Copy number estimation for de Bruijn graph *de novo* whole-genome shotgun assembly contigs

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Abstract

- High-throughput short reads widely used for reference-free whole genome assembly
- Multiplicity information for partly assembled sequences improves assembly quality
- However, a principled, general solution for multiplicity estimation had been unavailable
- Introduce novel, versatile copy number estimator for haploid & diploid genomes
- Reliable accuracy at resolving multiplicities up to a low maximum
  - Over a range of genome, sequencing, and assembly conditions
- Outperforms & far more versatile than closest alternatives

Background and vocabulary

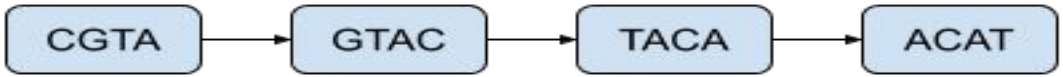
- Sequencing
  - **(high-throughput) short-read:** vs. long-read sequencing
  - **shotgun**: duplication and random fragmentation of genomic segments to generate overlapping reads
- Assembly
  - ***de novo**:* i.e. reference-free, vs. reference-based
  - **de Bruijn graph (DBG)**
- **contig**: partially assembled sequence
- **k-mer**: sequence of k characters (bases)
- **heterozygosity**: difference between homologous chromosomes at the same locus

De Bruijn graph (DBG) assembly

- Dominant paradigm for *de novo* whole-genome high-throughput short read assembly
- 3 stages
  - **k-mer de Bruijn graph construction**
  - **Contig building**
  - Scaffolding

k-mer DBG construction

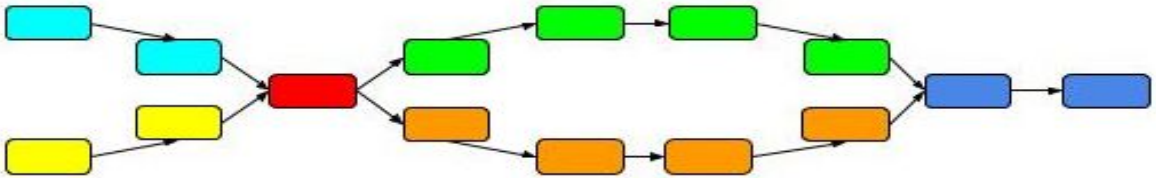
- Extract all distinct k-mers in read dataset
- Represent each distinct k-mer with a node
- Create a directed edge between each pair of nodes with a k-1 suffix-prefix overlap



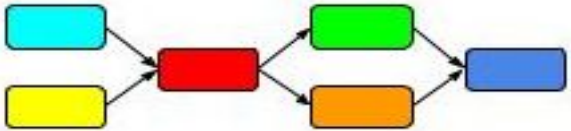
k-mer graph of the sequence "CGTACAT", with k = 4

Contig building

- Nodes are merged along "linear" (non-forking) paths in the DBG
- **Contig**: the resulting string for each path



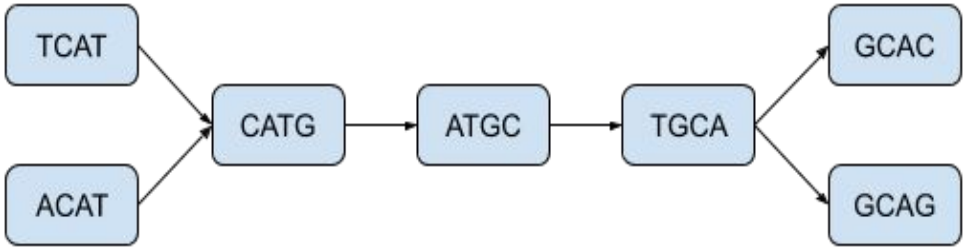
k-mer graph (k-mers omitted): Linear paths, each corresponding to a contig, colour coded



Contig graph: Linear path nodes merged into contigs; each node represents a contig.

Repeat resolution

- The core difficulty in de novo whole-genome assembly
- Which of the 4 possible paths here are correct???

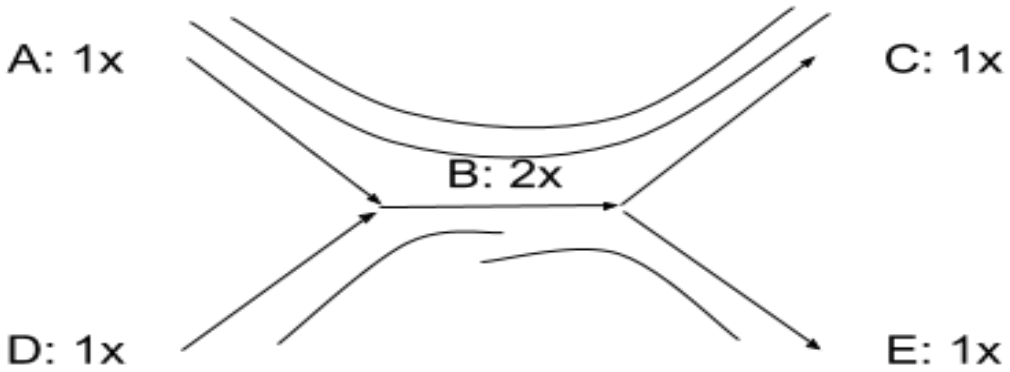


Motivation

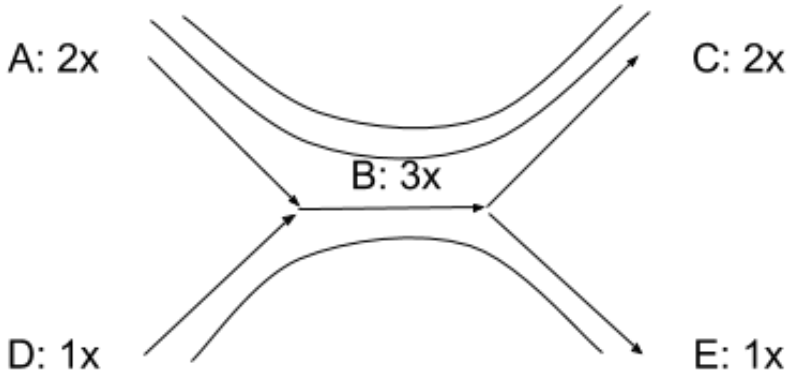
- Copy number with graph topology and long-range information can resolve repeats
  - i.e. resolve ambiguity in correct paths through any node with in- and out-degrees > 1
- Resulting in improvements to:
  - Genome coverage and size estimate accuracy
  - Structural and base-level correctness
  - Contiguity

Repeat resolution: copy number & graph topology

- ABC has sufficient long-range support, but DBE doesn't
- But B has "remaining" copy number 1
- Gives unambiguous support to DBE based on graph topology



Repeat resolution affects genome coverage



Resolved

Paths: ABC x2, DBE x1

Total path length:

$2L_A + 3L_B + 2L_C + L_D + L_E$

Unresolved

Paths: AB, DB, C, E

Total path length:

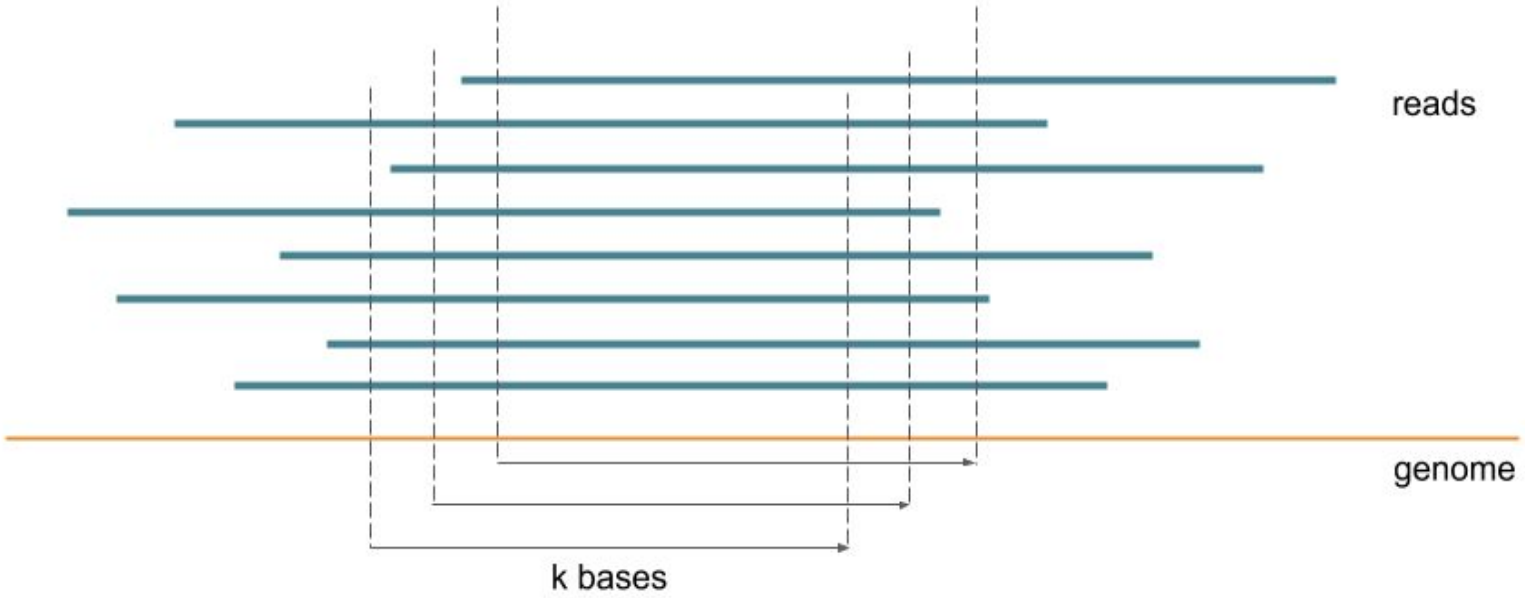
$L_A + 2L_B + L_C + L_D + L_E$

Missing path length:

$L_A + L_B + L_C$

Read coverage / depth

- The number of reads covering a given base or bases, e.g. a k-mer
- **Contig mean k-mer read depth**: mean # reads covering k-mers in a contig



Copy number accounting

- Any given sequence can occur in a genome 0, 1, 2, 3, 4, ... times
- Copy number in haploid genome: equals # occurrences
- Copy number in diploid genome: -> 0, 0.5, 1, 1.5, 2, ...
  - Only consider 0, 0.5, 1, 2, ...

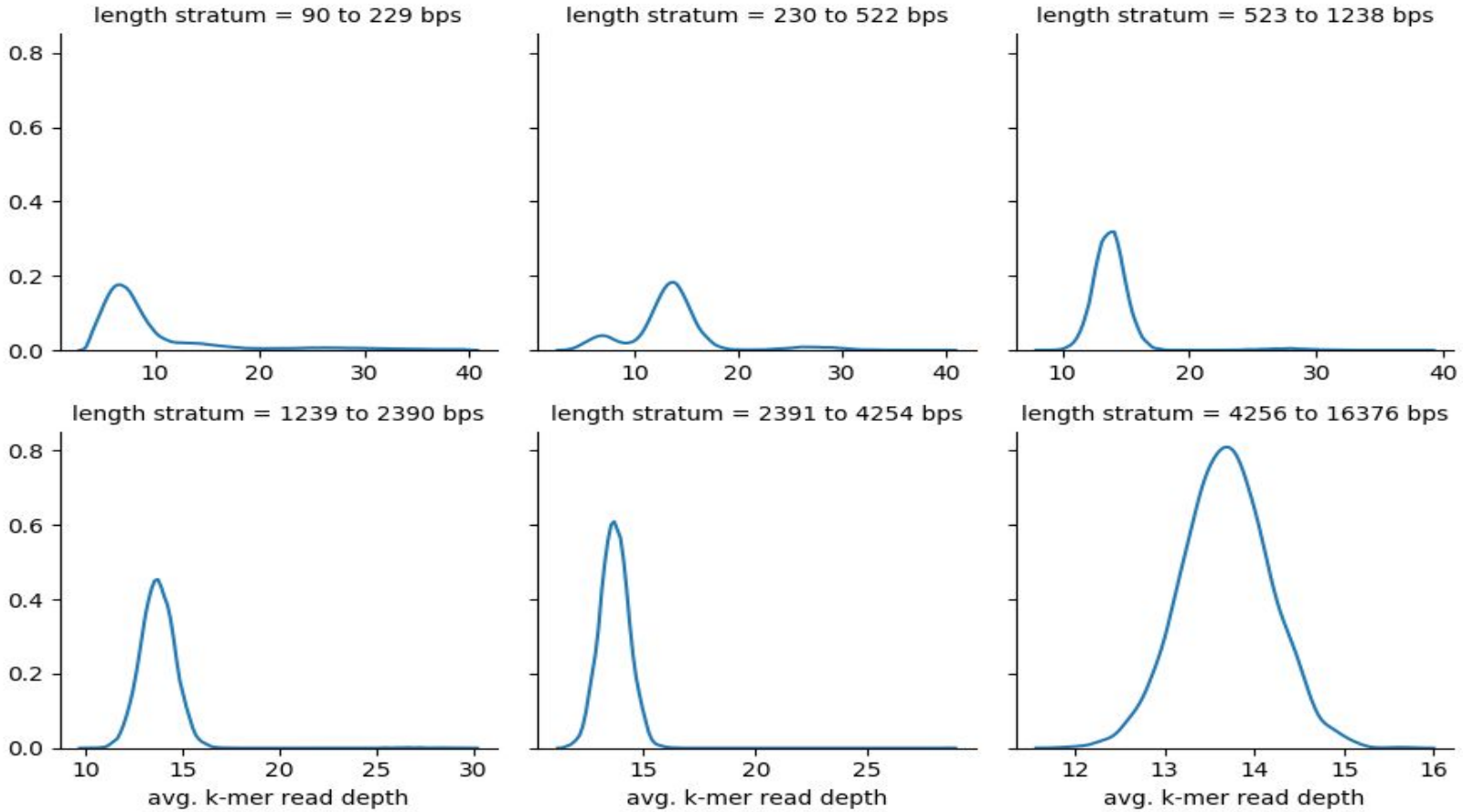
Literature gap

- Accurate, statistically informed copy number estimation in *de novo* WGS assembly
- *Unicycler*: naive usage of read depth in graph-based copy number propagation
- *GenomeScope*: mixture model of k-mer frequency as function of read depth & copy number (≤ 2)
- *Magnolya*: Poisson mixture model of read counts for long OLC contigs
- Shortcomings
  - Dependence on a narrow range of sequencing coverage (*Unicycler*)
  - Inflexible: do not accommodate arbitrary contig lengths (*GenomeScope*, *Magnolya*)

Methods

- Input: Contig sequences, lengths, mean k-mer read depths (coverage)
- **Repeat prevalence decreases rapidly as length increases**
- Partition contigs by length
  - Depth distributions differ across partitions: fit separate model on each
  - Flexible to differing length and depth joint distributions

Contig mean k-mer depth across length strata



Model formulation

- Mean k-mer read depth of sufficiently long contigs is approximately Gaussian
- Use for all contig lengths due to consistency, simplicity, and precedent
- μ: mean k-mer read depth of copy # 1 contigs across entire dataset
- Copy # 1 depth variance, σ^2, specific to each length stratum
- Within stratum, copy number i mean and variance are $i\mu$ and $i\sigma^2$
- Where applicable:
  - High copy numbers modelled in aggregate using gamma distribution
  - Copy number 0 (spurious) contigs modelled using exponential distribution

Model statement

- Within-stratum contig frequency, given mean k-mer depth:

$$f(x;\Theta) = G \cdot \{I_0 w_0 f_{exp}(x;r) + I_{0.5} w_{0.5} f_g(x; 0.5\mu, 0.5\sigma^2) + \sum_{i=1}^{c} w_i f_g(x; i\mu, i\sigma^2) + I_{c+1} w_{c+1} f_\gamma(x;\lambda, s, \theta)\}$$

Where

$\Theta \equiv \{r, \mu, \sigma^2, \lambda, s, \theta, I_0, I_{0.5}, I_\gamma, G, \mathbf{w}\}$ is the parameter set

f_{exp}, f_g, f_γ are the density functions of the exponential, Gaussian, and gamma distributions

r is the rate parameter of the exponential distribution

c is the highest copy number represented by a Gaussian in the stratum

λ, s, θ are gamma distribution location, shape, and scale parameters

$I_0, I_{0.5}$ are indicator variables for copy numbers 0 and 0.5 components

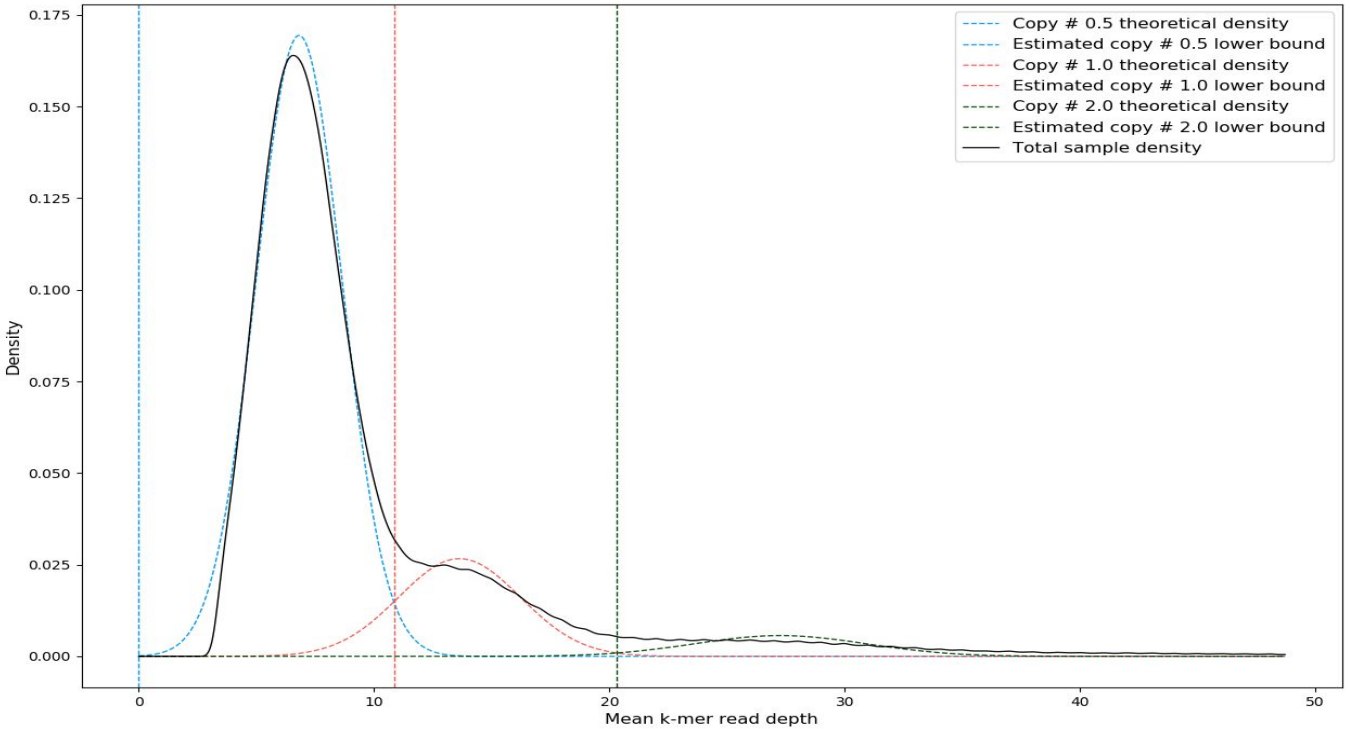
I_{c+1} is an indicator variable for the component aggregating all copy numbers > *c*

$\mathbf{w}$ is the set of component weights

Model implementation

- Longest contigs (almost) exclusively single-copy (copy # 0.5 or 1)
- Empirical depth density with peak very near or at copy # 0.5 or 1 mean
  - 0.5 for sufficiently heterozygous diploid data; 1 otherwise
  - Correct copy # specified by user or chosen using model fit with lower AIC
- First fit model on longest contig stratum for precise estimate of μ
- Iterate over strata in decreasing order of length, constraining μ
- Each contig assigned copy number with highest estimated density given its depth value

Copy number assignment boundaries



Workflow

For contigs in each length stratum:

Preprocess: Compute exact model (copy numbers & probability distributions) to fit

↓

Fit: contig frequency $f(x;\Theta)$, where $x \equiv$ mean contig k-mer read depth, $\Theta \equiv$ model parameters

↓

Post-process: To each contig, assign most probable copy number implied by Θ

Experiments: summary

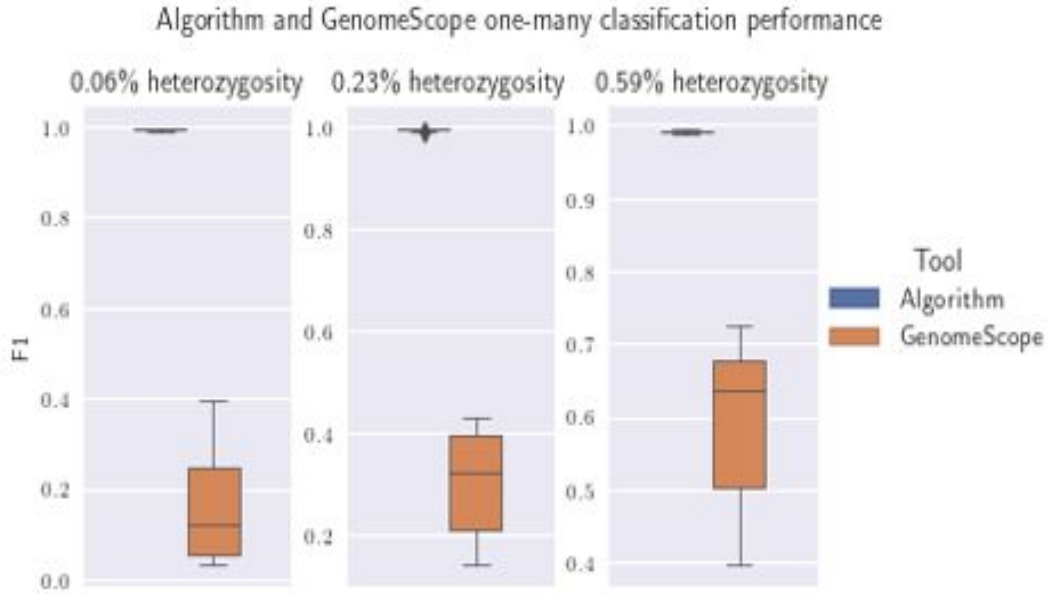
Data type	**Species**	**Heterozygosity rate, approx. (%)**	**Mean per-base read coverage**	***k* values**
Simulated	*E. coli*	N/A	30	60, 65, …, 115, 120
			50	65, 70, …, 120, 125
	C. elegans	0.0609	30	60, 65, …, 115, 120
			50	65, 70, …, 120, 125
		0.2430	30	60, 65, …, 115, 120
			50	65, 70, …, 120, 125
		1.1726	50	65, 70, …, 120, 125
	P. trichocarpa	0.0580	50	65, 70, …, 120, 125
		0.2338		
		0.5852		
	H. sapiens	0.0563	50	65, 70, …, 120, 125
		0.1148		
Real	*E. coli*	N/A	989	40, 45, …, 85, 90

Experiments: details and comparisons

- 2 types of classification performance evaluated:
  - Binary (1-many), multi-class (copy numbers [0, 0.5,] 1, 2+)
- Performance evaluated against:
  - GenomeScope 2.0 for diploid genomes
  - Unicycler for *E. coli* at odd *k* values

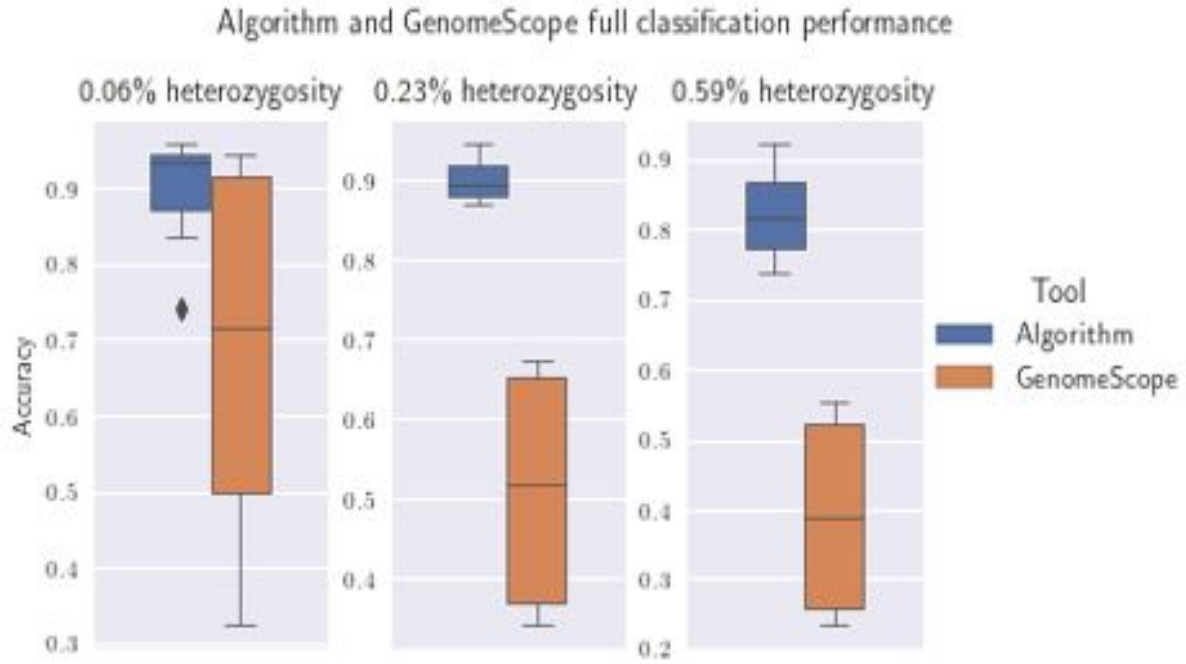
Results example 1: 1-many classification

- *P. trichocarpa*, simulated reads, across *k* values
- Note: GenomeScope sometimes doesn't converge, resulting in fewer data points

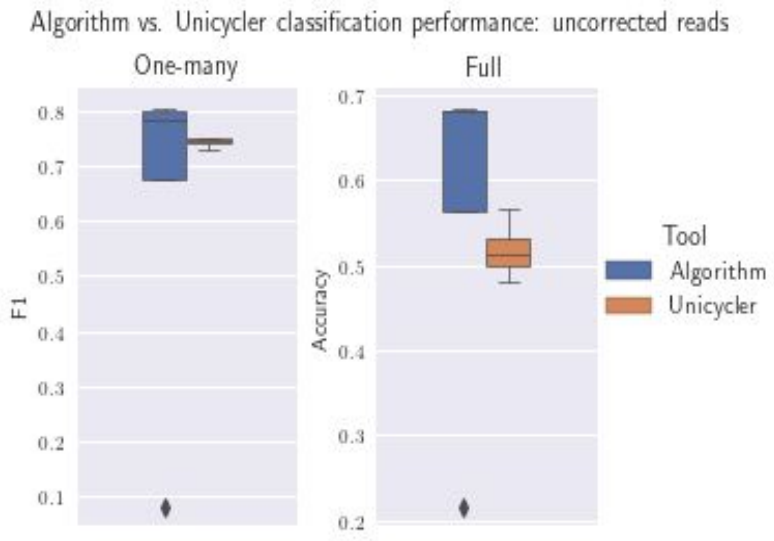


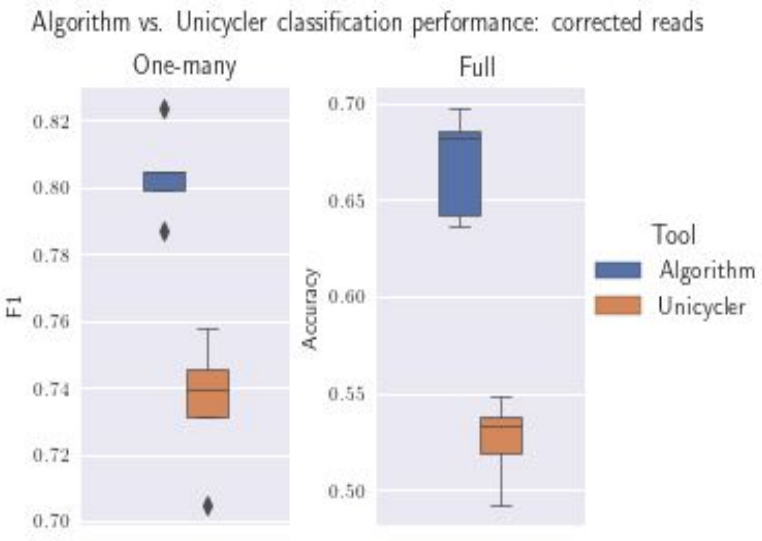
Results example 2: multiclass classification

- *P. trichocarpa*, simulated reads, across *k* values



Results example 3: real *E. coli* data





Discussion

- Performs reliably across a range of conditions
  - Genome characteristics: heterozygosity level, repeat content
  - Sequencing and assembly characteristics: read coverage, *k* value
- Outperforms closest existing alternatives, often by wide margin
- Performs better at 1-many than multiclass classification
- Shows that versatile, reliable DBG contig copy # estimation feasible

Limitations and further work

- Performs poorly on copy number 0: depth & length data inadequate
  - Spurious contigs arise from several error reads having high overlap with correct reads
- Simulated data experiments may overstate performance on real data
  - Real data depth distributions depart from theoretical assumptions underlying algorithm
- Suggests fundamental limitations to using only length and depth information
- Different data, maybe also model, needed for substantial improvement
  - E.g. one or more measures of GC content