A complete bacterial genome assembled de novo using only nanopore sequencing data

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Long Read Sequencing

Oxford Nanopore (this paper), Pacific Biosciences

- Uses:
  - Span large variations
  - Resolve tricky regions (repeats)
  - Speed, portability (ONT)
  - Direct base modification, RNA detection (ONT)

- Weaknesses
  - Error rate (ONT R7.3 chemistry: 15 - 20%)
  - Cost/Yield

Nanopore Data

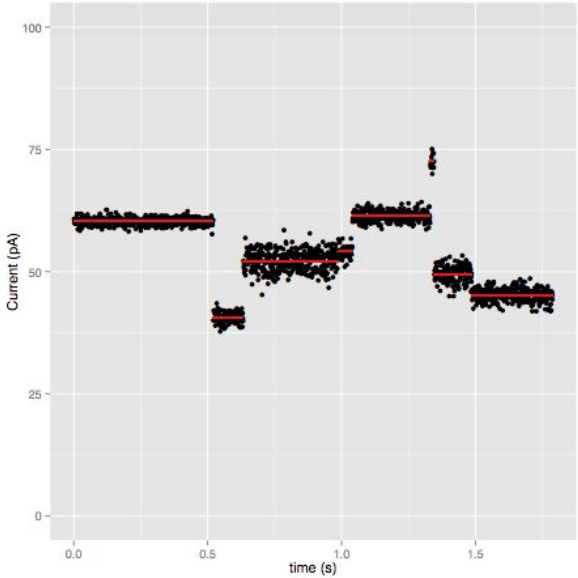


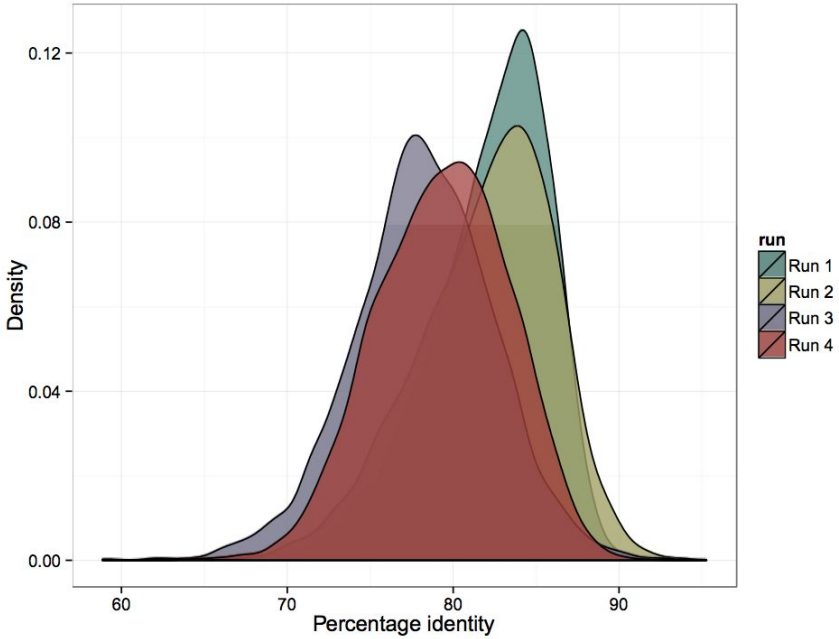
Figure S1: Simulated ideal signal data to illustrate the data that is input into our model. The black points are sampled current levels at a given time. The red lines are the events detected by a feature detection algorithm, which partitions the samples into discrete segments.

- Raw data
  - Ionic current readings as DNA passes through a protein pore
    - 5 bases occupy pore at once (now 6 with R9)
  - 4 kHz sampling rate
- Sequence data
  - Current readings segmented into events (edge detection)
    - Mean, standard deviation, duration
  - Translate into 5-mers
    - Metrichor (RNN, previously HMM)

Assembly of E. *coli* - Data

Four MinION runs:

- E. coli K-12, a genome of 4.6 Mb
- 22,270 reads
- 133.6 Mb ~ 29x coverage



Assembly of E. *coli* - Outline

Primary Assembly

- Compute Overlaps (Daligner)
- Multiple Sequence Alignment (POA)
- Celera Assembler

Polish

- Map reads to primary assembly (bwa-mem)
- Segment and anchor
- Compute consensus

Primary Assembly

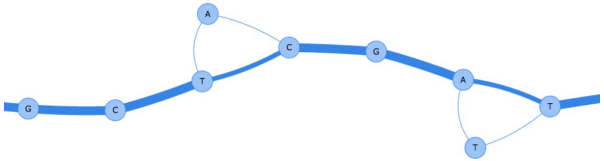
Compute read overlaps with Daligner

Consensus and correction with POA

Read GCTACGAT

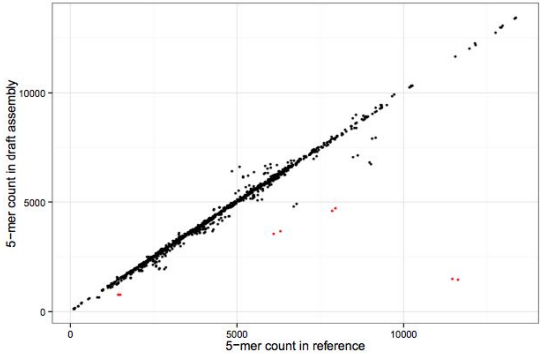
Add overlapping sequence GCTCGAT

Add overlapping sequence GCTCGATT



Assembly with Celera

Draft: 98.5% accuracy



Figures from Jared Simpson (LC 2015)

Polish - Outline

Map reads (bwa-mem with -x ont2d)

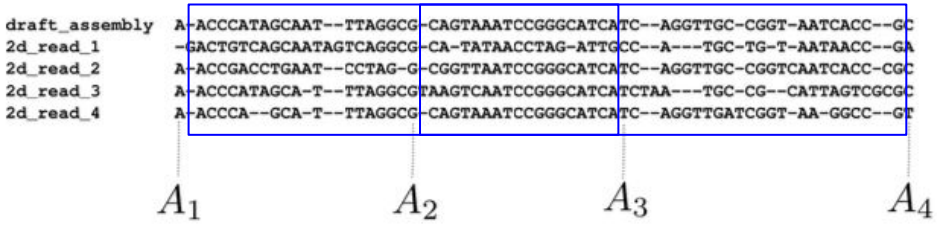
Segment and anchor

Mutate and evaluate

Probabilistic Model:

- Profile HMM
- Train Transitions

Segment and Anchor



Split alignment into 10kb segments that overlap by 200bp

Assign anchor points every 50bp - define a mapping between draft assembly and events in reads

Compute consensus for each segment between two anchors

Segment and Anchor

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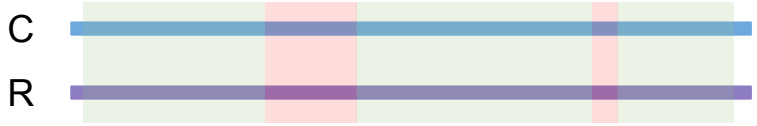
Assign anchor points every 50bp - define a mapping between draft assembly and events in reads

Compute consensus for each segment between two anchors

Mutate and Evaluate

Block replacement method



Add to set of candidates *C*:

Choose best candidate from *C*, repeat until convergence

Mutation method

Candidates are all sequences with edit distance 1 from current consensus

Choose best candidate to be new C, repeat until convergence

Update 10kb segment

Probabilistic Model

Choose best candidate:

$$C' = \underset{S \in \mathcal{C}}{\operatorname{argmax}}\, P(\mathcal{D}|S)$$

$$P(\mathcal{D}|S) = \prod_{k=1}^{r} P(e_{i,k}, e_{i+1,k}, \ldots, e_{j,k}|S, \Theta)$$

Forward Algorithm to compute over all paths in Profile HMM

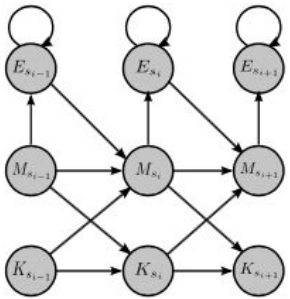


Figure S4: The state structure of the Profile Hidden Markov Model we use to calculate the probability of a sequence of events given a known sequence.

E: 5-mer has emitted an extra event
K: 5-mer has not emitted an event
M: 5-mer has emitted an event

Probabilistic Model - Transition Probabilities

$$t(M_{s_{i-1}} \to K_{s_i}) = f(|\mu_{s_{i-1}} - \mu_{s_i}|)$$
$$t(M_{s_{i-1}} \to E_{s_{i-1}}) = p_{me}(1 - t(M_{s_{i-1}} \to K_{s_i}))$$
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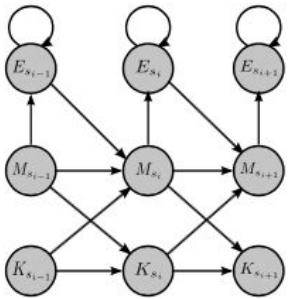

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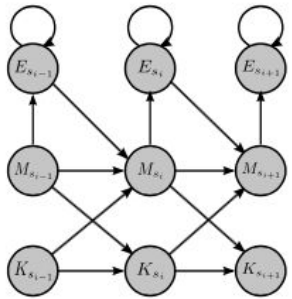

Figure S4: The state structure of the Profile Hidden Markov Model we use to calculate the probability of a sequence of events given a known sequence.

Function (lookup table) of expected event means. Calculated from alignment of reads to previous C using Viterbi, counting the types of transitions.

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Probabilistic Model - Transition Probabilities

$$t(M_{s_{i-1}} \to K_{s_i}) = f(|\mu_{s_{i-1}} - \mu_{s_i}|)$$
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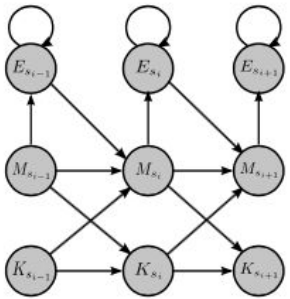

Figure S4: The state structure of the Profile Hidden Markov Model we use to calculate the probability of a sequence of events given a known sequence.

Leverage knowledge that you haven't gone to a K state, and scale.

E: 5-mer has emitted an extra event
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Probabilistic Model - Transition Probabilities

$$t(M_{s_{i-1}} \to K_{s_i}) = f(|\mu_{s_{i-1}} - \mu_{s_i}|)$$
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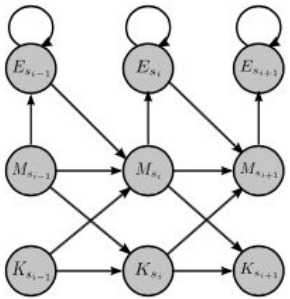

Figure S4: The state structure of the Profile Hidden Markov Model we use to calculate the probability of a sequence of events given a known sequence.

Not K state and not E state

E: 5-mer has emitted an extra event
K: 5-mer has not emitted an event
M: 5-mer has emitted an event

Probabilistic Model - Transition Probabilities

$$t(M_{s_{i-1}} \to K_{s_i}) = f(|\mu_{s_{i-1}} - \mu_{s_i}|)$$
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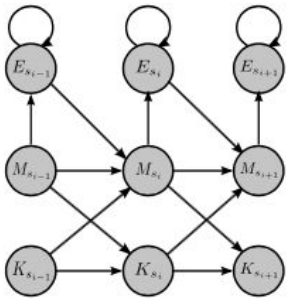

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Trained from sample of reads (Viterbi to previous C)

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M: 5-mer has emitted an event

Probabilistic Model - Transition Probabilities

$$t(M_{s_{i-1}} \to K_{s_i}) = f(|\mu_{s_{i-1}} - \mu_{s_i}|)$$
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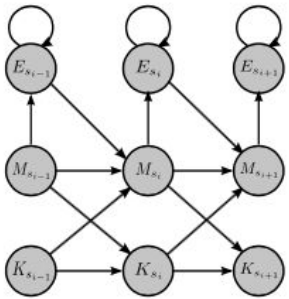

Figure S4: The state structure of the Profile Hidden Markov Model we use to calculate the probability of a sequence of events given a known sequence.

Did not stay in E state

E: 5-mer has emitted an extra event
K: 5-mer has not emitted an event
M: 5-mer has emitted an event

Probabilistic Model - Transition Probabilities

$$t(M_{s_{i-1}} \to K_{s_i}) = f(|\mu_{s_{i-1}} - \mu_{s_i}|)$$
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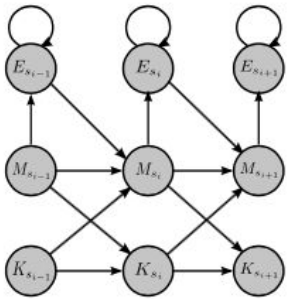

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Like before, not emitting an event is a function of expected current levels

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Probabilistic Model - Transition Probabilities

$$t(M_{s_{i-1}} \to K_{s_i}) = f(|\mu_{s_{i-1}} - \mu_{s_i}|)$$
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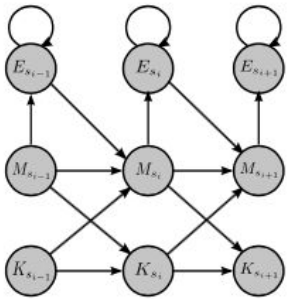

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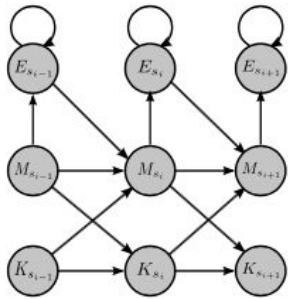
Has not gone to another K state

E: 5-mer has emitted an extra event
K: 5-mer has not emitted an event
M: 5-mer has emitted an event

Probabilistic Model - Emission Probabilities

$$P(e_i|\pi_k = (i, M_{s_j})) = \mathcal{N}(\mu_{s_j}, \sigma^2_{s_j})$$
$$P(e_i|\pi_k = (i, E_{s_j})) = \mathcal{N}(\mu_{s_j}, (v\sigma_{s_j})^2)$$

Emission distributions from M state: known



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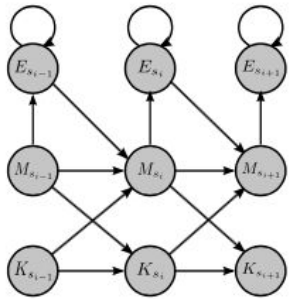
$$P(e_i|\pi_k = (i, E_{s_j})) = \mathcal{N}(\mu_{s_j}, (v\sigma_{s_j})^2)$$

Emission distributions from E state: same as distribution from M, but variance is scaled by v=1.75



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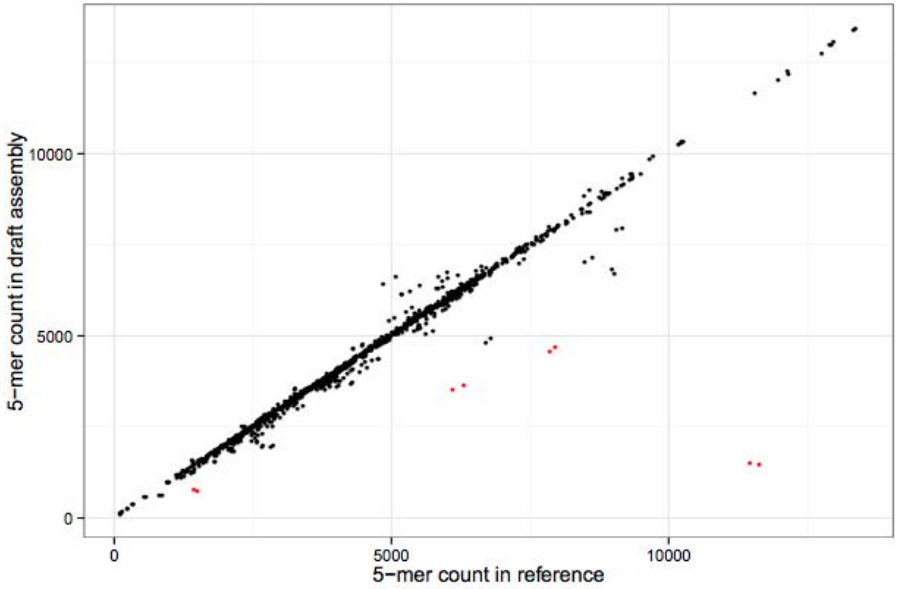
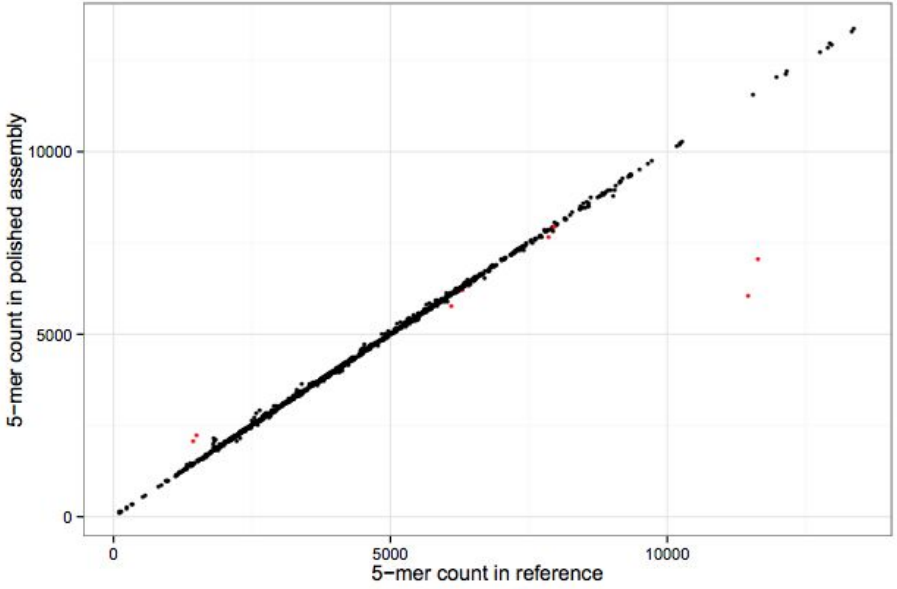
Results

Draft: 98.5% accuracy

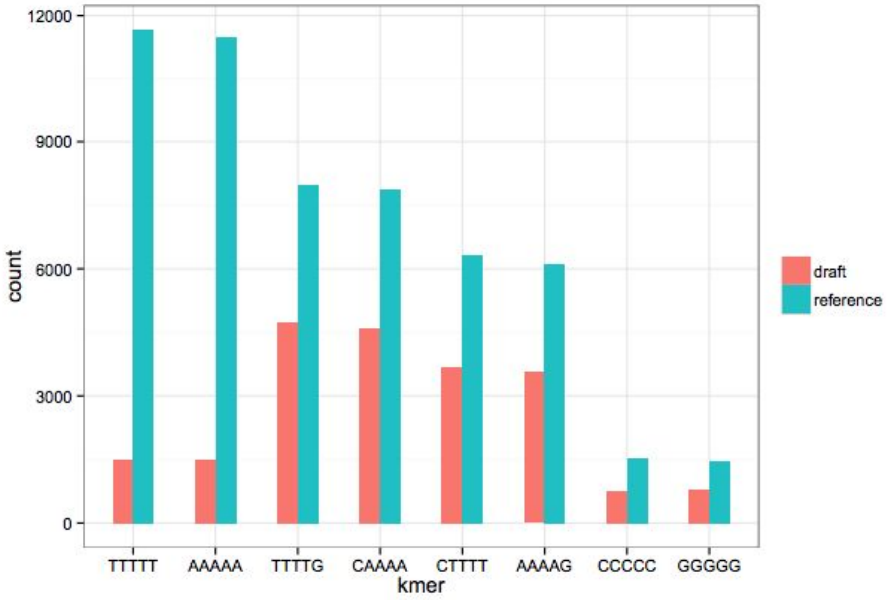


Polished: 99.5% accuracy



Figures from Jared Simpson (LC 2015)

Results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AAAAG
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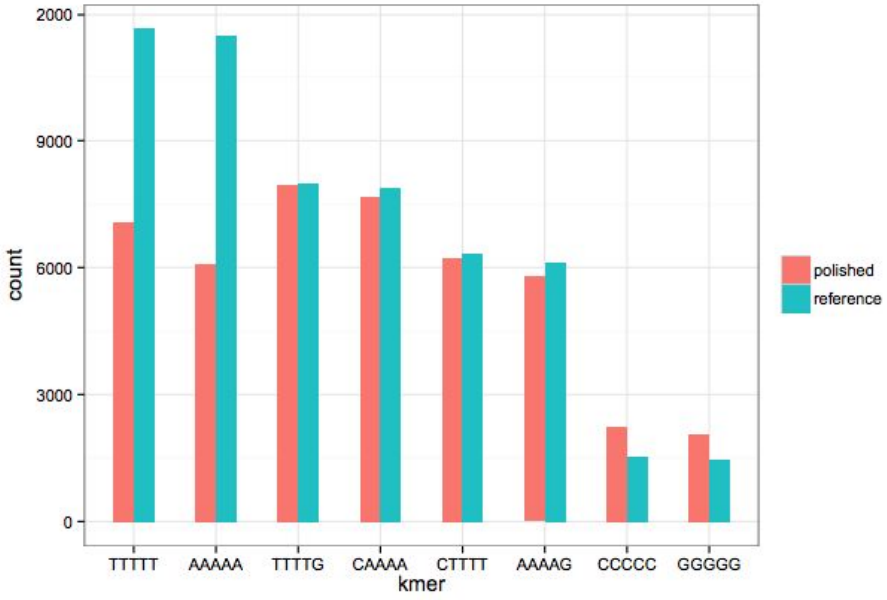
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CCCCC
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kmer
polished
reference