Portable DNA Sequencing: Analysis Methods and Applications

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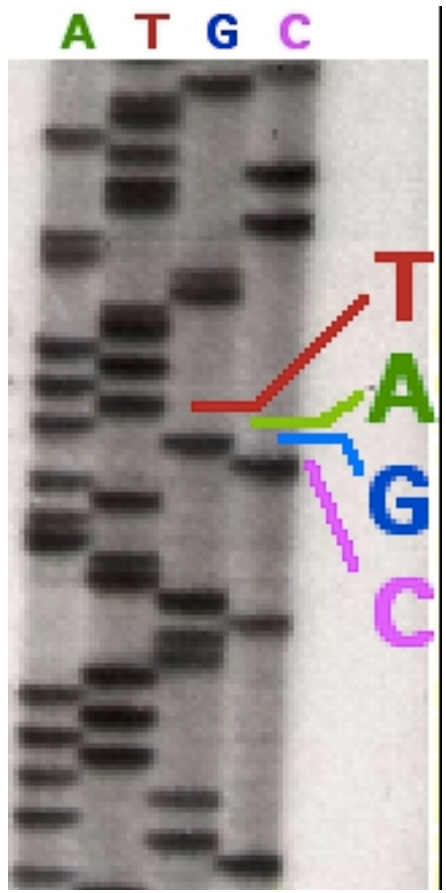
Timeline of DNA Sequencing

1970s

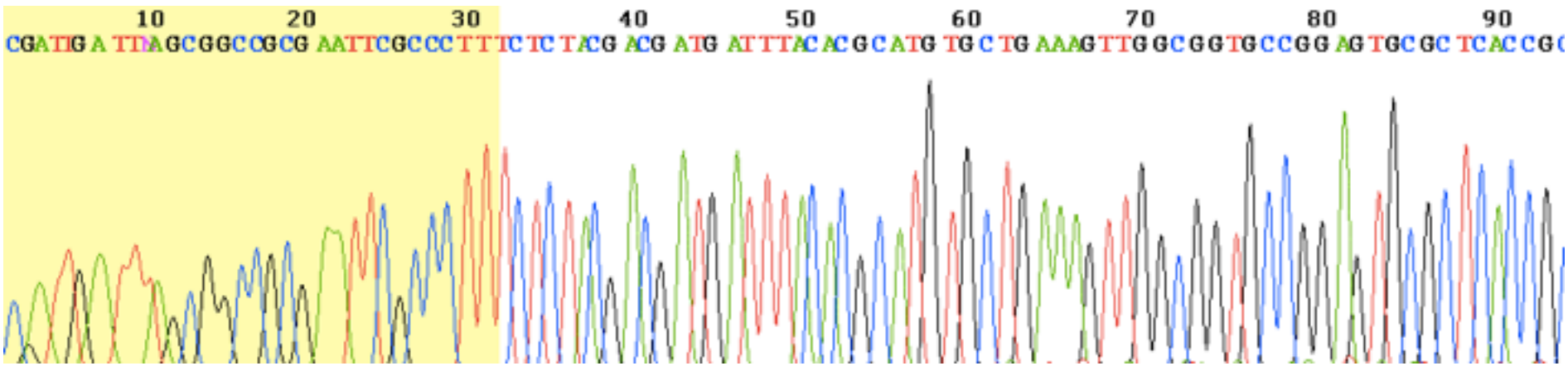
Sanger sequencing invented

Manual process; low throughput



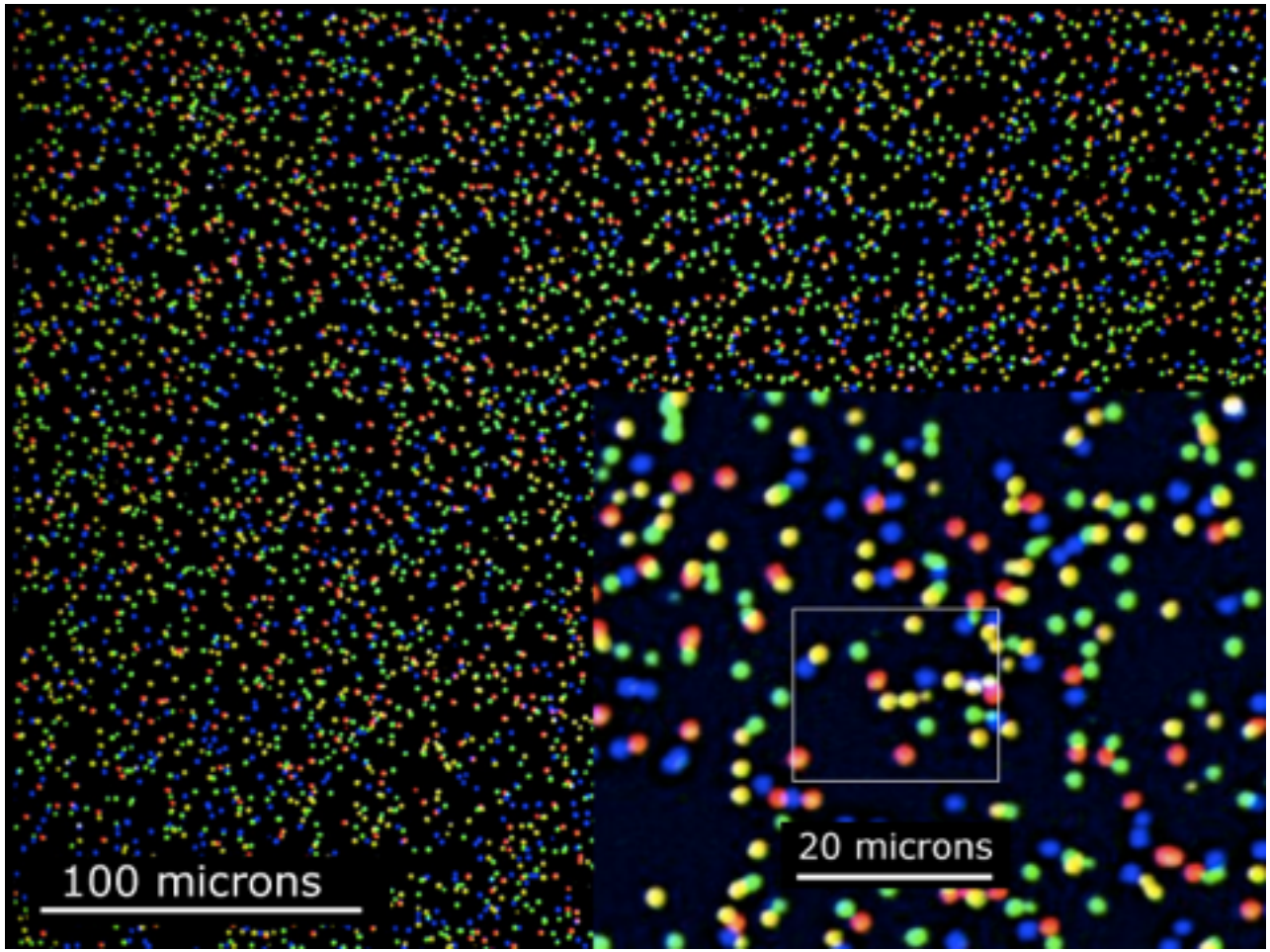
Timeline of DNA Sequencing

1980-1990s
Automation of Sanger sequencing
10-100 Megabases of data per run



Timeline of DNA Sequencing

2000s
Massively Parallel Sequencing
10-100s Gigabases per run



Timeline of DNA Sequencing

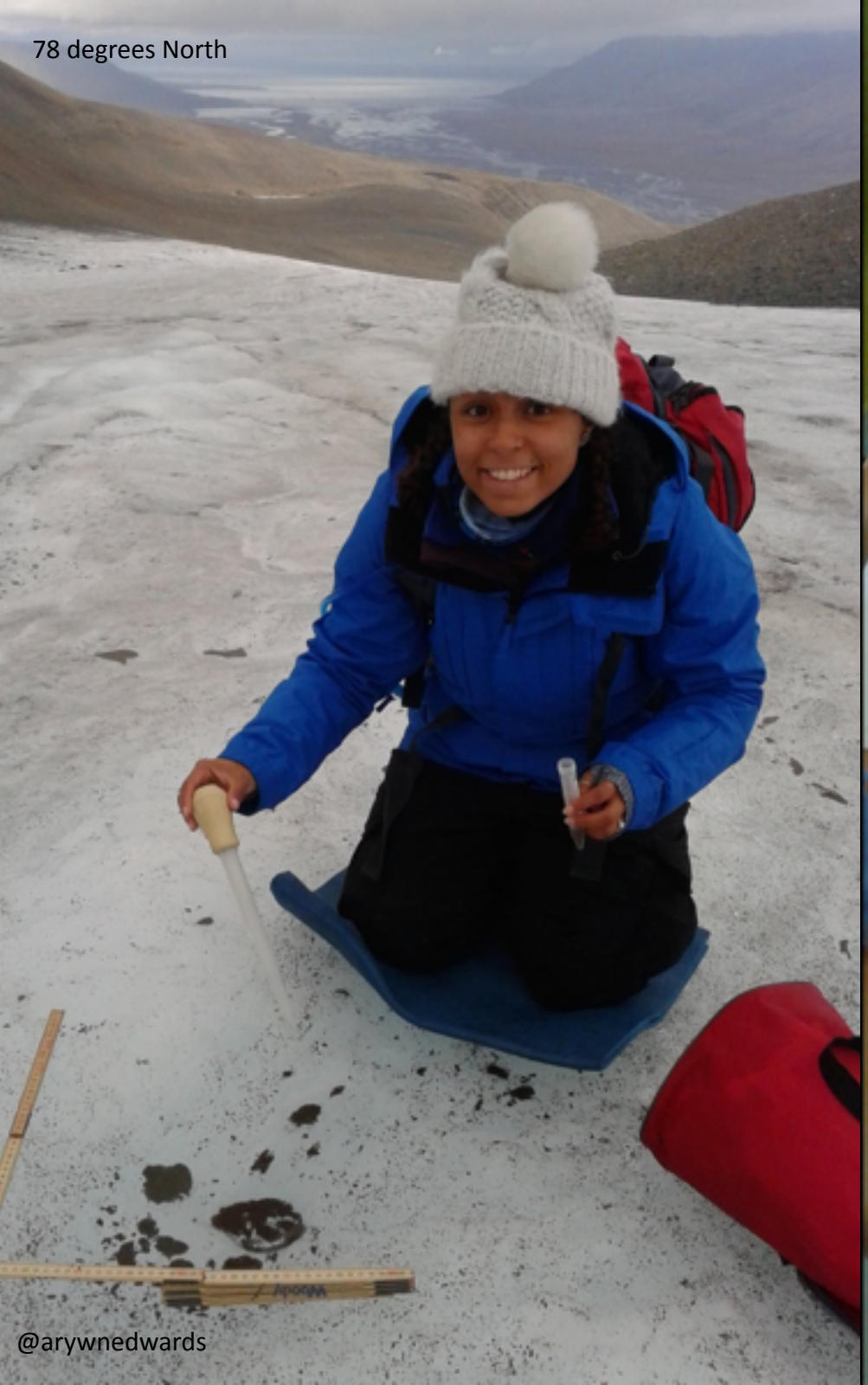
2010s

Centralisation of sequencing

18,000 human genomes/year

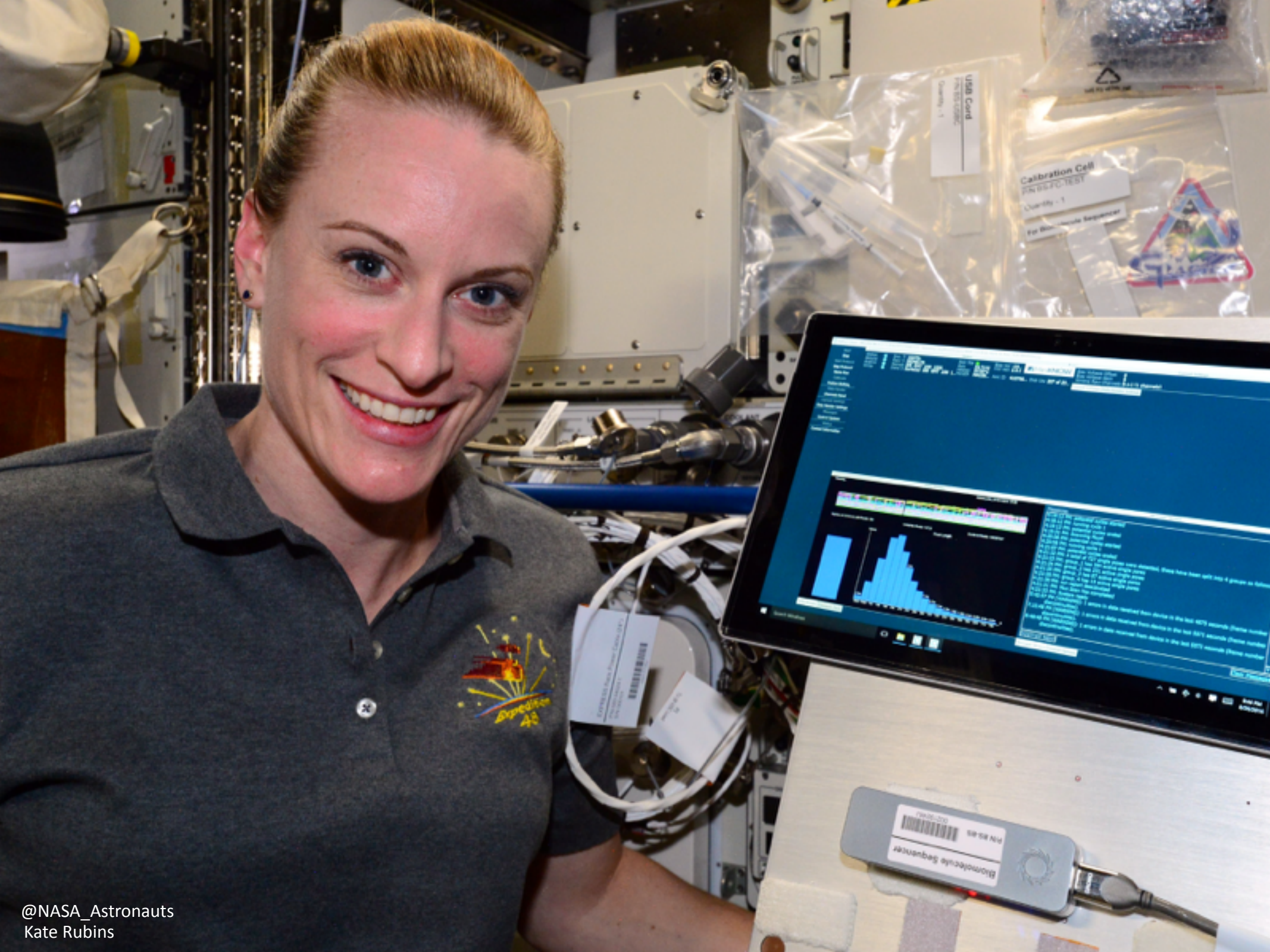
Miniature, Portable Sequencing

Joseph Bore running the MinION in Guinea, Quick et al Nature (2016)


78 degrees North
@arywnedwards


MinION


European astronaut centre
ESA CAVES
@explornaut



@NASA_Astronauts
Kate Rubins

Disclosure: ONT provides research funding to my lab

Why use genome sequencing during an outbreak?

- Sequence viral genomes to calculate mutation rate
- Identify host adaptations
- Characterise response to therapy/immunization
- Relate cases to each other and monitor geographic spread

Why use genome sequencing during an outbreak?

- Sequence viral genomes to calculate mutation rate
- Identify host adaptations
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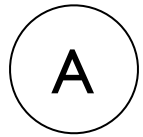
- **Relate cases to each other and monitor geographic spread**

Ebola Surveillance

Ebola is passed through direct contact



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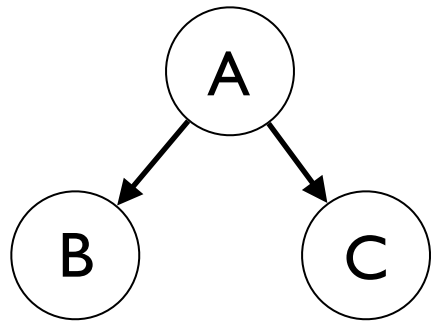

Person A transmits the virus to B and C

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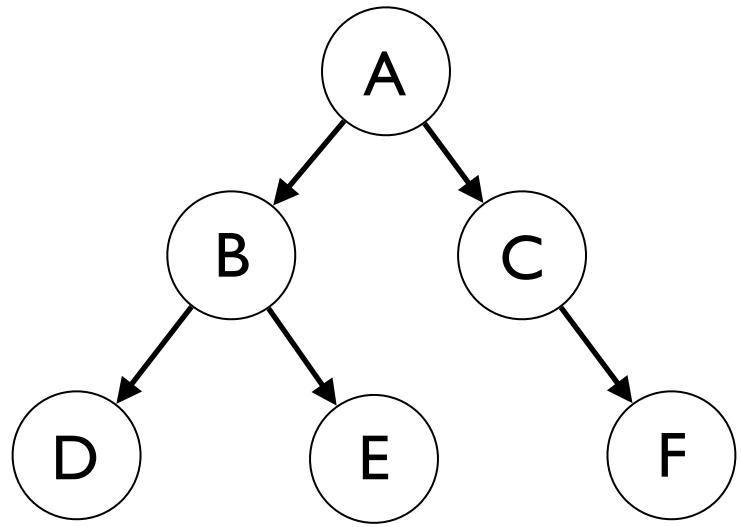
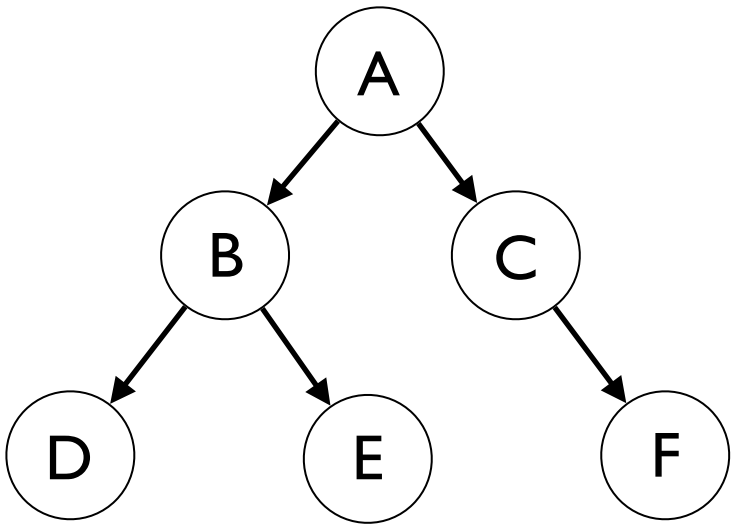


Person A transmits the virus to B and C
Person B transmits the virus to D and E
Person C transmits the virus to F

Ebola Surveillance

Ebola virus mutates at a rate of ~1.15 $\times 10^{-3}$ mutations/bp/year
These mutations allow us to track patterns of transmission




```
Ebola-A ...AGTAGCCTACGATACTACGATCGACTTA...
Ebola-B ...AGTAGCCTACGATATTACGATCGACTTA...
Ebola-C ...AGTAGCCGACGATACTACGATCGACTTA...
Ebola-D ...AGTTGCCTACGATATTACGATCGACTTA...
Ebola-E ...AGTAGCCTACGATATTACGATCGACATA...
Ebola-F ...AGTAGCCGACGATACTACGATGGACTTA...
```

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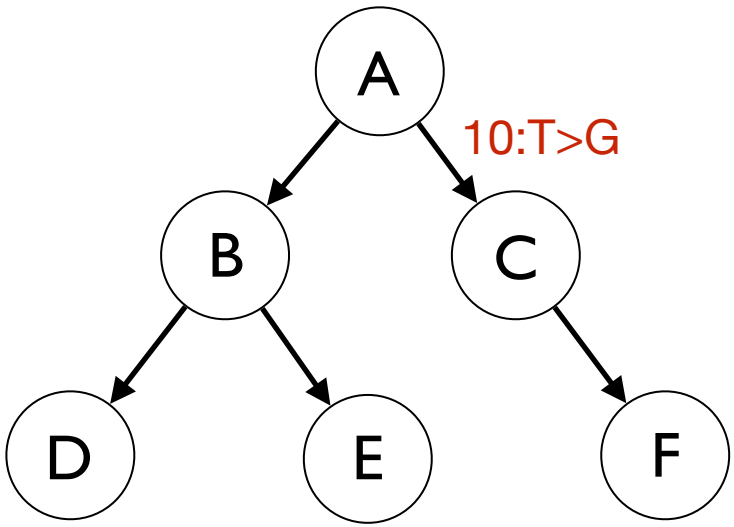


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Ebola-D ...AGTTGCCTACGATATTACGATCGACTTA...
Ebola-E ...AGTAGCCTACGATATTACGATCGACATA...
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```

T>G here indicates C/F lineage

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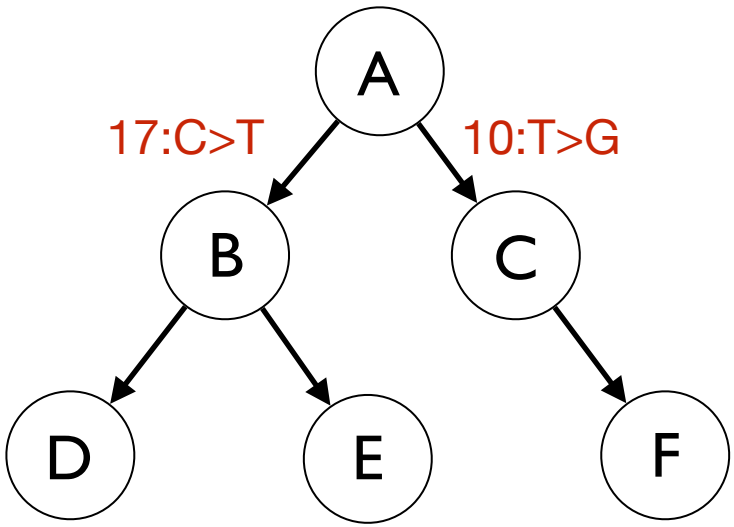


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```
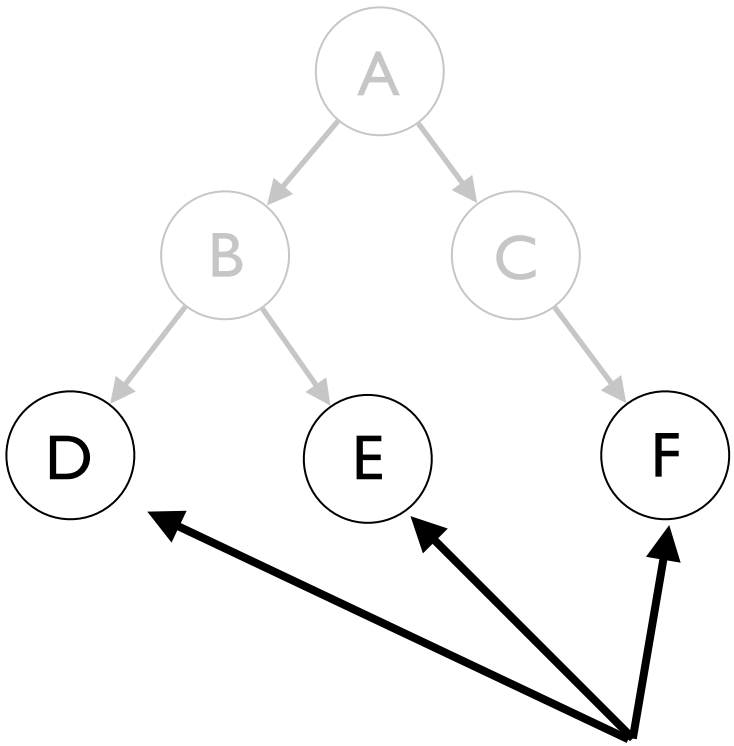

C>T here indicates B/D/E lineage

Ebola Surveillance

We can't sequence every case in the outbreak but by sampling enough cases we can build a picture of how the virus is spreading (e.g. geographically)




```
Ebola-D ...AGTTGCCTACGATATTACGATCGACTTA...
Ebola-E ...AGTAGCCTACGATATTACGATCGACATA...
Ebola-F ...AGTAGCCGACGATACTACGATGGACTTA...
```

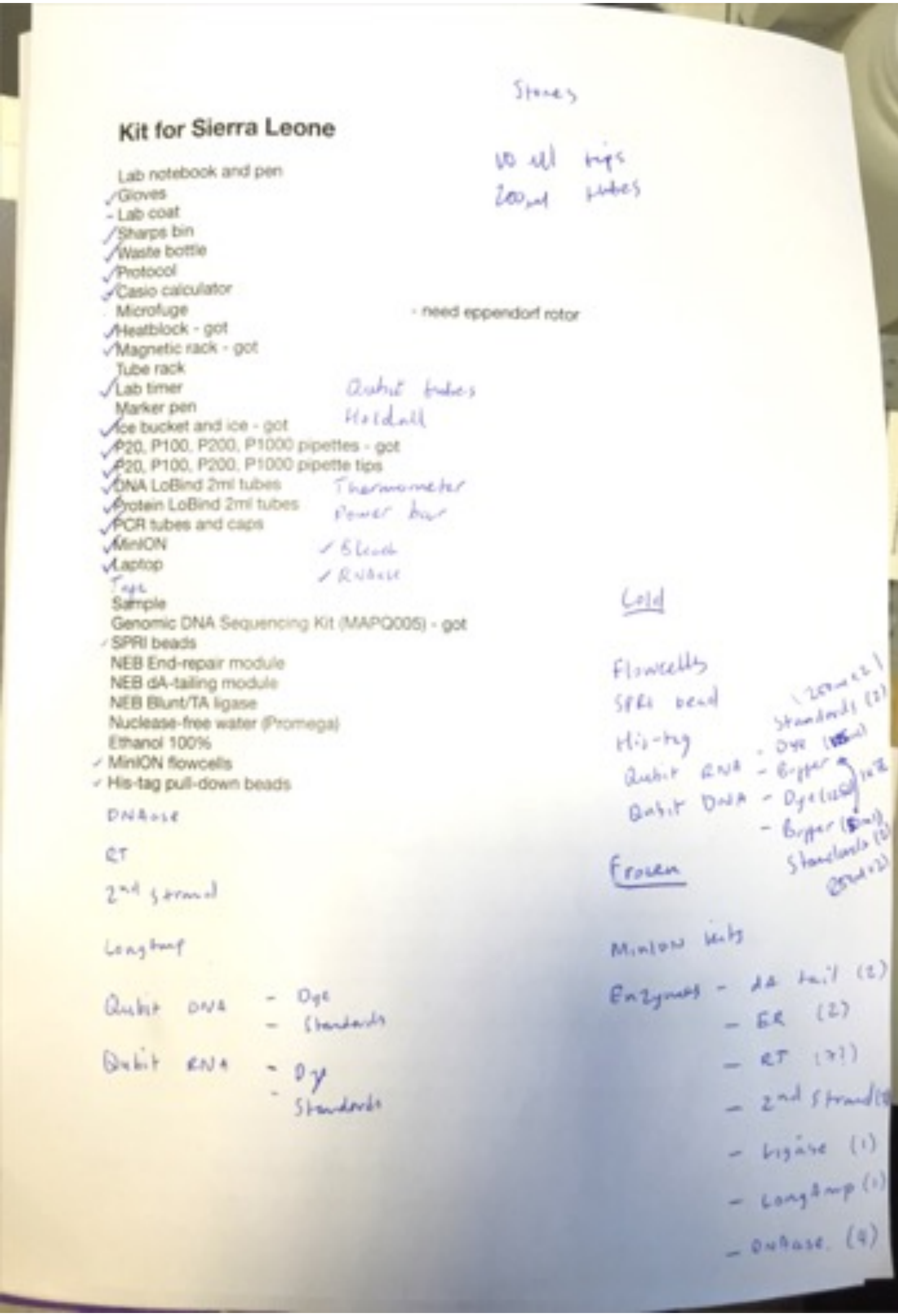
Sequence these cases; D and E share a mutation - possibly related?

Why use portable genome sequencing?

- Old process: take samples locally, send to lab in Europe/North America, sequence, return results to local epidemiologists
  - Slow, difficult to ship samples
- New process: use portable sequencers directly at the location they are needed

Image credit: Genome Research Limited


Heathrow
THE NORTH FACE



Kit for Sierra Leone

- Lab notebook and pen
- ✓ Gloves
- \- Lab coat
- ✓ Sharps bin
- ✓ Waste bottle
- ✓ Protocol
- ✓ Casio calculator
- Microfuge - need eppendorf rotor
- ✓ Heatblock - got
- ✓ Magnetic rack - got
- Tube rack
- ✓ Lab timer
- Marker pen
- ✓ Ice bucket and ice - got
- ✓ P20, P100, P200, P1000 pipettes - got
- ✓ P20, P100, P200, P1000 pipette tips
- ✓ DNA LoBind 2ml tubes
- ✓ Protein LoBind 2ml tubes
- ✓ PCR tubes and caps
- ✓ MinION
- ✓ Laptop
- Tape
- Sample
- Genomic DNA Sequencing Kit (MAPQ005) - got
- ✓ SPRI beads
- NEB End-repair module
- NEB dA-tailing module
- NEB Blunt/TA ligase
- Nuclease-free water (Promega)
- Ethanol 100%
- ✓ MinION flowcells
- ✓ His-tag pull-down beads

DNAase

RT

2nd strand

LongAmp

Qubit DNA - Dye - Standards

Qubit RNA - Dye - Standards

Stores

10 ul tips

200 ul tubes

Qubit tubes

Holdall

Thermometer

Power bar

✓ Bleach

✓ RNAase

Cold

Flowcells

SPRI bead

His-tag

Qubit RNA - Dye - Buffer - Standards (2)

Qubit DNA - Dye - Buffer - Standards (2)

Frozen

MinION kits

Enzymes - dA tail (2)

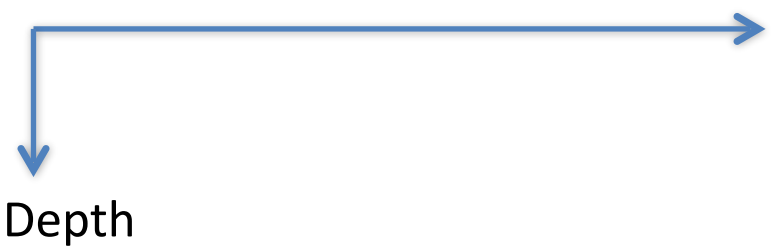
- ER (2)
- RT (?)
- 2nd strand
- ligase (1)
- LongAmp (1)
- DNAase (4)

Porton Down validation set, 89.1% coverage

Guinea 19 reactions v1, 98.1% coverage

Guinea 11 reactions v1, 95.9% coverage

Guinea 11 reactions v2, 98.4% coverage



Calculating a consensus sequence

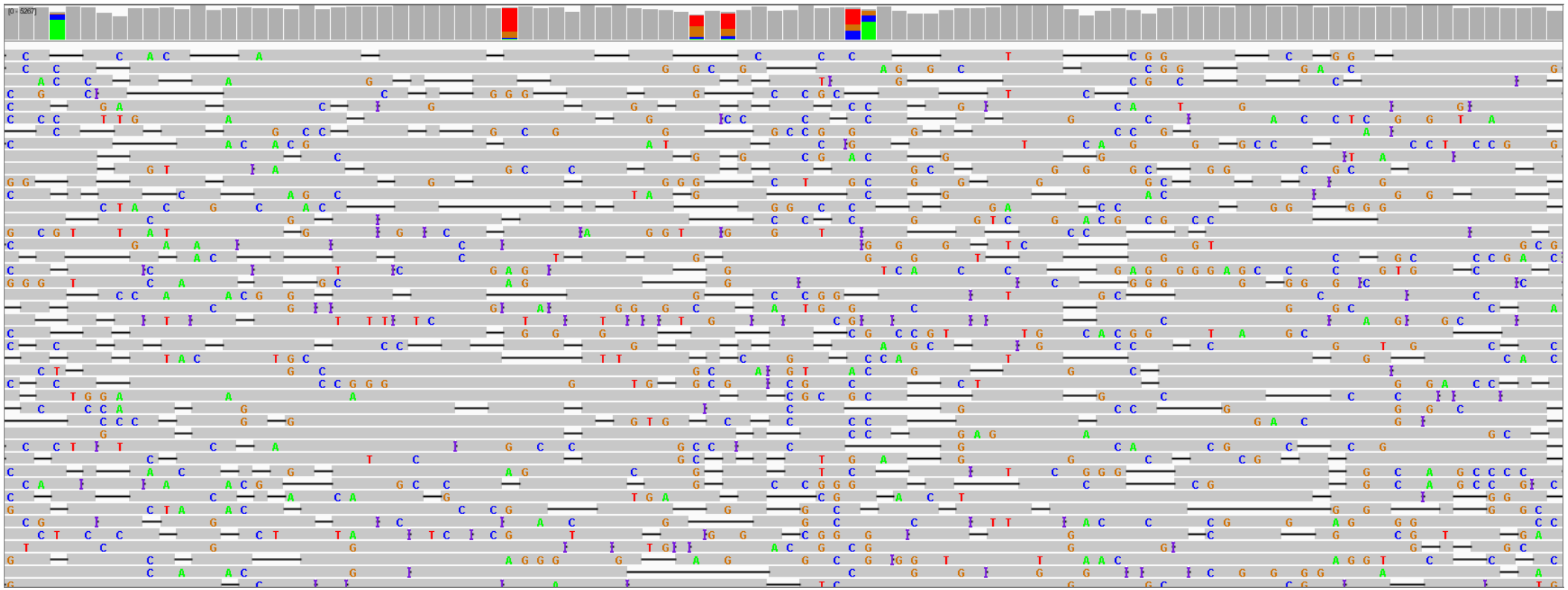
- Input: a set of nanopore reads ($r_1, r_2, \ldots, r_n$) from an Ebola genome (g)
- Output: the sequence of the Ebola genome, g

r_1 CAGATAGTCGGATGTTATGAACCAGATATATA
r_2 CAGACAGTCGGATGTTATGATCCAGATATGTA
r_3 CAGATAGTCGGATGTTATAATCCAGATATATA

---

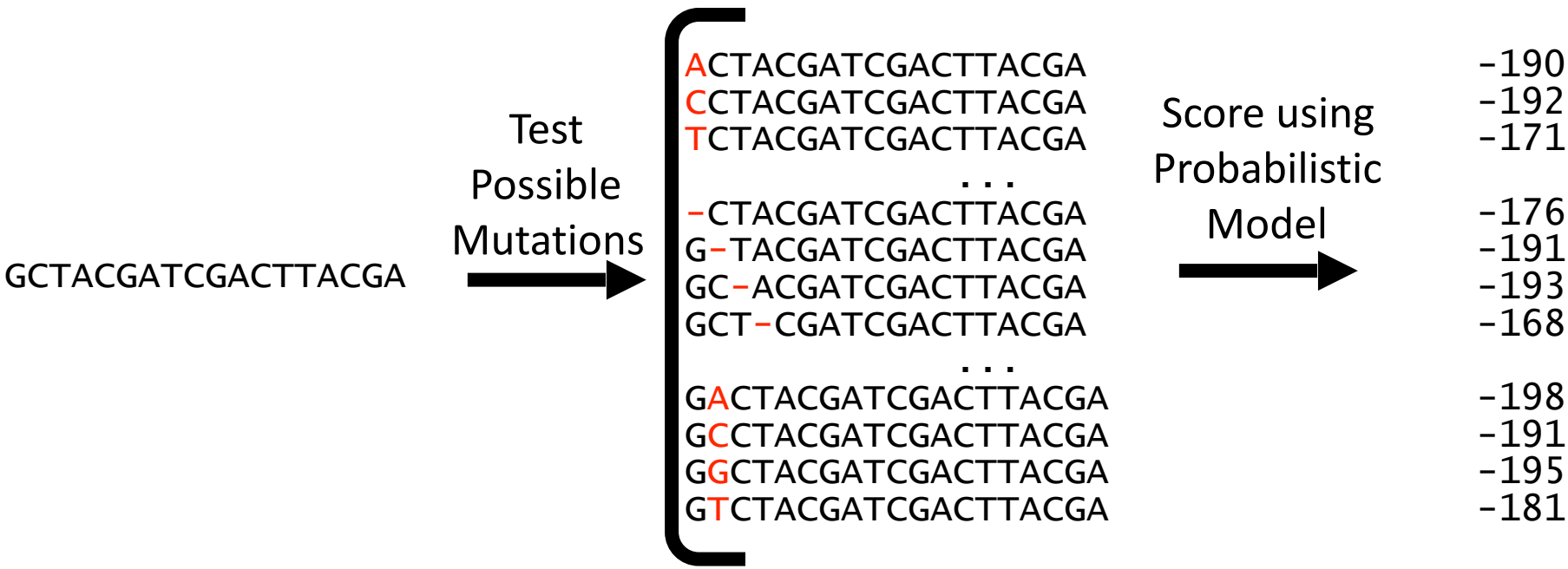
g CAGATAGTCGGATGTTATGATCCAGATATATA

Calculating a consensus sequence



Main challenge: the base-calling error rate is quite high (~10%)

Calculating a consensus sequence



Nanopore Sequencing

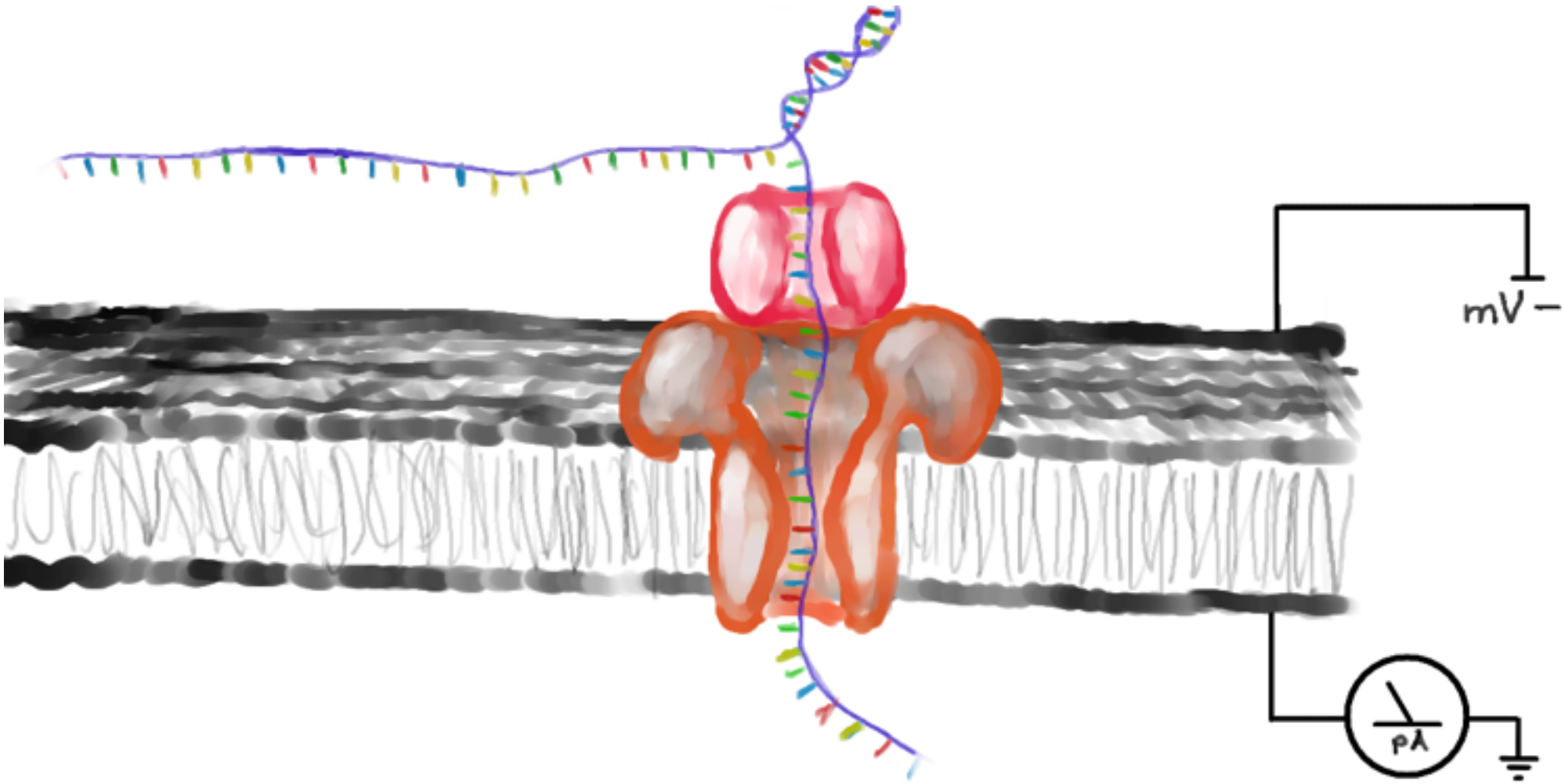
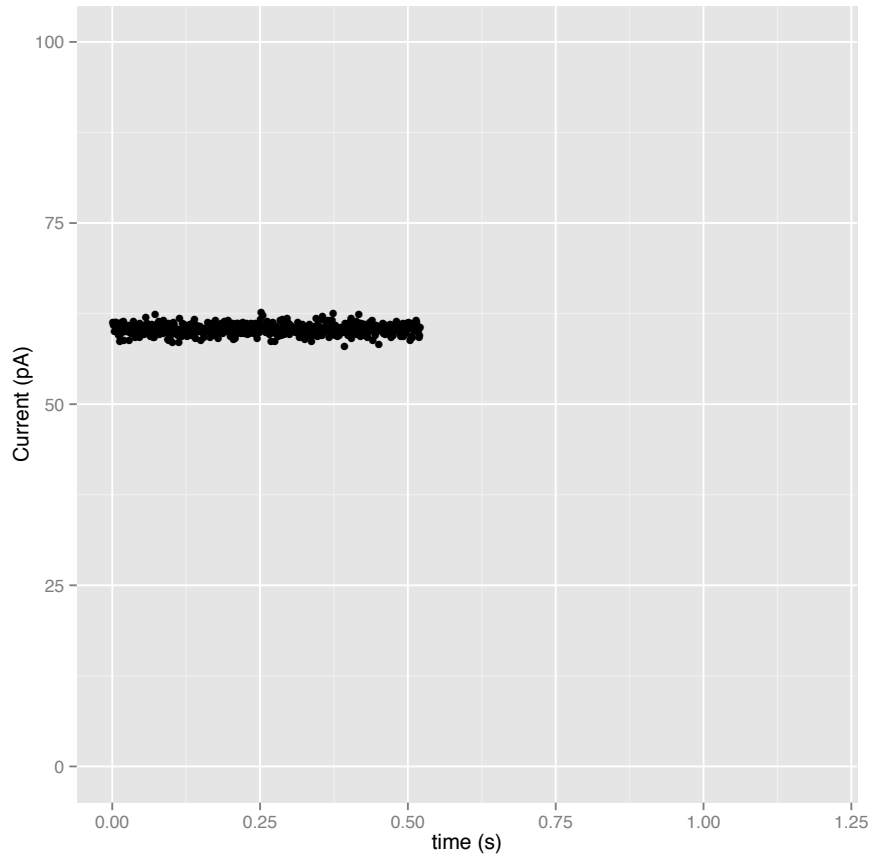


Illustration by David Eccles

Nanopore Sequencing



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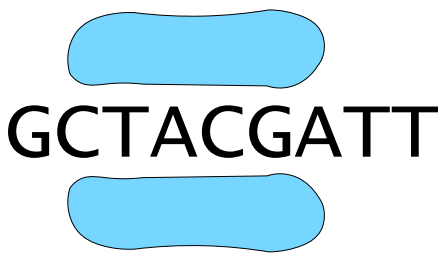
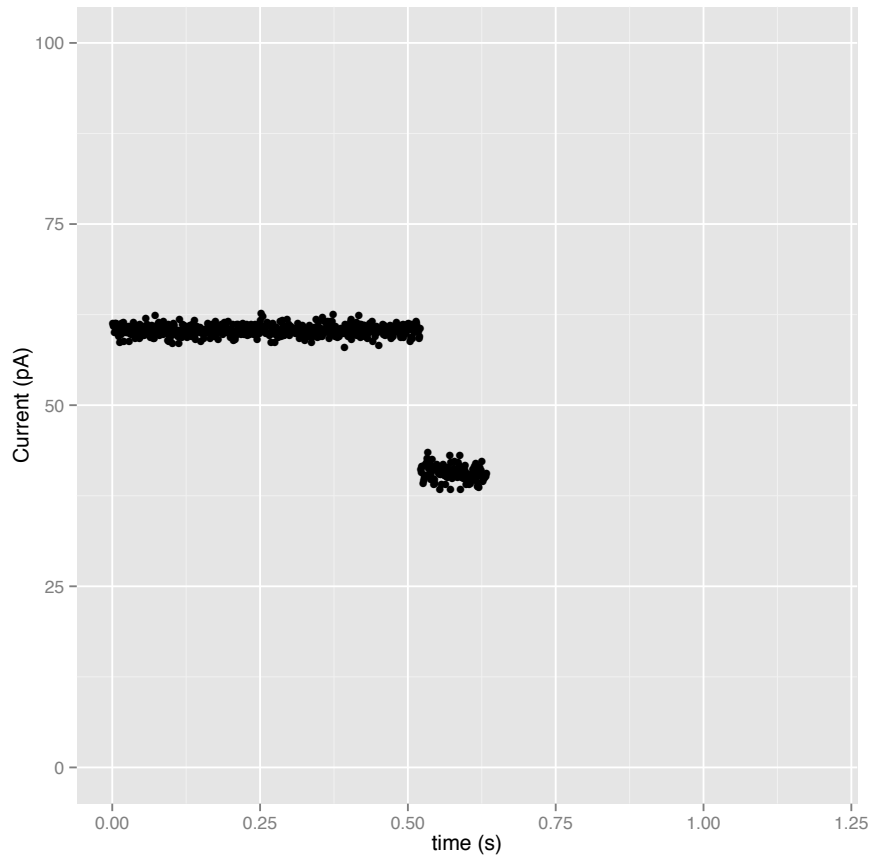


Sample Current



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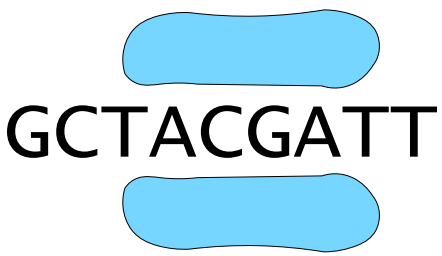

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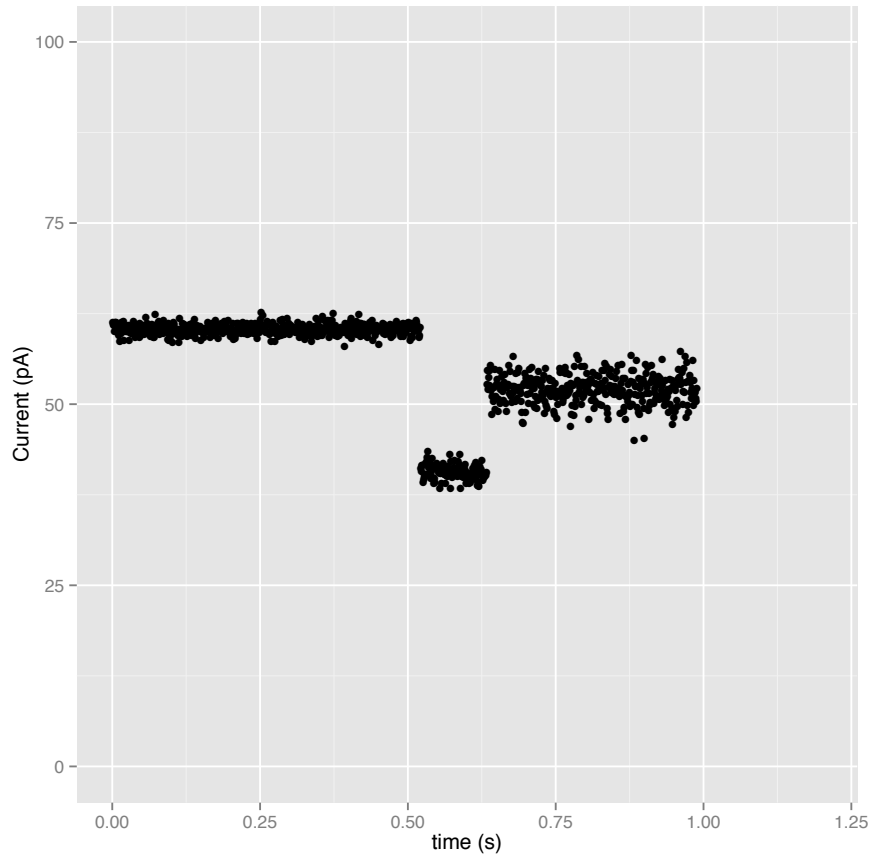

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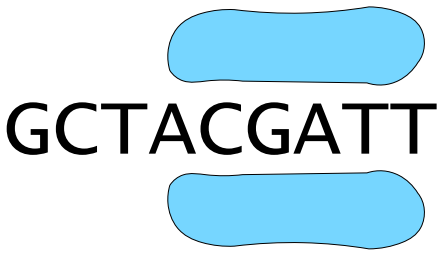

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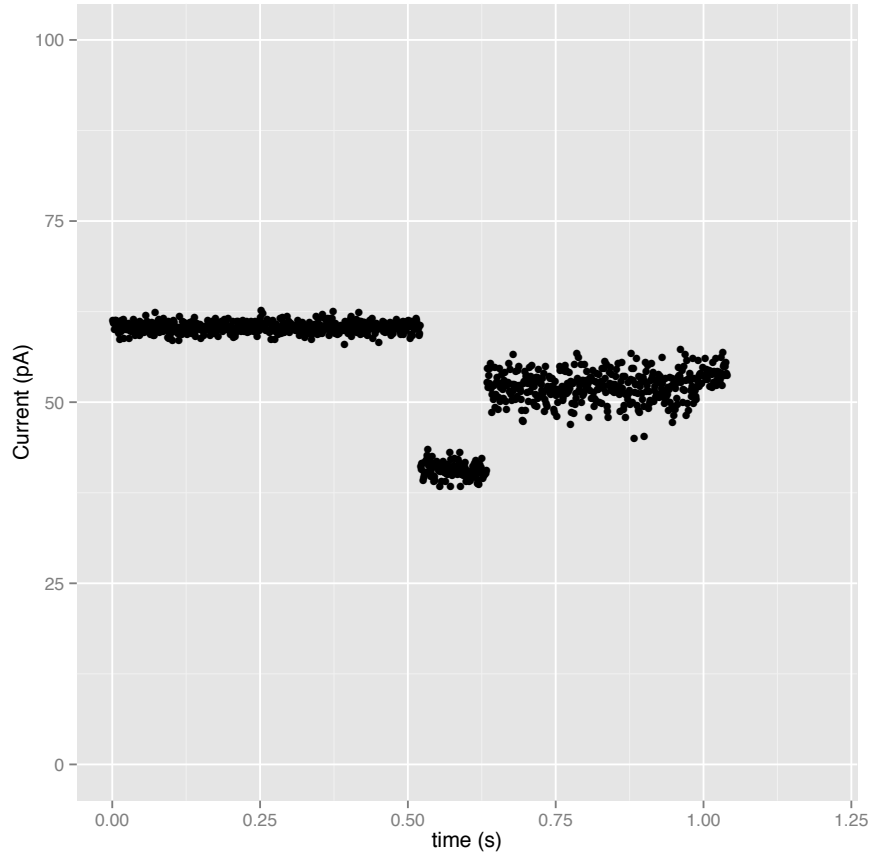

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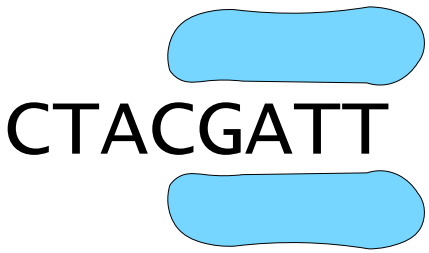

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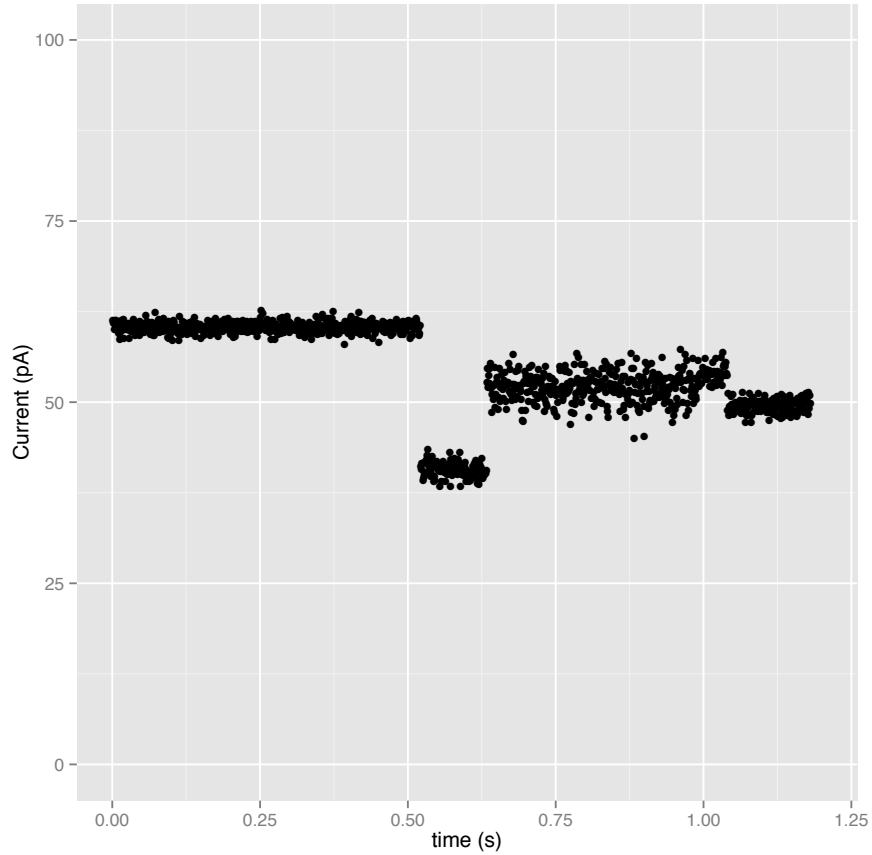
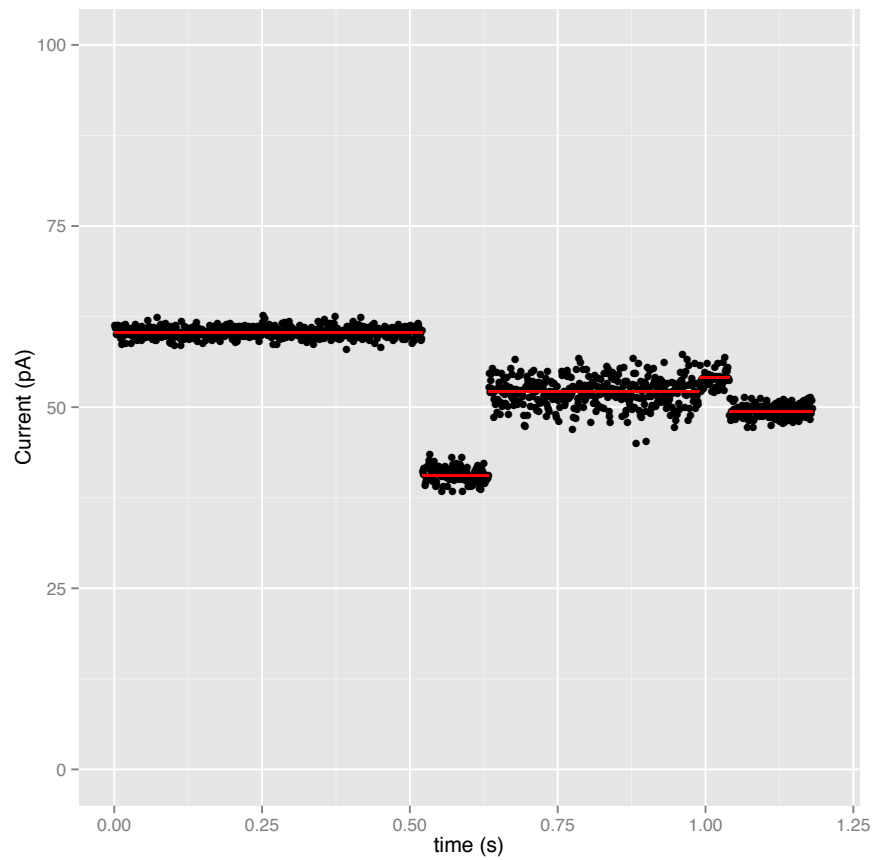
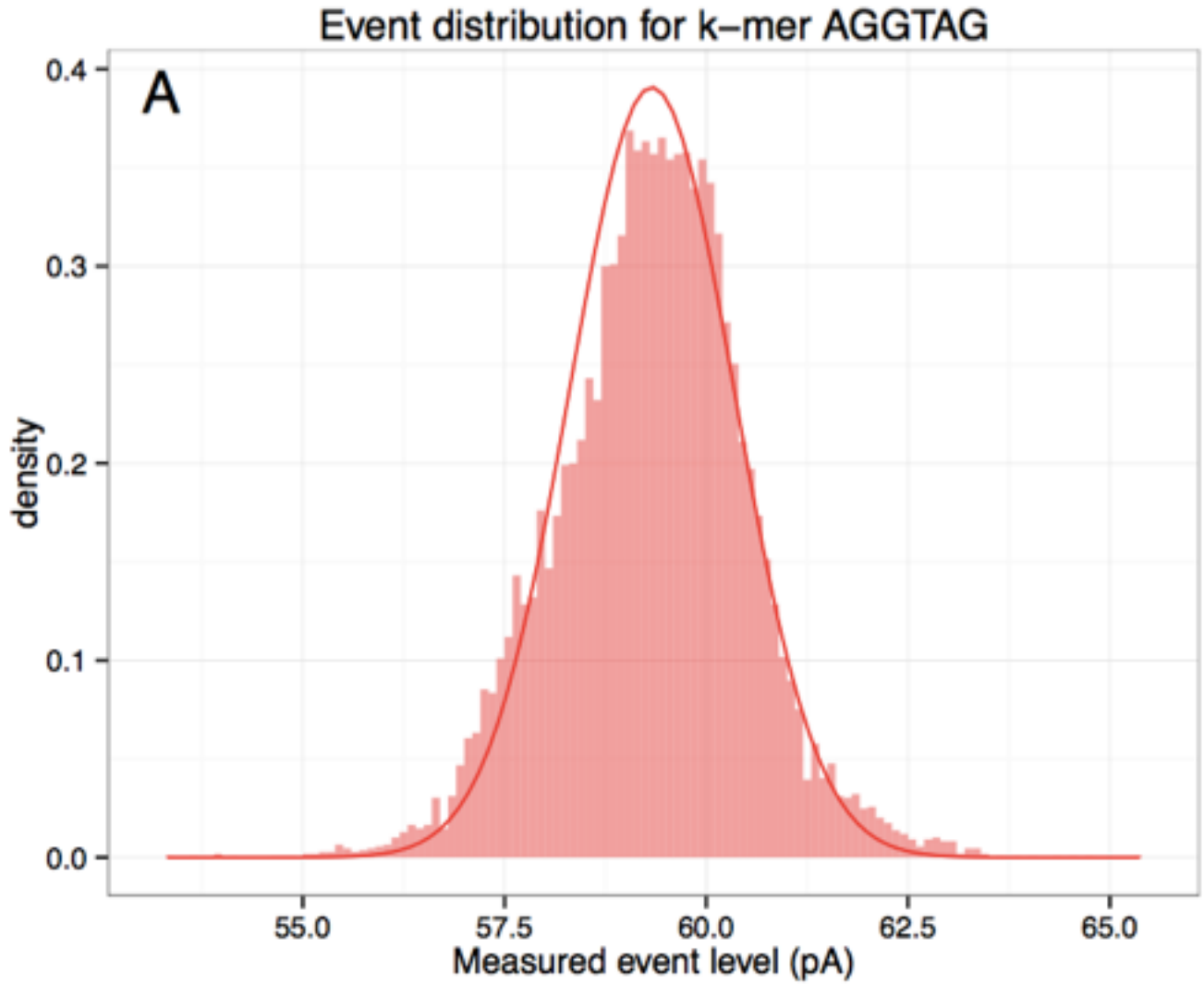


Event Detection

Event	mean current (pA)	current stdv	duration (s)
1	60.3	0.7	0.521
2	40.6	1.0	0.112
3	52.2	2.0	0.356
4	54.1	1.2	0.291
5	49.5	1.5	0.141



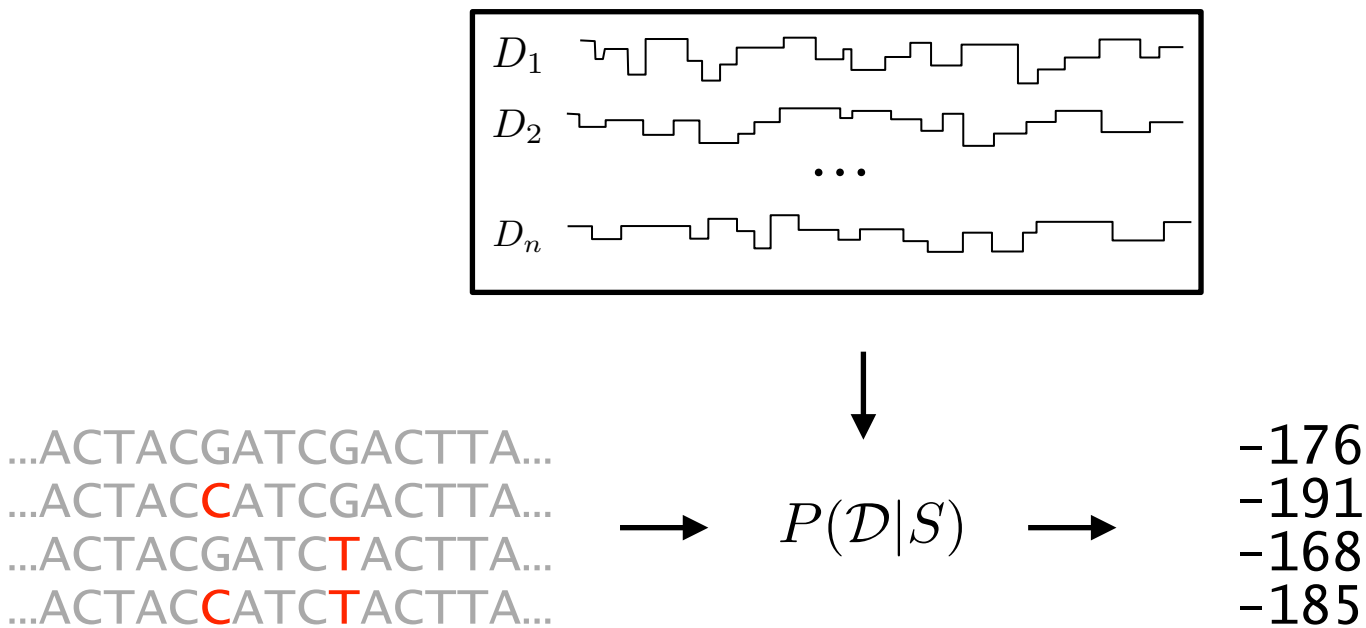
Event levels are k-mer dependent



Pore Models

6-mer	μ_k	σ_k
AAAAAA	54.2	1.2
AAAAAC	56.4	0.9
…	…	…
TTTTTG	61.7	1.1
TTTTTT	62.3	0.8

SNP Calling Pipeline

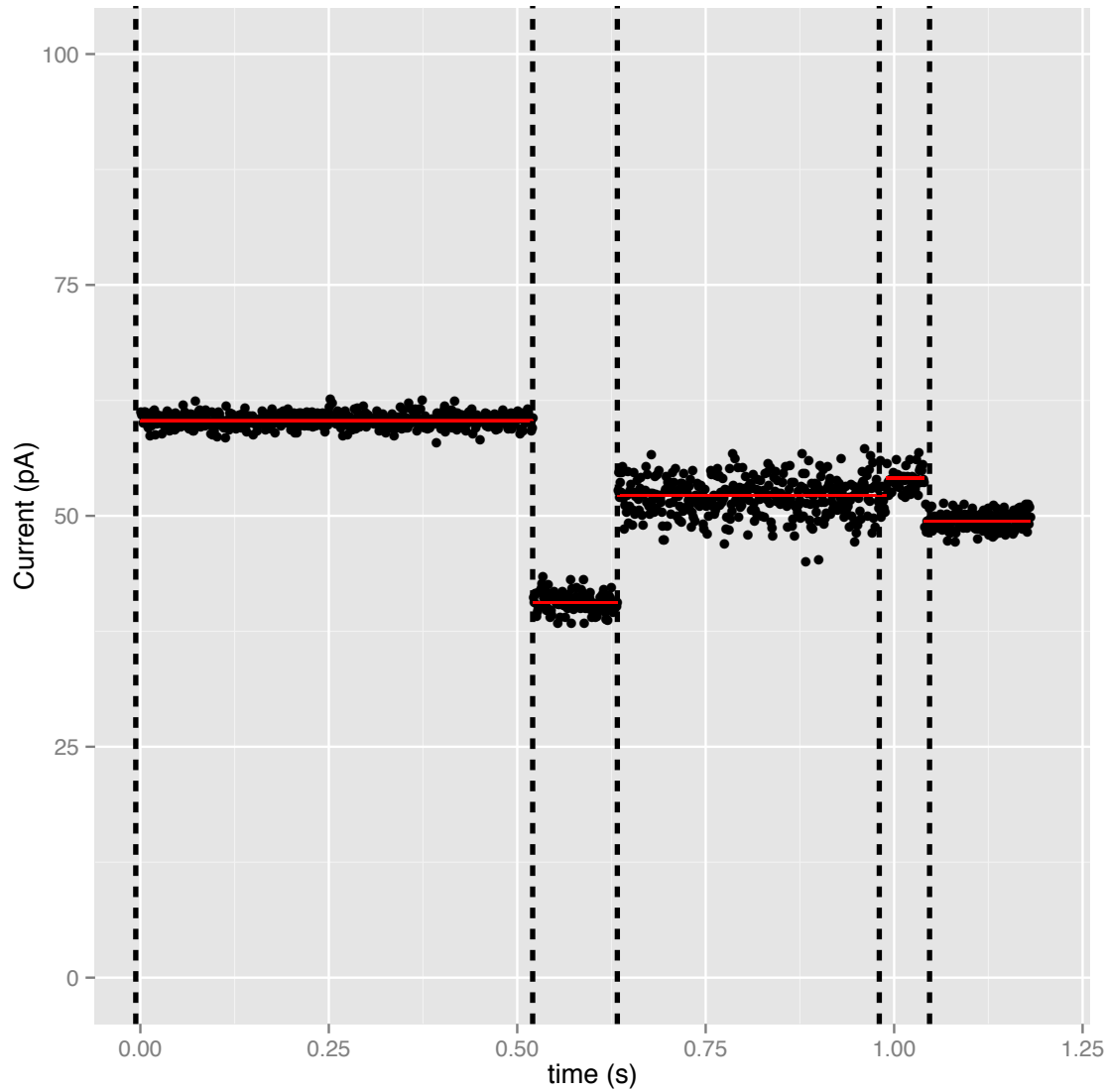


We need a function $P(\mathcal{D}|S)$ that calculates the probability of observing some nanopore data (D) given a proposed sequence (S).

A first model

Unrealistic assumption:

- every base generates exactly one event



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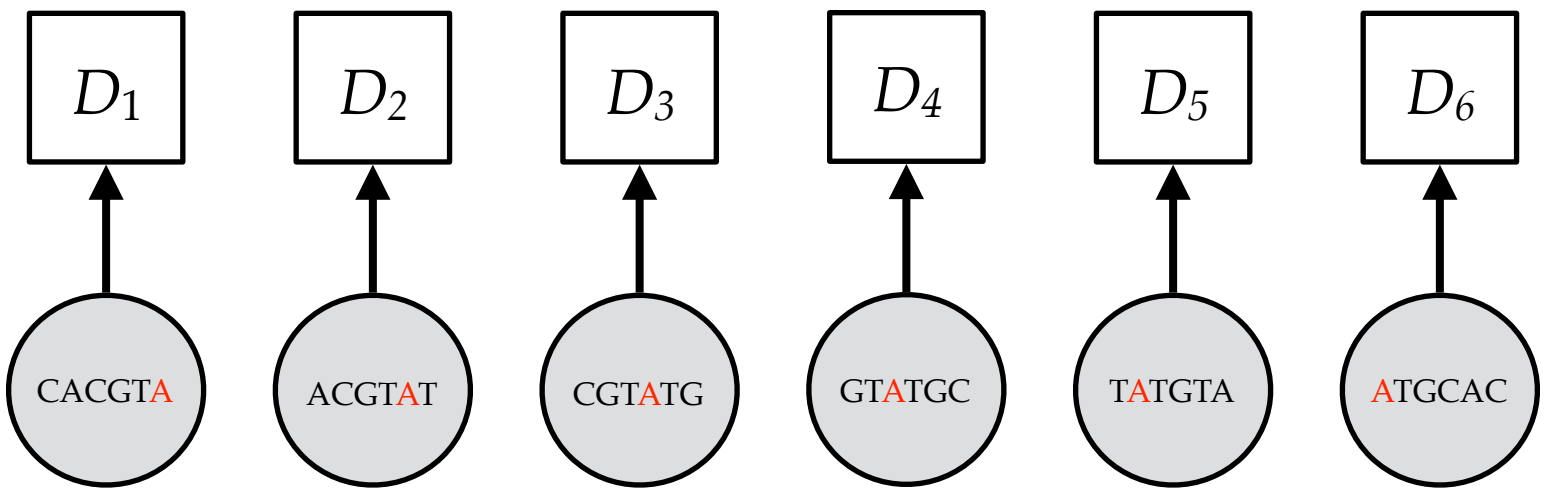

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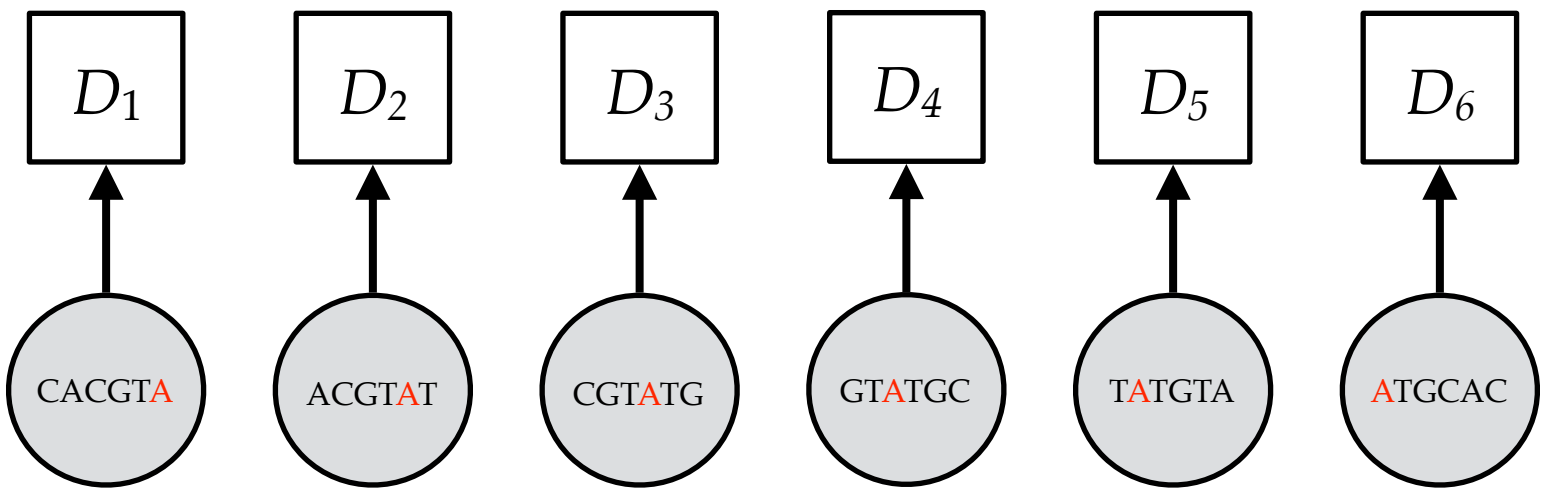
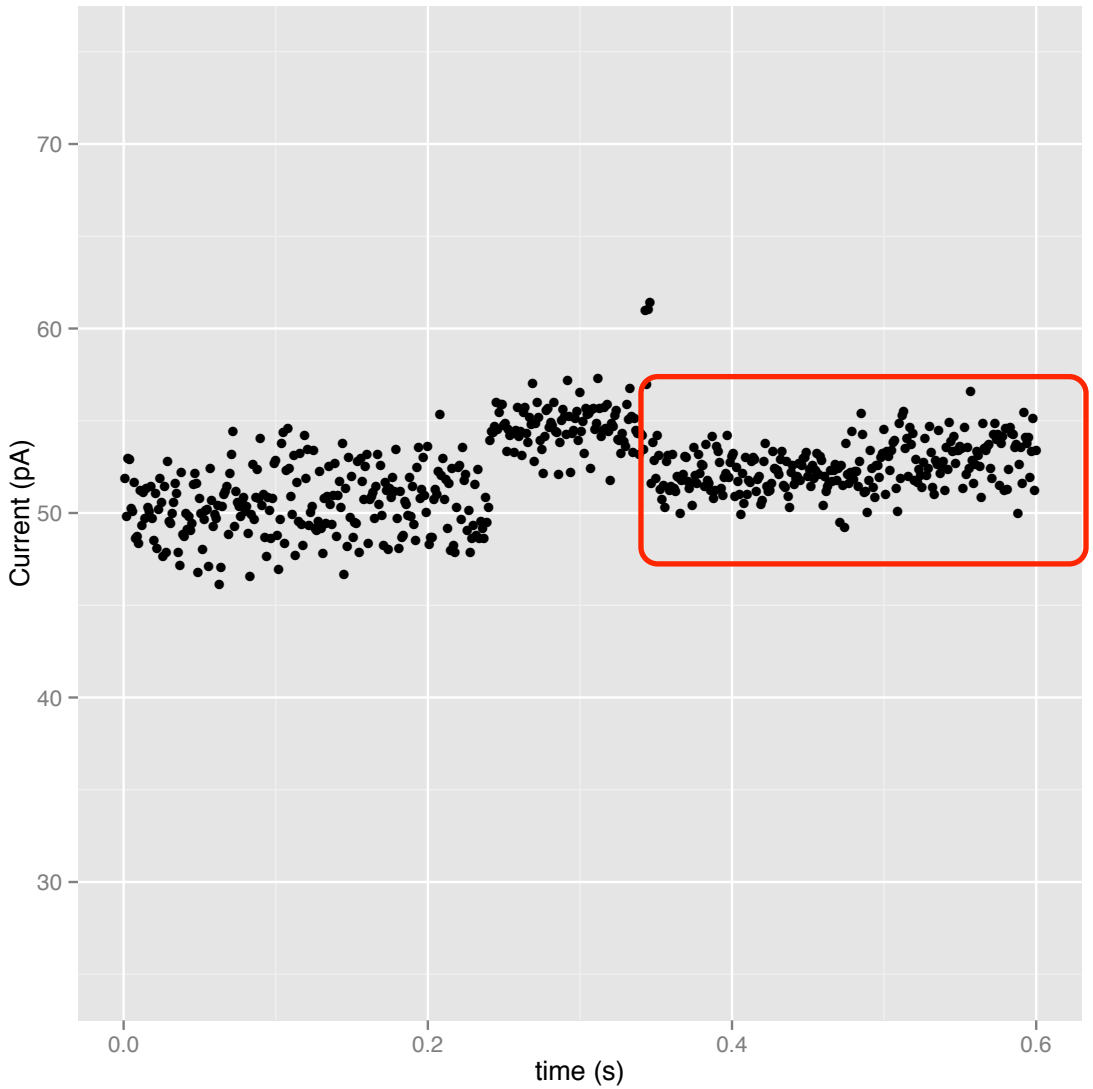


$$P(\mathcal{D}|S_h) = \prod_{i=1}^{n} P(D_i|S_{h,i})$$

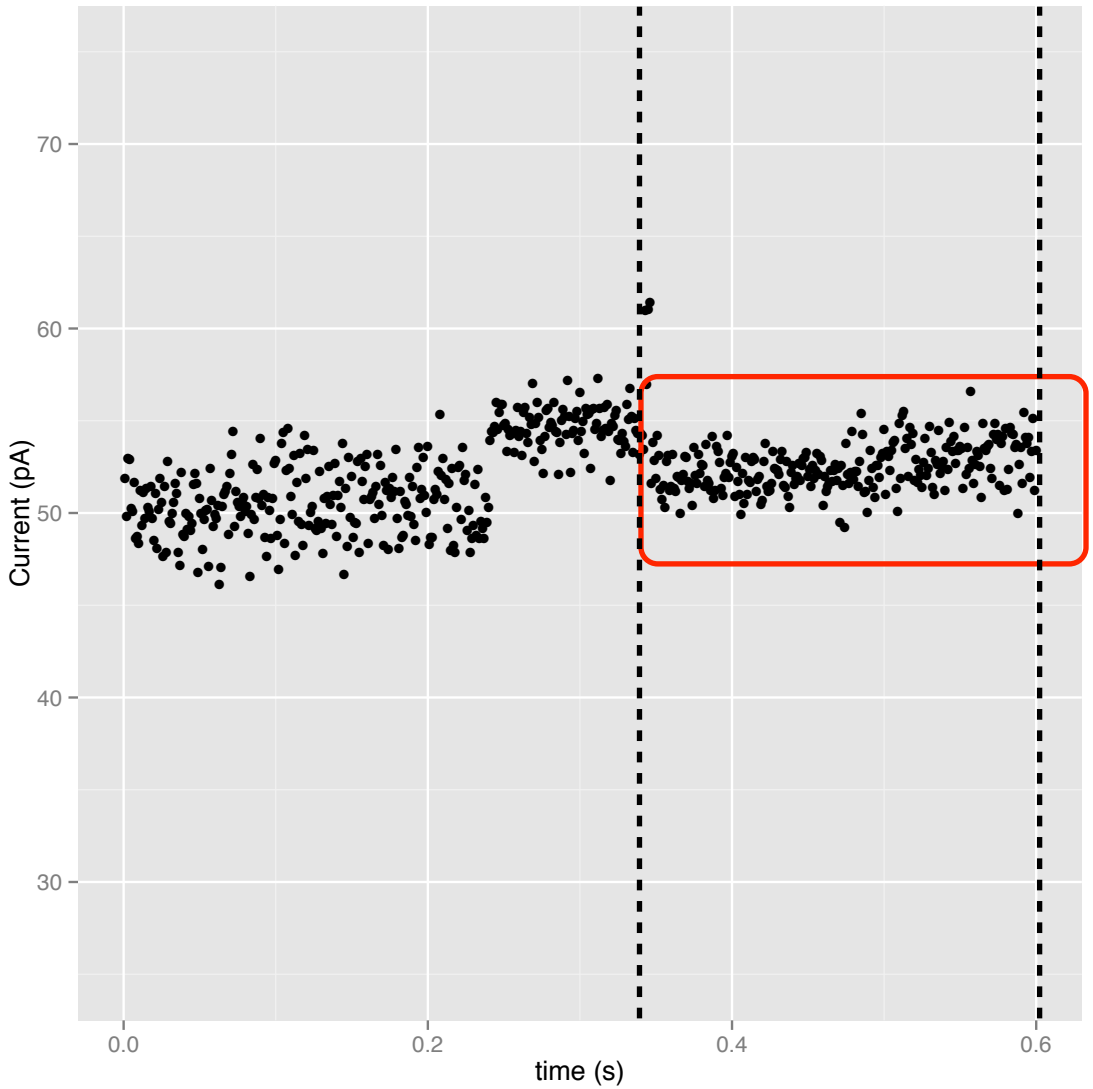
$$P(\mathcal{D}_i|S_{h,i}) = \mathcal{N}(\mu_{S_{h,i}}, \sigma^2_{S_{h,i}})$$

Complications - Segmentation Errors



One event or two ?

Complications - Segmentation Errors



One event or two ?

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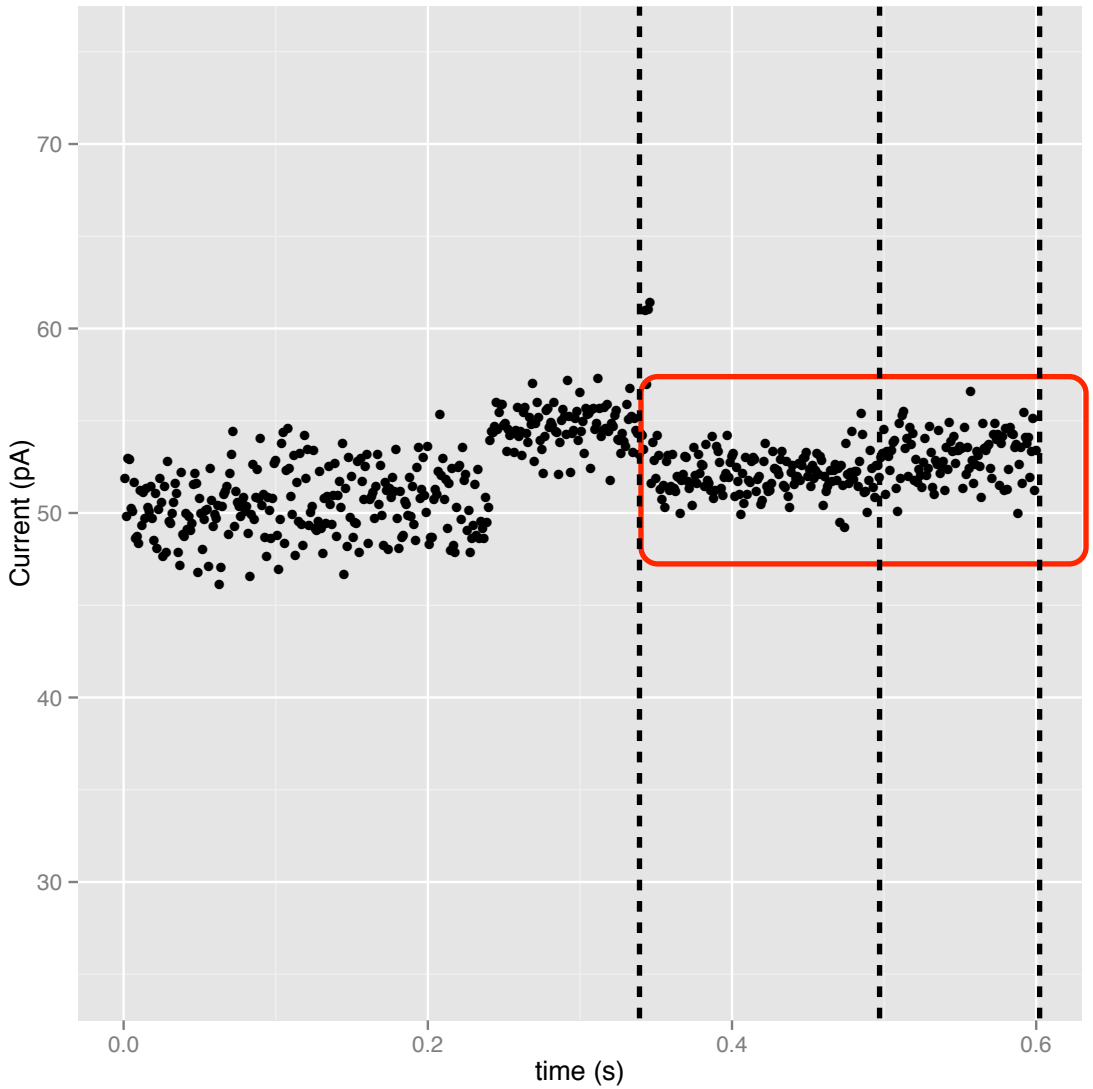
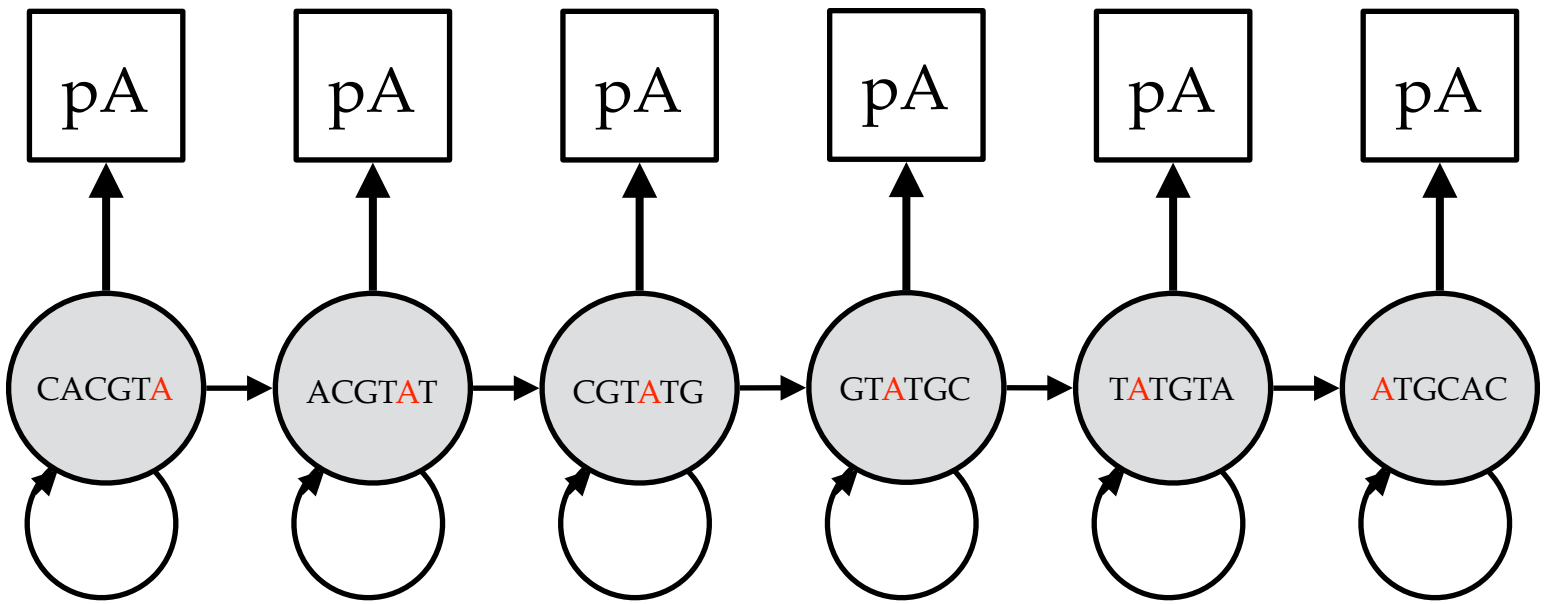


One event or two ?

Hidden Markov Model

Slightly more realistic assumption:

- every base generates at least one event



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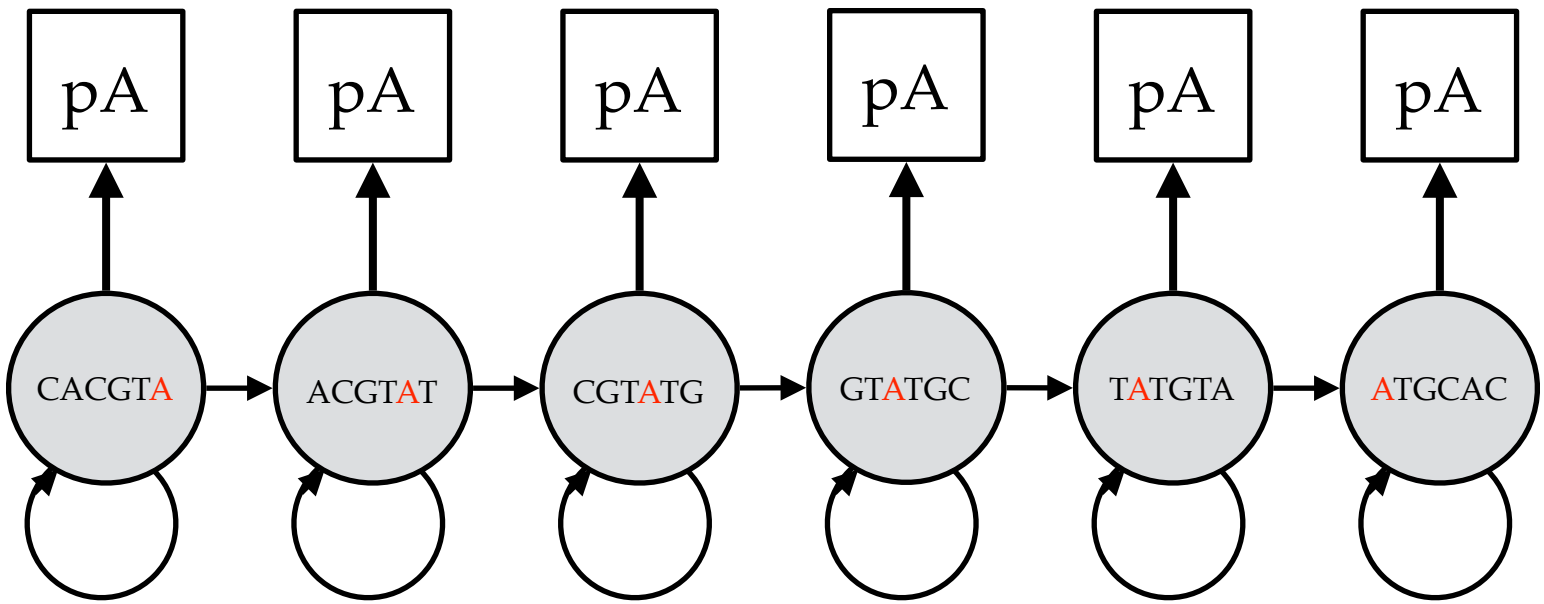

$$P(\pi, D|S_h) = \prod_{i=1}^{n} P(D_i|\pi_i, \mu_{S_{h,i}}, \sigma_{S_{h,i}}) P(\pi_i|\pi_{i-1}, S_h)$$

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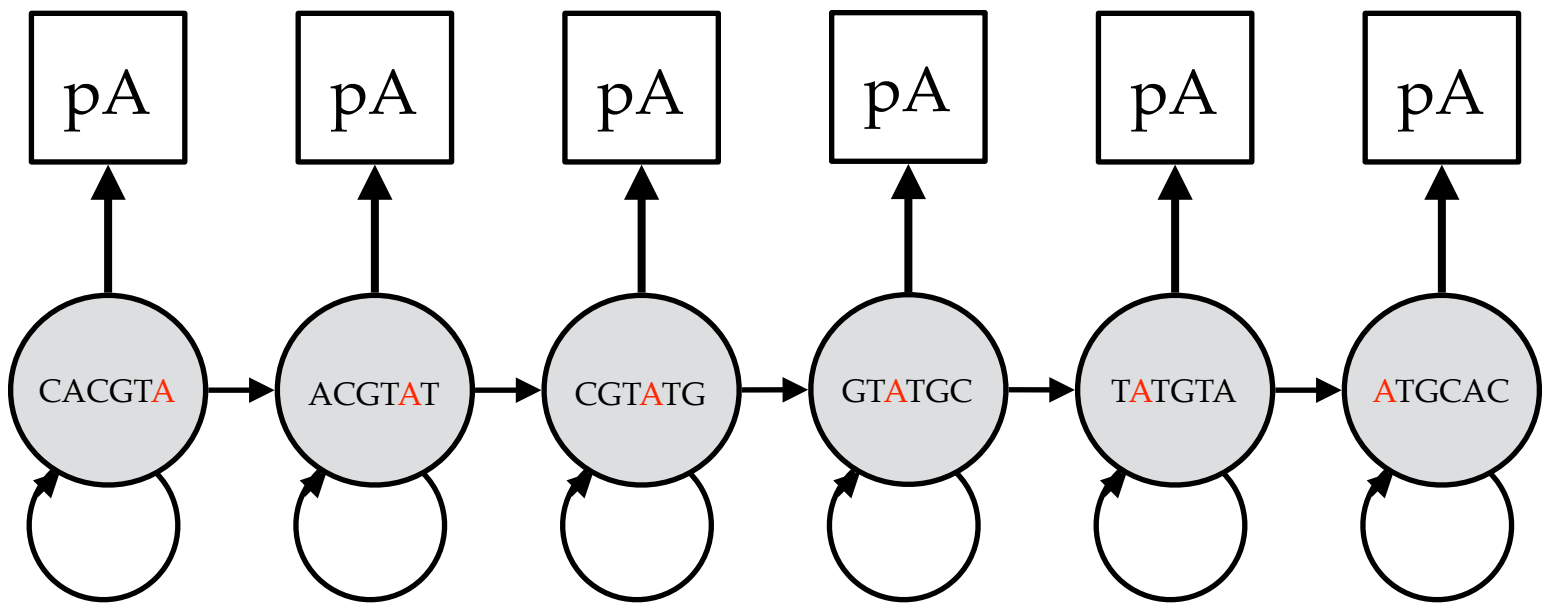
- every base generates at least one event



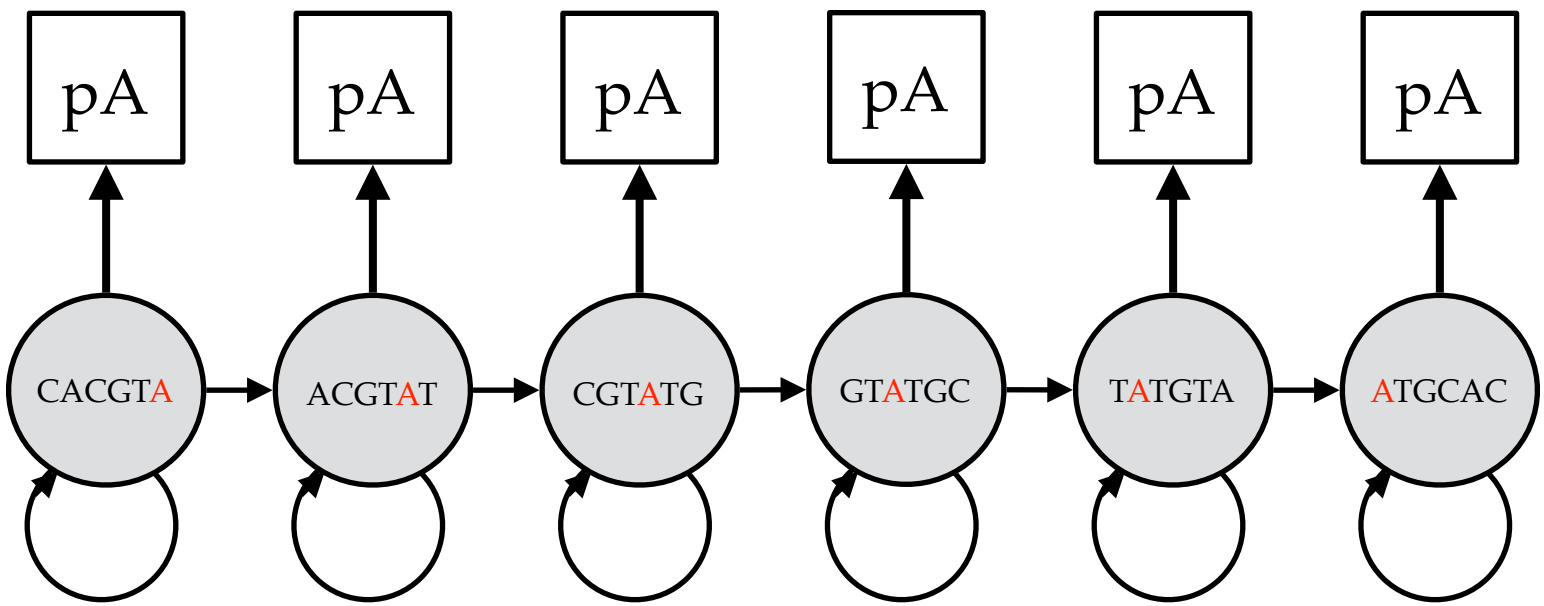
$$P(\pi, D|S_h) = \prod_{i=1}^{n} P(D_i|\pi_i, \mu_{S_{h,i}}, \sigma_{S_{h,i}}) P(\pi_i|\pi_{i-1}, S_h)$$

State path Emission function Transition function

Hidden Markov Model

Slightly more realistic assumption:

- every base generates at least one event



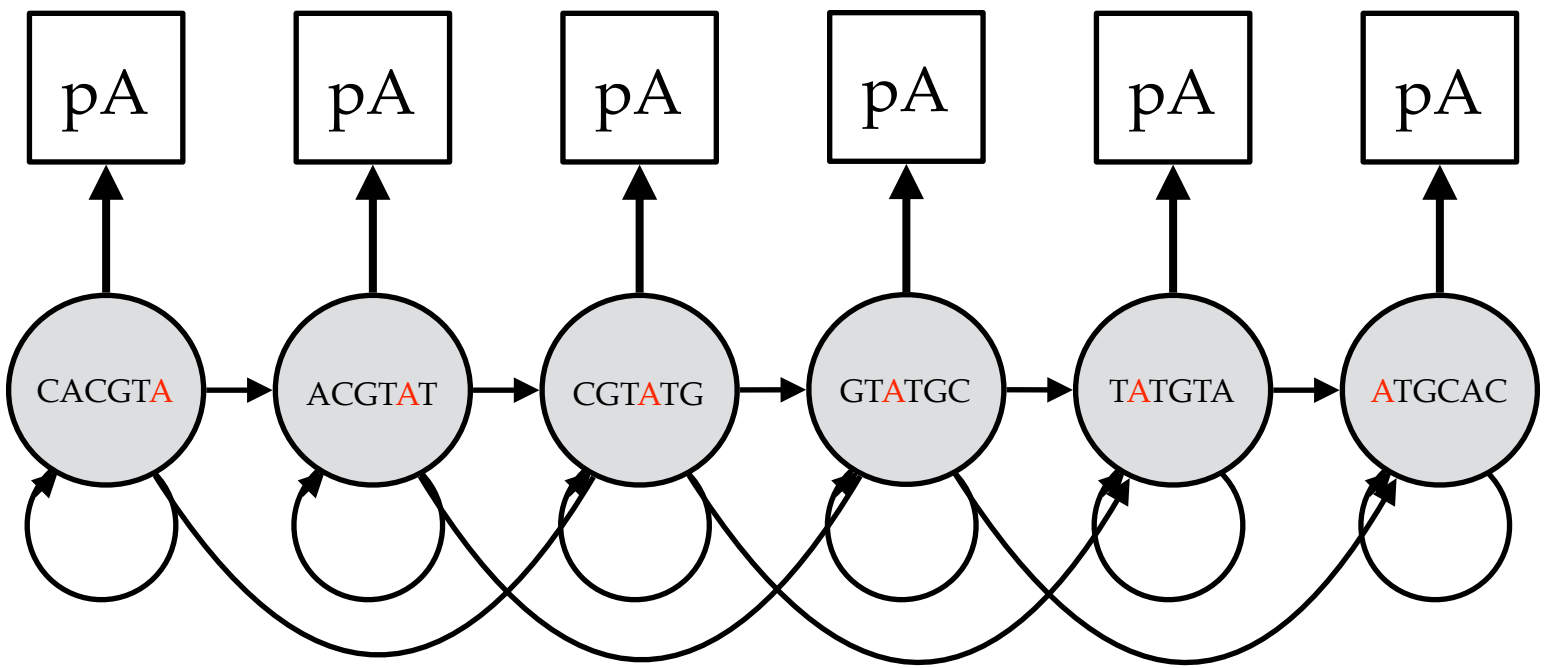
$$P(\pi, D|S_h) = \prod_{i=1}^{n} P(D_i|\pi_i, \mu_{S_{h,i}}, \sigma_{S_{h,i}}) P(\pi_i|\pi_{i-1}, S_h)$$

$$P(D|S_h) = \sum_{\pi} P(\pi, D|S_h)$$

Realistic model

Assumptions:

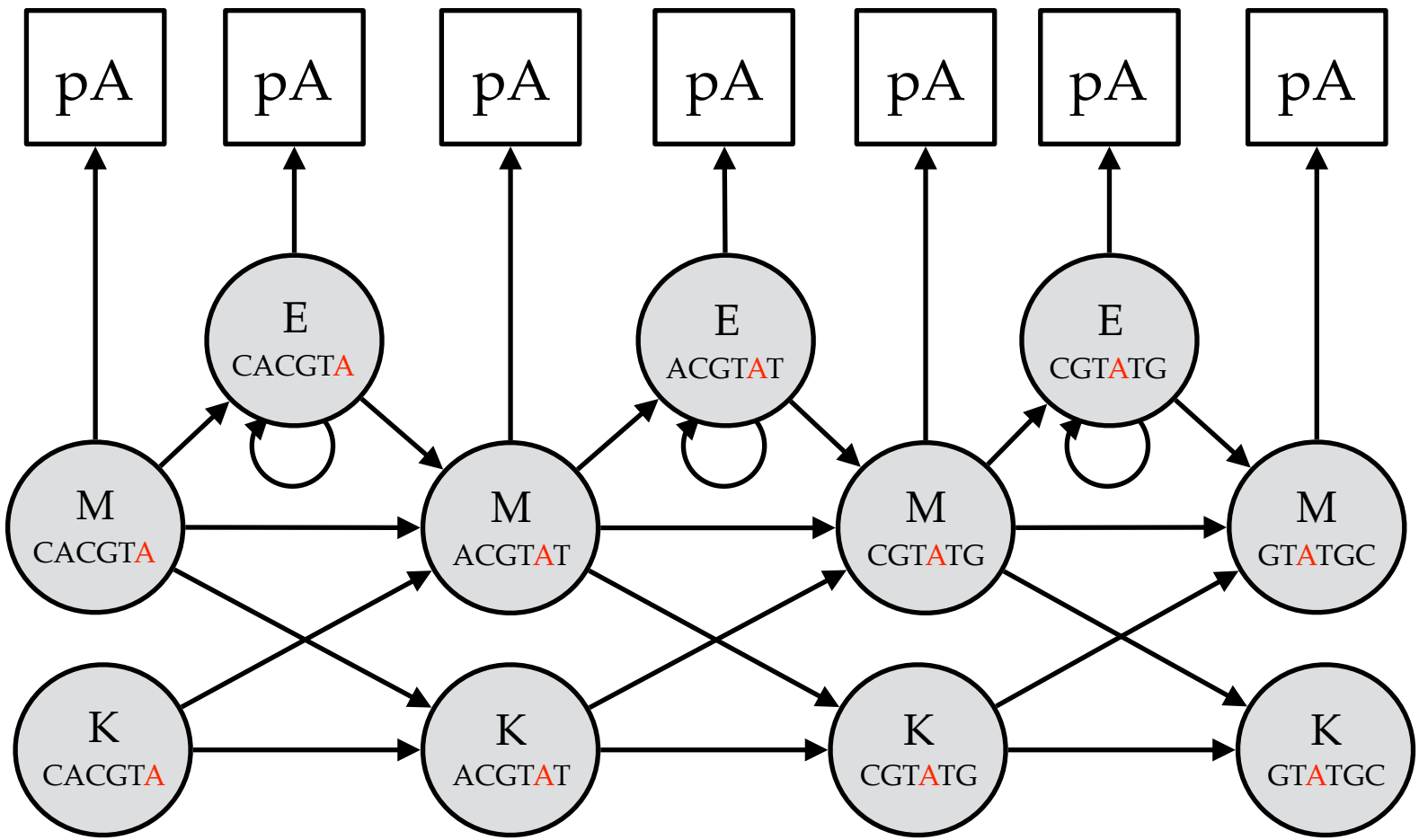
- ~~every base generates at least one event~~



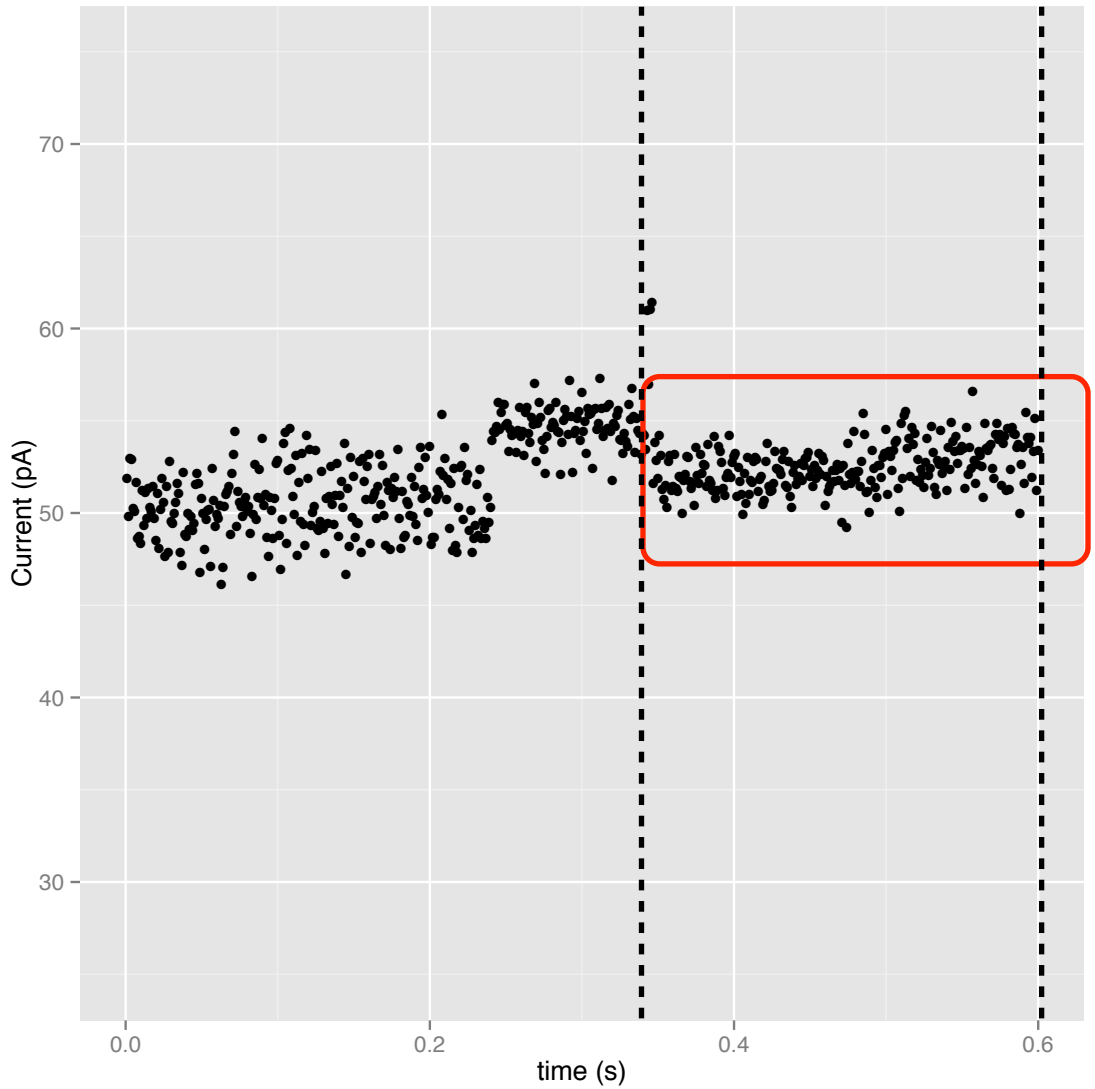
Realistic model

Assumptions:

- ~~every base generates at least one event~~



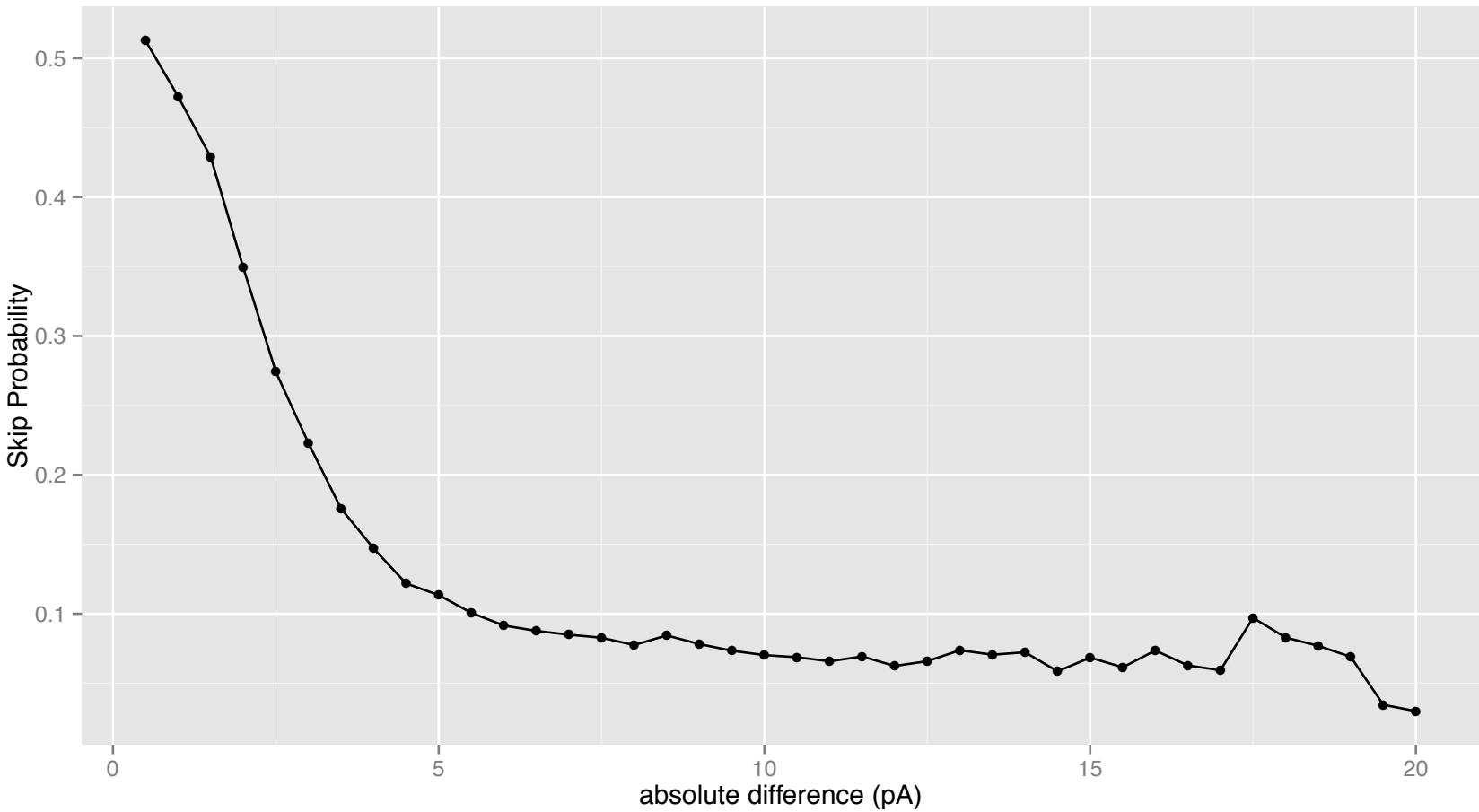
Transition Probabilities



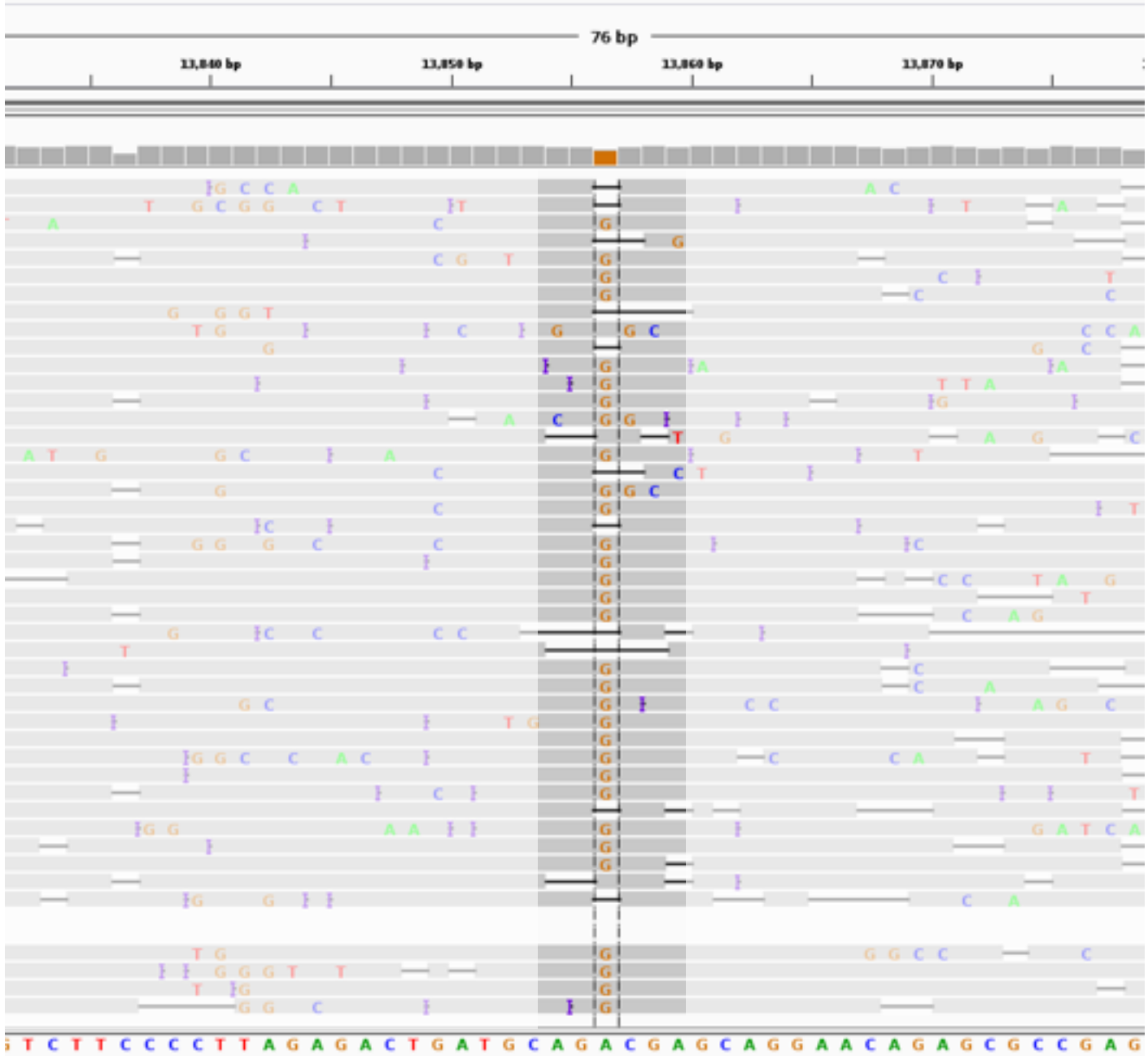
One event or two ?

Transition Probabilities

- Transition probabilities depend on how similar the event levels of adjacent *k*-mers are:

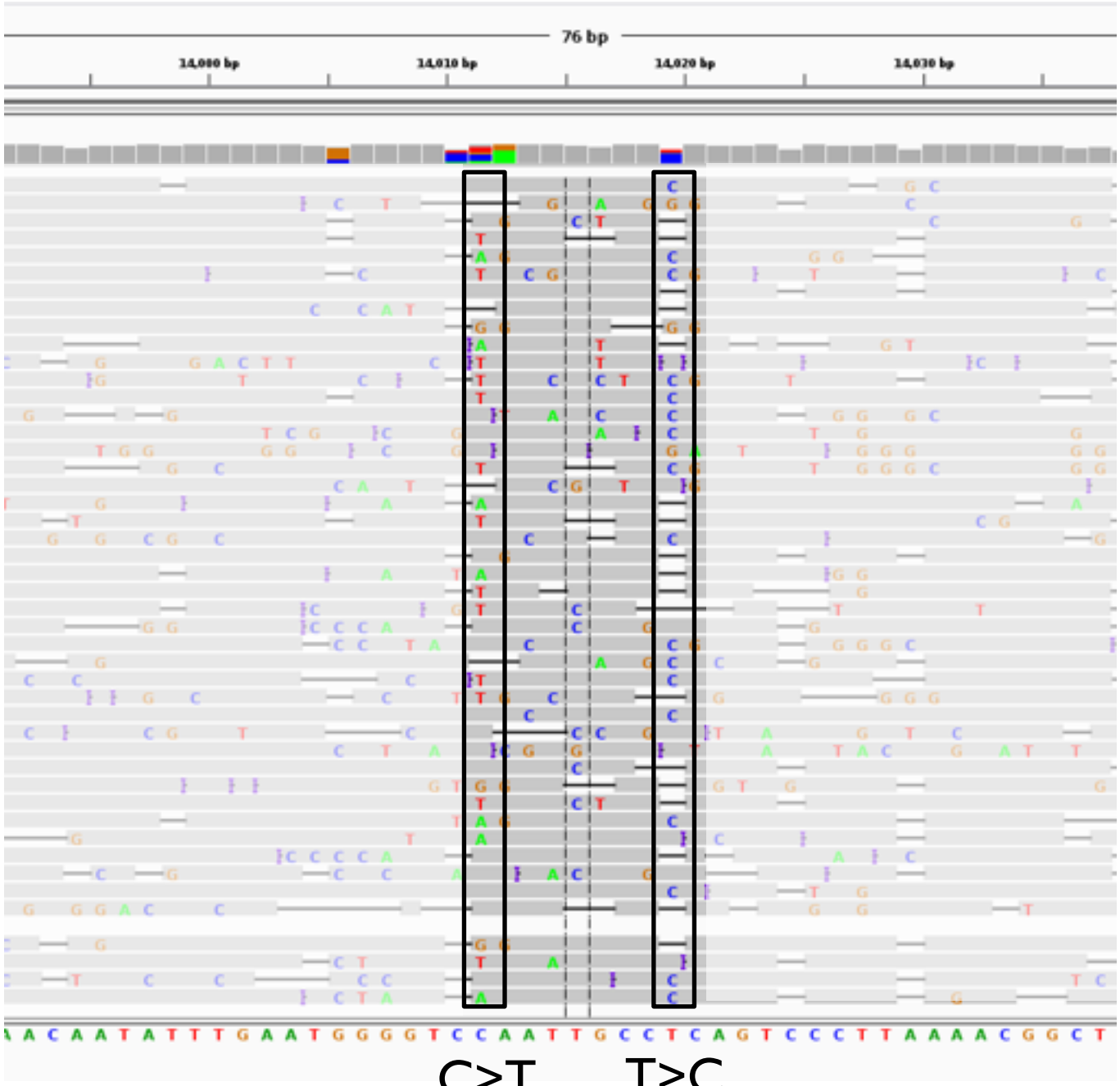


SNP Calling



A>G

SNP Calling



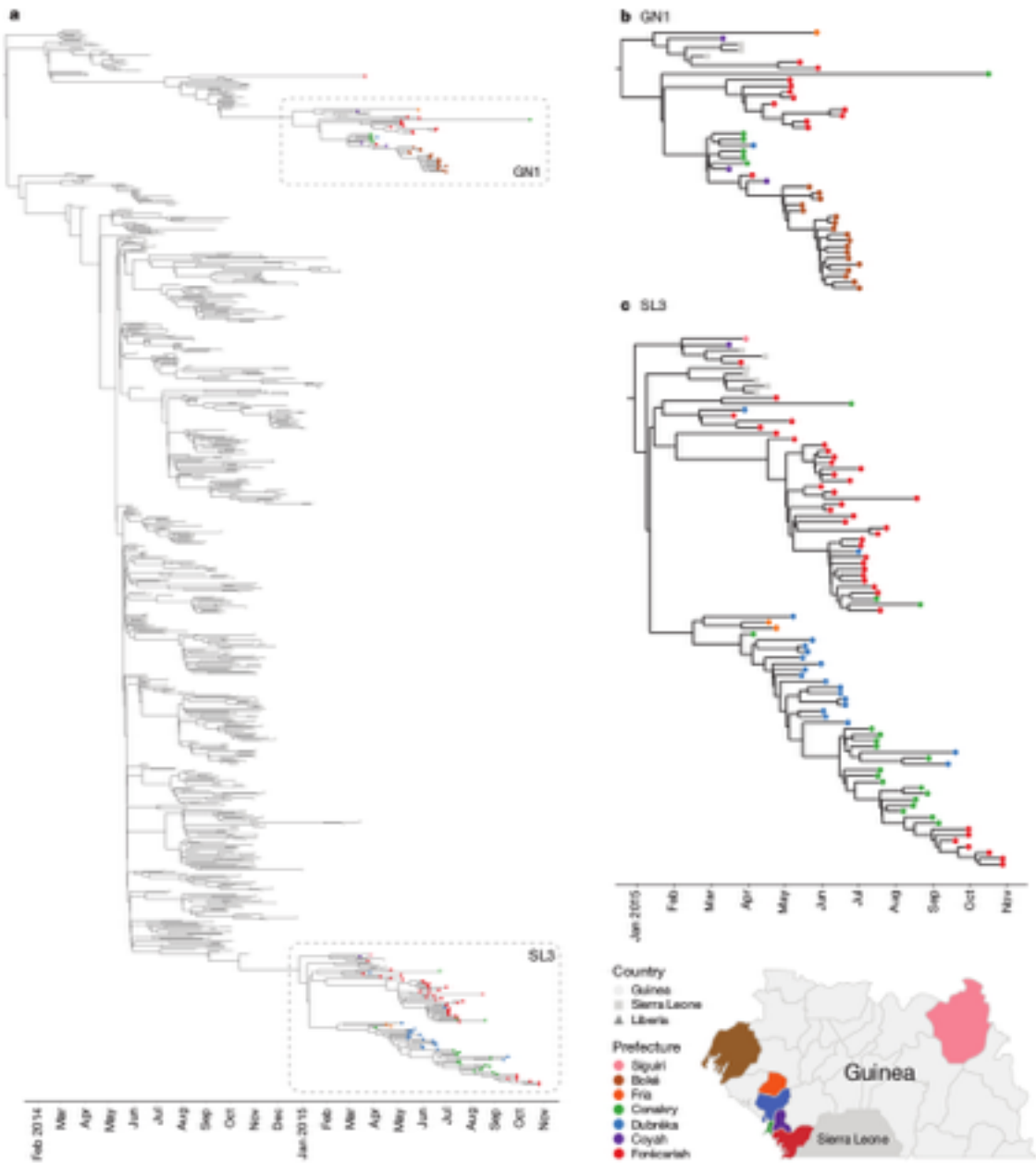
Ebola Genomes

We sequenced and reconstructed 142 Ebola genomes using the MinION and our hidden Markov model-based analysis software (nanopolish)

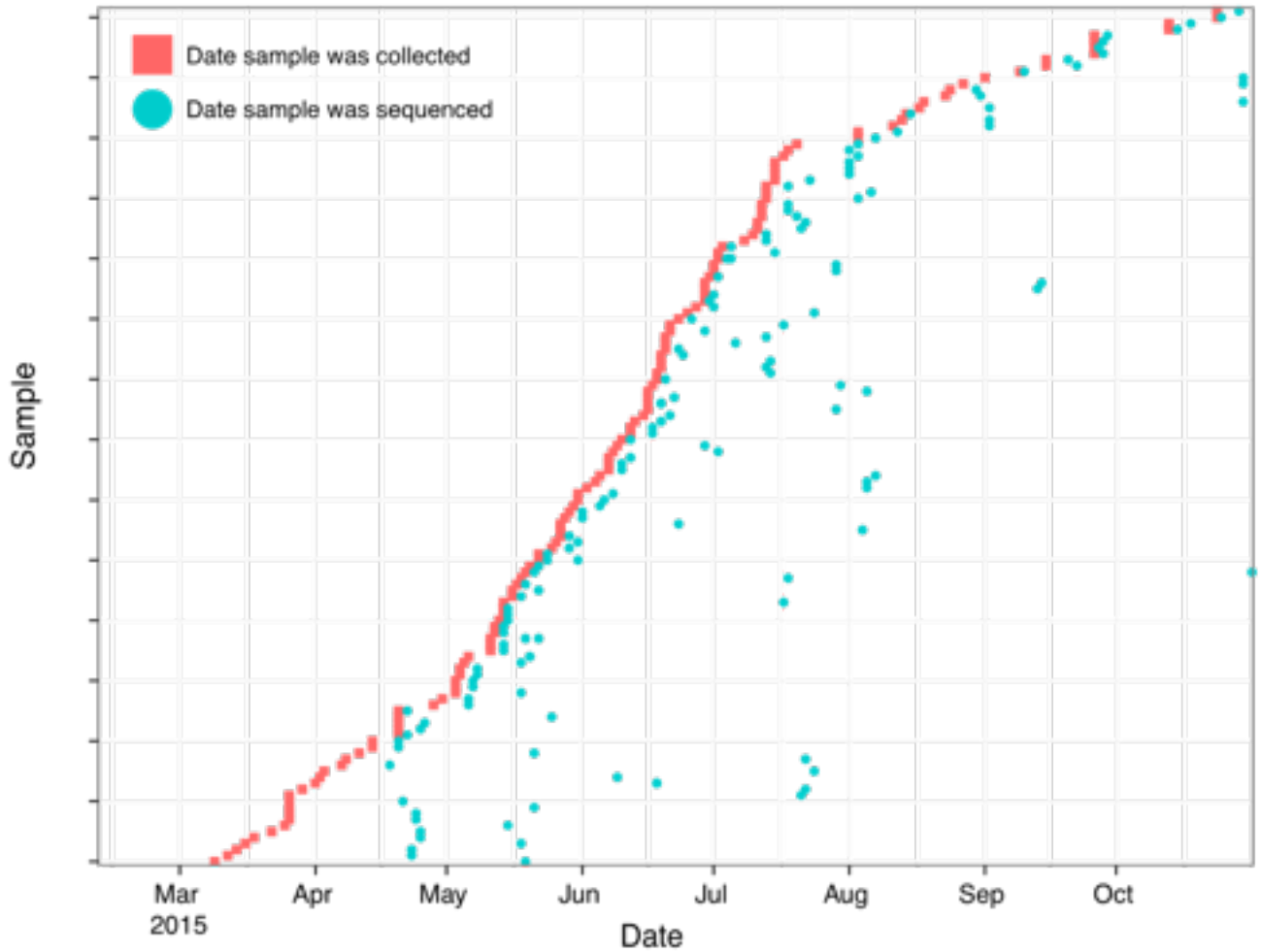
```
Ebola-1     ...AGTAGCCTACGATACTACGATCGACTTA...
Ebola-2     ...AGTAGCCTACGATATTACGATCGACTTA...
Ebola-3     ...AGTAGCCGACGATACTACGATCGACTTA...
Ebola-4     ...AGTTGCCTACGATATTACGATCGACTTA...
                          ...
Ebola-141   ...AGTAGCCTACGATACTACGATCGAGTTA...
Ebola-142   ...AGTAGGCTACGATACTACGATCGACTTA...
```

Next computational challenge: Arrange these genomes into a phylogenetic tree

Ebola Phylogeny



Rapid sequencing



MRC Medical Research Council

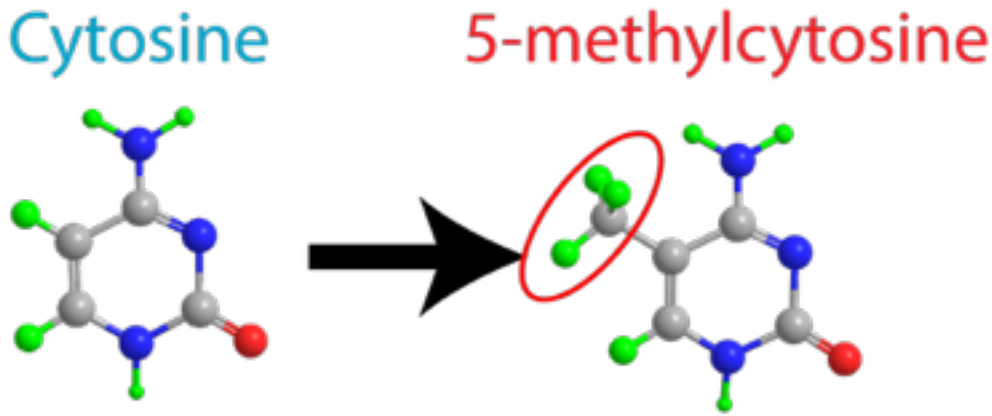
zibraproject.org

ZIBRA

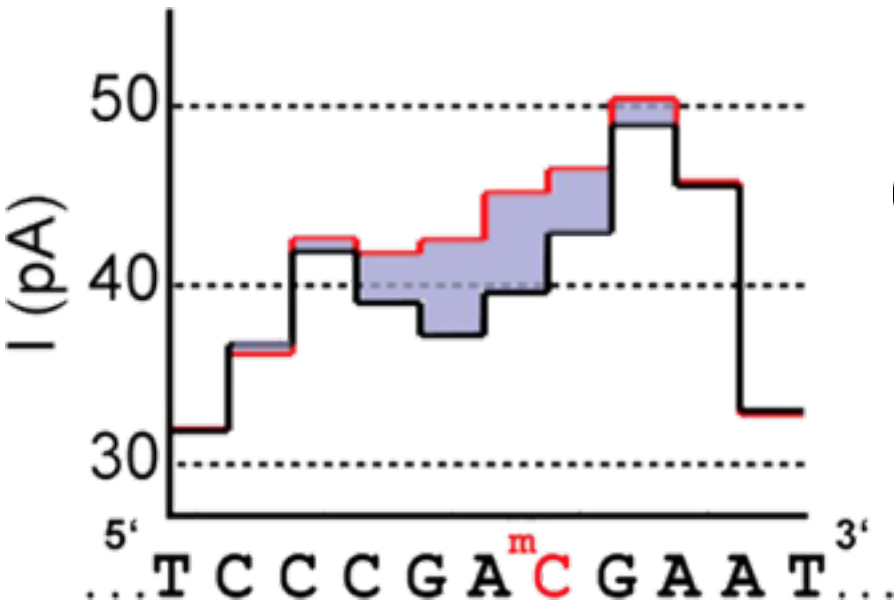
ZIKA IN BRAZIL REAL TIME ANALYSIS


CIÊNCIA NA ESTRADA

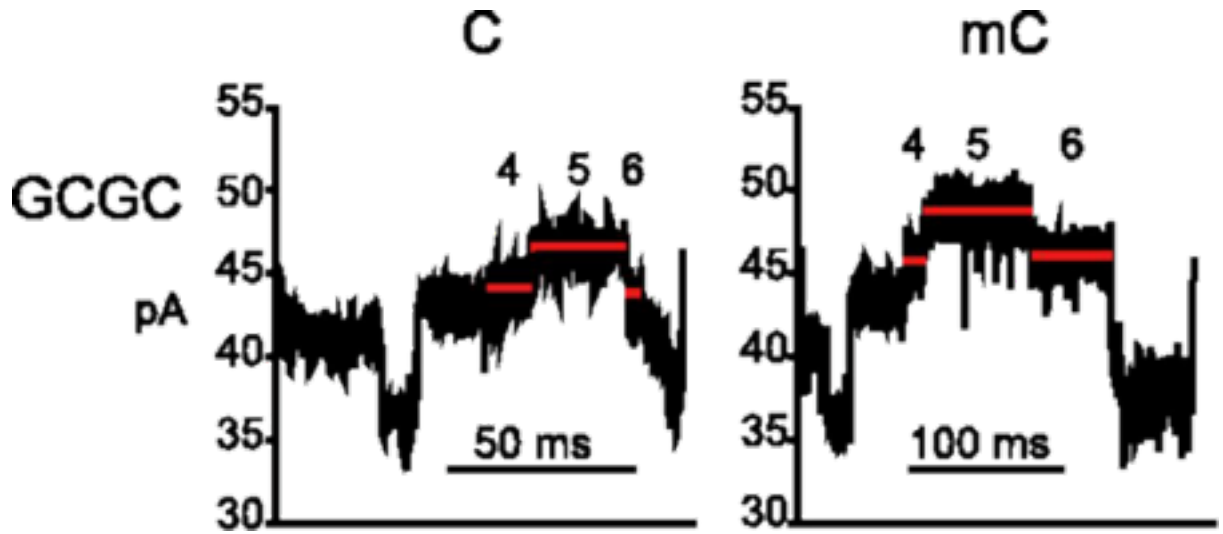
Nanopore Methylation



Laszlo, et al. *PNAS* (2013)



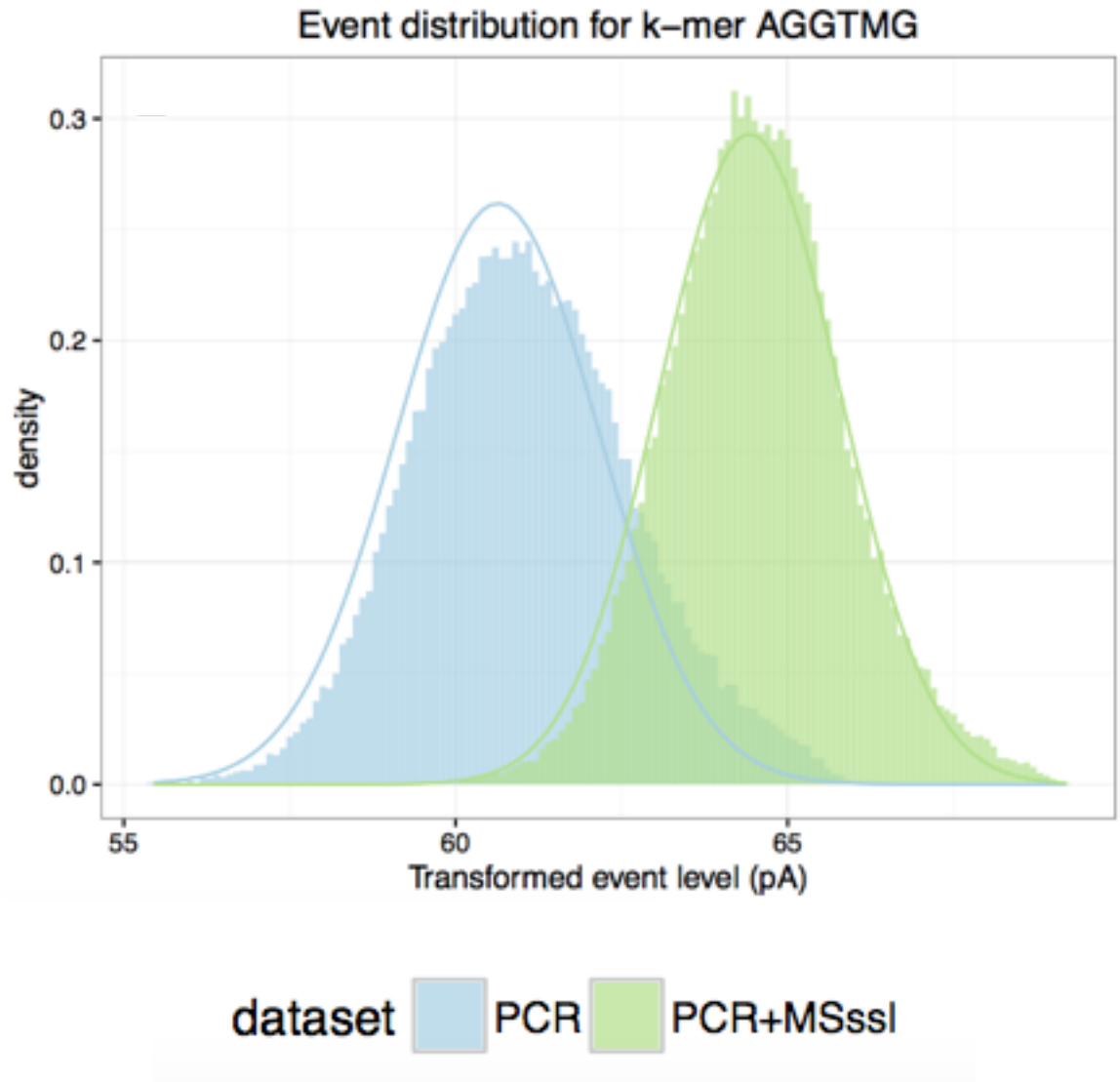
Schreiber, et al. *PNAS.* (2013)



Slide courtesy of Winston Timp

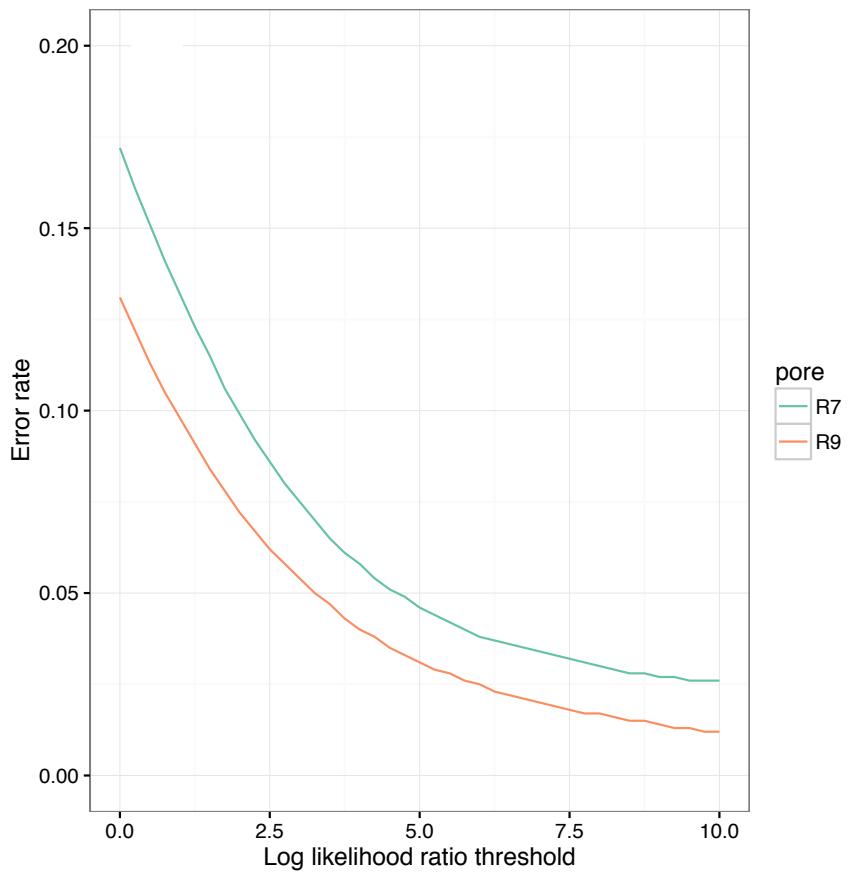
Training Methylation Models

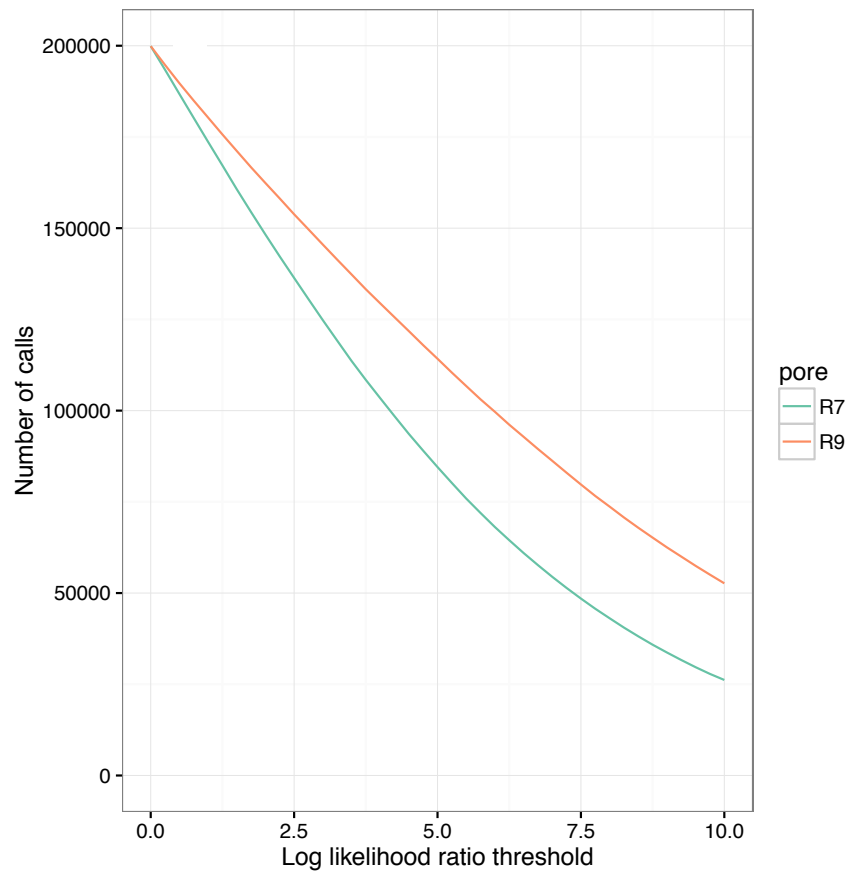
Learn emissions for *k*-mers over expanded alphabet using synthetically methylated DNA (w/ M.SssI)



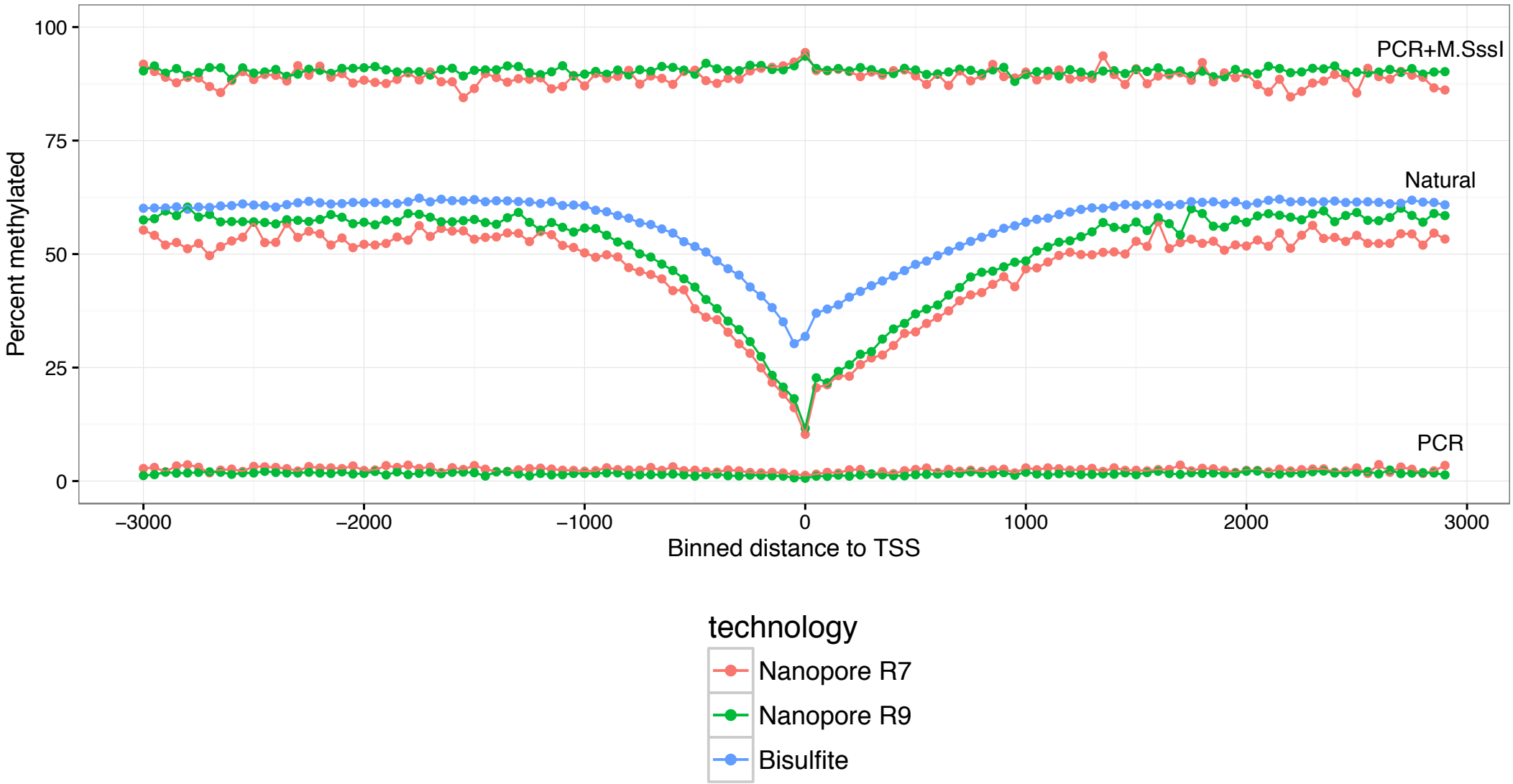
Evaluating Accuracy

- We test our model by calling methylation at randomly sampled sites from the human positive/negative controls
- Accuracy: 82% (R7) 87% (R9)





Low CpG methylation near TSS



Consensus accuracy improvement

Version	Coverage	Percent Identity	# Mismatches	# Indels
R7-SQK005	29X	99.47%	1,363	22,702
R7-SQK006	30X	99.81%	239	8,659
R9-SQK007	30X	99.89%	693	4,467
R9-SQK007	88X	99.95%	325	2,263

* nanopolish after CA/canu assembly

Summary/Outlook

- Overview of portable sequencing & applications
- Data is challenging to work with but similar to classic machine learning problems
- Human genome sequencing now possible (four human genomes were announced last week)

Jonathan Dursi

Matei David

Phil Zuzarte

Nick Loman

Josh Quick

Winston Timp

Rachael Workman

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