

# Nanopore sequencing

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# Introduction to nanopore

- MinION is the size of a:
  - USB stick
  - “around the same length as an iPhone” [1]
  - “no larger than a typical smartphone” [2]
  - Fits in your pocket [3]
  - “10 cm × 2 cm × 3.3 cm; approximately 90 g” [4]
  - Office stapler [5]
  - Ruler
    - A 4” ruler [6]
  - Chocolate bar [7]



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+ Follow

Size comparison of one of the @nanopore MinIONs and a banana. Actually, search youtube for 'minion and banana'. :-)



[1] Loman et al. Bioinformatics 2014, [2] Quick et al. GigaScience 2014, [3] Urban et al. bioRxiv 2015, [4] Wang et al. Front. Microbiol 2015 [5] Risse, Watson et al. bioRxiv 2015, [6] Unpublished, probably forever, [7] Manuscript under preparation

# Priming

- Nanopore sequencing starts when:
  - Fuel is added to flow cell
    - ATP makes ratcheting enzyme work
    - More ATP – faster speed, more data
    - Currently 35bp/s, due to change to 70bp/s soon
    - Potentially 500 bp/s or higher?
  - Electrical potential applied
    - Movement of ions
    - “Ionic battery”
    - Controlled by Python scripts
      - Bias voltage changes over time but quality drops off

# QC

- MinION Mk I flow cell is actually
  - 2000 wells, theoretically each containing 1 pore
  - But Poisson loading means some wells >1 pore (unusable – “multiple”) or 0 pores (“None”)
  - 1 sensor for each 4 well cluster – can address one
  - Each sensor is a miniature axopatch
  - Pores divided into groups
  - “Remuxing” after length of time
    - Again controlled by Python

# Interrogating MinKNOW

- Read length histogram
  - Counts ‘events’ – events  $\sim$  1base (but in practice  $<1$  due to skips)
  - If read is 2D then 2x events- don’t get too excited
- Pore view

# Poretools

# Progress monitoring

# Metrichor



# Real-time analysis

What's in my pot?

# Extract FASTA files

# Hybrid assembly with SPAdes

Compare with Illumina only assembly

# Nanopore-only assembly

- Is possible!
  - Nanocorrect
  - Celera
  - Nanopolish
  - Resulting assembly is 99.5% accurate at 30x – mainly homopolymer errors
  - Rather time consuming at present

QUAST again

# Inspecting alignments with BWA and IGV



Do it again