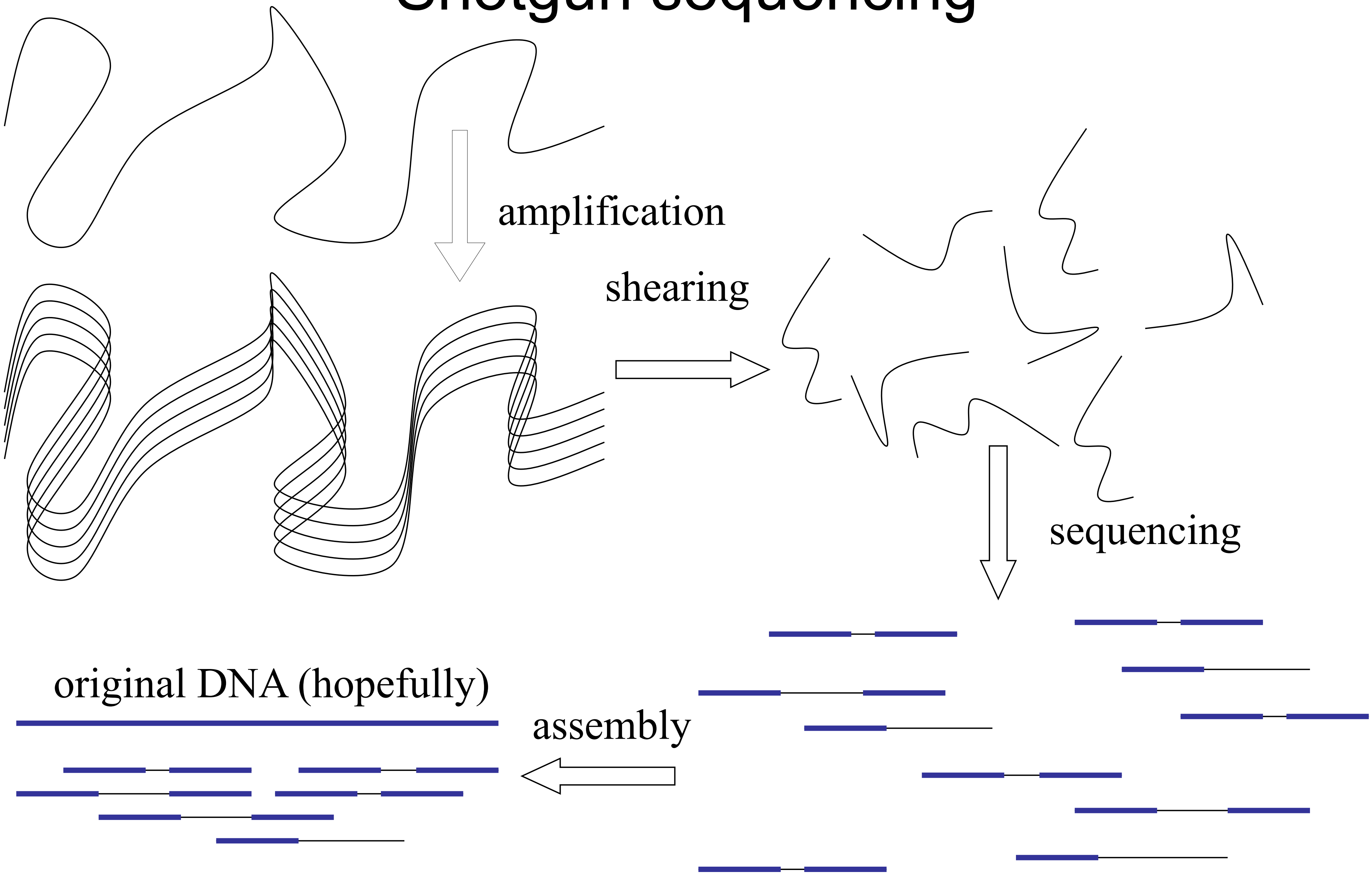
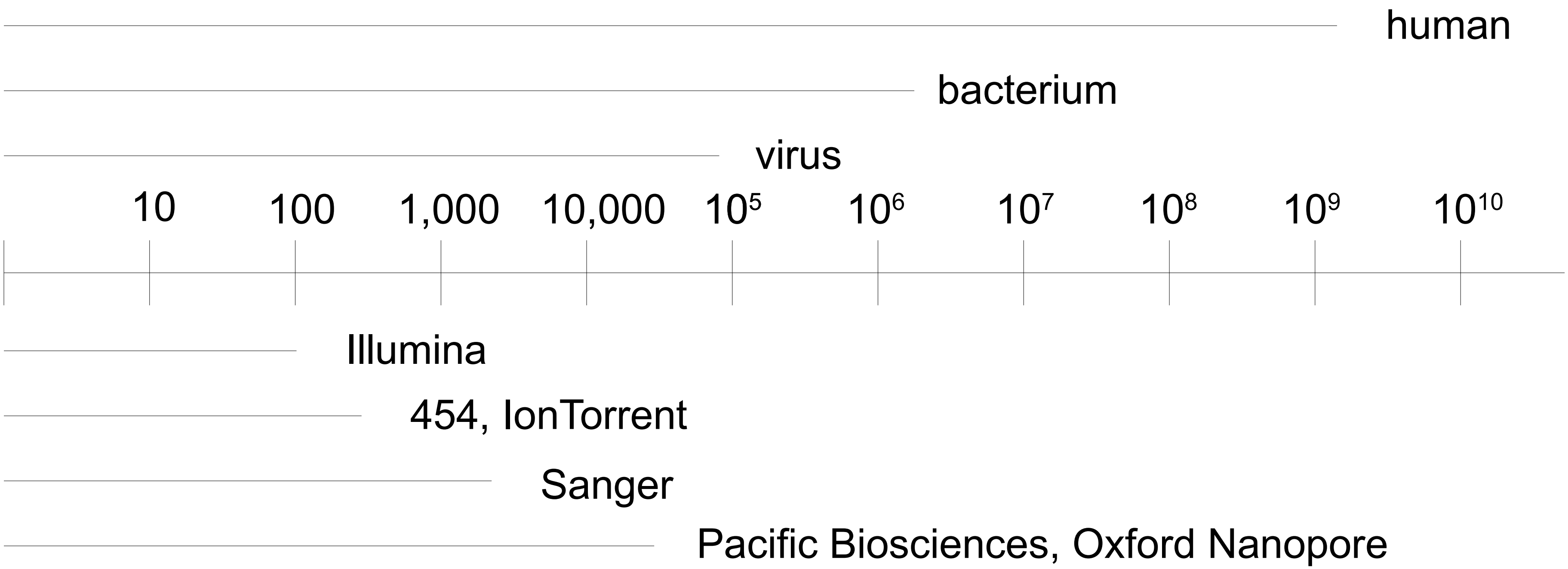
Introduction to Shotgun Sequencing

Mihai Pop

Shotgun sequencing



Why shotgun sequencing?



human

bacterium

virus

10 100 1,000 10,000 10^5 10^6 10^7 10^8 10^9 10^{10}

Illumina

454, IonTorrent

Sanger

Pacific Biosciences, Oxford Nanopore

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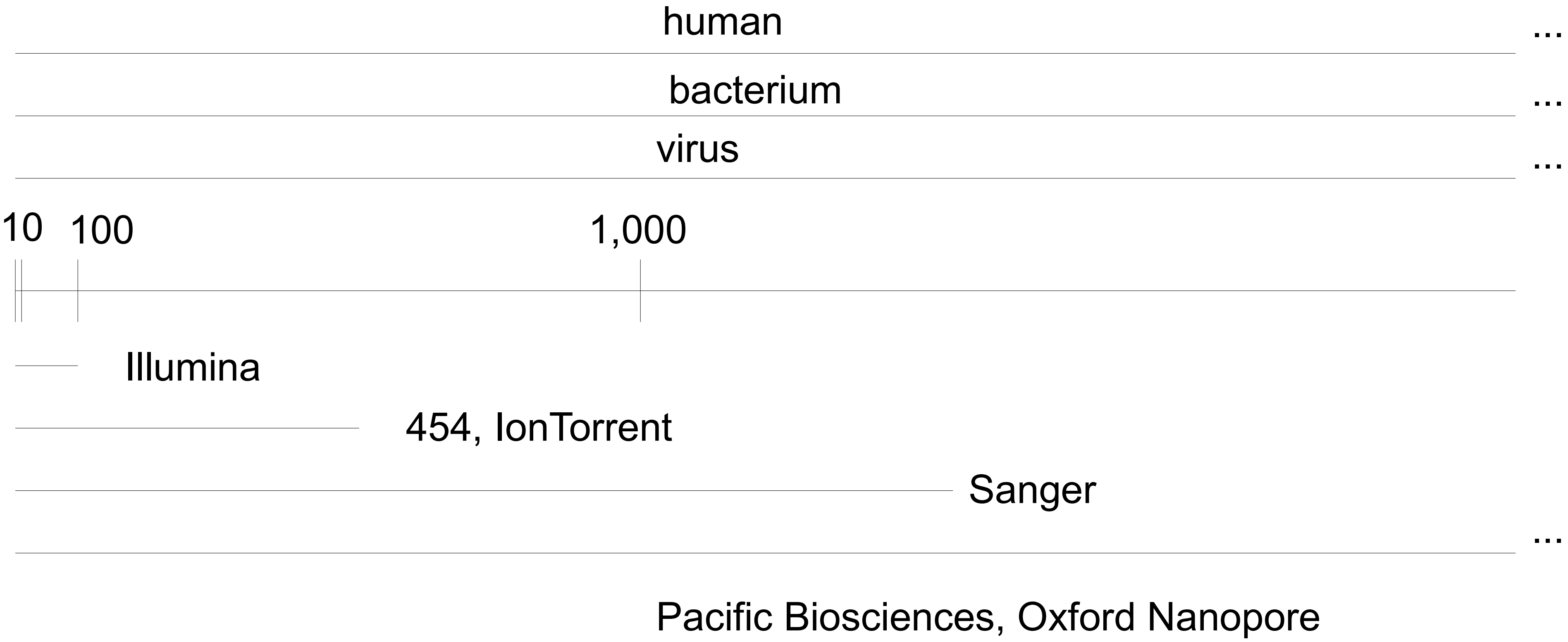

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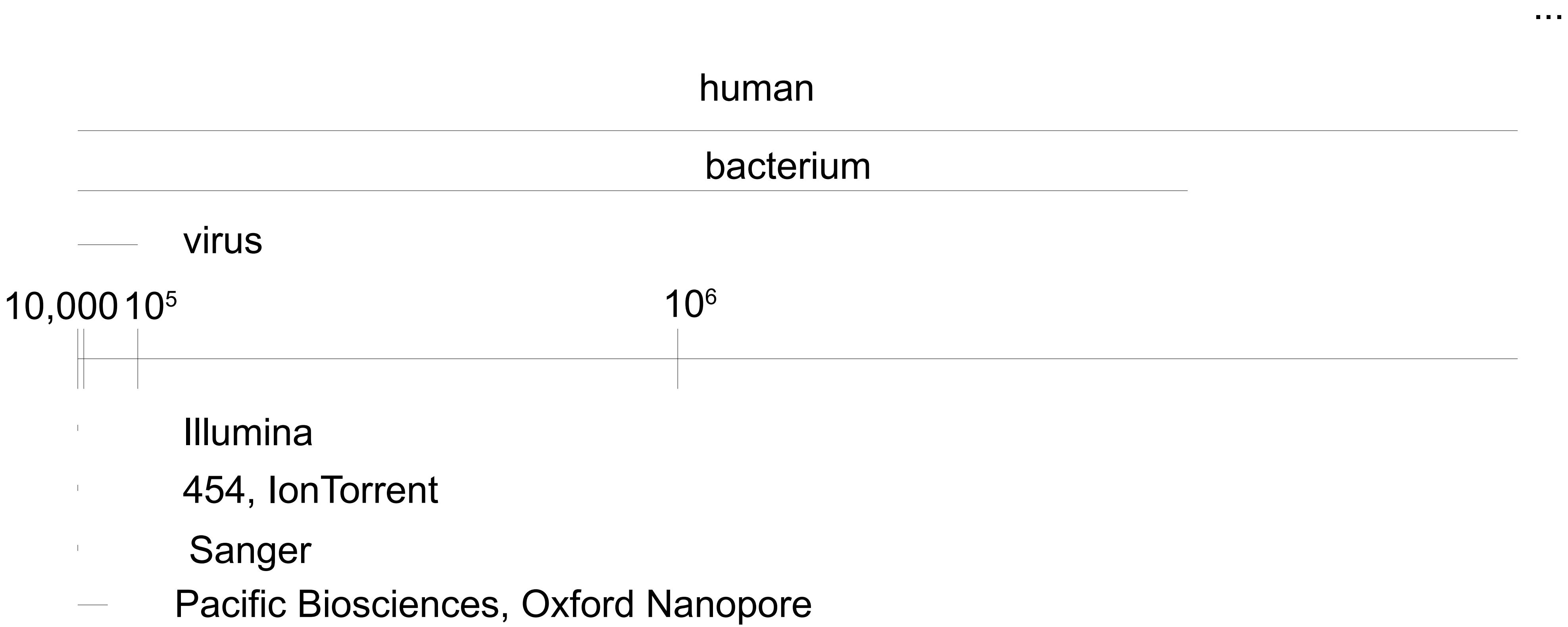

...

human

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10,000 10^5 10^6

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Stop and Think!

- Why is it necessary to have multiple copies of the original DNA?

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- Why is it necessary to have multiple copies of the original DNA?

- If adjacent DNA fragments do not share any information, it is impossible to reconstruct the original order.

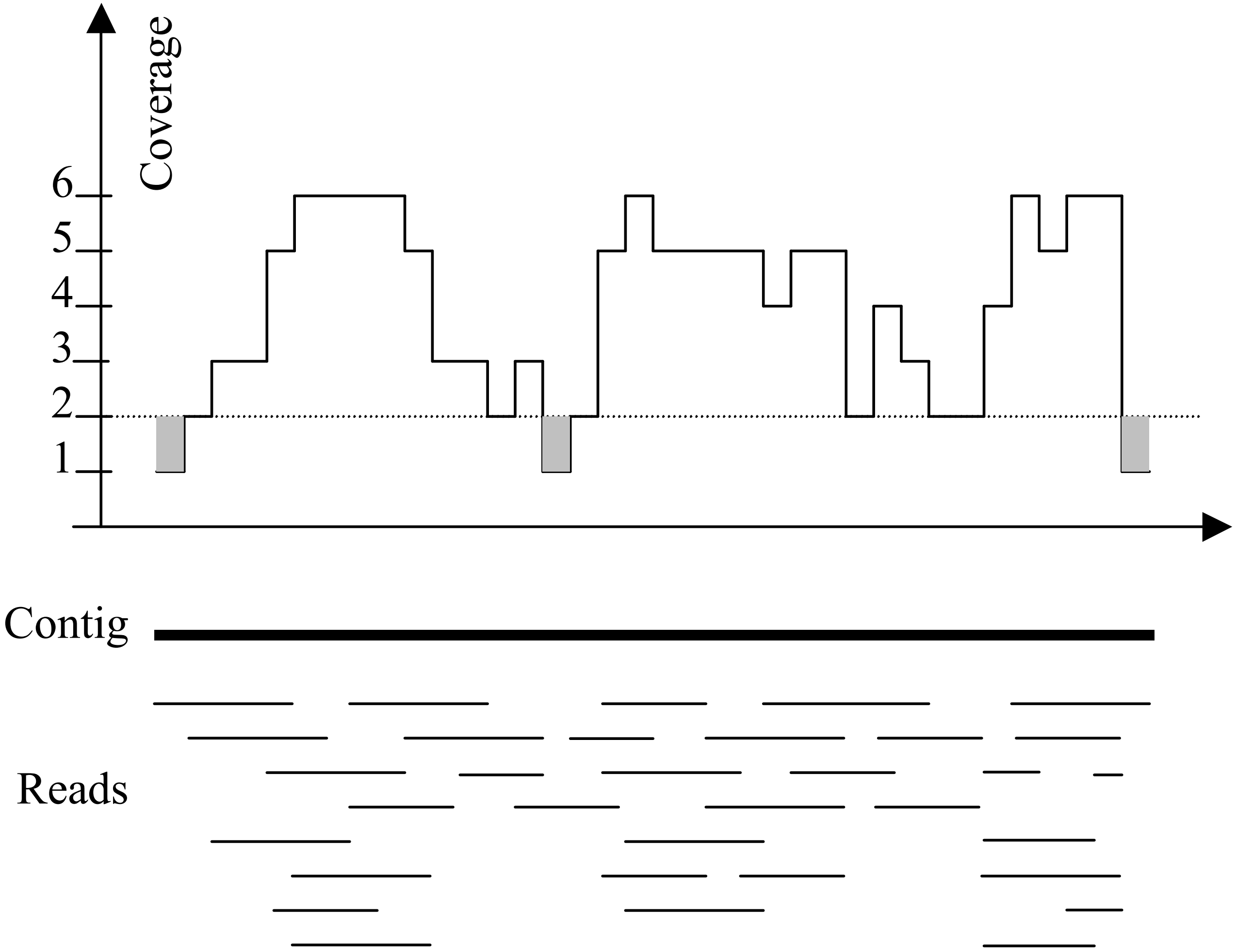
GCAACAT   TTCAGT   CCGCCGT ATCACAG

Is assembly even possible?

- If we randomly sequence will we ever cover every base in the genome?

- How much DNA do we need to sequence to cover every base in the genome?

Impact of randomness – non-uniform coverage

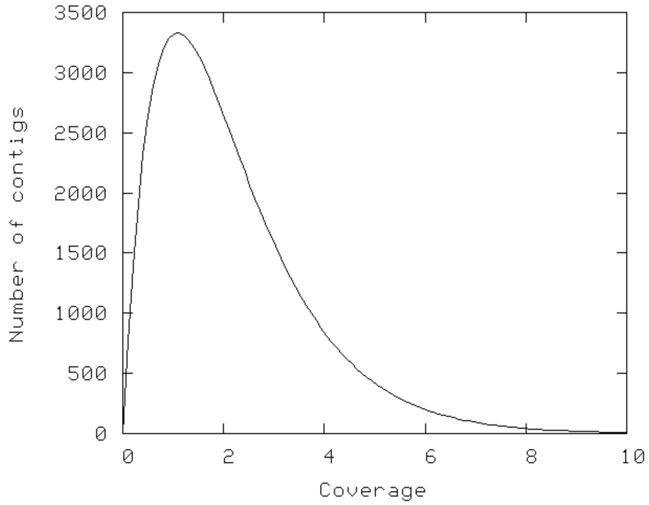


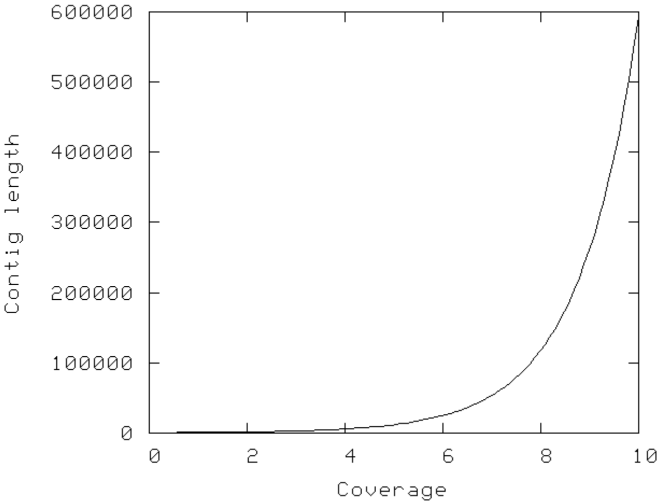
Imagine raindrops on a sidewalk

Lander-Waterman statistics

L = read length
T = minimum overlap
G = genome size
N = number of reads
c = coverage (NL / G)
$\sigma = 1 - T/L$

E(#islands) = $Ne^{-c\sigma}$
E(island size) = $L(e^{c\sigma} - 1) / c + 1 - \sigma$
contig = island with 2 or more reads





Next: assembly algorithms