Genomic Technologies

Michael Schatz

January 30, 2019

Lecture 2: Applied Comparative Genomics

Welcome!

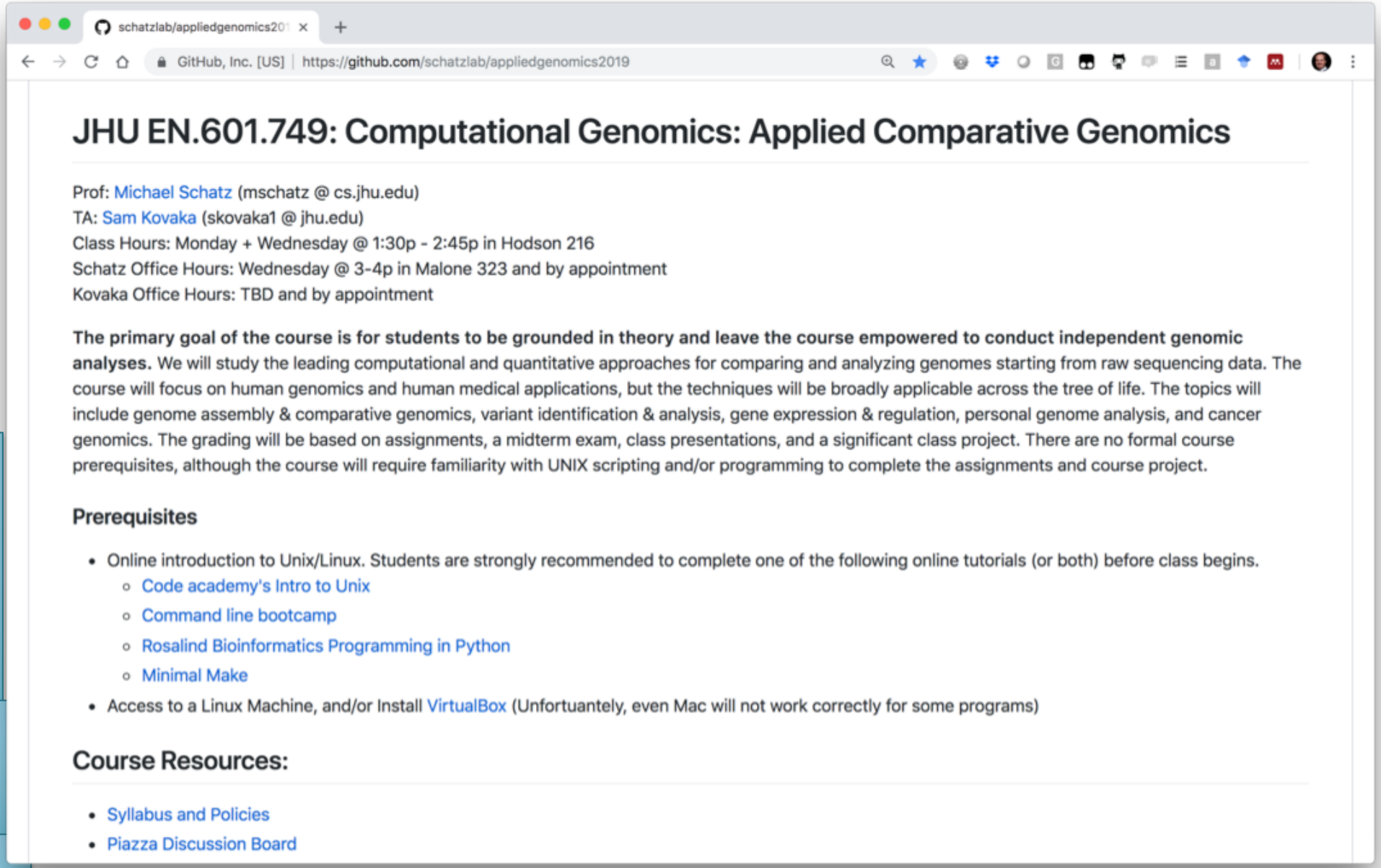
The primary goal of the course is for students to be grounded in theory and leave the course empowered to conduct independent genomic analyses.

- We will study the leading computational and quantitative approaches for comparing and analyzing genomes starting from raw sequencing data.
- The course will focus on human genomics and human medical applications, but the techniques will be broadly applicable across the tree of life.
- The topics will include genome assembly & comparative genomics, variant identification & analysis, gene expression & regulation, personal genome analysis, and cancer genomics.

Course Webpage:	https://github.com/schatzlab/appliedgenomics2019
Course Discussions:	http://piazza.com
Class Hours:	Mon + Wed @ 1:30p – 2:45p, Hodson 216
Schatz Office Hours:	Wed @ 3-4p and by appointment
Kovaka Office Hours:	Wed @ 4-5p and by appointment

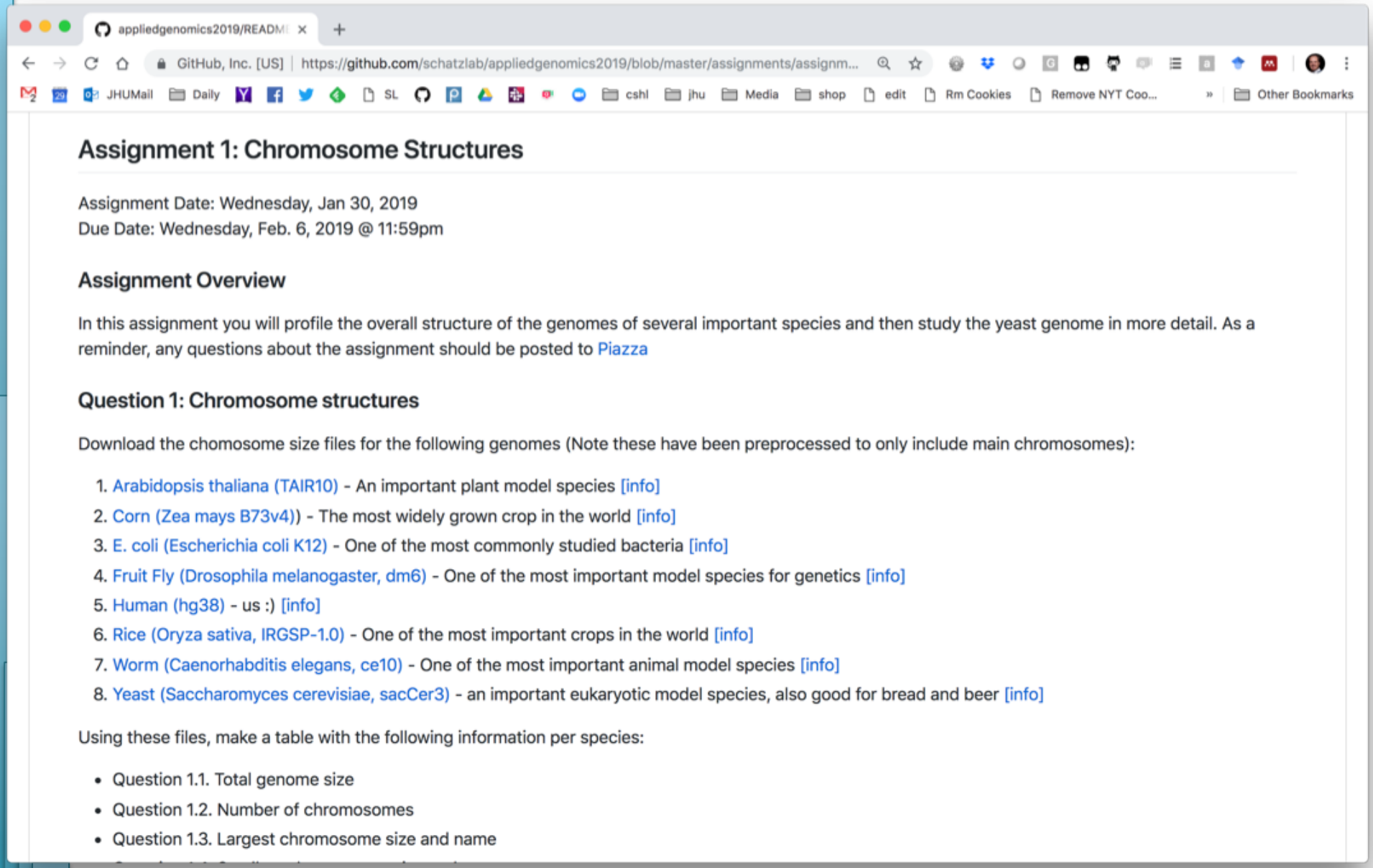
Please try Piazza first!

Course Webpage



https://github.com/schatzlab/appliedgenomics2019

Assignment 1: Chromosome Structures
Due Feb 6 @ 11:59pm



Assignment 1: Chromosome Structures

Assignment Date: Wednesday, Jan 30, 2019
Due Date: Wednesday, Feb. 6, 2019 @ 11:59pm

Assignment Overview

In this assignment you will profile the overall structure of the genomes of several important species and then study the yeast genome in more detail. As a reminder, any questions about the assignment should be posted to Piazza

Question 1: Chromosome structures

Download the chomosome size files for the following genomes (Note these have been preprocessed to only include main chromosomes):

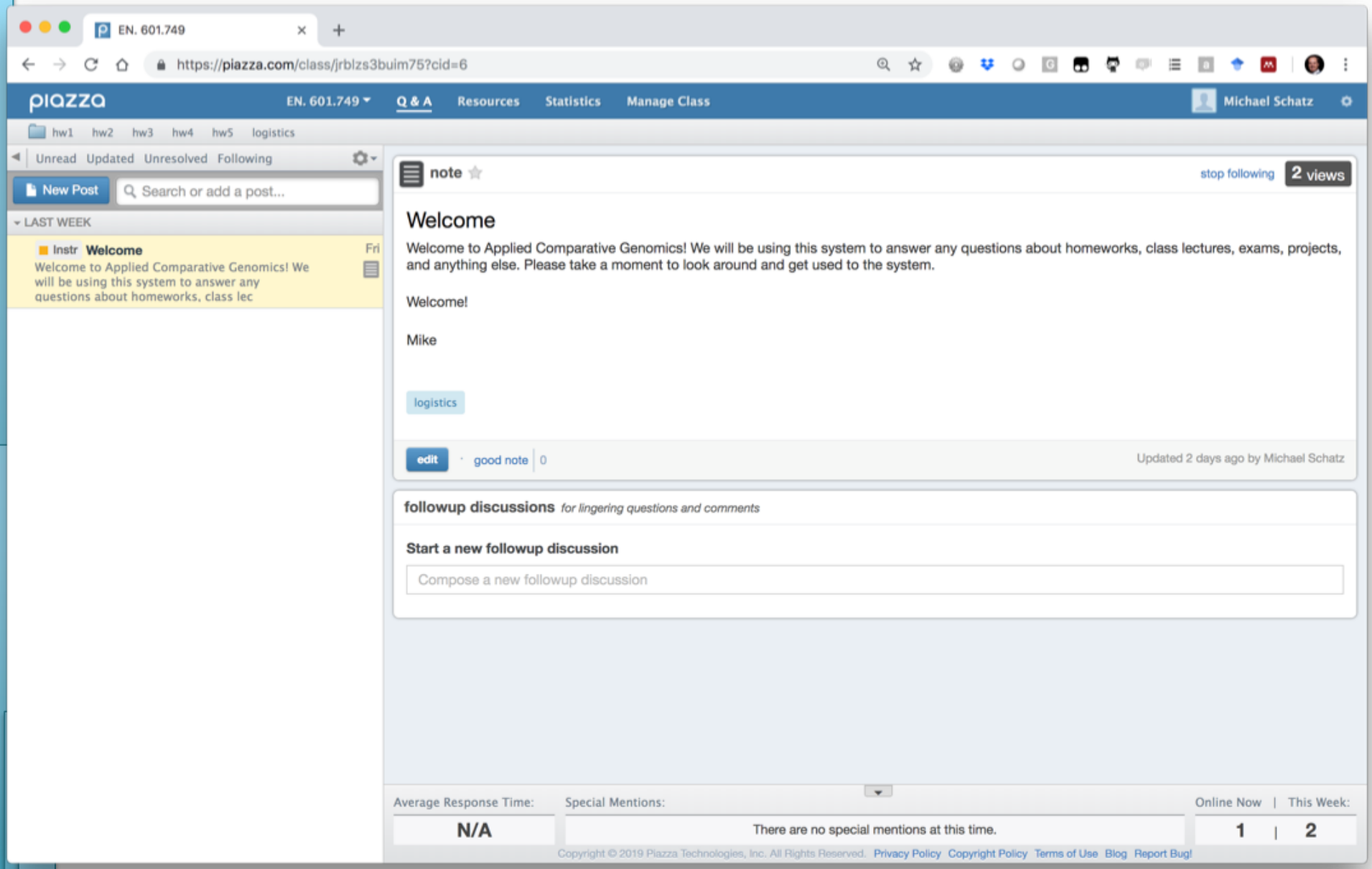
1. Arabidopsis thaliana (TAIR10) - An important plant model species [info]
2. Corn (Zea mays B73v4)) - The most widely grown crop in the world [info]
3. E. coli (Escherichia coli K12) - One of the most commonly studied bacteria [info]
4. Fruit Fly (Drosophila melanogaster, dm6) - One of the most important model species for genetics [info]
5. Human (hg38) - us :) [info]
6. Rice (Oryza sativa, IRGSP-1.0) - One of the most important crops in the world [info]
7. Worm (Caenorhabditis elegans, ce10) - One of the most important animal model species [info]
8. Yeast (Saccharomyces cerevisiae, sacCer3) - an important eukaryotic model species, also good for bread and beer [info]

Using these files, make a table with the following information per species:

- Question 1.1. Total genome size
- Question 1.2. Number of chromosomes
- Question 1.3. Largest chromosome size and name

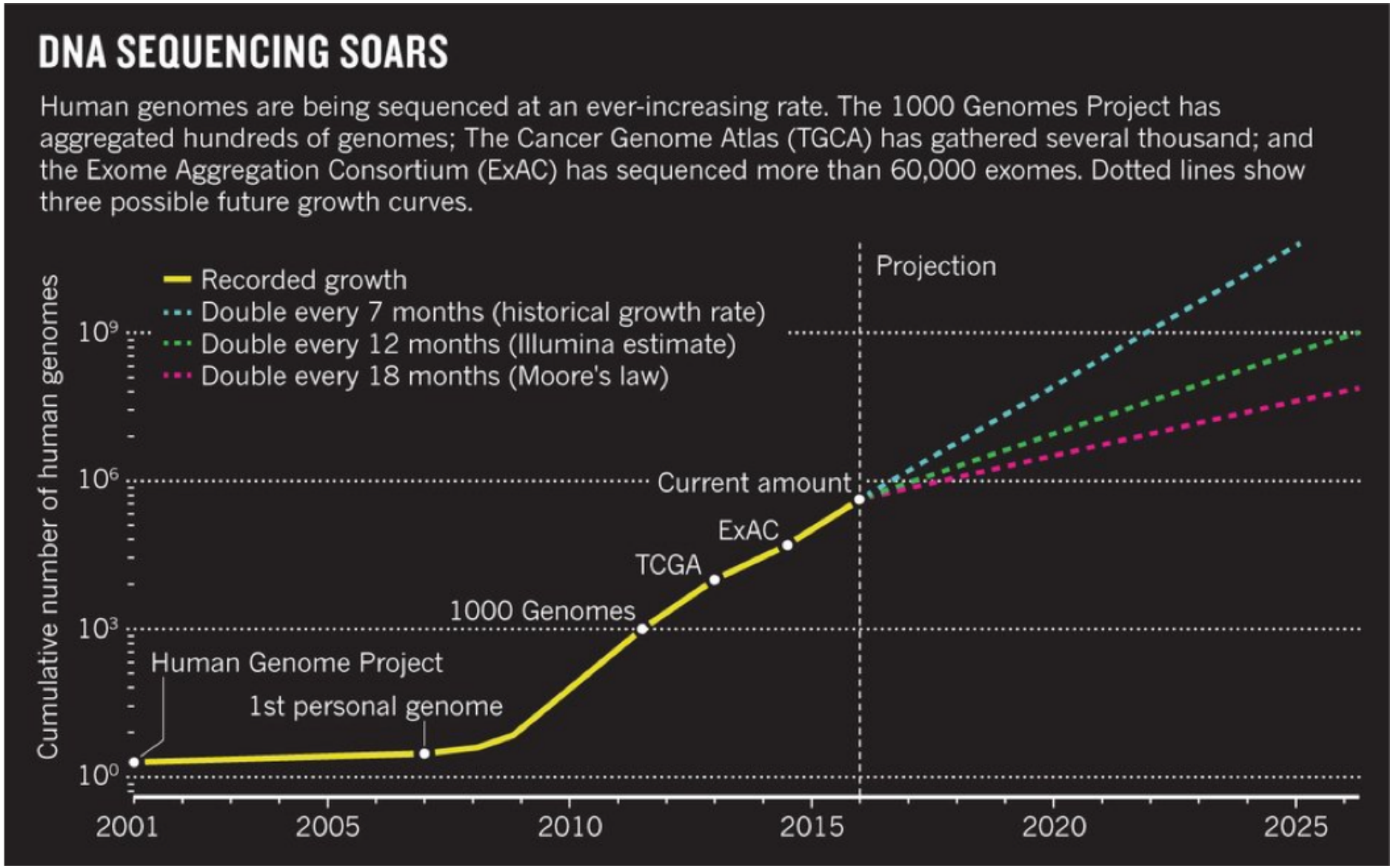
https://github.com/schatzlab/appliedgenomics2019

Piazza



http://piazza.com/jhu/spring2019/en601749

Sequencing Capacity



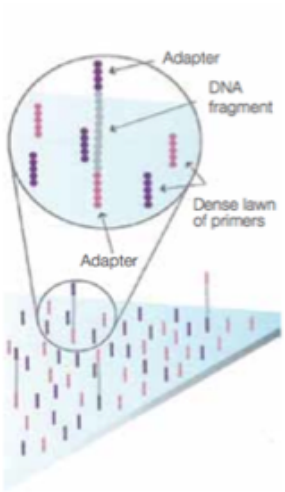
Big Data: Astronomical or Genomical?

Stephens, Z, et al. (2015) PLOS Biology DOI: 10.1371/journal.pbio.1002195

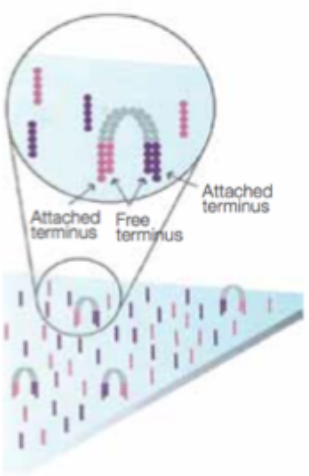
Second Generation Sequencing

Illumina NovaSeq 6000
Sequencing by Synthesis

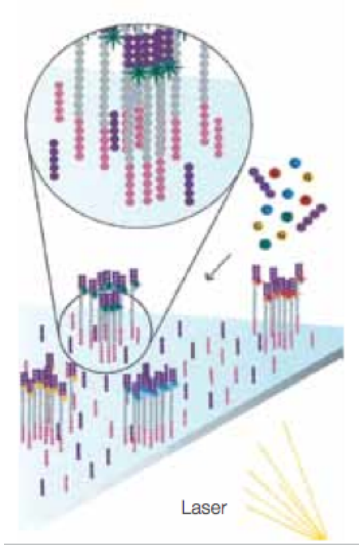
>3Tbp / day



1. Attach



2. Amplify



3. Image

Metzker (2010) Nature Reviews Genetics 11:31-46
https://www.youtube.com/watch?v=fCd6B5HRaZ8

Question?

We would love to generate
longer and longer reads with this technology

What can we do?

Genome Hacking

BIOINFORMATICS ORIGINAL PAPER

Vol. 29 no. 12 2013, pages 1492–1497
doi:10.1093/bioinformatics/btt178

Genome analysis

Advance Access publication May 22, 2013

Assembling the 20 Gb white spruce (*Picea glauca*) genome from whole-genome shotgun sequencing data

Inanc Birol[1,2,3,*], Anthony Raymond[1], Shaun D. Jackman[1], Stephen Pleasance[1], Robin Coope[1], Greg A. Taylor[1], Macaire Man Saint Yuen[4], Christopher I. Keeling[4], Dana Brand[1], Benjamin P. Vandervalk[1], Heather Kirk[1], Pawan Pandoh[1], Richard A. Moore[1], Yongjun Zhao[1], Andrew J. Mungall[1], Barry Jaquish[5], Alvin Yanchuk[5], Carol Ritland[6], Brian Boyle[7], Jean Bousquet[7,8], Kermit Ritland[6], John MacKay[7,8], Jörg Bohlmann[4,6], Steven J.M. Jones[1,2,9]

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Associate Editor: Michael Brudno

ABSTRACT

White spruce (*Picea glauca*) is a dominant conifer of the boreal forests of North America, and providing genomics resources for this commercially valuable tree will help improve forest management and conservation efforts. Sequencing and assembling the large and highly repetitive spruce genome though pushes the boundaries of the current technology. Here, we describe a whole-genome shotgun sequencing strategy using two Illumina sequencing platforms and an assembly approach using the ABySS software. We report a 20.8 giga base pairs draft genome in 4.9 million scaffolds, with a scaffold N50 of 20 356 bp. We demonstrate how recent improvements in the sequencing technology, especially increasing read lengths and paired end reads from longer fragments have a major impact on the assembly contiguity. We also note that scalable bioinformatics tools are instrumental in providing rapid draft assemblies.

Availability: The *Picea glauca* genome sequencing and assembly data are available through NCBI (Accession#: ALWZ01000000000 PID: PRJNA83435). http://www.ncbi.nlm.nih.gov/bioproject/83435.

Contact: ibirol@bcgsc.ca

Supplementary information: Supplementary data are available at *Bioinformatics* online.

Received on March 20, 2013; revised on April 10, 2013; accepted on April 11, 2013

1 INTRODUCTION

The assembly of short reads to develop genomic resources for non-model species remains an active area of development (Schatz *et al.*, 2012). The feasibility of the approach and its scalability to large genomes was demonstrated by (Simpson *et al.*, 2009) using human and was later used to assemble the SOAPdenovo tool (Li *et al.*, 2010). high quality results, as demonstrate *et al.*, 2012; Ladner *et al.*, 2013; Rib been successfully applied numerous ti omes (Chan *et al.*, 2011; Chu *et al.*, 2 2011; Godel *et al.*, 2012; Swart *et al.*,

Estimated at 20 giga base pairs (Gi cing and assembly of the genome of t the pine (*Pinaceae*) family present uni generation end, those challenges inclu whole-genome shotgun sequencing da ing reduced representation resources tude of the problem. On the bioin massive sequencing datasets is extrem ing cycles, memory usage, storage req programming implementations on co

We addressed the data representati and sequencing multiple whole-genom HiSeq 2000 and MiSeq sequencers CA, USA). Compared with localized as building and sequencing fosmi approach of isolating ~10 kb DNA sequencing fragments in high through CA, USA), a shotgun only sequencing sequence data effectively covering th that can be an order of magnitude les especially substantial when sequencin

In this work, we demonstrate that at this scale remains viable and pro

*To whom correspondence should be addressed.

assemble the spruce genome, we used the ABySS algorithm (Simpson *et al.*, 2009), which captures a representation of read-to-read overlaps by a distributed de Bruijn graph and uses parallel computations to build the target genome. The modular nature of the tool allowed us to execute a large number of tests to tune the message passing interface for a successful execution, train the assembly parameters for an optimal assembly and quantify the utility of long reads for large genome assemblies. To the best of our knowledge, the ABySS algorithm is unique in its ability to enable genome assemblies of this scale using whole-genome shotgun sequencing data.

2 METHODS

2.1 Sample collection

Apical shoot tissues were collected in April 2006 from a single white spruce (*Picea glauca*, genotype PG29) tree at the Kalamalka Research Station of the British Columbia Ministry of Forests and Ranges, Vernon, British Columbia, Canada. Genomic DNA was extracted from 60 gm tissue by BioS&T (http://www.biost.com), Montreal, QC, Canada) using an organelle exclusion method yielding 300 µg of high quality purified nuclear DNA.

2.2 Library preparation and sequencing

DNA quality was assessed by spectrophotometry and gel electrophoresis before library construction. DNA was sheared for 45 s using an E210 sonicator (Covaris) and then analysed on 8% PAGE gels. The 200–300 bp (for libraries with 250 bp insert size) or 450–550 bp (for libraries with 500 bp insert size) DNA size fractions were excised and eluted from the gel slices overnight at 4°C in 300 µl of elution buffer [5:1 (vol/vol) LoTE buffer [3 mM Tris-HCl (pH 7.5), 0.2 mM EDTA)/7.5 M ammonium acetate] and was purified using a Spin-X Filter Tube (Fisher Scientific) and ethanol precipitation. Genomic libraries were prepared using a modified paired-end tag (PET) protocol supplied by Illumina Inc. This involved DNA end repair and formation of 3′ adenosine overhangs using the Klenow fragment of DNA polymerase I (3′–5′ exonuclease minus) and ligation to Illumina PE adapters (with 5′ overhangs). Adapter-ligated products were purified on QIAquick spin columns (Qiagen) and amplified using Phusion DNA polymerase (NEB) and 10 PCR cycles with the PE primer 1.0 and 2.0 (Illumina). PCR products of the desired size range were purified from adapter ligation artifacts using 8% PAGE gels. DNA quality was assessed and quantified using an Agilent DNA 1000 series II assay (Agilent) and Nanodrop 7500 spectrophotometer (Nanodrop). DNA was subsequently diluted to 8 nM. The final concentration was confirmed using a Quant-iT dsDNA HS assay kit and Qubit fluorometer (Invitrogen).

The mate pair (MPET, a.k.a. jumping) libraries were constructed using 4 µg of genomic DNA with the Illumina Nextera Mate-Pair library construction protocol and reagent (FC-132-1001). The genomic DNA sample was simultaneously fragmented and tagged with a biotin containing mate pair junction adapter, which left a short sequence gap in the tagmented DNA. The gap was filled by a strand-displacement reaction using a polymerase to ensure that all fragments were flush and ready for circularization. After an AMPure Bead cleanup, size selection was done on a 0.6% agarose gel to excise 6–9 kb and 9–13 kb fractions, which were purified using a Zymoclean Large Fragment DNA Recovery Kit. The fragments were circularized by ligation, followed by a digestion to remove any linear molecules and left circularized DNA for shearing. The sheared DNA fragments that contain the biotinylated junction adapter (mate pair fragments) were purified by means of binding to streptavidin magnetic beads, and the unwanted unbiotinylated molecules were washed away. The DNA fragments were then end repaired and A-tailed following the protocol and ligated to indexed TruSeq adapters. The final library was enriched by a 10-cycle PCR and purified by AMPure bead clean-up. Library quality and size were assessed by Agilent DNA 1000 series II assay and KAPA Library Quantification protocol. The two fractions were pooled for sequencing paired end 100 bp using Illumina HiSeq2000.

The construction of the 12 kb mate pair libraries was achieved by a hybrid 454/Illumina procedure. Briefly, 50 µg of genomic DNA was [illegible] mented for 20 cycles at speed code 12 using a Hydroshear (Digilab, Marlborough, MA) equipped with a large assembly module. The sheared DNA was loaded on a 1% agarose gel, and fragments [illegible] 18 kb were extracted. Biotinylated circularization adapters [illegible] Titanium Paired-end Adaptor set (454 Life Sciences/Roche, Branford, CT) were added to ends of the gel-extracted fragments. [illegible] recombination of the ends was performed with Cre recombinase (New England Biolabs, Ipswich, MA), and linear molecules remaining after digestion were removed with Plasmid Safe (Epicentre, Madison, WI). The circular molecules were fragmented using GS Rapid Library Nebulization System (454 Life Sciences/Roche, Branford, CT), and fragment end-repair followed by A-tailing was performed with the GS Rapid Library preparation kit (454 Life Sciences/Roche, Branford, CT). TruSeq Adaptors (Illumina, Inc., Carlsbad, CA) were ligated to the repaired/A-tailed ends. Biotinylated fragments were enriched using Streptavidin-coupled Dynabeads (Life Technologies, Grand Island, NY) and amplified by PCR using Illumina primers.

Random bacterial artificial chromosome (BAC) sequencing was performed using DNA from the same genotype on 454 Titanium with 6 kb paired-end libraries at the PlateForme Génomiques of the Institute for Systems and Integrative Biology (Université Laval, Quebec City, QC). A single paired-end library prepared on a pool of 15 BACs (equimolar concentrations) was used as described earlier in the text with the following modifications: 15 µg of DNA was fragmented using a Hydroshear with a standard assembly program at speed code 18, 6–10 kb fragments were extracted from the gel, and GS-FLX library adaptors were ligated to the repaired/A-tailed fragments. GS-FLX sequencing using the titanium chemistry was performed according to manufacturer's instructions (454 Life Sciences/Roche, Branford, CT). Sanger sequencing method was used to obtain BAC sequencing data as previously described (Hamberger and Bohlmann, 2006; Keeling *et al.*, 2010).

2.3 MiSeq modification

In sequencing the spruce genome, we generated longer read data by modifying the MiSeq platform. The MiSeq uses a clamshell style cartridge (Supplementary Fig. S1A) to hold reagent tubes in an array accessed by the MiSeq's sippers. Most of the reagents are used in a single length independent steps such as denaturation and cluster generation. The three reagents, the Scan, Cleavage and Incorporation mixes, are consumed at each cycle. Although the MiSeq allows any read length to be specified in the control software, the reagent cartridge cannot be changed during the run without stopping it. Increasing the read length therefore requires increasing the quantity of the length-dependent reagents in the cartridge. This led to the solution of combining the length-dependent reagents of two kits into one.

A tool was designed that opens the snap-hook latches that hold the cartridge together (Supplementary Figs S1B and S2), giving access to the reagent tubes, yet allowing the cartridge to be put back on the sippers without damage to its components (Supplementary Fig. S1C). Of the 40 ml, the stock length-dependent reagent containers allow a maximum of ~650 cycles in total. To maximize the potential of the modified kit approach, a new reagent tray with 70 ml wells was designed and placed in a modified clamshell base.

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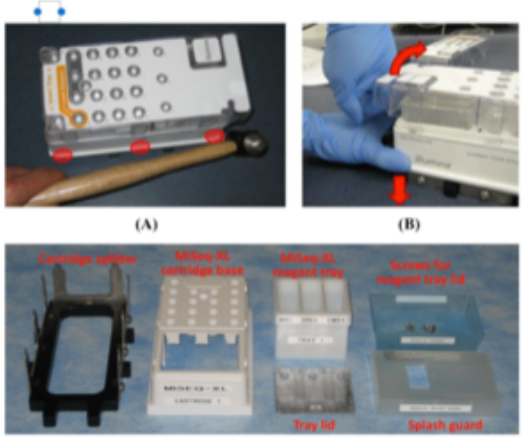
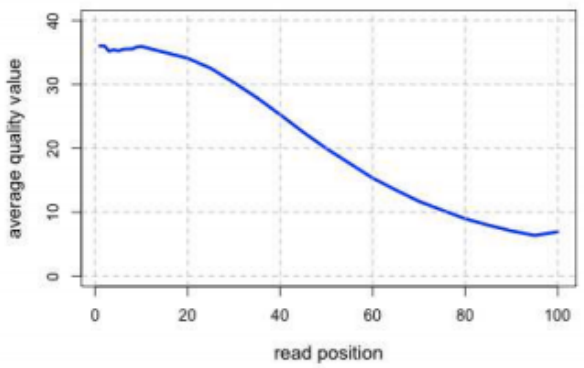


Figure S1. Modification of the MiSeq cartridge. MiSeq reagent cartridge was modified to allow for longer read lengths. **(A, B)** Opening of the clamshell style cartridge. **(C)** Contents of the modified cartridge. This was initially used to combine two PE150 kits for PE300 runs. When Illumina introduced the P250 kit, the same apparatus was used to enable PE500 runs.

Illumina Quality

QV	p_{error}
40	1/10000
30	1/1000
20	1/100
10	1/10

$$Q_{\text{sanger}} = -10 \log_{10} p$$

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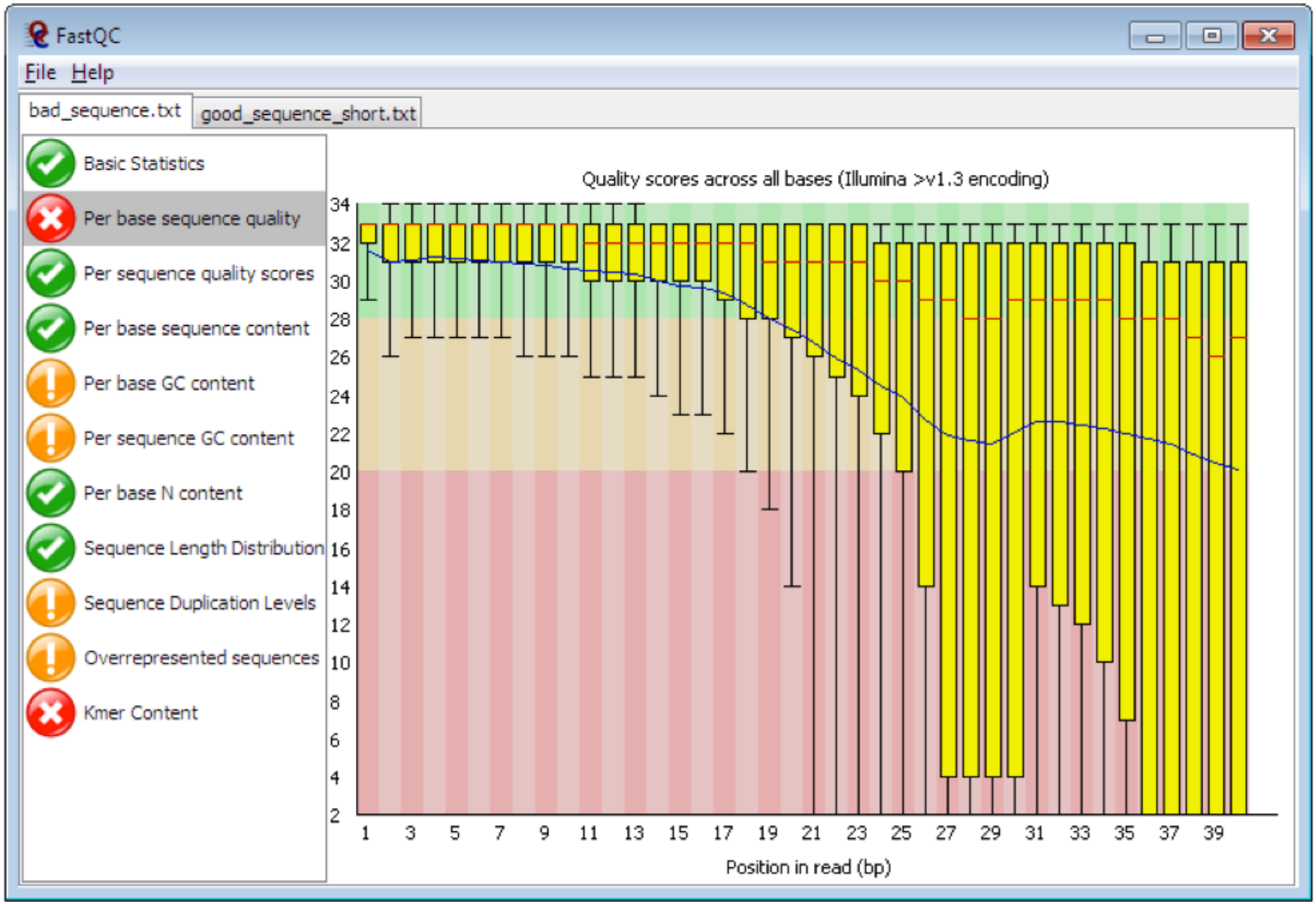
  SSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSS.....................................................
  ........................XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX.....................
  ...............................IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII.....................
  ................................JJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJ.....................
  LLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLL.....................................................
  !"#$%&'()*+,-./0123456789:;<=>?@ABCDEFGHIJKLMNOPQRSTUVWXYZ[\]^_`abcdefghijklmnopqrstuvwxyz{|}~
  |                         |    |        |                              |                     |
 33                        59   64       73                            104                   126

 S - Sanger        Phred+33,  raw reads typically (0, 40)
 X - Solexa        Solexa+64, raw reads typically (-5, 40)
 I - Illumina 1.3+ Phred+64,  raw reads typically (0, 40)
 J - Illumina 1.5+ Phred+64,  raw reads typically (3, 40)
    with 0=unused, 1=unused, 2=Read Segment Quality Control Indicator (bold)
    (Note: See discussion above).
 L - Illumina 1.8+ Phred+33,  raw reads typically (0, 41)
```

http://en.wikipedia.org/wiki/FASTQ_format

FASTQC: Is my data any good?



http://www.bioinformatics.babraham.ac.uk/projects/fastqc/

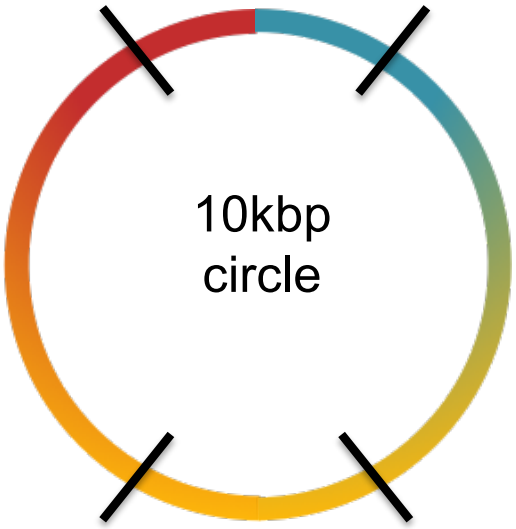
Paired-end and Mate-pairs

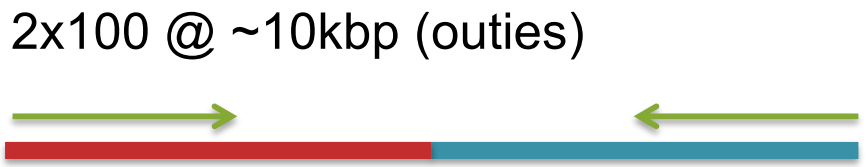
Paired-end sequencing

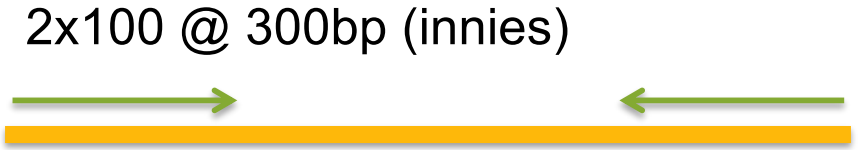
- Read one end of the molecule, flip, and read the other end
- Generate pair of reads separated by up to 500bp with inward orientation

Mate-pair sequencing

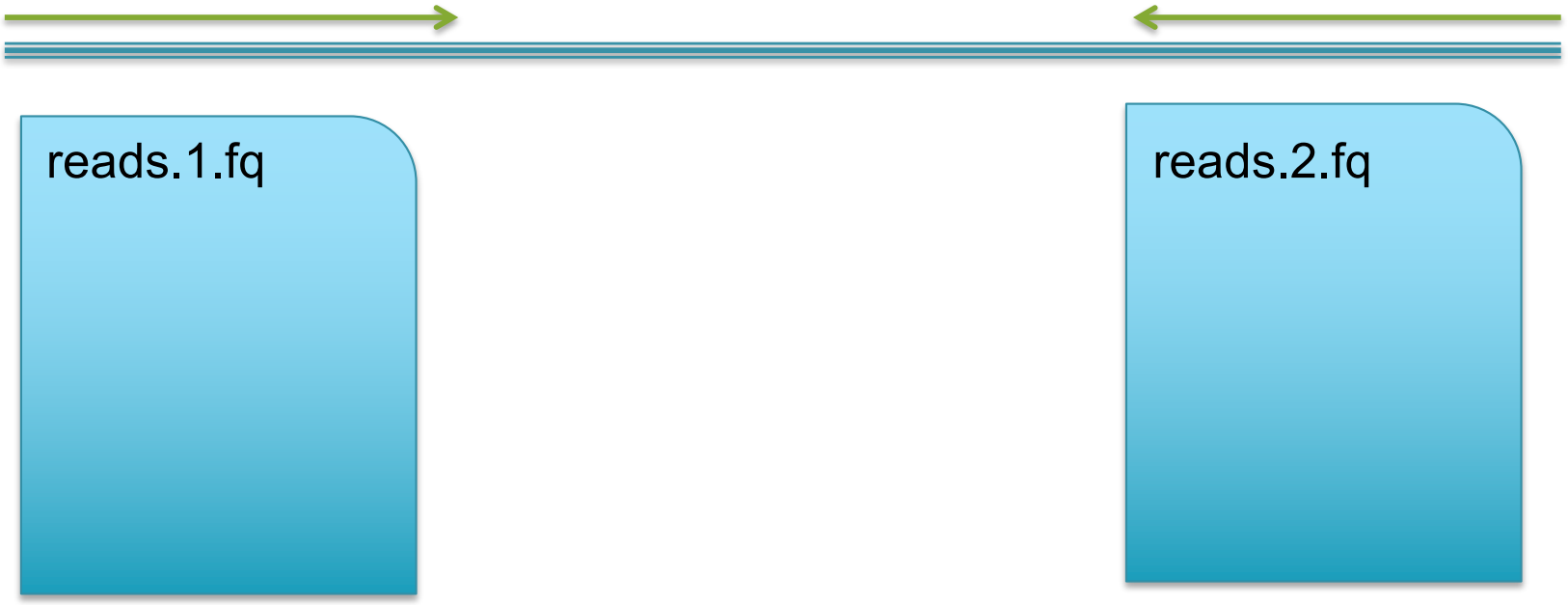
- Circularize long molecules (1-10kbp), shear into fragments, & sequence
- Mate failures create short paired-end reads







FASTQ Files



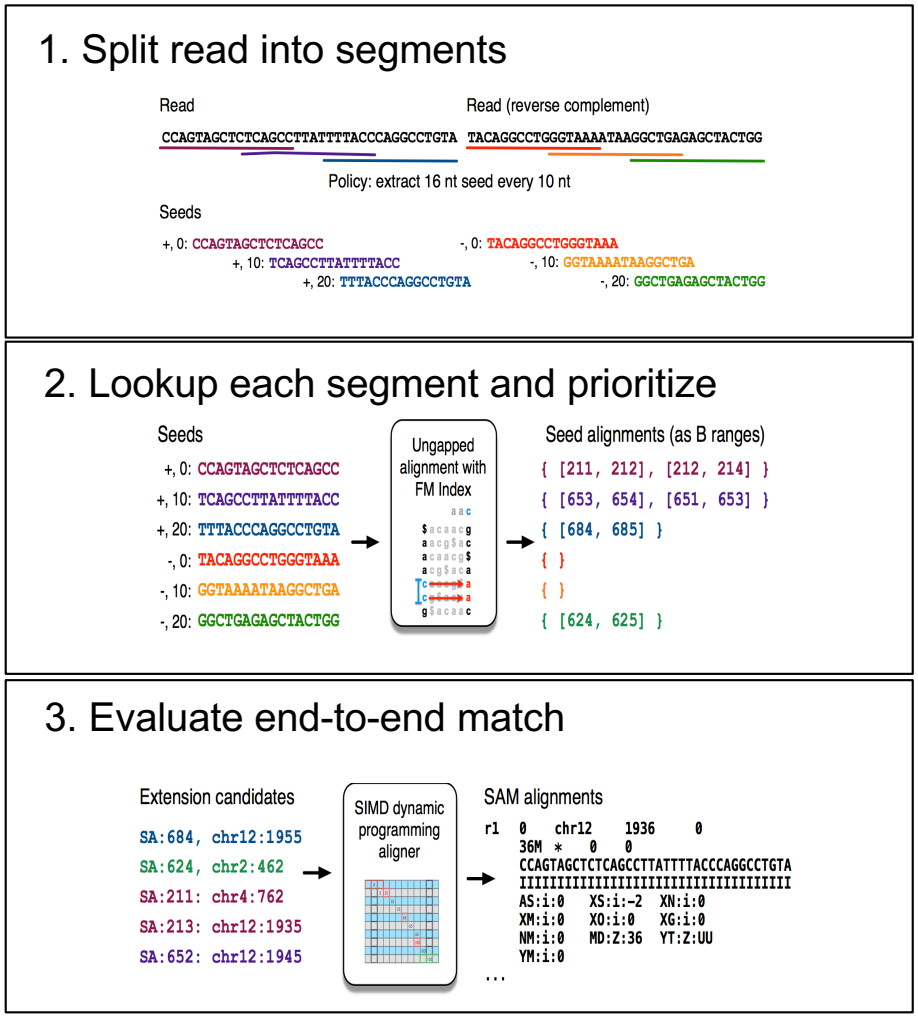

```
@SEQ_ID
GATTTGGGGTTCAAAGCAGTATCGATCAAATAGTAAATCCATTTGTTCAACTCACAGTTT
+
!''*((((***+))%%%++)(%%%%).1***-+*''))**55CCF>>>>>>CCCCCCC65
```

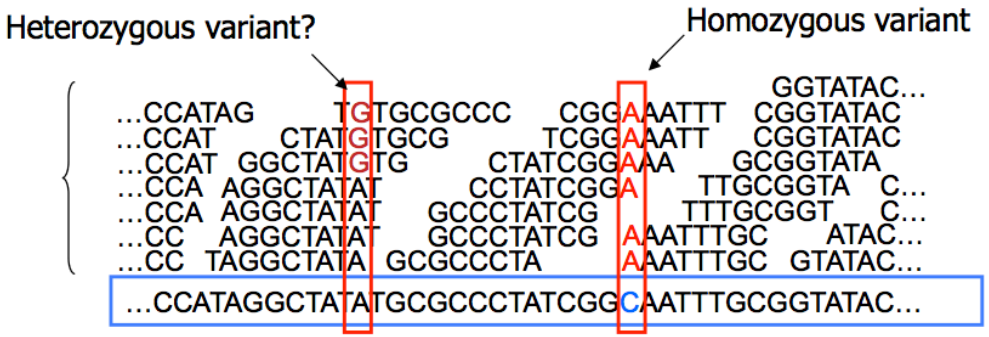
@Identifier
Sequence
+Separator
Quality Values
…

http://en.wikipedia.org/wiki/FASTQ_format

Assembly, Mapping & Genotyping

Week 2/3/4



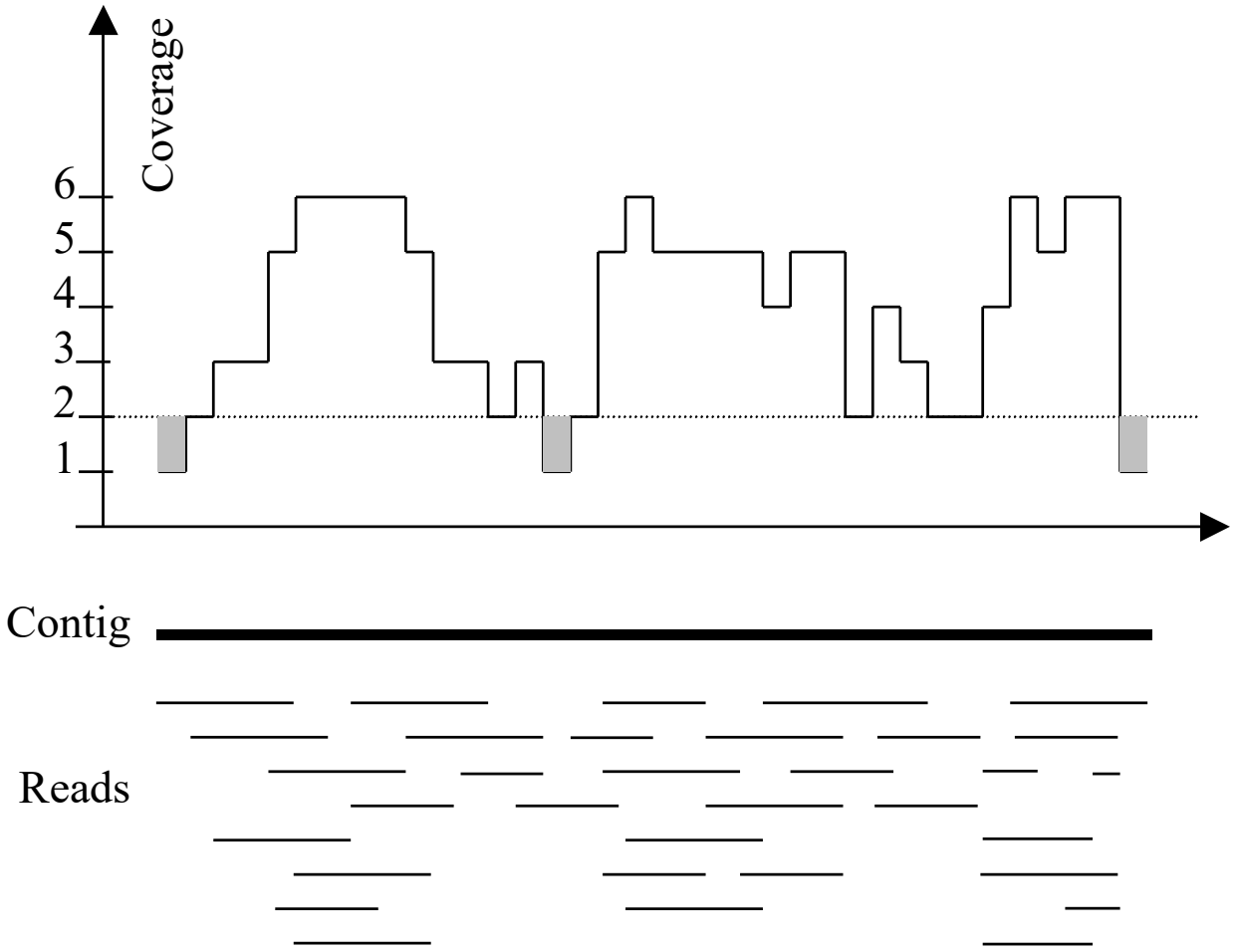


- Distinguishing SNPs from sequencing error typically a likelihood test of the coverage
  - Hardest to distinguish between errors and heterozygous SNP.
  - Coverage is the most important factor!
    - Target at least 10x, 30x more reliable

Fast gapped-read alignment with Bowtie 2
Langmead & Salzberg. (2012) *Nature Methods*. 9:357-359.

The Sequence Alignment/Map format and SAMtools
Li H et al. (2009) *Bioinformatics*. 25:16 2078-9

Typical sequencing coverage

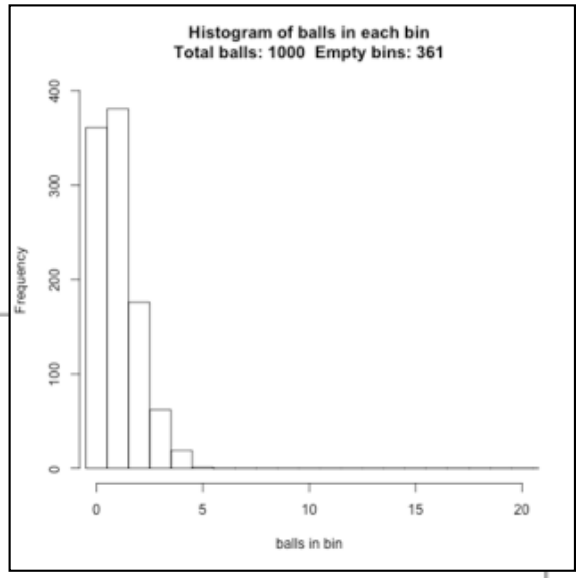


Imagine raindrops on a sidewalk

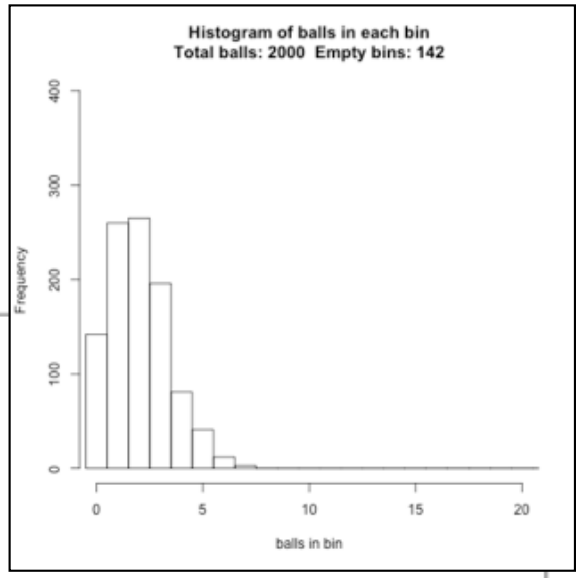
We want to cover the entire sidewalk but each drop costs $1

If the genome is 10 Mbp, should we sequence 100k 100bp reads?

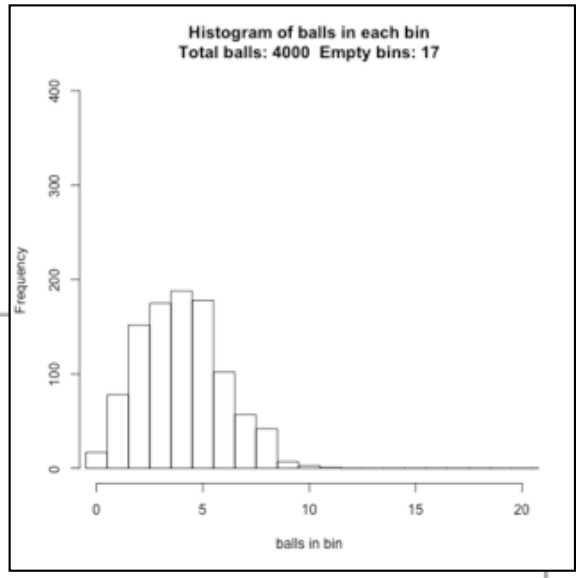
1x sequencing



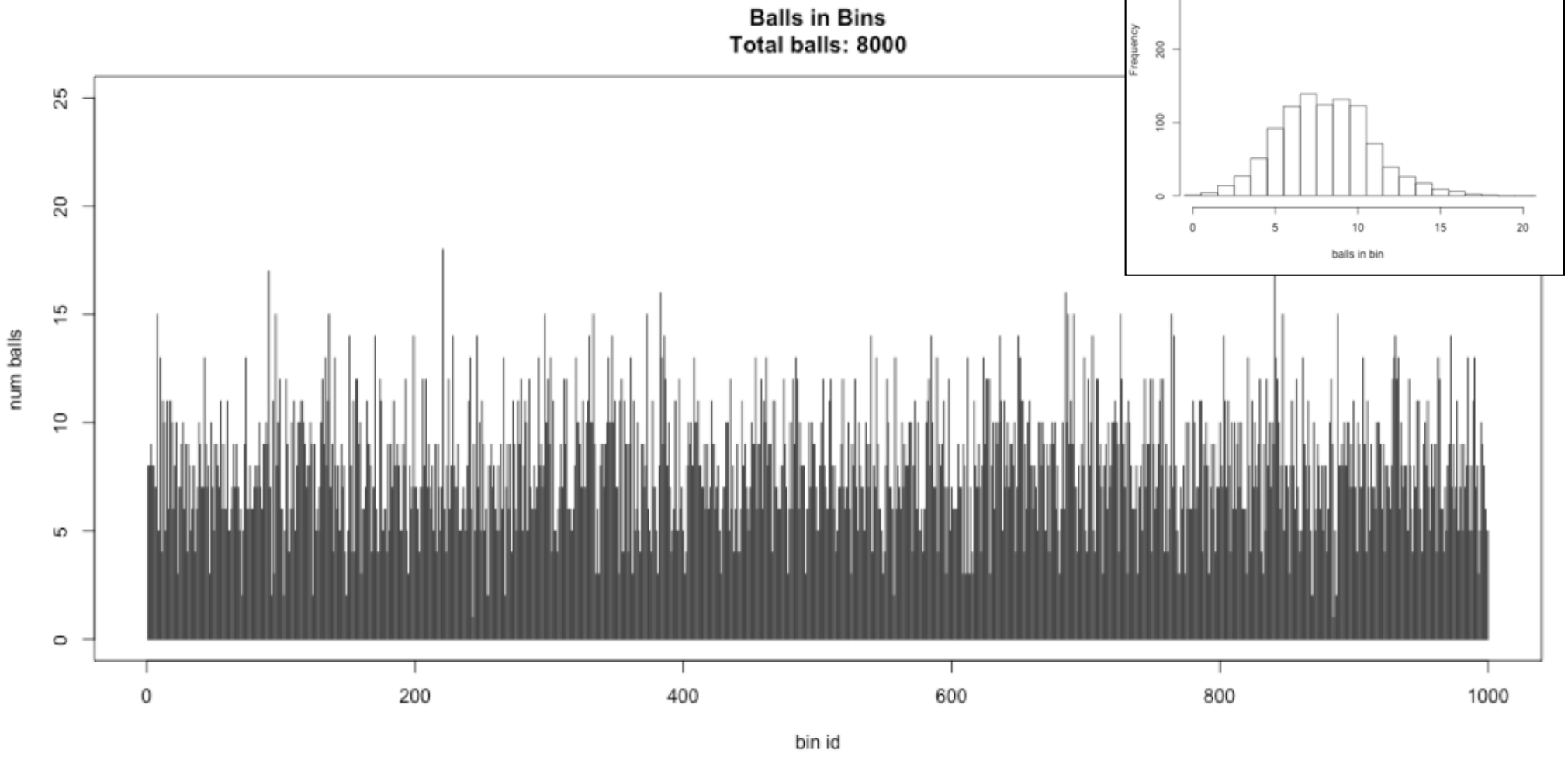
2x sequencing



4x sequencing



8x sequencing



Poisson Distribution

The probability of a given number of events occurring in a fixed interval of time and/or space if these events occur with a known average rate and independently of the time since the last event.

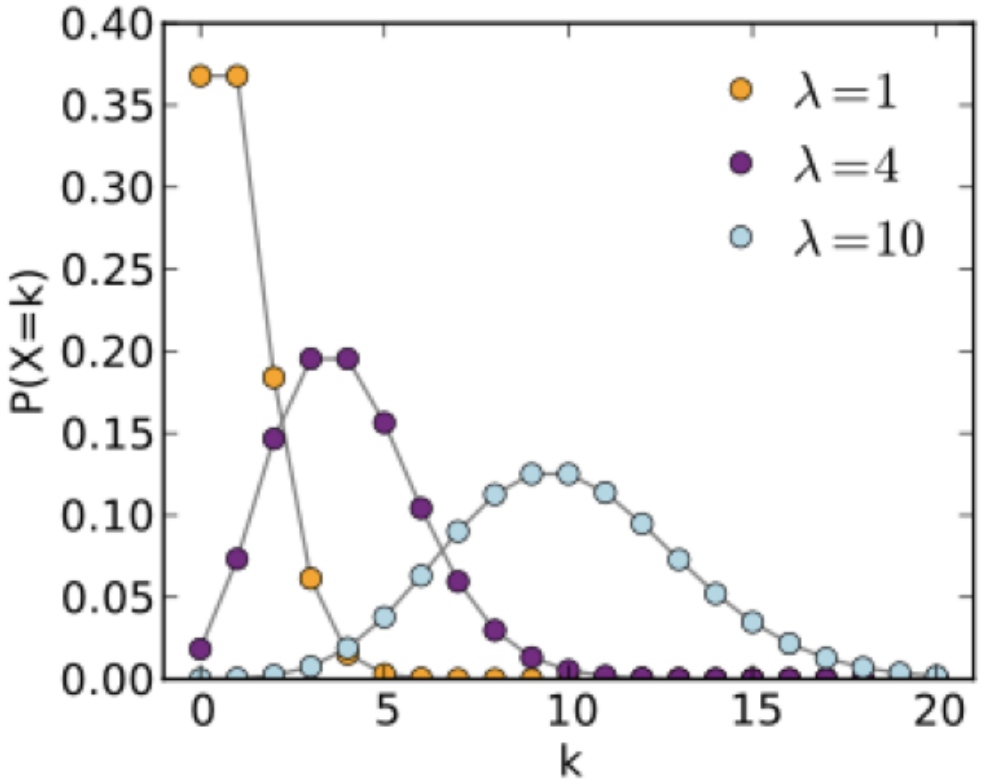
$$P(k) = \frac{\lambda^k}{k!} e^{-\lambda}$$

Formulation comes from the limit of the binomial equation

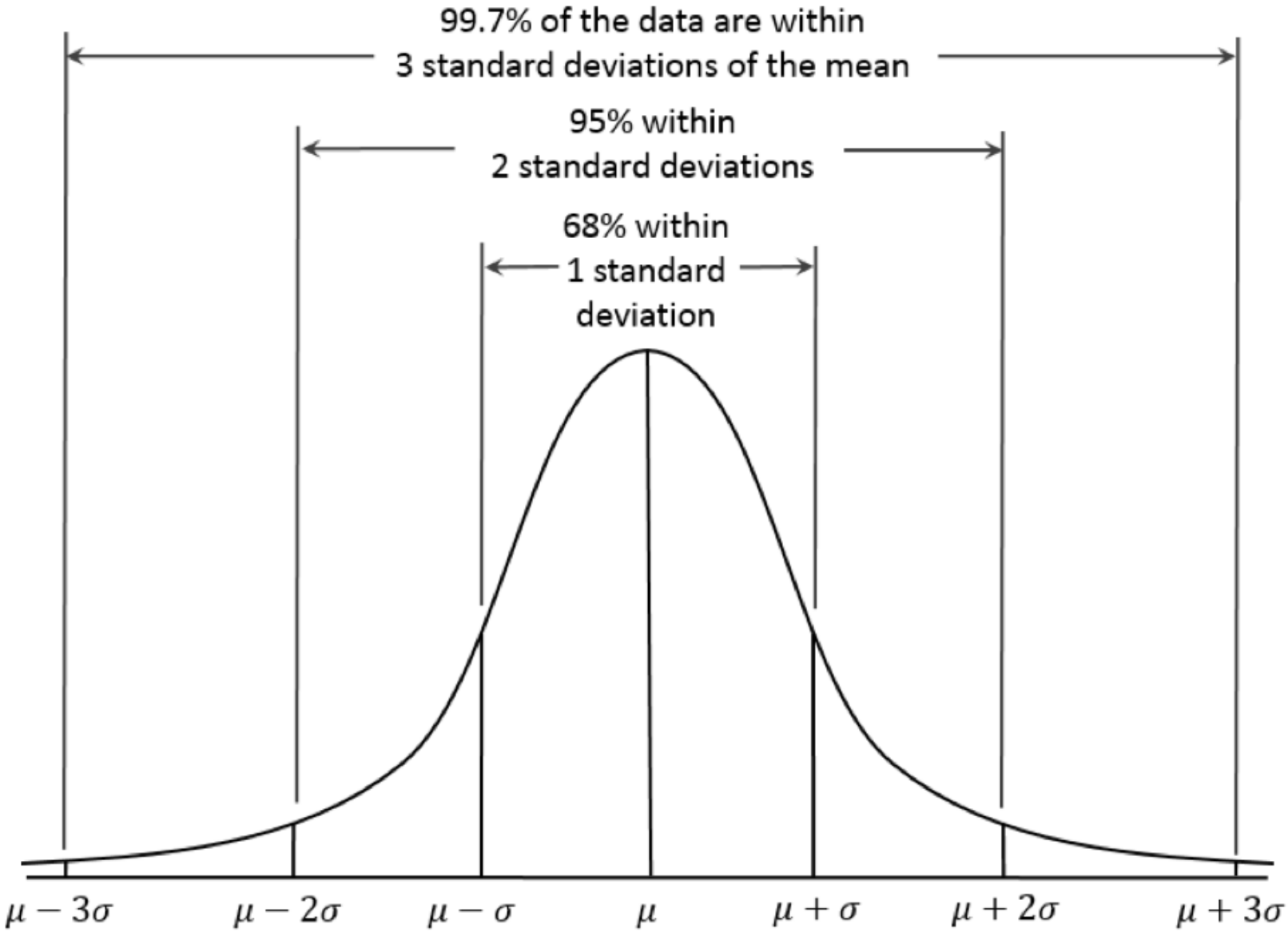
Resembles a normal distribution, but over the positive values, and with only a single parameter.

Key properties:

- ***The standard deviation is the square root of the mean.***
- ***For mean > 5, well approximated by a normal distribution***



Normal Approximation



Can estimate Poisson distribution as a normal distribution when $\lambda > 10$

Next Steps

1. Reflect on the magic and power of DNA ☺
2. Check out the course webpage
3. Register on Piazza
4. Work on Assignment 1
    1. Set up Linux, set up Virtual Machine, set up Ubuntu
    2. Set up Dropbox for yourself!
    3. Get comfortable on the command line