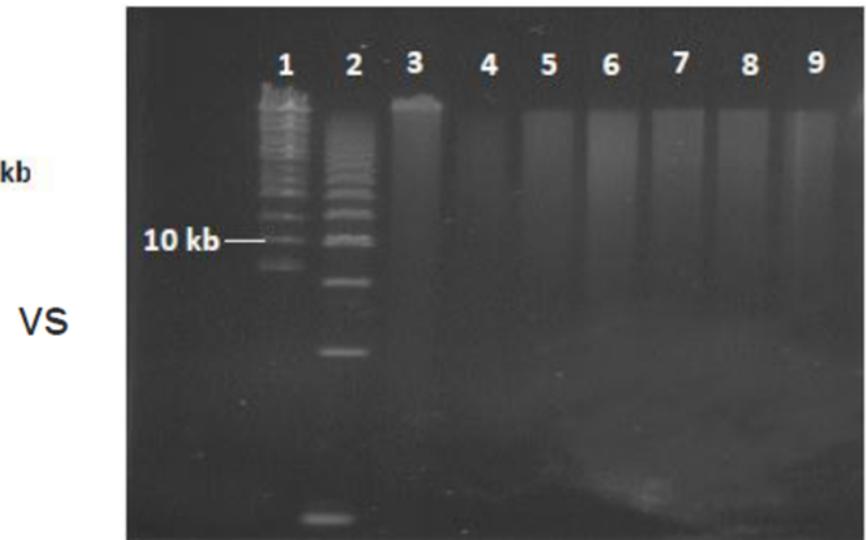
# DNA Quality/Integrity

## Is my agarose gel lying to me?

- Low percentage agarose gel can be misleading.
- Use Field Inversion or Pulse Field gel electrophoresis for accurate assessment of DNA integrity.



1.2% Lonza Gel



FIGE



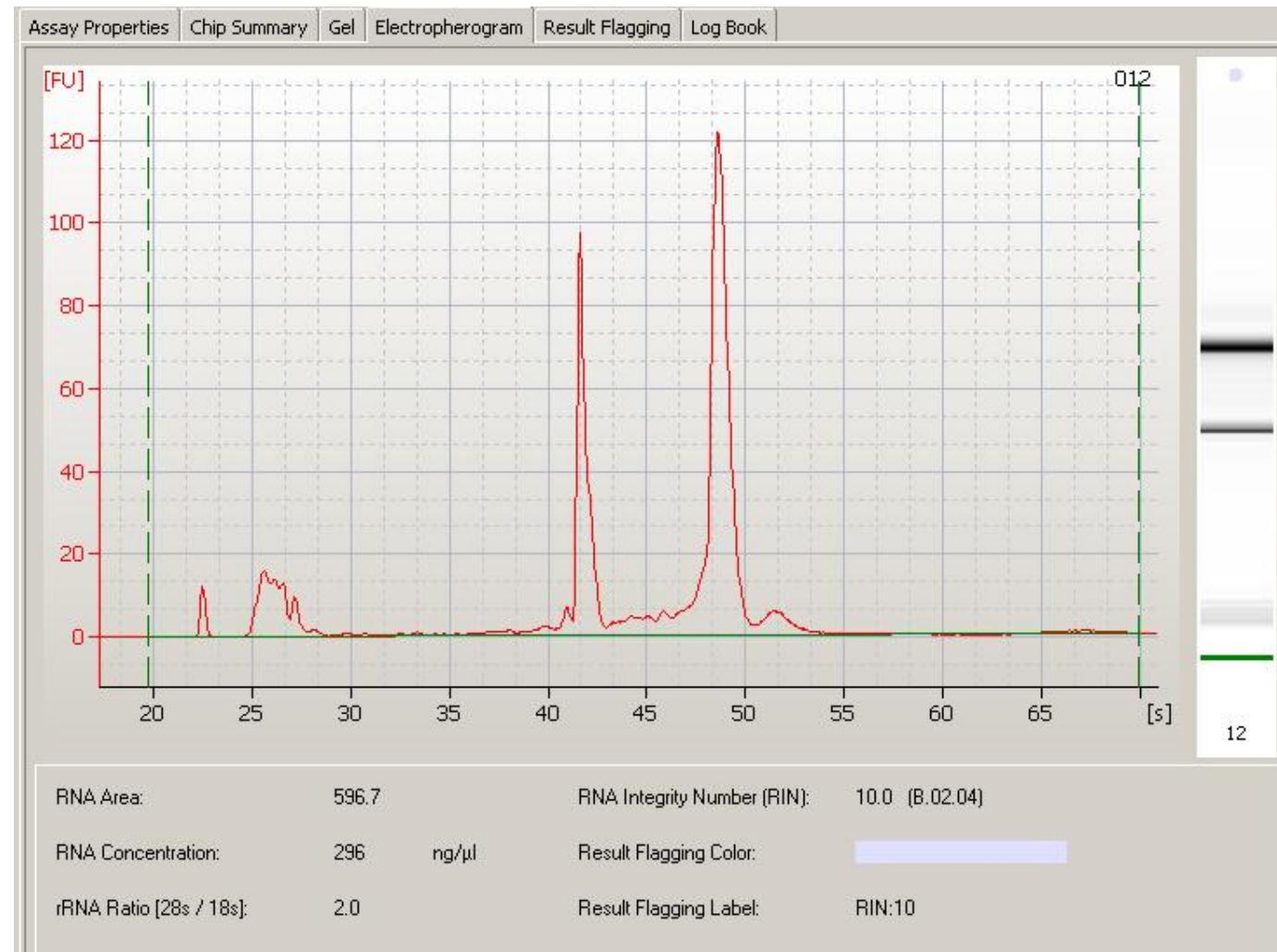
Ramaciotti Centre  
for Genomics

# RNA Quality/Integrity- Bioanalyzer

Intact RNA

RNA Integrity

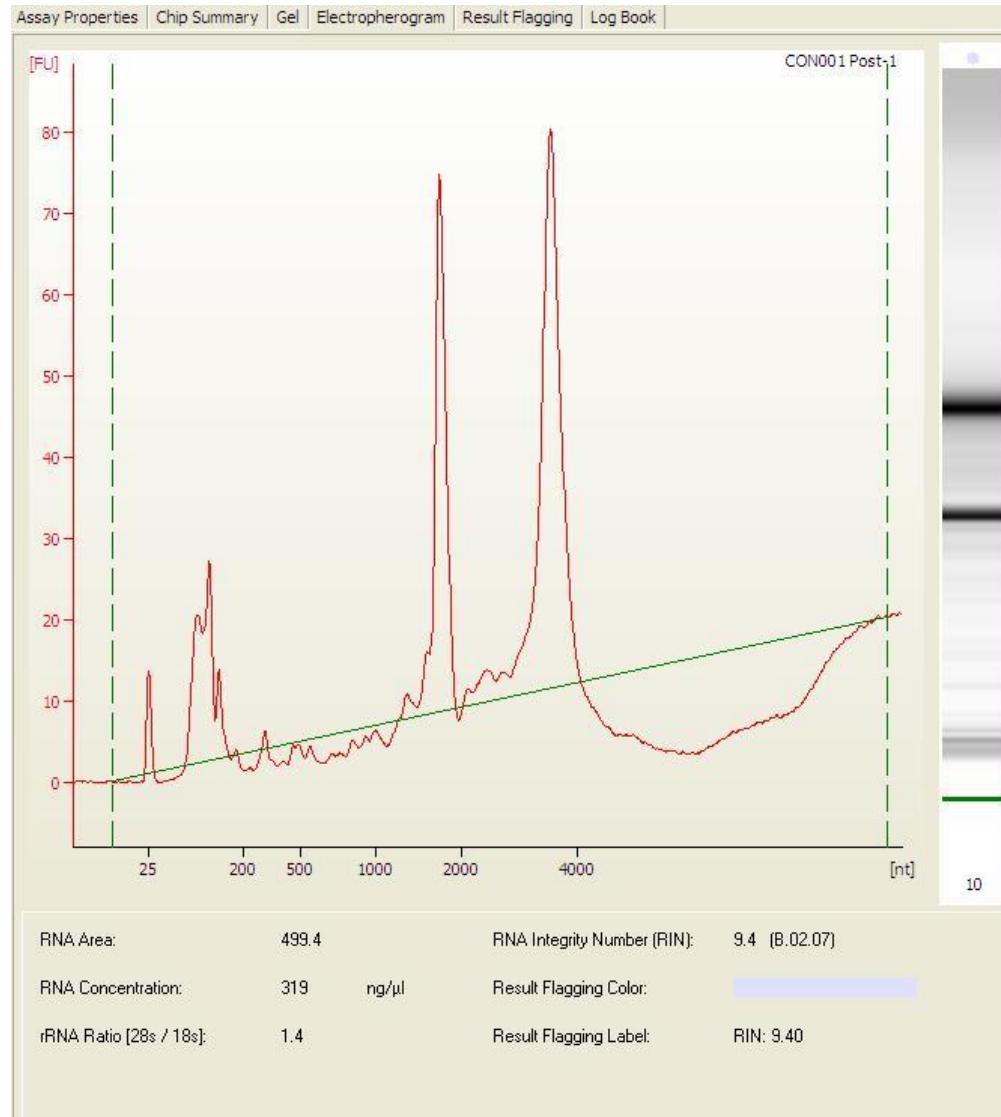
Number (RIN): 9-10





Ramaciotti Centre  
for Genomics

# DNA contamination of RNA





# How to obtain Quality DNA/RNA

- ❶ Species dependent. May need multiple extraction methods.
- ❷ Do your research and be prepared for some trial and error.
- ❸ Some commercial extraction/clean up kit columns will shear DNA below 50kb.
- ❹ <https://www.protocols.io/> (Ben Schwessinger)
- ❺ Number of high molecular weight extraction kits on the market.
- ❻ Many Phenol/Chloroform protocols for long read sequencing on the internet.

# DNA Extraction Methods

- ⌚ Standard Spin Column Kits: 20-50kb
  - ⌚ QIAgen DNeasy
- ⌚ Commercial high molecular weight kits: Up to ~150kb
  - ⌚ QIAgen MagAttract HMW
  - ⌚ QIAgen Genomic tip
- ⌚ Phenol/Chloroform methods: Up to ~200kb
  - ⌚ Must ensure DNA contains no remaining contaminates from extraction procedure.
- ⌚ Agarose plug: Up to ~1000kb
  - ⌚ Tricky procedure.



Ramaciotti Centre  
for Genomics

# Tips for Handling DNA/RNA



- ❶ Avoid too many freeze thaw cycles.
- ❷ Avoid vortexing: Flick mix or slow pipetting with wide bore tips.
- ❸ Avoid excessive heat exposure.
- ❹ Do not use UV/Ethidium Bromide for gel visualisation if you need to gel purify your DNA/RNA.
- ❺ Resuspend in a neutral buffer such as Tris HCL. Do not use EDTA.
- ❻ RNA: Maintain RNase free work conditions.

# Thank You!



# Introduction to the MinKNOW software



Tim Kahlke  
[tim.kahlke@uts.edu.au](mailto:tim.kahlke@uts.edu.au)  
<https://github.com/timkahlke>  
Twitter: @AdvancedTwigTec

# MinKNOW

Main Interface to your MinION sequencer to

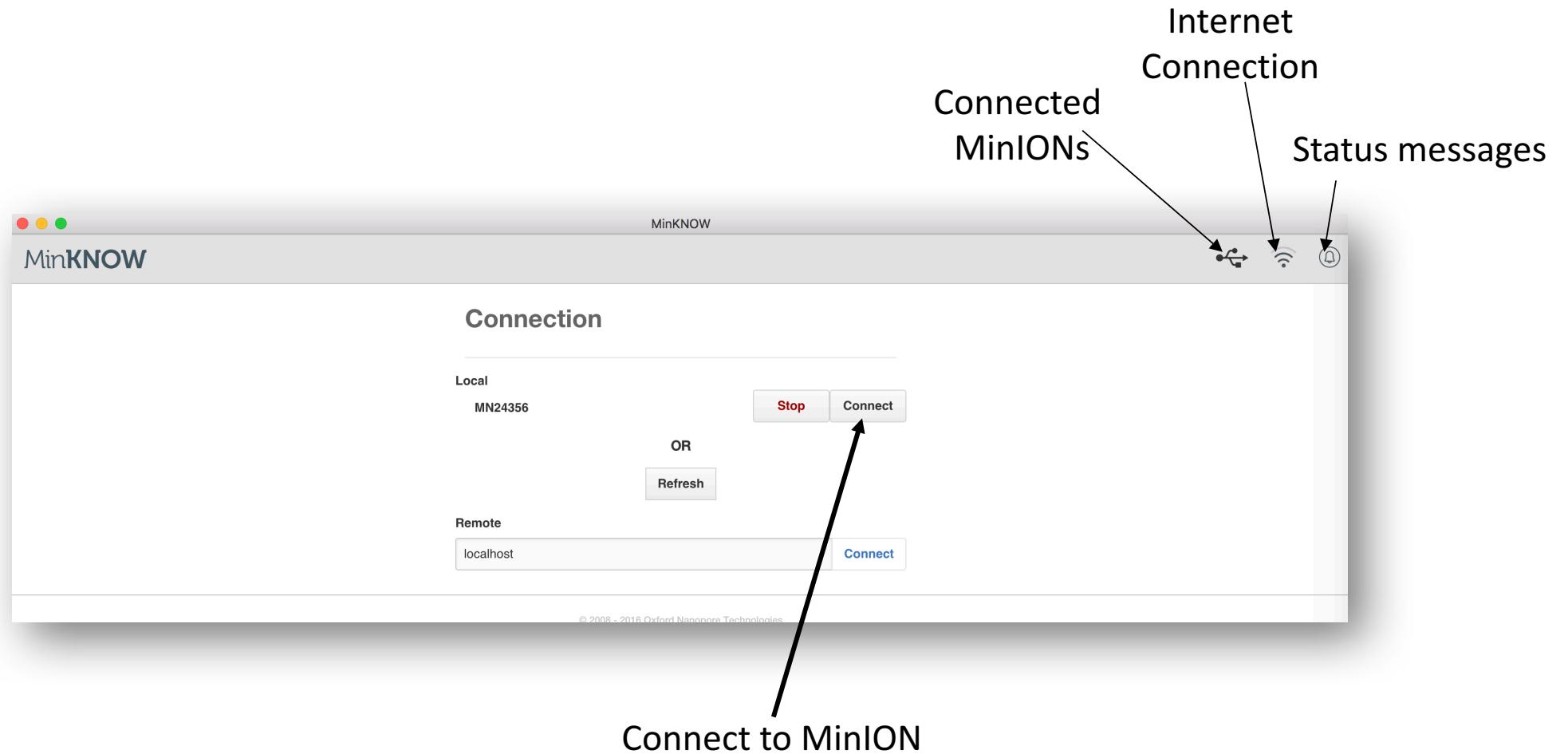
- Conduct flow Cell QC
- Configure and monitor the sequencing run
- Perform base calling
- Store the sequencing data

# MinKNOW

- Free software
- Available only through the Oxford Nanopore Community
- Supports Windows, Mac and Linux
- Regularly updated

# MinKNOW GUI

# Main Menu



# Main Menu

The screenshot shows the MinKNOW software interface. On the left, five sections are labeled with blue lines pointing to specific areas on the right:

- General Information**: Points to the "Connection" section, which includes fields for Local (MN24356) and Remote (localhost) connections, and Flow Cell Connection (3306102685 and 1774103).
- Sample / Experiment identifier**: Points to the "Label Experiment" section, which includes fields for Sample ID (with validation message: "Must only contain letters, numbers, white spaces, '-' and be between 1 and 50 characters long") and Flow Cell ID.
- Flow Cell ID**: Points to the "Disk Space" section, which shows available disk space (16777220 (236.478Mb free)).
- Available memory**: Points to the "Choose Operation" section, which includes radio buttons for Configuration Test Cell (CTC), Platform Quality Control (QC), Sequencing Run, and Control Experiment. The "Platform Quality Control (QC)" option is selected. It also shows the flow cell product code (FLO-MIN106) and a script choice (INC\_Platform\_QC\_FLO-MIN106).
- MinION Operation**: Points to the "Execute" button in the "Choose Operation" section.

# MinION Operation

1. Configure Minion using the Test Flow cell that comes with the MinION
2. Check your Flow Cell, i.e., how many pores are active
3. List of sequencing scripts for actual sequencing
4. Specific parameters for provided control experiment

**Choose Operation**

---

Configuration Test Cell (CTC)  Platform Quality Control (QC)  Sequencing Run  Control Experiment  expert

**Flow cell product code**

FLO-MIN106  FLO-MIN107

There is 1 script choice:

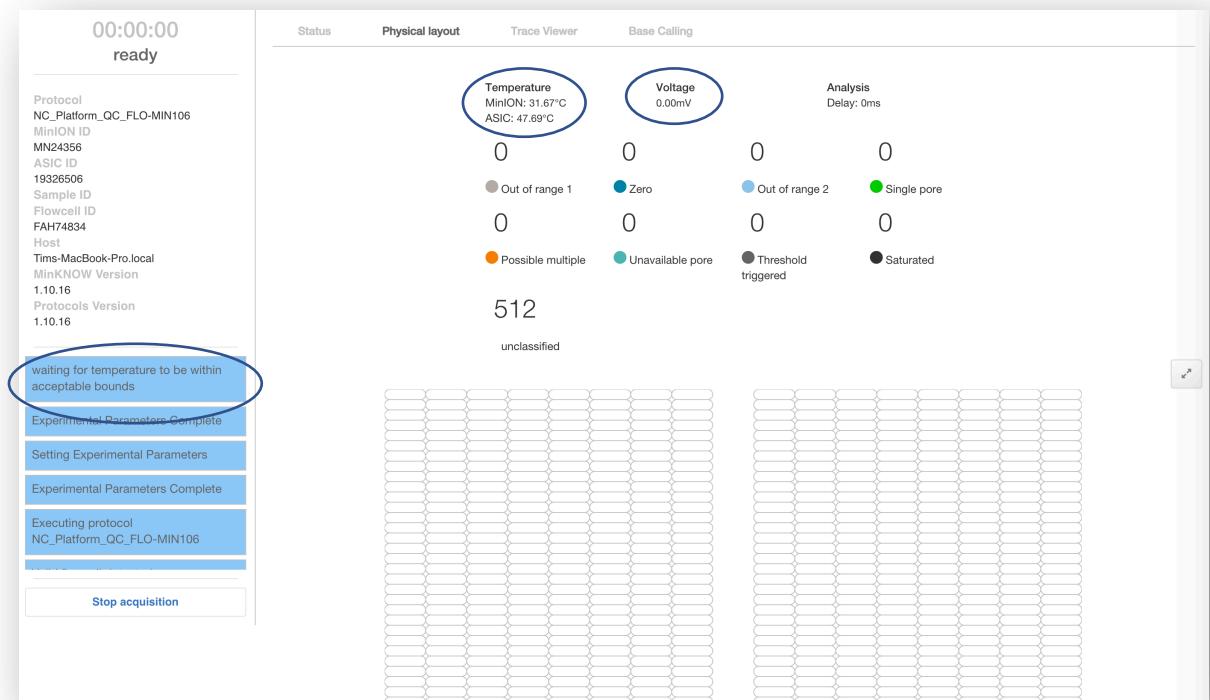
NC\_Platform\_QC\_FLO-MIN106 ▼

**Execute**

# MinKNOW GUI – Flow Cell QC

# Flow Cell QC

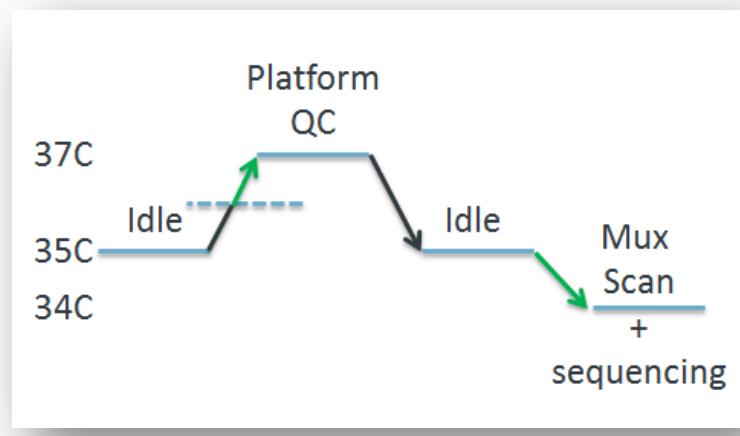
- Should be performed before every sequencing run
- Temperature has to be reached
- A series of current changes form positive (reversed) to negative -180mA to maximise number of free active pores



# Flow Cell QC run

## Temperature

- Higher temperature increase mobility of charged particles



## Voltage

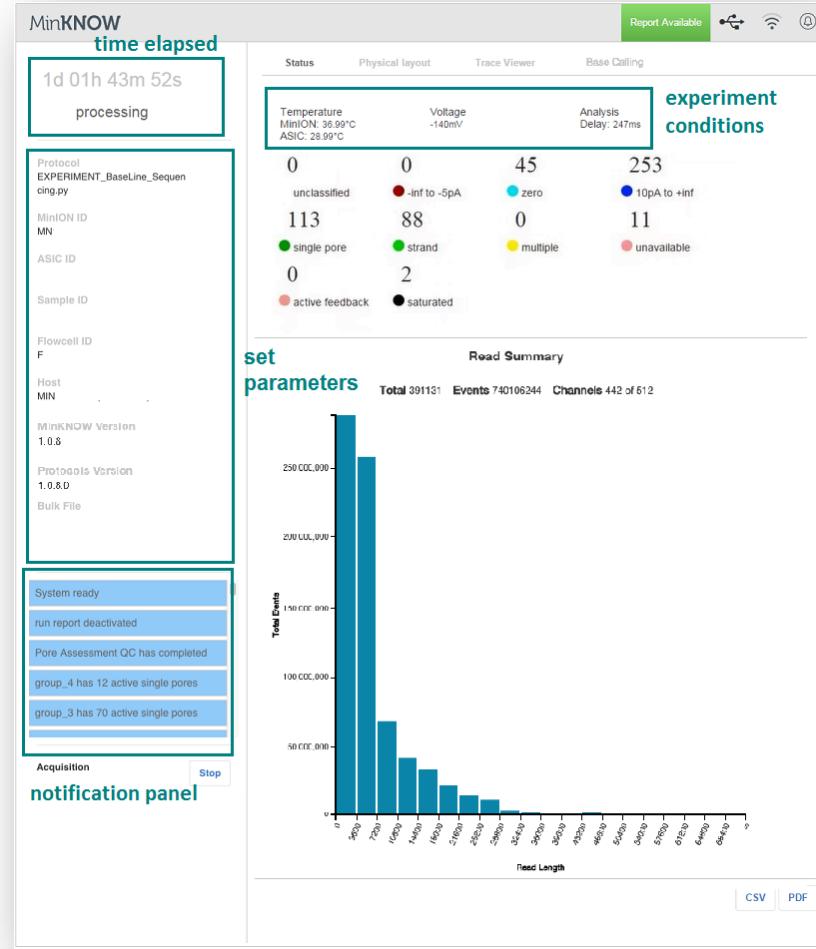
- Positive / reverse voltage to clear pores
- Over time current is decreased in 5mV steps to account for loss of potential (drift)



# MinNOW GUI – Sequencing Run

# MinION – Status Widget

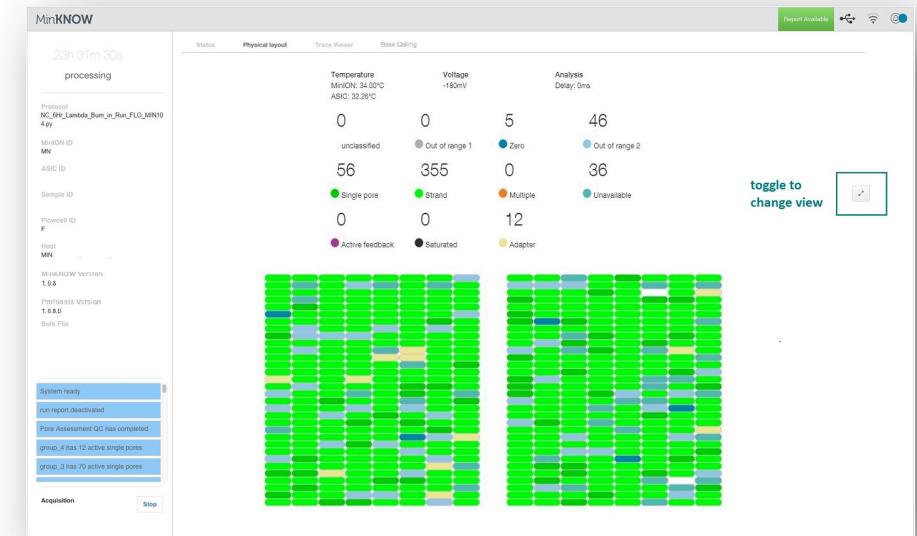
- Sequencing yield overview
- Events ± nucleotides
  - Per event ~1.7 (R9 Flow cells)
  - nucleotides pass through one pore



Source: <https://community.nanoporetech.com>

# MinION – Physical Layout Widget

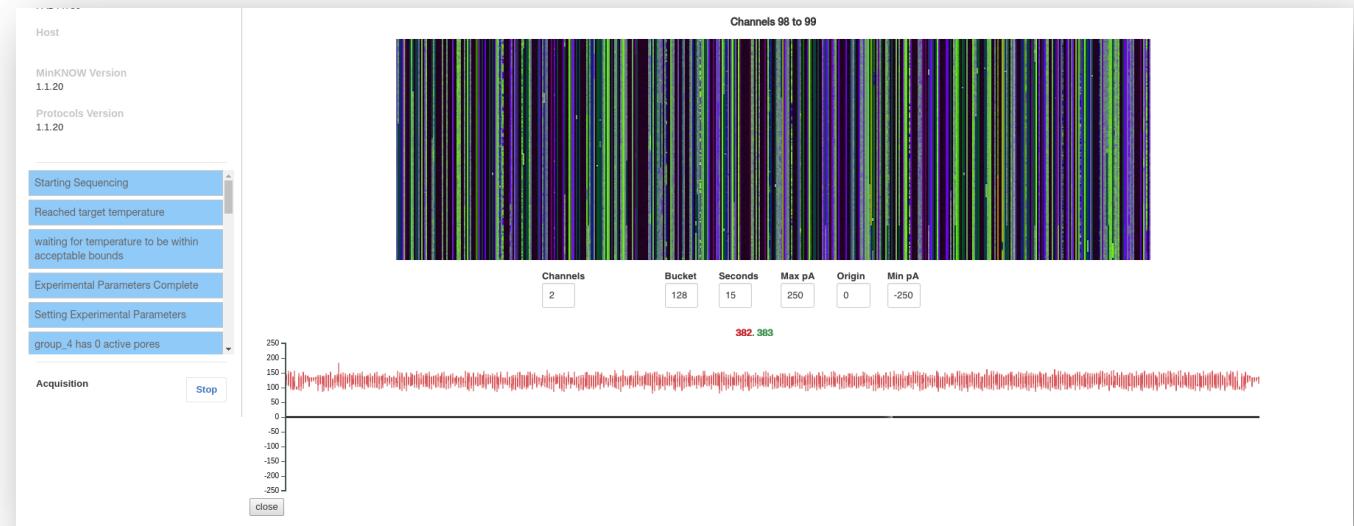
Single pore	Open but empty pore
Strand	DNA translocating through pore, i.e., sequencing
Adapter	Pore sequencing unligated adapter oligo
Multiple	The current levels indicate that more than one pore is active
Active feedback	The current is flicked, e.g., to remove a stalled strand
Saturated	Current outside detector range and channel is switched off and won't be used for sequencing anymore



Source: <https://community.nanoporetech.com>

# MinION – Trace Viewer Widget

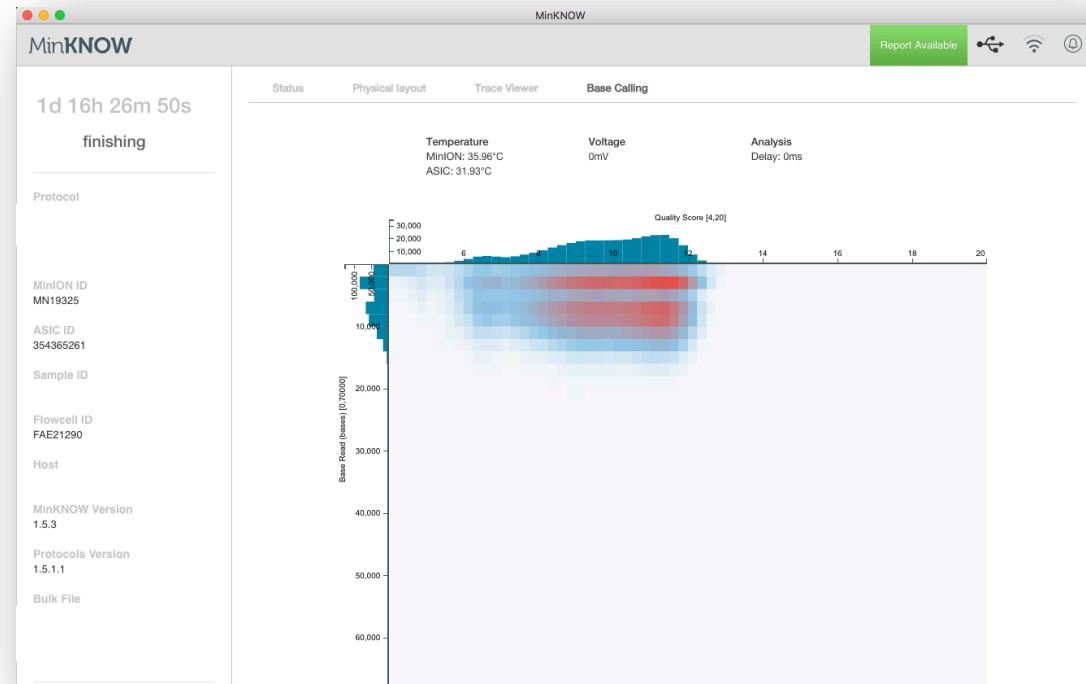
- Visualisation of channels
- Each channel is represented by one pixel in waterfall plot



Source: <https://community.nanoporetech.com>

# MinION – Base Calling Widget

- Overview over local base calling
- Plot showing quality score of base-called reads in relation to read length



Source: <https://community.nanoporetech.com>

# Questions?