

# CH2: Genome Assembly and Lander-Waterman Statistics

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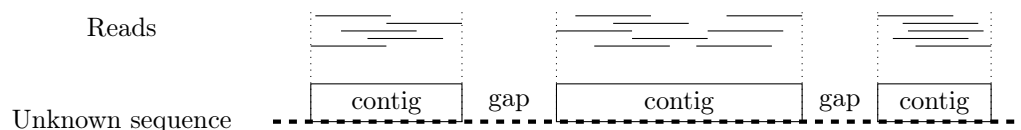
*Reach out to the TAs on Ed for any clarifications or corrections.*

## Overview of Genome Assembly

Genome assembly is the process of inferring a sequence from many fragments. Sophisticated algorithms have been developed to align and merge large numbers of genomic fragments (“reads”) obtained from shotgun sequencing or other experimental methods, with the goal of reconstructing the original genomic sequence which produced the fragments. Sequence assembly was the central challenge of the Human Genome Project, which succeeded in assembling the first reference human genome in 2001.

## General Methods

A schematic for genome assembly is shown below:



Broadly, large numbers of *reads* (short genomic strands) are sequenced and aligned to each other to infer the unknown target sequence which produced them. *Contigs*, or contiguous regions, represent preliminary consensus sequences based on aligned reads; these are eventually merged to produce the final full-length alignment. *Gaps* are regions of the target sequence which cannot be inferred due to insufficient reads.

In addition to the difficulty inherent in determining the orientation and order of reads, there are several other complexities that arise in sequencing which complicate the process of genome assembly. Genomic sequences which are repeated will yield identical reads, which may obscure information about important genomic features. Low sequencing depth or insufficient coverage of the target genome can yield gaps in the inferred sequence, as shown in the schematic above. Read length is also a consideration; short reads may not provide enough information to fully reconstruct the target sequence, while long reads may include a higher proportion of errors due to missequencing of bases.

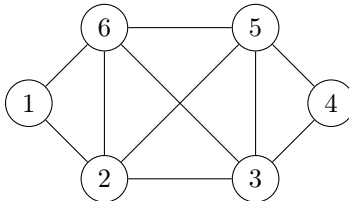
*Sanger sequencing* was the primary method of sequencing genomic material during the first several decades of the field. It was capable of producing reads of several hundred bases in length. *Next-generation sequencing* methods are the standard used today; these produce shorter reads of 100 – 200 base pairs. The Human Genome Project involved a sequencing depth of 8x; human DNA was sheared and cloned into plasmids

for amplification and extracted via restriction enzymes for sequencing and assembly. This process introduced issues of contamination (via plasmid DNA) and correction (via sequencing errors), both of which are significant considerations in any genome assembly workflow.

## Graph Theory

**Defn:** An *Eulerian circuit (cycle)* is a path in a graph which visits edge exactly once and ends at the starting vertex.

**Defn:** An *Eulerian graph* is a graph which contains an Eulerian circuit.



Example of an Eulerian graph

**Thm:** A graph  $G$  is Eulerian if and only if it is a connected graph and all nodes have even degree. If  $G$  is connected and Eulerian, it has at least one Eulerian cycle. If  $G$  has  $r$  connected components and is Eulerian, then it has at least one Eulerian cycle for every component.

## Sequencing Coverage

**Defn:** The sequencing *coverage* (also referred to as sequencing depth) of a genomic sequence is

$$a = \frac{NL}{G}$$

where  $G$  is the length of the target sequence,  $L$  is the read length, and  $N$  is the number of reads.

For the Human Genome Project,  $a = 8$ .

This equation can help inform the approximate number of reads necessary to achieve a desired coverage. For example, to achieve 10x coverage of a bacterial artificial chromosome (BAC) of length 125 kb, the number of 480 bp-long reads required is

$$N = a \left( \frac{G}{L} \right) = (10) \left( \frac{125,000}{480} \right) \approx 2,604.$$

However, since the orientation (i.e., strandedness) of each read is unknown, 10x coverage of both strands would require twice as many reads (in this case, about 5,200).

## Lander-Waterman Statistics

*Lander-Waterman statistics* provide a theoretical means of inferring information about the parameters involved in genome assembly. Proposed by Eric Lander and Michael Waterman (the latter of whom also developed the Smith-Waterman algorithm for local sequence alignment, along with Temple Smith) in the late 1980s, this statistical theory relies on properties of several well-established probability distributions, reviewed briefly below.

## Review of Probability Distributions

### The Bernoulli Distribution

**Defn:** A **Bernoulli trial** is a trial with two outcomes, “success” and “failure”, where  $p$  is the probability of success and  $q = 1 - p$  is the probability of failure. By definition,  $p + q = 1$ .

The random variable  $Y_1$  can be used to model the number of successes for a single Bernoulli trial (i.e., either 0 or 1), with probability mass function

$$p_{Y_1}(y) = p^y(1 - p)^{1-y}$$

$$= \begin{cases} p & \text{if } y = 1 \\ q & \text{if } y = 0 \end{cases}$$

### The Binomial Distribution

**Defn:** A **binomial random variable** gives the number of successes in  $n$  independent Bernoulli trials with the same probability of success ( $p$ ) in every trial.

The random variable  $Y_2$  can be used to model the number of successes (ranging from 0 to  $n$ ), with probability mass function

$$p_{Y_2}(y) = \binom{n}{y} p^y (1 - p)^{n-y}$$

### The Poisson Distribution

**Defn:** A **Poisson distribution** gives the probability of a given number of events happening within a fixed interval, provided that they occur with a constant mean rate  $\lambda > 0$ .

A random variable  $Y_3$  has a Poisson distribution if its probability mass function is

$$p_{Y_3}(y) = \frac{e^{-\lambda} \lambda^y}{y!}$$

where the number of events is  $y = 0, 1, 2, \dots$ . Calculus can be used to show that this is a probability mass function since

$$\sum_{m=0}^{\infty} \frac{\lambda^m}{m!} = e^{\lambda}$$

The Poisson distribution is a particularly relevant one for genome assembly statistics because it is a limiting form of the binomial distribution. If  $n$  (the number of trials) is large and  $p$  (the probability of success per trial) is small, then the quantity  $np$  is of moderate size and the binomial distribution can be approximated as a Poisson distribution with parameter  $\lambda = np$ . This leads to the following result:

**Theorem:** For any  $k \in \{0, 1, \dots, n\}$ , the binomial distribution  $\text{Bin}(k; n, p)$  approaches the Poisson distribution  $\text{Pois}(k; \lambda)$  as  $n \rightarrow \infty$  and  $p \rightarrow 0$ .

*Proof.* Manipulating the binomial probability distribution and recalling that  $\lambda = np \implies p = \frac{\lambda}{n}$ ,

$$\begin{aligned}
\text{Bin}(k; n, p) &= \binom{n}{k} p^k (1-p)^{n-k} \\
&= \frac{n(n-1) \cdots (n-k+1)}{k!} p^k (1-p)^{n-k} \\
&= \frac{np \cdot (n-1)p \cdots (n-k+1)p}{k!} (1-p)^{n-k} \\
&= \frac{np \cdot (n-1)p \cdots (n-k+1)p}{k!} (1-p)^n (1-p)^{-k} \\
&= \frac{n(\frac{\lambda}{n}) \cdot (n-1)(\frac{\lambda}{n}) \cdots (n-k+1)(\frac{\lambda}{n})}{k!} \left(1 - \frac{\lambda}{n}\right)^n \left(1 - \frac{\lambda}{n}\right)^{-k}
\end{aligned}$$

Now, noting that

$$\lim_{n \rightarrow \infty} (n-i) \frac{\lambda}{n} = \lambda \qquad \lim_{n \rightarrow \infty} \left(1 - \frac{\lambda}{n}\right)^n = e^{-\lambda} \qquad \lim_{n \rightarrow \infty} \left(1 - \frac{\lambda}{n}\right)^{-k} = 1$$

it follows that

$$\lim_{n \rightarrow \infty} \text{Bin}(k; n, p) = \frac{\lambda^k}{k!} e^{-\lambda}$$

□

## Additional Distributions

### The Geometric Distribution

The **geometric distribution** models the number of successes up until (but not including) the first failure in a series of Bernoulli trials with probability of success  $p$ . A geometrically-distributed random variable  $Y_4$  has probability mass function

$$p_{Y_4}(y) = p^y (1-p)$$

for  $y = 0, 1, 2, \dots$

### The Exponential Distribution

The **exponential distribution** well-approximates the geometric distribution (note that the exponential distribution is continuous, while the geometric distribution is discrete). An exponentially-distributed random variable  $Y_5$  has probability mass function

$$f_{Y_5}(y) = \lambda e^{-\lambda y}$$

for  $y \geq 0$ . Note that the mean of this distribution is  $\frac{1}{\lambda}$  and that the cumulative distribution function is

$$F_{Y_5}(y) = 1 - e^{-\lambda y}$$

## Poisson Processes

**Defn:** A sequence of events that occur within some interval (of time) is a **homogeneous Poisson process** if the following two conditions hold:

- The occurrence of any event in the time interval  $(a, b)$  is independent of the occurrence of any event in the time interval  $(c, d)$  if  $(a, b)$  and  $(c, d)$  are disjoint (i.e.,  $(a, b) \cap (c, d) = \emptyset$ ).
- There is a constant,  $\lambda > 0$ , such that for any sufficiently small time interval  $(t, t + h)$ ,  $h > 0$ , the probability that a single event occurs within the interval  $(t, t + h)$  is independent of  $t$  and has the formula  $\lambda h + o(h)$ , where  $o(h)$  is a function that is sublinear with respect to  $h$  (i.e.,  $\lim_{h \rightarrow \infty} \frac{o(h)}{h} = 0$ ). Additionally, the probability that more than one event occurs in the interval  $(t, t + h)$  must also be  $o(h)$  (i.e., sublinear with respect to  $h$ ). This property is called *time homogeneity*.

If the conditions above hold, the number of events ( $N$ ) that occur up to time  $t$  has a Poisson distribution with parameter  $\lambda t$  (it is a *homogeneous Poisson process*). These two conditions taken together are often referred to as the “randomness assumption” in other contexts.

## Relevance to Genome Assembly

There are three major questions that Lander-Waterman statistics can help to answer:

1. What is the percentage of the target DNA region covered by the assembly?
2. What is the mean number of contigs in the assembly?
3. What is the mean length of a given contig in the assembly?

### Solution to Question 1

Recall that coverage is defined as  $a = \frac{NL}{G}$ . Assuming that all reads are sampled uniformly at random from the region of interest and that  $G \gg L$  (so that “end effects” at the boundaries may be disregarded), the left-hand ends of the reads are independently and uniformly distributed over the interval  $[0, G]$ . Then each left-hand end falls within an arbitrary interval  $[x, x + h)$  with probability  $\frac{h}{G}$ , and the distribution of the number of left-hand ends falling in this interval has a binomial distribution with (Bernoulli) probability of success  $p = \frac{h}{G}$  and mean  $\frac{Nh}{G}$ . If  $N$  is large and  $h$  is small, this distribution can be approximated by a Poisson distribution with parameter  $\lambda = Np = \frac{Nh}{G}$  (the mean of the aforementioned binomial distribution).

Let  $Y$  be a random variable equal to the number of fragments whose left-hand ends fall within a particular interval of length  $L$  to the left of a randomly-chosen point. Then  $Y$  has a Poisson distribution with mean  $\lambda = a$  (the coverage). This means that the probability of at least one read covering this interval is

$$1 - \mathbb{P}(Y = 0) = 1 - e^{-a} \cdot \frac{a^0}{0!} = 1 - e^{-a}$$

The table below summarizes the relationship between coverage (sequencing depth) and percent of target sequence covered with the technology available at the time of the original Human Genome Project:

Coverage ( $a$ )	% target region
2	86%
4	98%
6	99.8%
8	99.97%
10	99.995%
12	99.999%

## Solution to Question 2

Each contig has a unique rightmost read by definition. The probability that no reads cover an arbitrary region of length  $L$  is  $e^{-a}$  as shown above. This quantity is therefore the probability that no other read has a left-hand end within the length  $L$  defined by a given read; i.e., the probability of “success” ( $p$ ) for a Bernoulli trial. Modeling the number of unique contigs as a binomial distribution (where “success” indicates that a given read is the rightmost read of a unique contig), it follows that the mean number of contigs is

$$Np = Ne^{-a}$$

A summary of values is given in the table below (note that  $N \propto a$ ):

Coverage ( $a$ )	mean # contigs
0.5	60.7
0.7	69.5
1.0	73.6
1.5	66.9
2.0	54.1
3.0	29.9
4.0	14.7
5.0	6.7
6.0	3.0
7.0	1.3

Note that as coverage increases, the mean number of contigs increases to a point and then begins to decrease. This is because at low coverage additional reads will cover “new” areas of the region (thus generating additional contigs), while at higher coverage additional reads will cause existing contigs to merge together.

## Genome Assembly

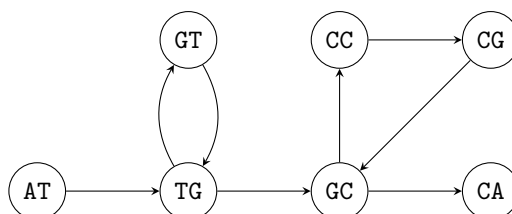
A robust field of theory underlies the process of assembling a genome from vast numbers of reads. Central to this algorithmic process are *de Bruijn graphs*.

### de Bruijn Graphs

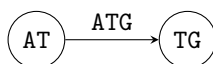
Consider the following sequence:

ATGTGCCGCA

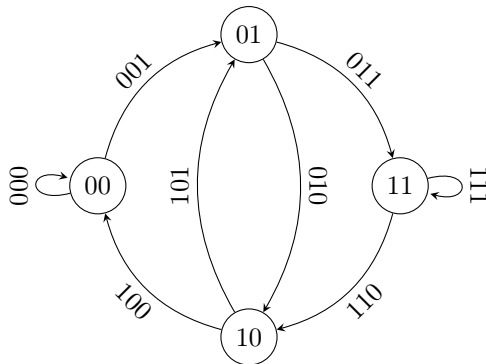
This sequence can be represented as a directed graph whose nodes are labeled with  $k$ -tuples, where edges are drawn between all pairs of  $k$ -tuples which “overlap” in the sequence. For  $k = 2$ , such a graph is



Edges can then be labeled with the appropriate  $(k + 1)$ -tuple; for example,



The following is a *de Bruijn graph* for the set  $\{000, 001, 010, 011, 100, 101, 110, 111\}$  (with  $k = 2$ ):



## The Idury-Waterman Algorithm

In 1995, Ramana Idury and Michael Waterman introduced an algorithm for assembling DNA sequences from reads obtained via shotgun sequencing. Their algorithm assumes *ideal data*; i.e., no sequencing errors, full coverage of the genome, and overlap between adjacent reads of at least  $k$  for some constant  $k > 0$ .

**Input:**  $N$  reads  $(\{f_1, f_2, \dots, f_N\})$ ; positive integer  $k$

**Output:** Full genome sequence which produced the reads  $f_{1:N}$

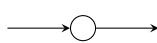
## Overview of the Idury-Waterman Algorithm

1. Construct the union of the *spectra* of all the reads and their reverse complements where the spectrum of a given read  $f_i$  is the set of all  $k$ -tuples in  $f_i$
2. From the union spectra created in step 1, construct the de Bruijn graph with each distinct  $(k - 1)$ -tuple as a node and each  $k$ -tuple as an edge
3. Perform a variant of an Eulerian tour through the de Bruijn graph and infer the assembled sequence(s)
4. Align all reads to the assembled sequence(s) inferred from step 3

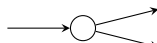
Refer to the [original paper](#) and [algorithm slides](#) on the website for a more detailed procedure.

## Complexities of de Bruijn Graphs

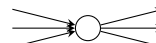
Ideally, de Bruijn graphs enable unambiguous reconstruction of a sequence of interest. However, this is rarely possible from the outset, due to sequencing errors and the presence of repeated subsequences. In general, there are 3 main types of nodes within an unsimplified de Bruijn graph: *singletons*, *forks* and *crosses*.



singleton



fork



cross

Singletons account for approximately 90% of nodes, and enable straightforward simplification of the de Bruijn graph around them. Many heuristics involved in de Bruijn graph-based genome assembly are therefore focused on reducing forks and crosses to singletons, by eliminating erroneous or extraneous edges and resolving cycles induced by repeated subsequences.

## Statistics of de Bruijn Graphs

de Bruijn graph-based genome assembly relies upon several assumptions:

- Errors are uniformly distributed, both over all reads and across the length of each read
- The global error rate is small (i.e., no more than 1 – 2%)
- The number of reads covering a given position of the sequence is a Poisson random variable with mean  $a = \frac{NL}{G}$ , so that  $P(\text{base } t \text{ is covered by } k \text{ reads}) = \frac{e^{-a} a^k}{k!}$
- There are no repeats of length  $k$  or greater
- The only sequencing errors are substitution errors (i.e., a single base substituted for another)

Using  $G$ ,  $N$ , and  $k$  as previously introduced, the following additional variables can be defined:

$$\begin{aligned}
L &= \text{length of a read} \\
U &= \# \text{ of } k-1 \text{ base pair regions in all the reads} = N(L - k + 2) \\
r &= \text{error rate of a single DNA base} \\
G' &= G - k + 2 \\
R &= 1 - (1 - r)^{k-1}
\end{aligned}$$

Denoting  $V$ ,  $S$  and  $F$  as the sets of vertices, singletons, and forks, respectively, the following holds:

**Theorem:** The de Bruijn graph obtained from applying the Idury-Waterman algorithm has

$$\begin{aligned}
\mathbb{E}(|V|) &= RU + [1 - e^{-a(1-R)}]G' \\
\mathbb{E}(|S|) &= RU + e^{-a(1-R)}[e^{a(1-R)(1-r)^2} + a(1-R)r(2-r) - 1]G' \\
\mathbb{E}(|F|) &= 2e^{-a(1-R)}[e^{a(1-R)(1-r)} - e^{a(1-R)(1-r)^2} - a(1-R)r(2-r)]G'
\end{aligned}$$

Note that the first term in the formula for  $\mathbb{E}(|V|)$  is the expected number of “false”  $k-1$ -mers, while the second term is the expected number of “true”  $k-1$ -mers.

Slides 78-82 of the [algorithm slides](#) outline the proof of  $\mathbb{E}(|V|)$ .