Genome Assembly Using de Bruijn Graphs

Biostatistics 666

Previously:
Reference Based Analyses

- Individual short reads are aligned to reference
- Genotypes generated by examining reads overlapping each position
- Works very well for SNPs and relatively well for other types of variant

Shotgun Sequence Reads

- Typical short read might be <25-100 bp long and not very informative on its own

- Reads must be arranged (*aligned*) relative to each other to reconstruct longer sequences

Read Alignment

GCTAGCTGATAGCTAGCTAGCTGATGAGCCCGA

Short Read (30-100 bp)

5’-ACTGGTCGATGCTAGCTGATAGCTAGCTAGCTGATGAGCCCGATCGCTGCTAGCTCGACG-3’

Reference Genome (3,000,000,000 bp)

- The first step in analysis of human short read data is to align each read to genome, typically using a hash table based indexing procedure
- This process now takes no more than a few hours per million reads …
- Analyzing these data without a reference human genome would require much longer reads or result in very fragmented assemblies

Mapping Quality

- Measures the confidence in an alignment, which depends on:
  - Size and repeat structure of the genome
  - Sequence content and quality of the read
  - Number of alternate alignments with few mismatches

- The mapping quality is usually also measured on a "Phred" scale

- Idea introduced by Li, Ruan and Durbin (2008) *Genome Research* **18:**1851-1858

Shotgun Sequence Data

TAGCTGATAGCTAGATAGCTGATGAGCCCGAT

ATAGCTAGATAGCTGATGAGCCCGATCGCTGCTAGCTC

ATGCTAGCTGATAGCTAGCTAGCTGATGAGCC

AGCTGATAGCTAGCTAGCTGATGAGCCCGATCGCTG

GCTAGCTGATAGCTAGCTAGCTGATGAGCCCGA

Sequence Reads

5’-ACTGGTCGATGCTAGCTGATAGCTAGCTAGCTGATGAGCCCGATCGCTGCTAGCTCGACG-3’

Reference Genome

Reads overlapping a position of interest are used to calculate genotype likelihoods and Interpreted using population information.

Limitations of Reference Based Analyses

- For some species, no suitable reference genome available
- The reference genome may be incomplete, particularly near centromeres and telomeres
- Alignment is difficult in highly variable regions
- Alignment and analysis methods need to be customized for each type of variant

Assembly Based Analyses

- Assembly based approaches to study genetic variation
  - Implementation, challenges and examples
- Approaches that naturally extend to multiple variant types

De Bruijn Graphs

- A representation of available sequence data
- Each *k*-mer (or short word) is a node in the graph
- Words linked together when they occur consecutively

Short Sequence

AATCGACAGCCGG

De Bruijn Graph Representation

AATC → ATCG → TCGA → CGAC → GACA → ACAG → CAGC → AGCC → GCCG → CCGG

Iqbal (2012)

Effective Read Depth

- Overlaps must exceed *k*-mer length to register in a de Bruijn graph
- This requirement effectively reduces coverage
- Give read read length L, word length k, and expected depth D …

$$D_{effective} = D\frac{L - k + 1}{L}$$

Cleaning

- De Bruijn graphs are typically “cleaned” before analysis
- Cleaning involves removing portions of the graph that have very low coverage
- For example, most paths with depth = 1 and even with depth <= 2 are likely to be errors

Variation in a de Bruijn Graph

- Variation in sequence produces a bubble in a de Bruijn graph

- Do all bubbles represent true variation? What are other alternative explanations?

Iqbal (2012)

Effective Read Depth - Consequences

- Consider a simple example where L = 100
- With k = 21 …
  - Each read includes 80 words
  - Each SNP generates a bubble of length 22
  - A single read may enable SNP discovery
- With k = 75 …
  - Each read includes 26 words
  - Each SNP generates a bubble of length 76
  - Multiple overlapping reads required to discover SNP

Properties of de Bruijn Graphs

- Many useful properties of genome assemblies (including de Bruijn graphs) can be studied using results of Lander and Waterman (1988)

- Described number of assembled contigs and their lengths as a function of genome size, length of fragments, and required overlap

Lander and Waterman (1988) Notation

- The genome size G
- The number of fragments in assembly N
- The length of sequenced fragments L
  - The fractional overlap required for assembly Θ
- The depth of coverage $c = NL/G$
- Probability a clone starts at a position $\alpha = N/G$

Number of Contigs

$Ne^{-c(1-\Theta)}$

- Consider the probability that a fragment starts is not linked to another before ending

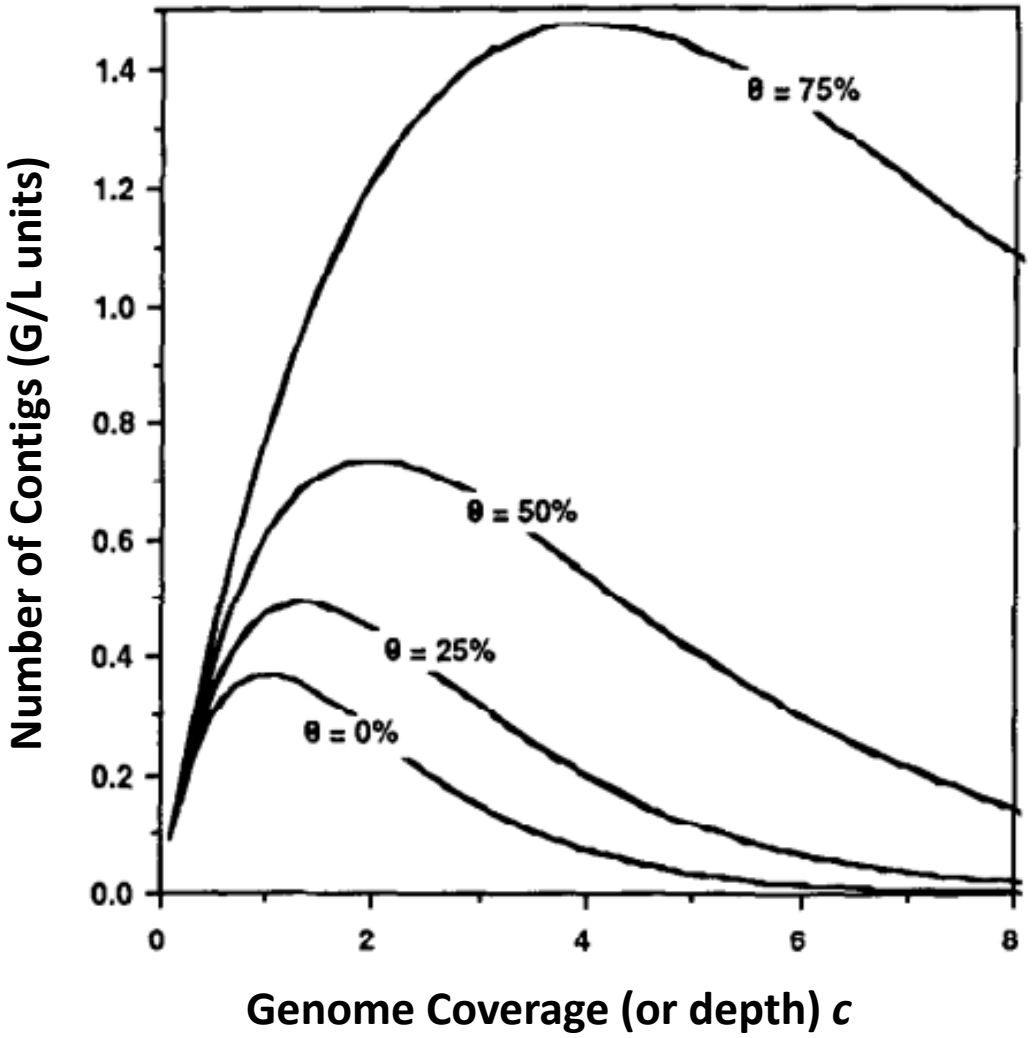
$$\alpha(1-\alpha)^{L(1-\theta)} = \alpha(1-N/G)^{\frac{Gc}{N}(1-\theta)} = \alpha e^{-c(1-\theta)}$$

- Then, the expected number of fragments that are not linked to another is

$$G\alpha e^{-c(1-\theta)} = Ne^{-c(1-\theta)}$$

- This is also the number of contigs!

Number of Contigs



Number of contigs peaks when depth $c = (1 - \alpha)^{-1}$

Contig Lengths

- Probability a fragment ends the contig:

$$e^{-c(1-\theta)}$$

- Probability of contig with exactly *j* fragments:

$$\left(1 - e^{-c(1-\theta)}\right)^{j-1} e^{-c(1-\theta)}$$

- The number of contigs with *j* fragments is:

$$Ne^{-c(1-\theta)}\left(1 - e^{-c(1-\theta)}\right)^{j-1}$$

- How many contigs will have 2+ fragments?

Contig Lengths (in bases)

- The expected contig length, in fragments, is

$$E(J) = e^{c(1-\theta)}$$

- Each fragment contributes X bases …

$$P(X = m) = (1-\alpha)^{m-1}\alpha \text{ for } 0 < m \leq L(1-\theta)$$
$$P(X = L) = (1-\alpha)^{L(1-\theta)}$$

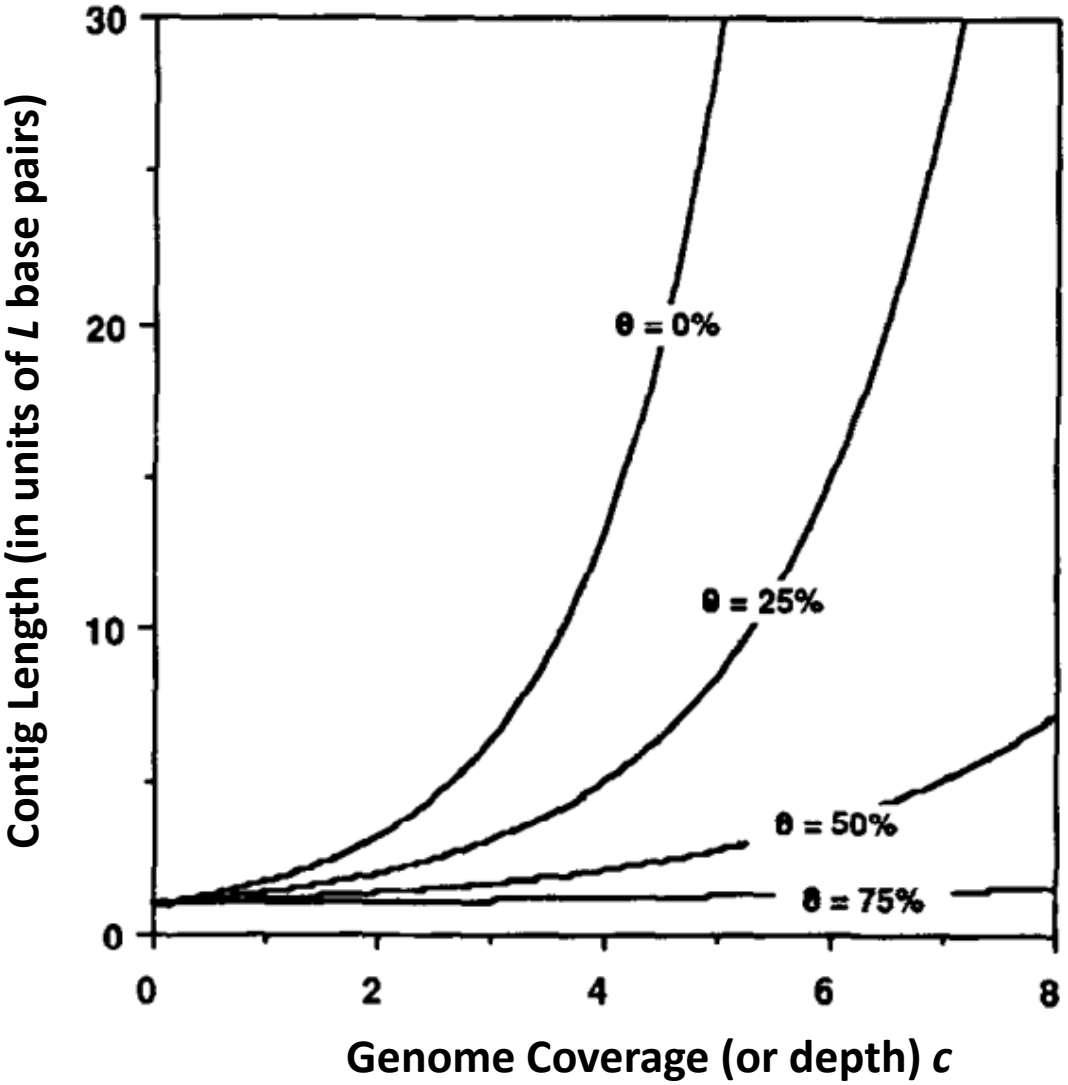
- After some algebra:

$$E(X) = L[\frac{1-e^{-c(1-\theta)}}{c} - \theta e^{-c(1-\theta)}]$$

- The expected contig length in bases is *E(X) E(J)*

$$L[\frac{e^{c(1-\theta)}-1}{c} - \theta]$$

Contig Lengths



Lander and Waterman also studied gap lengths

Enhanced De Bruijn Graphs

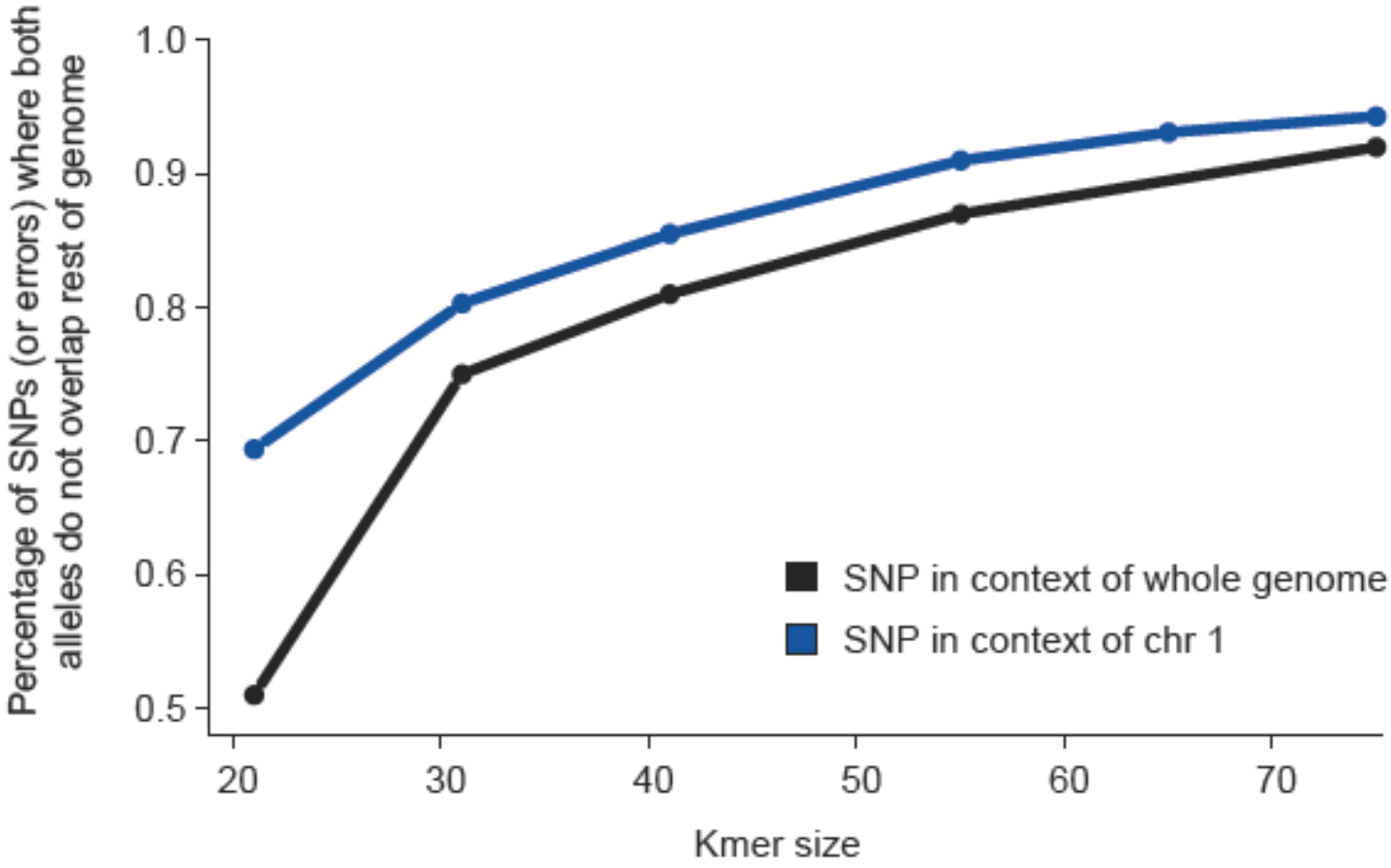
- Usefulness of a de Bruijn graph increases if we annotate each node with useful information
- Basic information might include the number of times each word was observed
- More detailed information might include the specific individuals in which the word was present

Variant Analysis Algorithm 1: "Bubble Calling"

- Create a de Bruijn graph of reference genome
  - Bubbles in this graph are paralogous sequences
- Using a different label, assemble sample of interest
- Systematically search for bubbles
  - Nodes where two divergent paths eventually connect

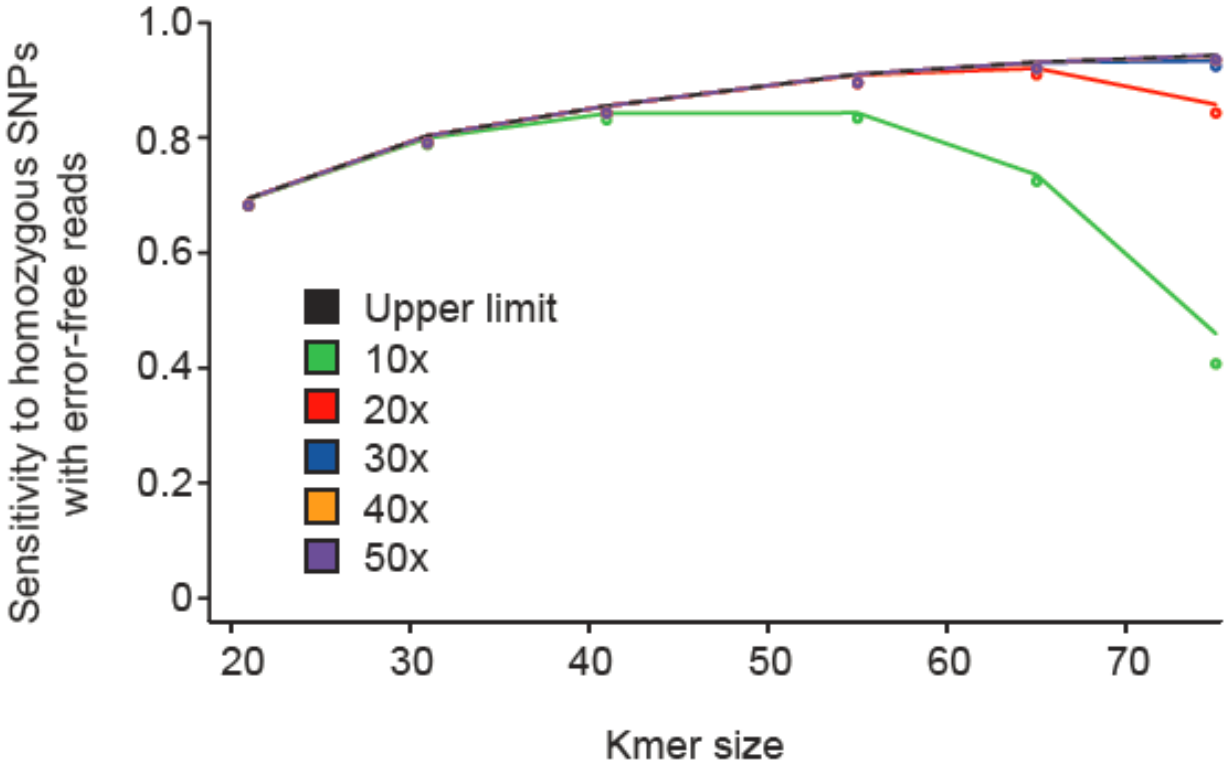
Iqbal (2012)

Word size *k* and Accessible Genome



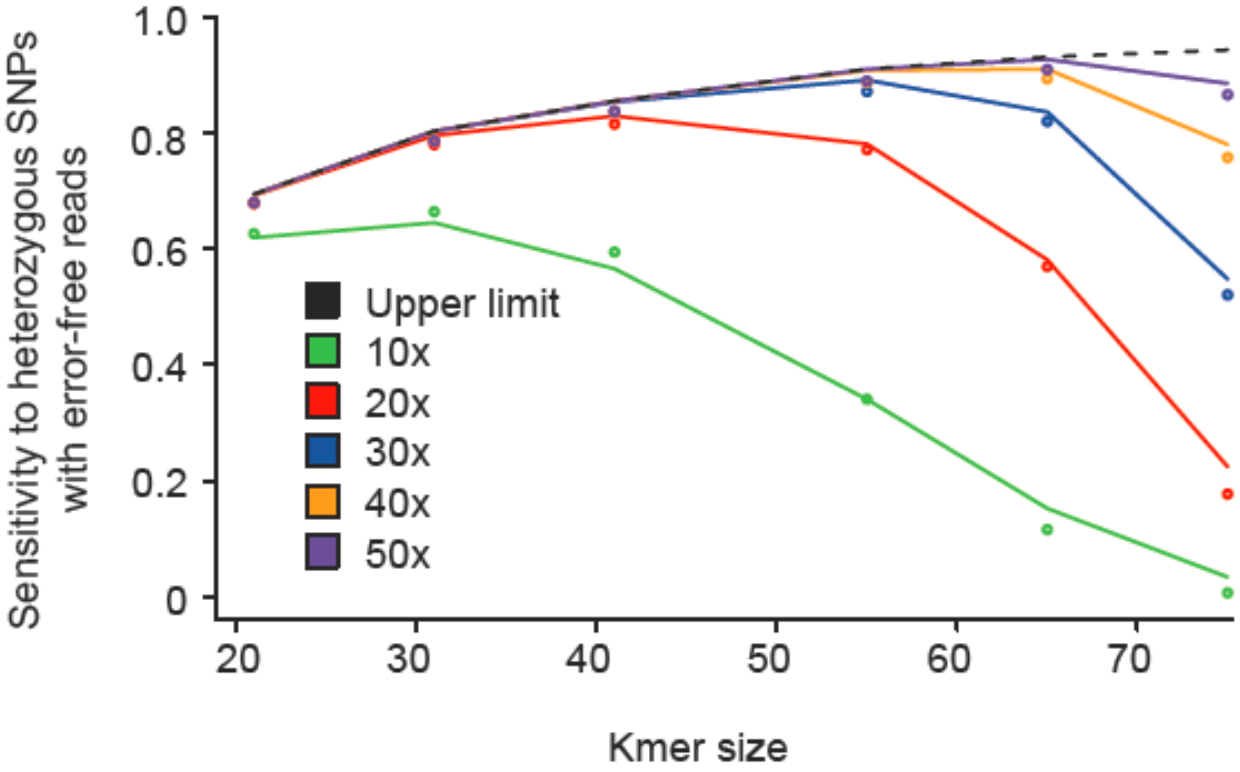
Iqbal (2012)

Power of Homozygous Variant Discovery (100-bp reads, no errors)



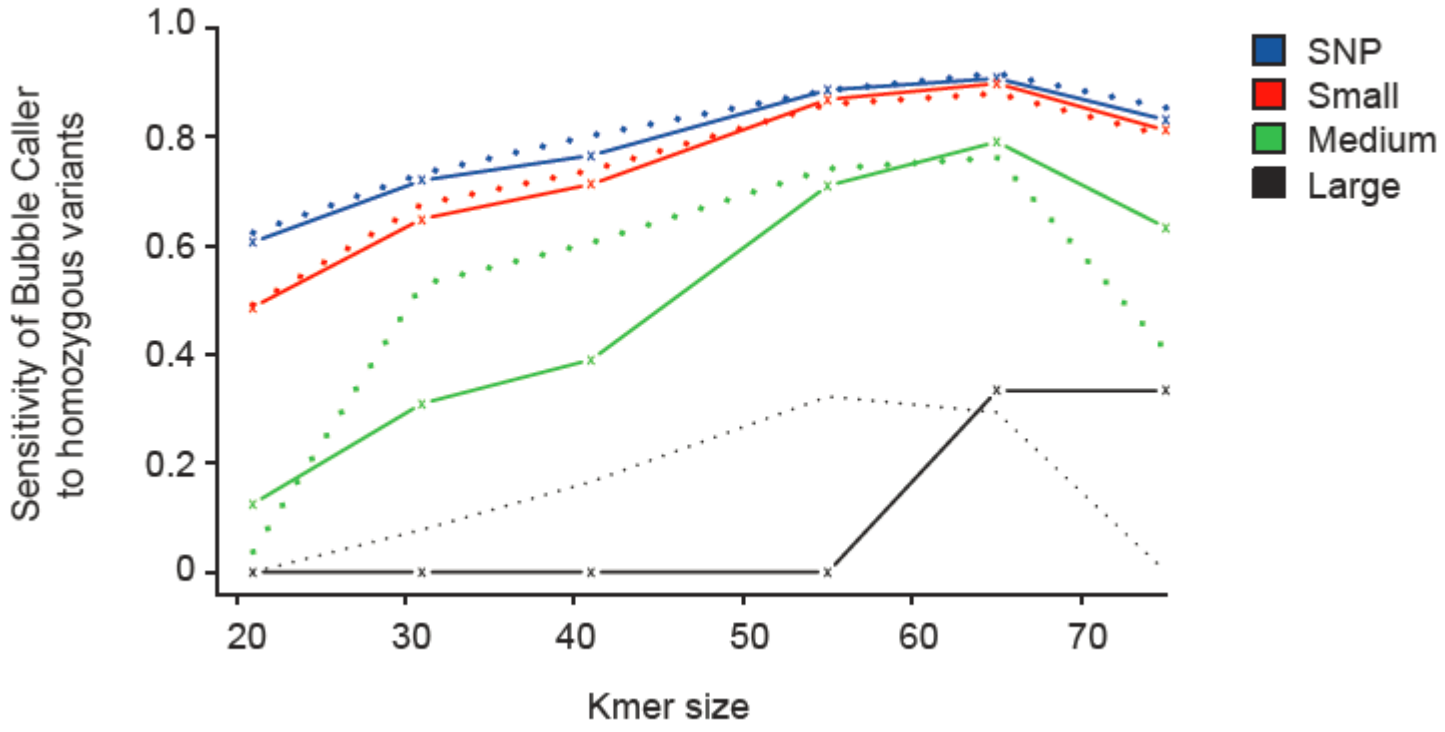
Iqbal (2012)

Power of Heterozygous Variant Discovery (100-bp reads, no errors)



Iqbal (2012)

Power of Homozygous Variant Discovery
(Simulated 30x genomes, 100-bp reads)



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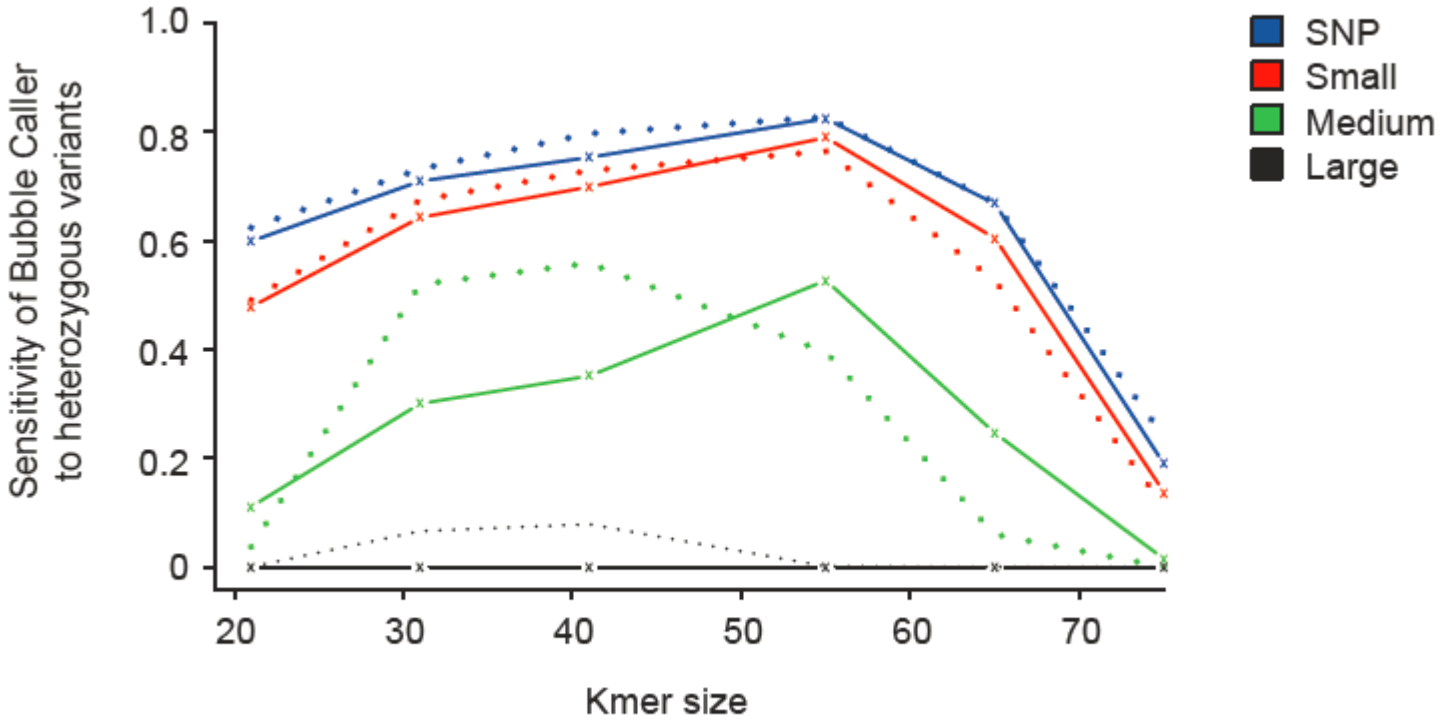
Power of Heterozygous Variant Discovery
(Simulated 30x genomes, 100-bp reads)



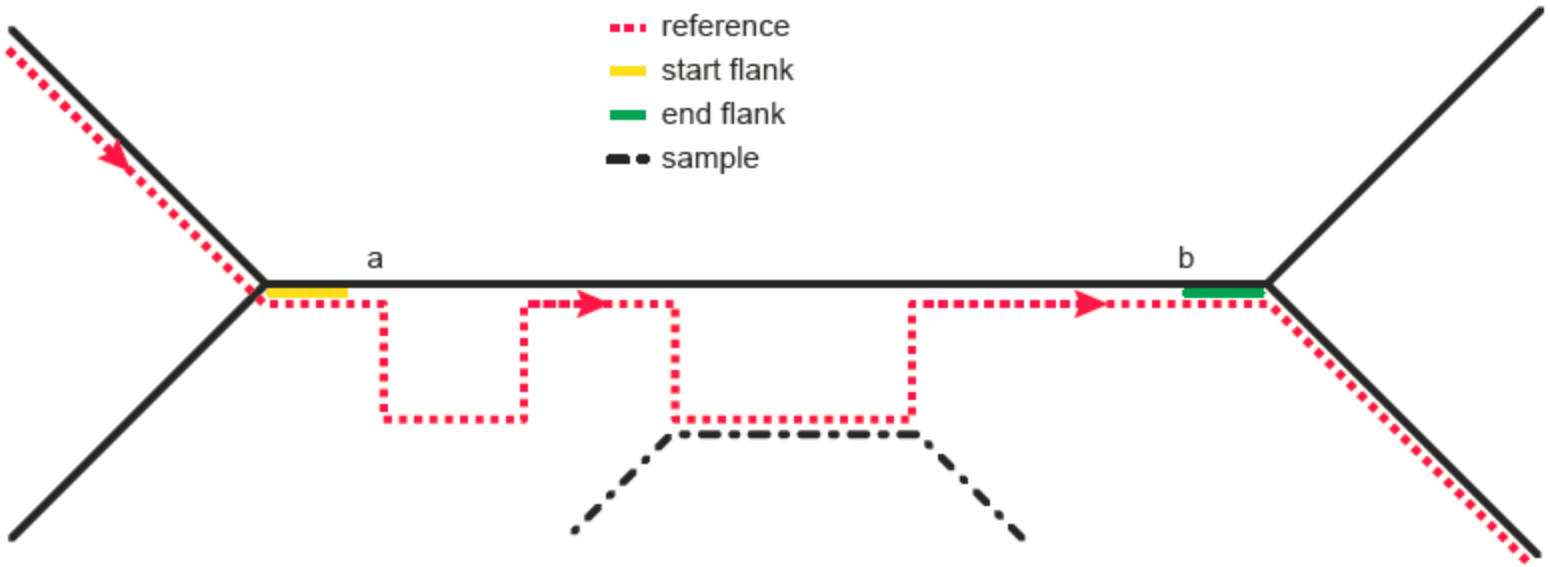
Dotted lines (…) refer to theoretical expectations.
Solid lines (---) refer to simulation results.

Iqbal (2012)

Variant Analysis Algorithm 2: Path Divergence

- Bubble calling requires accurately both alleles
  - Power depends on word length *k*, allele length, genome complexity and error model
  - Low power for the largest events
- Path divergence searches for regions where a sample path differs from the reference
- Especially increases power for deletions
  - Deletion often easier to assemble than reference

Path Divergence Example

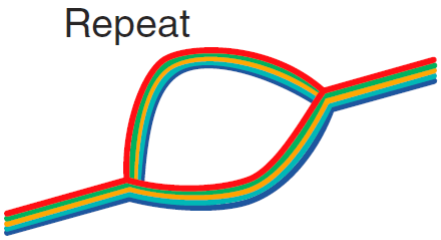


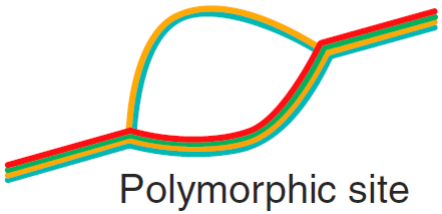
Black line represents assembly of sample.
We can infer a variant between positions a and b,
because the path between them differs from reference.

Variant Analysis Algorithm 3: Multi-Sample Analysis

- Improves upon simple bubble calling by tracking which paths occur on each sample

- Improved ability to distinguish true variation from paralogous sequence and errors





Iqbal (2012)

Classifying Sites

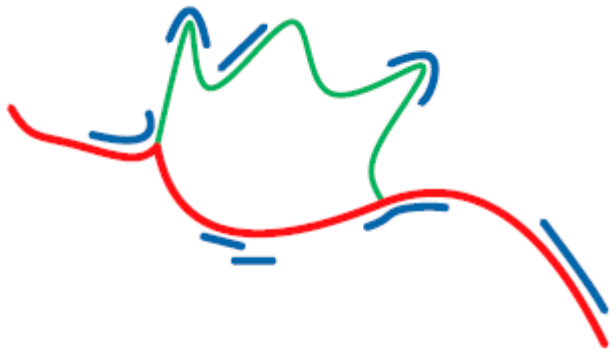
- Evaluate ratio of coverage along the two branches of each bubble and in each individual

- If the ratio is uniform across individuals …
  - **Error:** Ratio consistently low for one branch
  - **Repeat:** Ratio constant across individuals

- If the ratio varies across individuals …
  - **Variant:** Ratio clusters around 0, ½ and 1 with probability of these outcomes depending on HWE

Variant Analysis Algorithm 4: Genotyping

- Calculate probability that a certain number of k-mers cover each path
- To improve accuracy, short duplicate regions within a path can be ignored.
- Allows likelihood calculation for use in imputation algorithms

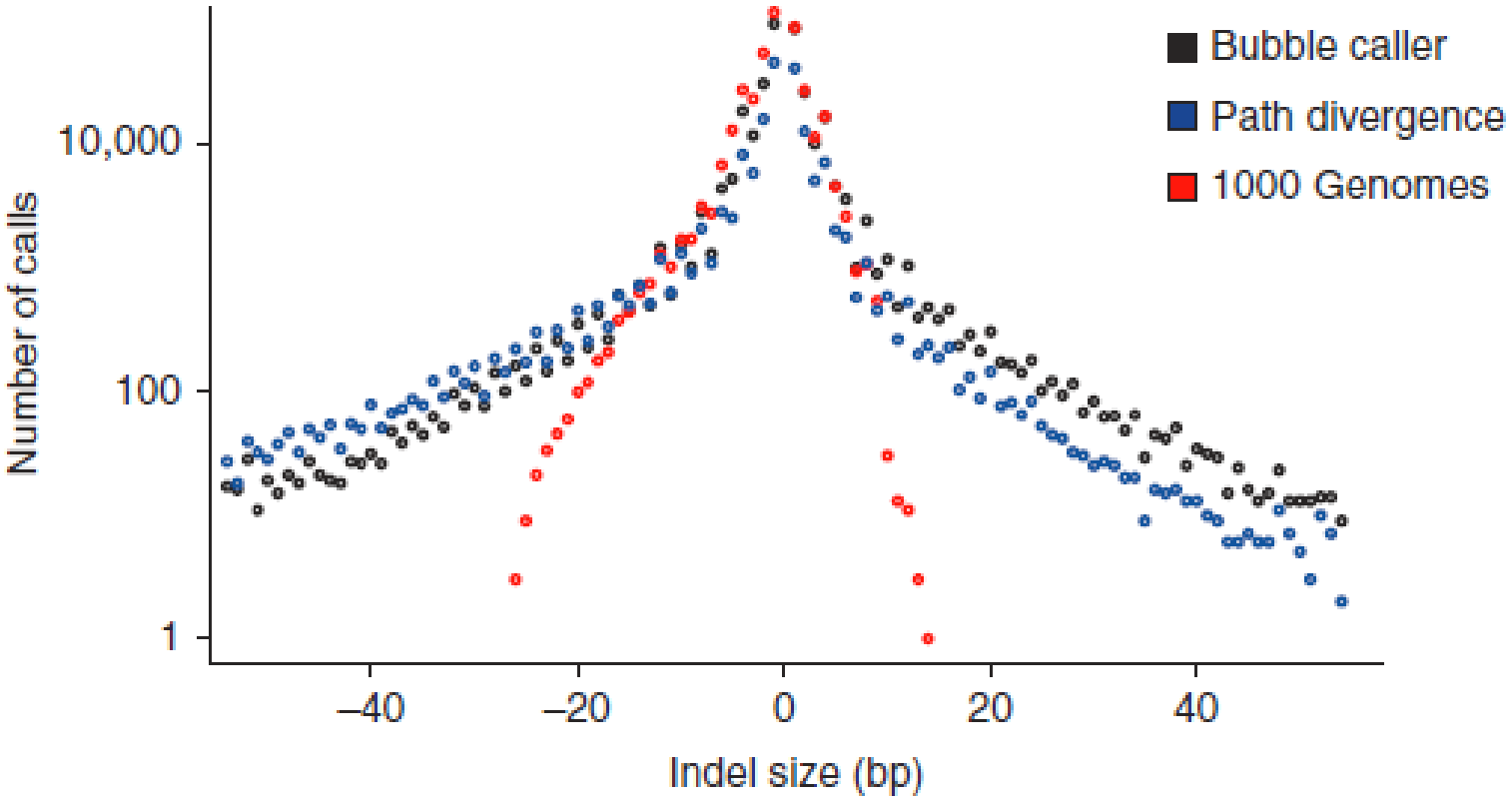


Iqbal (2012)

Example Application to High Coverage Genome

- 26x, 100-bp reads, k = 55
- 2,777,252,792 unique k-mers
  - 2,691,115,653 also in reference
  - 23% more k-mers before cleaning
- 2,686,963 bubbles found by Bubble Caller
  - 5.6% of these also present in reference
- 528,651 divergent paths
  - 39.8% of these also present in reference
- 2,245,279 SNPs, 361,531 short indels, 1,100 large or complex events
  - Reproduces 67% of heterozygotes from mapping (87% of homozygotes)

Comparison to Mapping Based Algorithms



Summary

- Assembly based algorithms currently reach about 80% of the genome
- These algorithms can handle different variant types more conveniently than mapping based approaches
- Incorporating population information allows repeats to be distinguished from true variation

Recommended Reading

- Iqbal, Caccamo, Turner, Flicek and McVean (2012) *Nature Genetics* **44:**226-232

- Lander and Waterman (1988) *Genomics* **2**:231-239