# *De novo* whole genome assembly

## Lecture 1

**Qi Sun**

**Bioinformatics Facility**
**Cornell University**

# Data generation

**Sequencing Platforms**

- Short reads: Illumina
- Long reads: PacBio; Oxford Nanopore

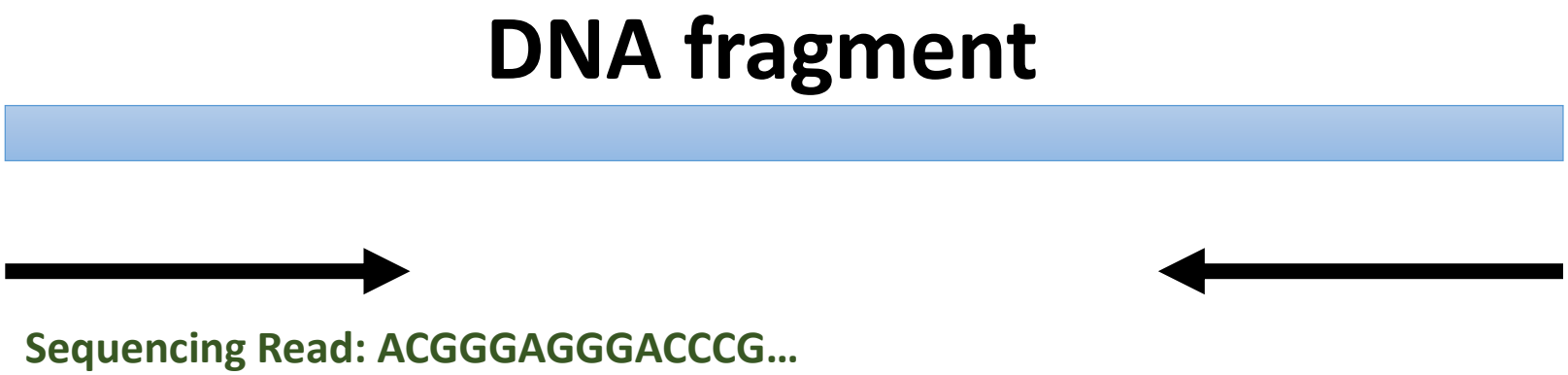
**Contiging/Scaffolding Platforms**

- Linked-Reads: 10X Chromium
- Optical Mapping: BioNano
- Hi-C: Dovetail; Phase Genomics

# DNA fragment length

## vs

# Sequencing read length



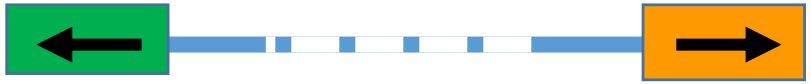
# Short-read Sequencing Platform: Illumina

## Paired-end

**Fragment:** <500bp fragment
**Read:** 100-250 bp

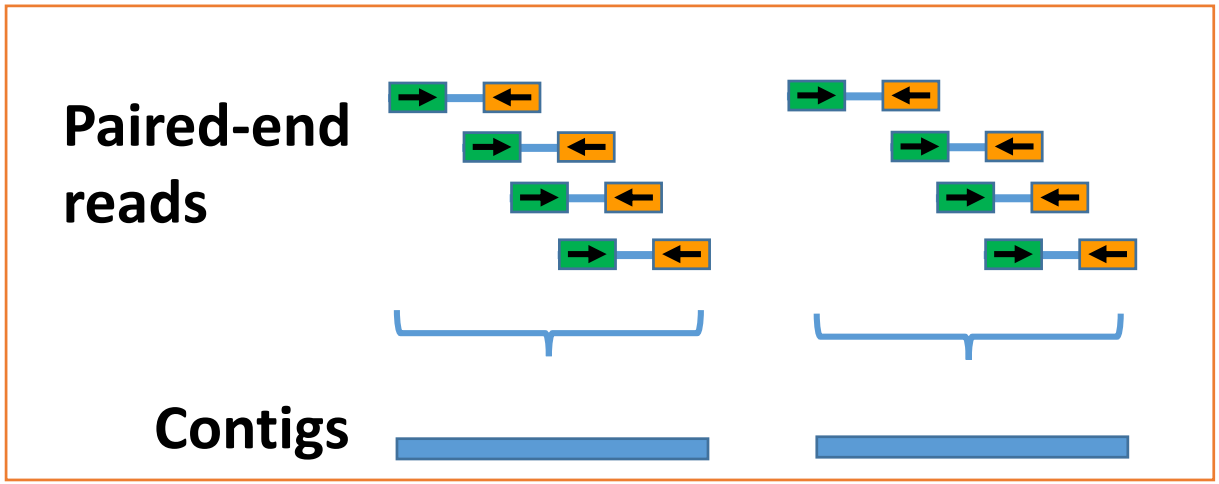
## Mate pair reads

**Fragment:** 5kb, 8kb or 15kb
**Read:** 50 bp

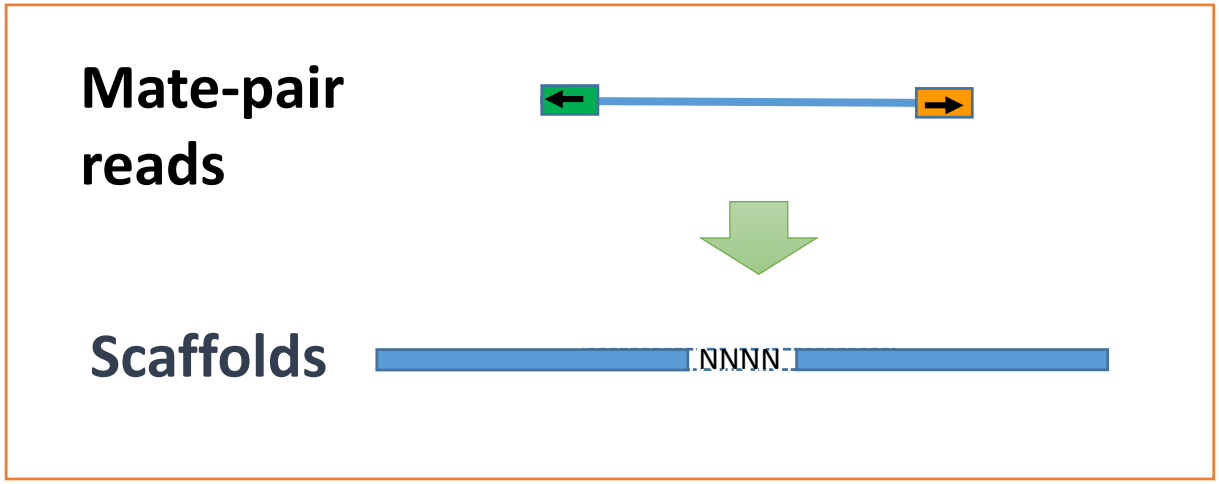


# Illumina Reads Assembly Strategy

**Contiging**

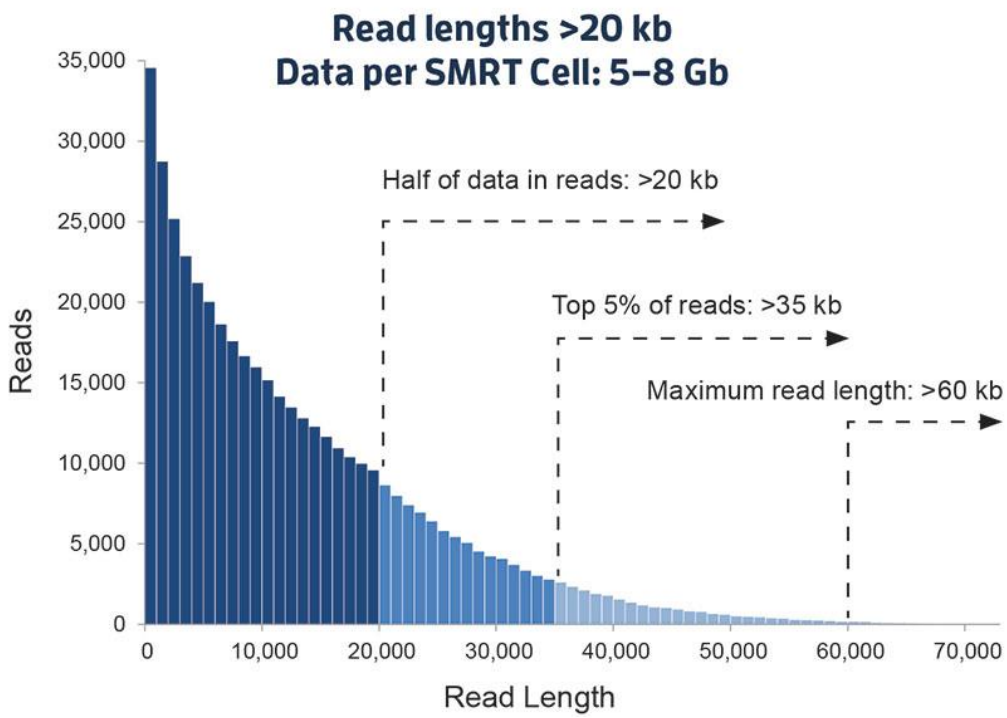



**Scaffolding**



# Long-read Sequencing Platform: PacBio

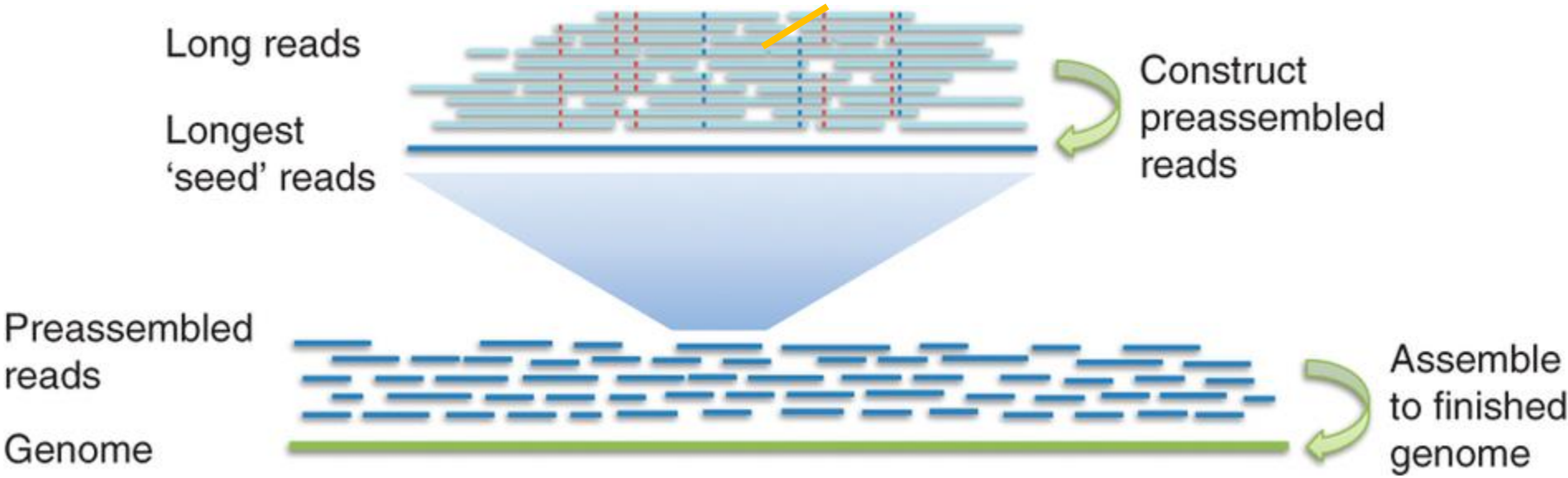
Fragment: >>10kb
Read: 10-20 kb



# PacBio: Long read (>10kb); High error rate (>10%)

**Error correction methods**

- Self error correction (100x depth)
- By Illumina reads




Chin et al.(2013) Nature Methods 10:563

# Oxford Nanopore

**Error rate is catching up to PacBio but much much cheaper**

**MinION**

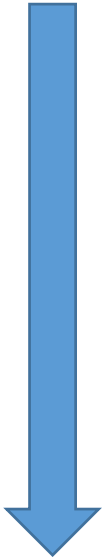
# Software for short-read assembly

**SOAP de novo: easy to run; relatively fast**

**Discovar: require longer reads (>=250bp);**

**MaSuRCA: high quality assembly; slow and memory intensive**

Easy

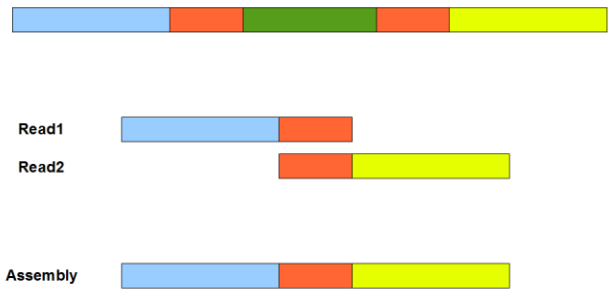


difficult

# Two categories of contiging strategies

Long reads

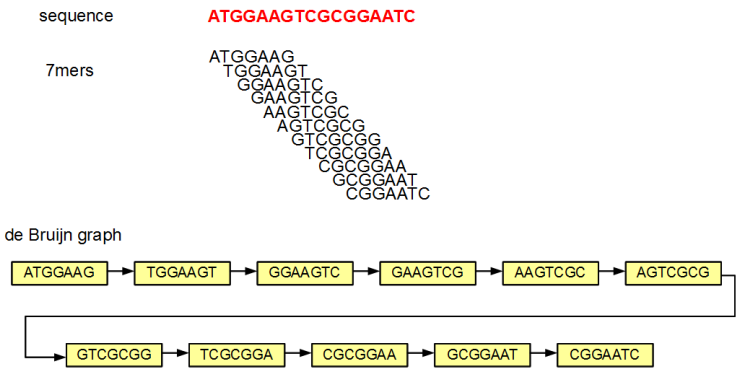
**overlap–layout–consensus**




Canu, Falcon, Celera et al.

Short reads

**de-bruijn-graph**




Velvet, Soap-denovo, Abyss, Trinity et al

source: http://www.homolog.us/Tutorials/index.php

# de-bruijn-graph for contiging short reads

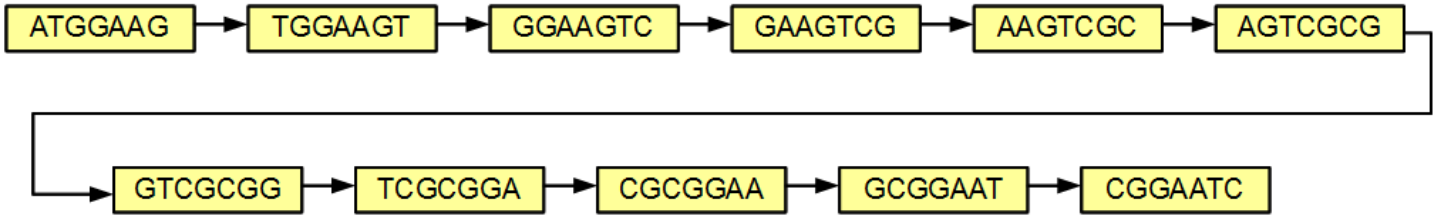
sequence **ATGGAAGTCGCGGAATC**

7mers

```
ATGGAAG
  TGGAAGT
    GGAAGTC
      GAAGTCG
        AAGTCGC
          AGTCGCG
            GTCGCGG
              TCGCGGA
                CGCGGAA
                  GCGGAAT
                    CGGAATC
```

Kmers

de Bruijn graph

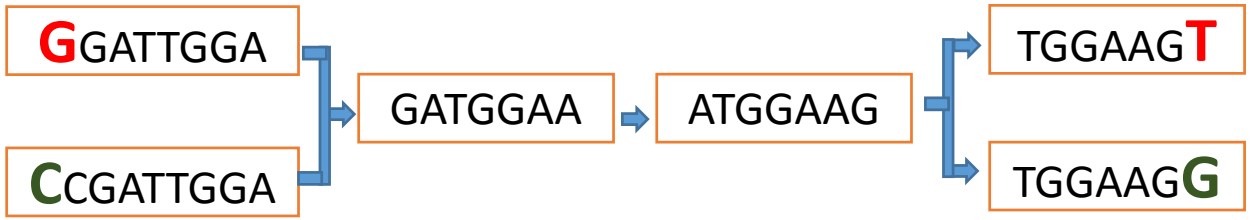



source: http://www.homolog.us/Tutorials/index.php

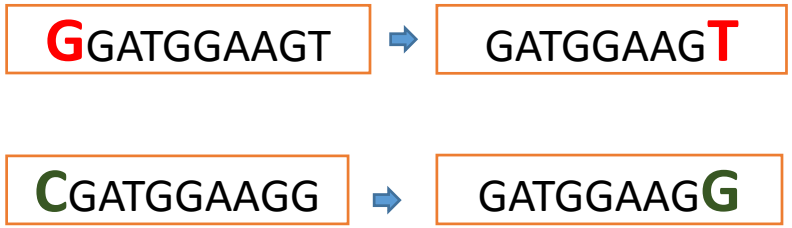
## Kmer Paths in paralogous regions

Paralogous regions **G**GATGGAAG**TCG**..... **C**GATGGAAG**GAT**

7mer

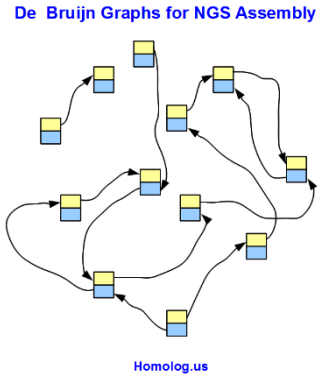



9mer



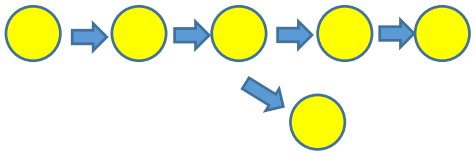
## Sequencing errors and

# Tips, bubbles and crosslinks

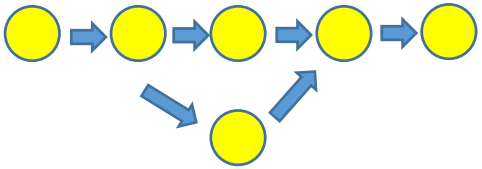



source: http://www.homolog.us/Tutorials/index.php

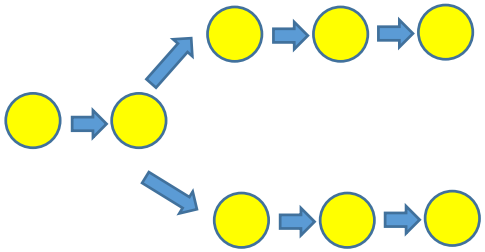
**Tips**



**Bubbles**



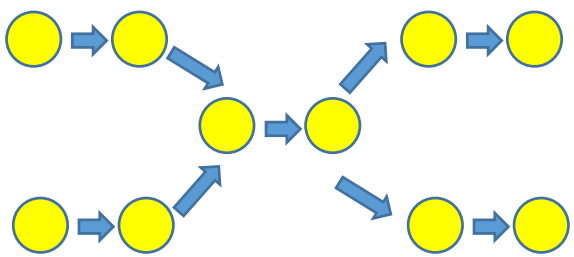
**Crosslinks**



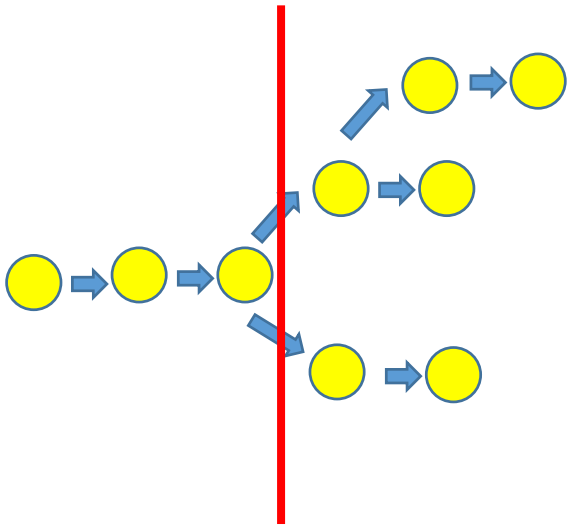
**Deal with sequencing errors and repetitive regions**

1. **Sequencing errors**
   - **Remove low depth kmers in a bubble;**
   - **Too long kmers would cause coverage problem;**

2. **Repetitive region**
   - **Longer kmers**



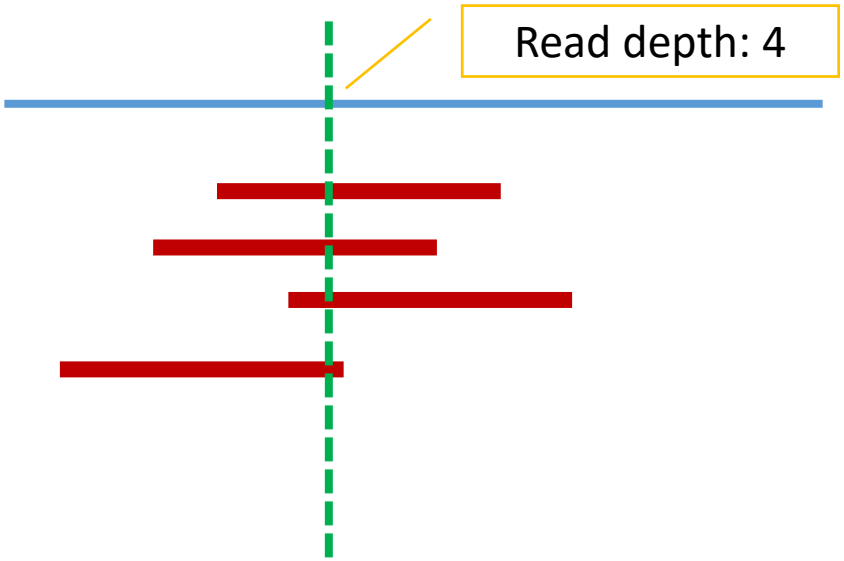
Tiny repeat:
Separate the path



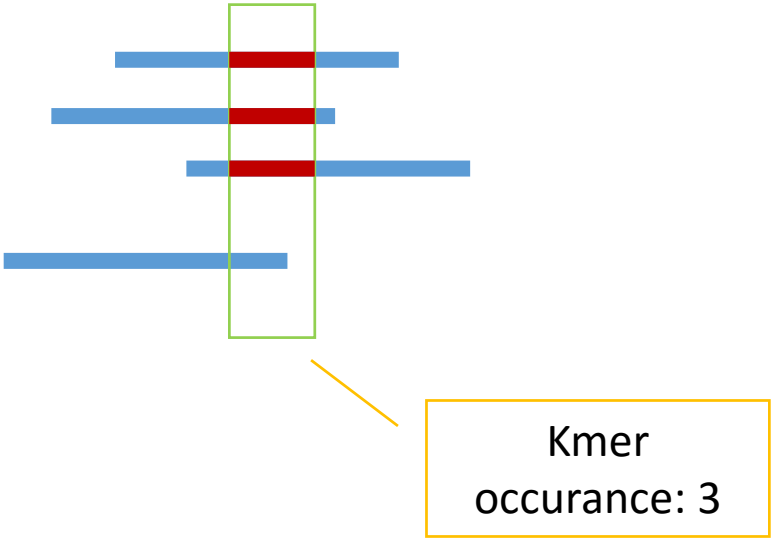
Break boundary between low and high copy regions

# Read depth vs Kmer depth

**Read depth: number of reads at a genome position**



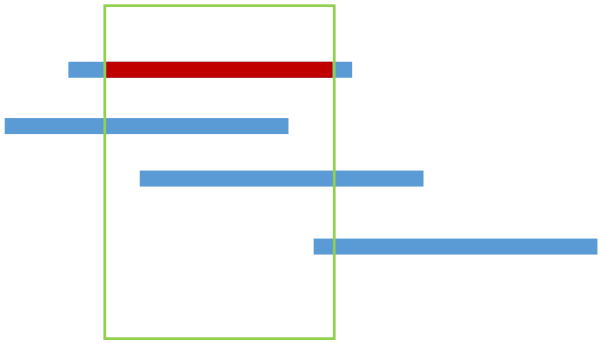

**Kmer depth: number of occurrence of an identical kmer**



# Impact of kmer-length (2)

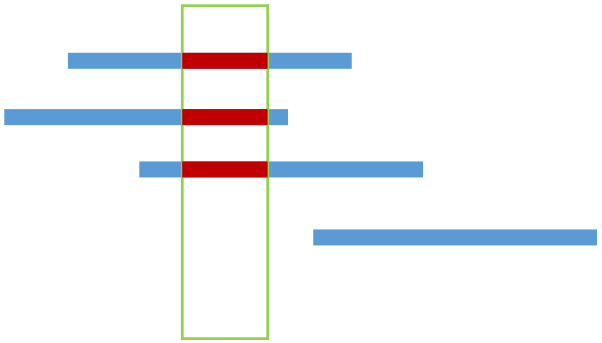
## Read depth vs Kmer depth

80mer



Kmer depth =1

30mer



Kmer depth =3

**Read depth: 3**

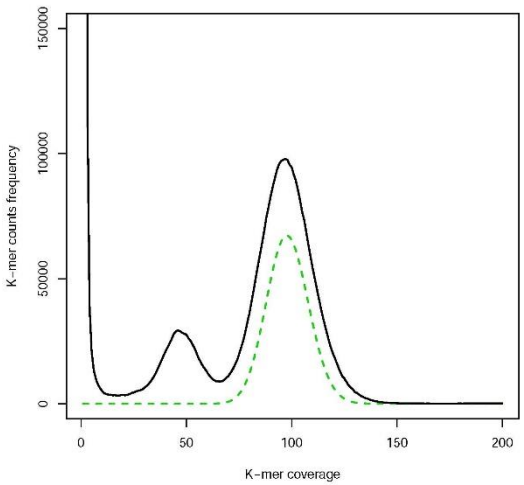
**Longer kmers could results in too little kmer depth**

## Kmer counting from sequencing reads

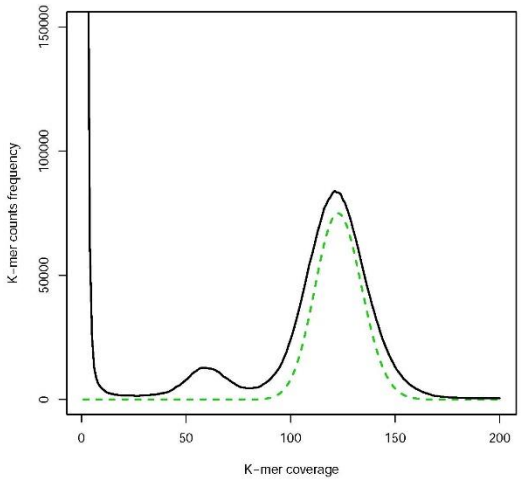
```
AATTTGTGAATGGCT     1
ATGCAAGACCAATTG     128
ACAAATAGGGGTAGT     1
AACACAAAATAATAA     1
GCTGTAAGCTTTAAC     1
AACCTATGAGTGAAA     1
ATTCGTAGATCTTCC     101
CAGGTATCAGGCATG     146
TATCAGCTGGAGTCA     60
ACCAGAGATATAAAA     1
GGTCCATTTTGTTTA     1
GTCAAAAAATTACGA     1
ACATTCCCTCGGTAA     1
AGCATTTCTTAACAC     1
TAAAAACCATCTTTA     137
ATTCGATTTGTCAAG     62
ACATTGGAAAAAACT     1
AATGGAAATACAATA     2
…
CATTCGTTAGATCAA     1
…
CATTCGTGAGATCAA     128
…
CCTTGTCATTAACTC     667
…
```

(15mers)

Kmer depth distribution plots




31mer




15mer

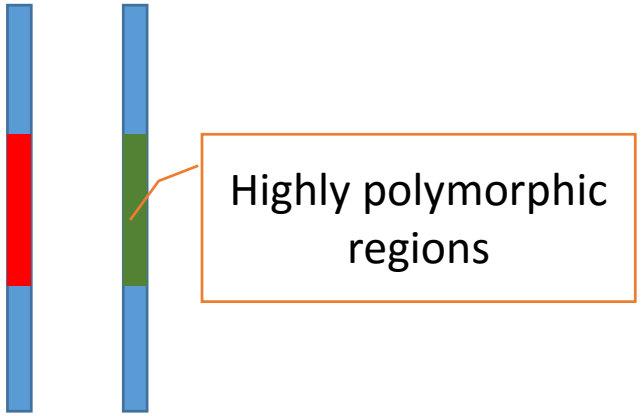
# Heterozygous genomes

**Experimentally:**

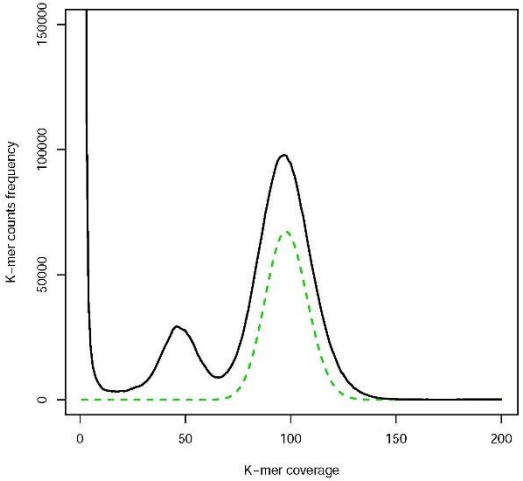
- Create inbred lines or haploid cell culture.
- Assembly of clonal fragments, merging allelic regions.

**Assembly**

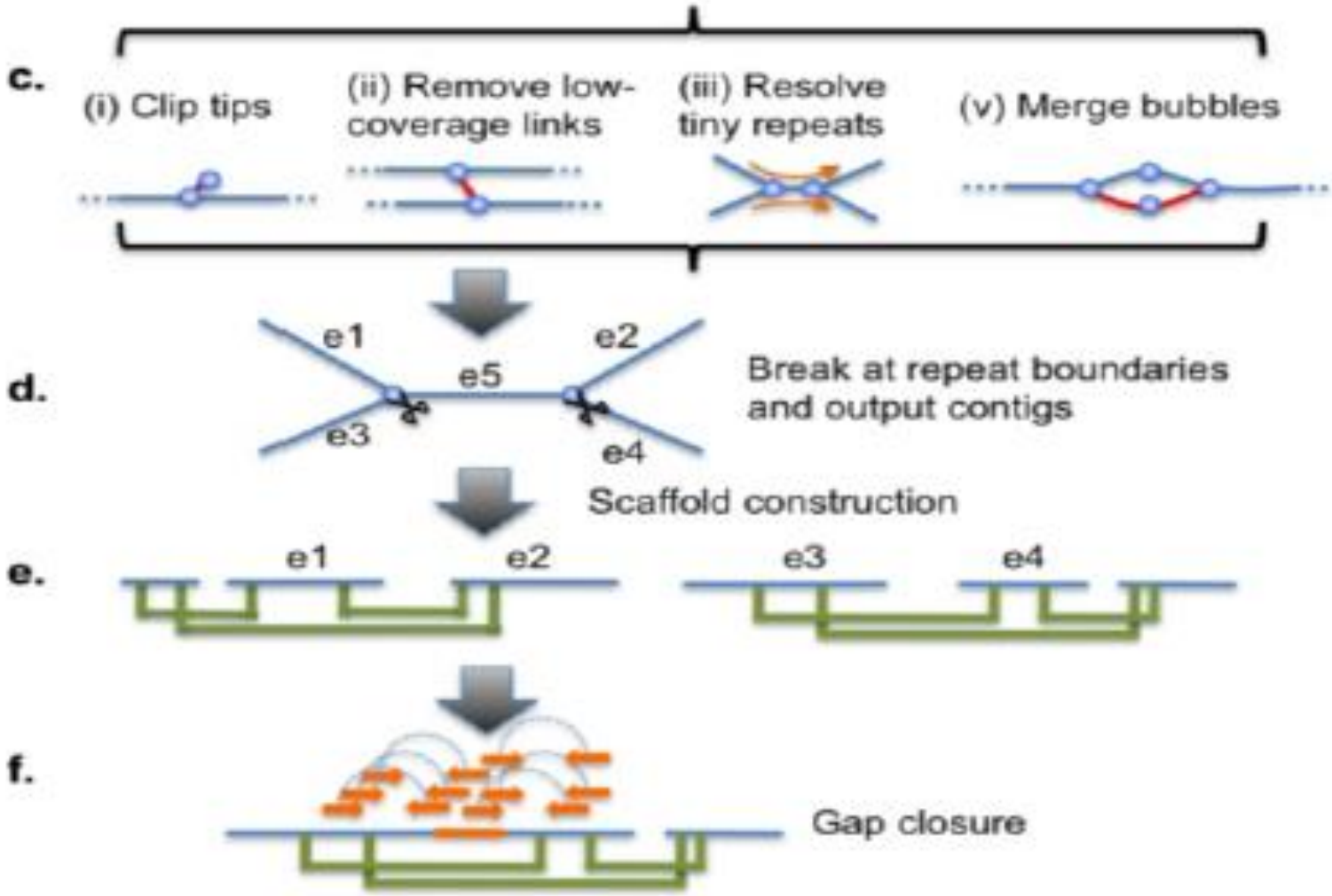
- SNP: merge bubbles
- Highly polymorphic regions



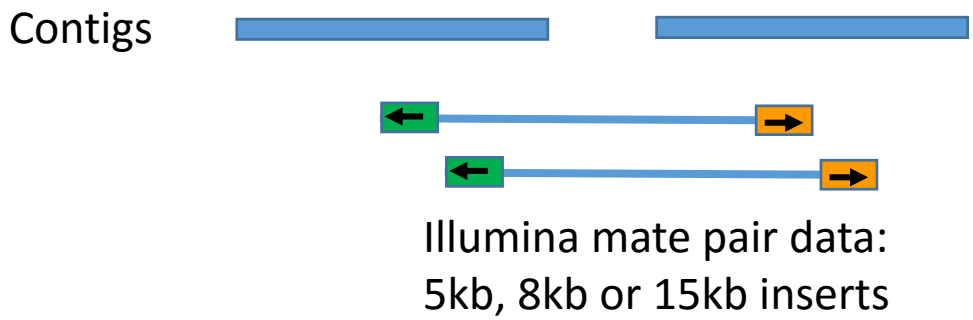

Kmer coverage distribution



# Examine an assembly software 1. Soap denovo

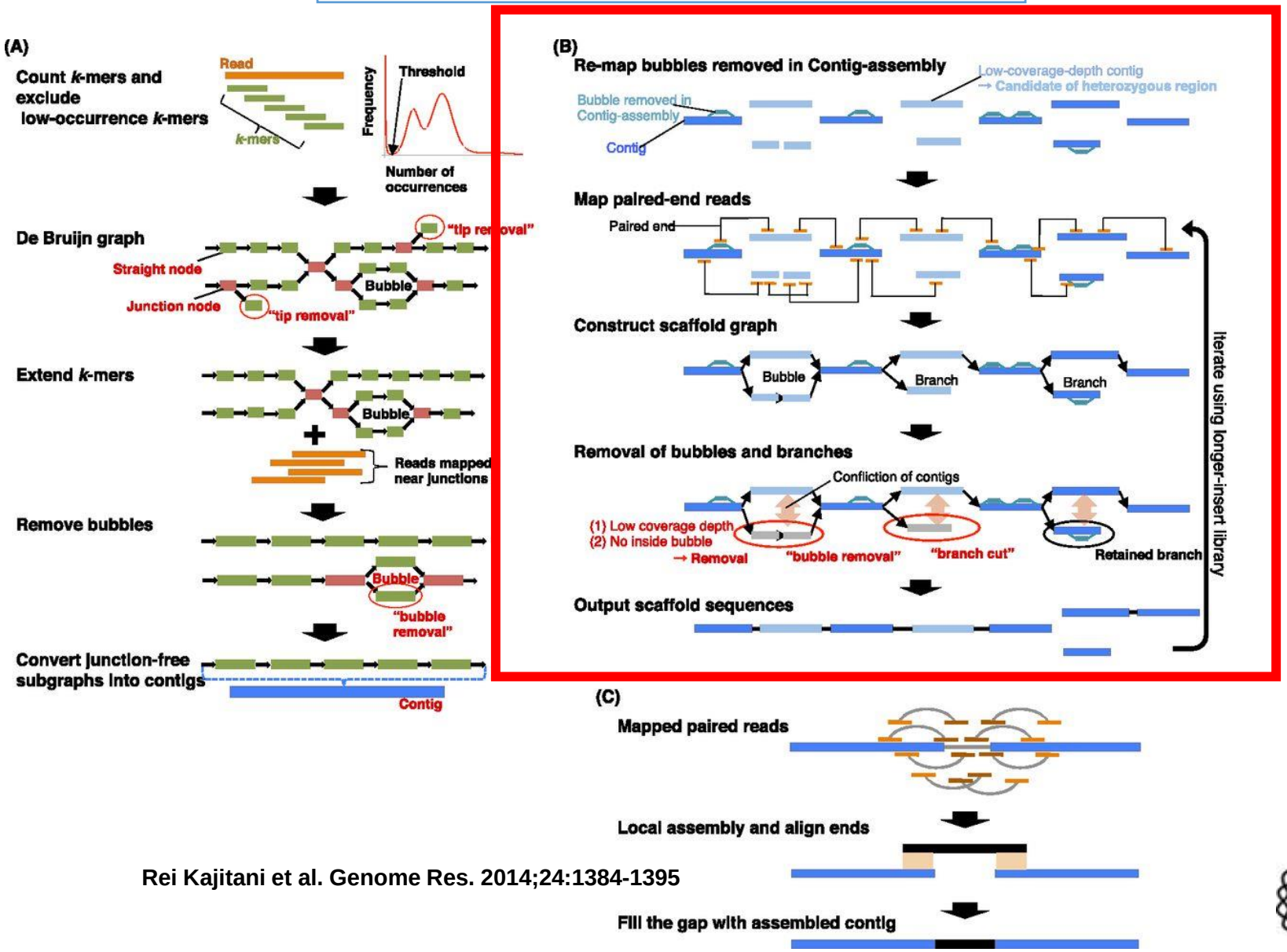


# Scaffolding strategies 1. Mate-pair reads



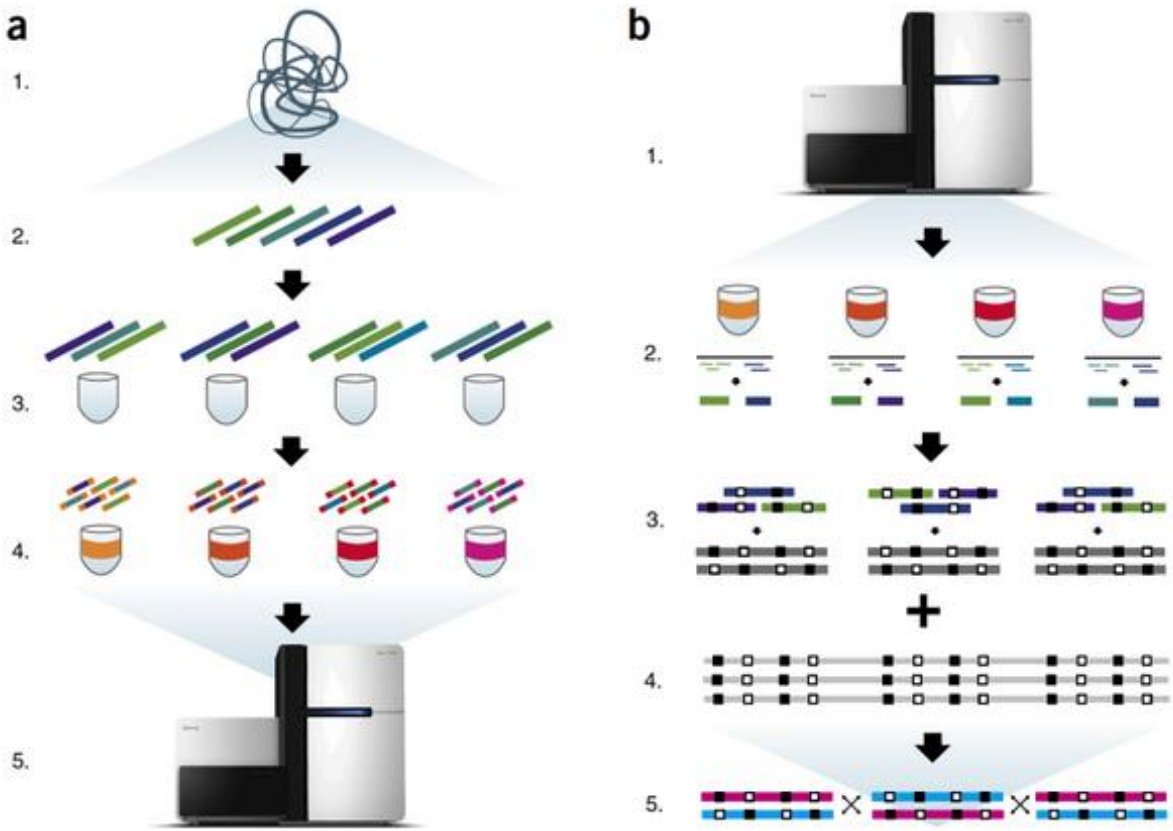

Software: Soap denovo, et al

# Examine an assembly software 2. Platanus




Rei Kajitani et al. Genome Res. 2014;24:1384-1395

## Scaffolding strategies 2. Illumina Moleculo and 10X

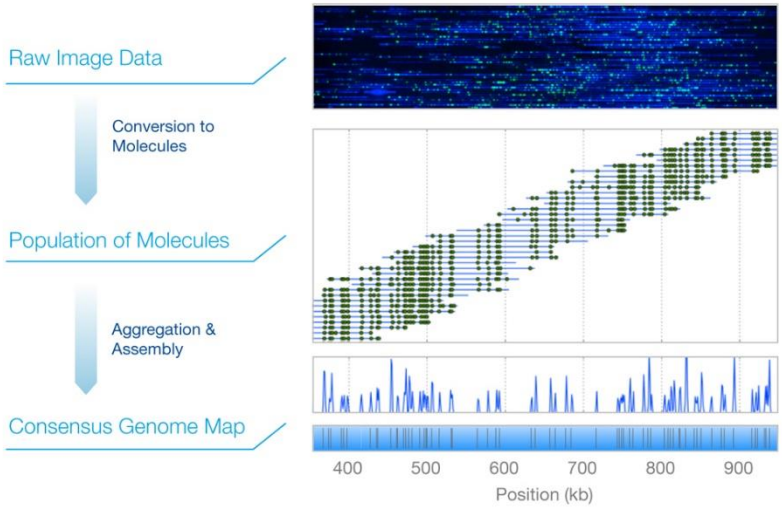



**Illumina Moleculo**

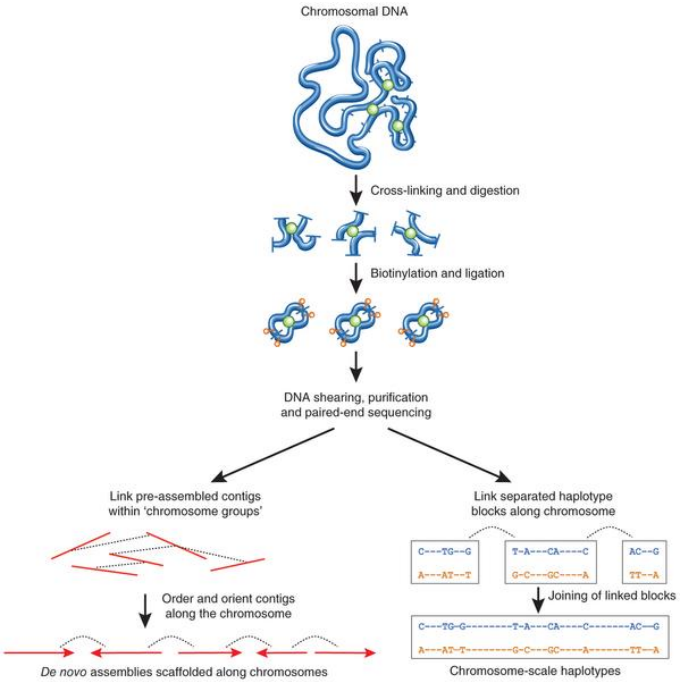
- Scaffolding contigs
- Phasing

# Scaffolding strategies 3. Physical maps

BioNano:
Generating high-quality genome maps by labeling specific 7-mer nickase recognition sites in a genome with a single-color fluorophore




Hi-C:
Sequence cross-linked and ligated DNA fragments



# Eucaryotic genome assembly of  in 2016

Small and Inbred

Illumina
**paired-end** + **mate-pair**

OR

Highly heterozyougs/Polyploidy

PacBio  long reads

Scaffolding by physical mapping

- BioNano
- Hi-C
- 10X

Pseudo-chromsoomes through genetics mapping

# Exercise: Estimate genome size based on kmer distribution

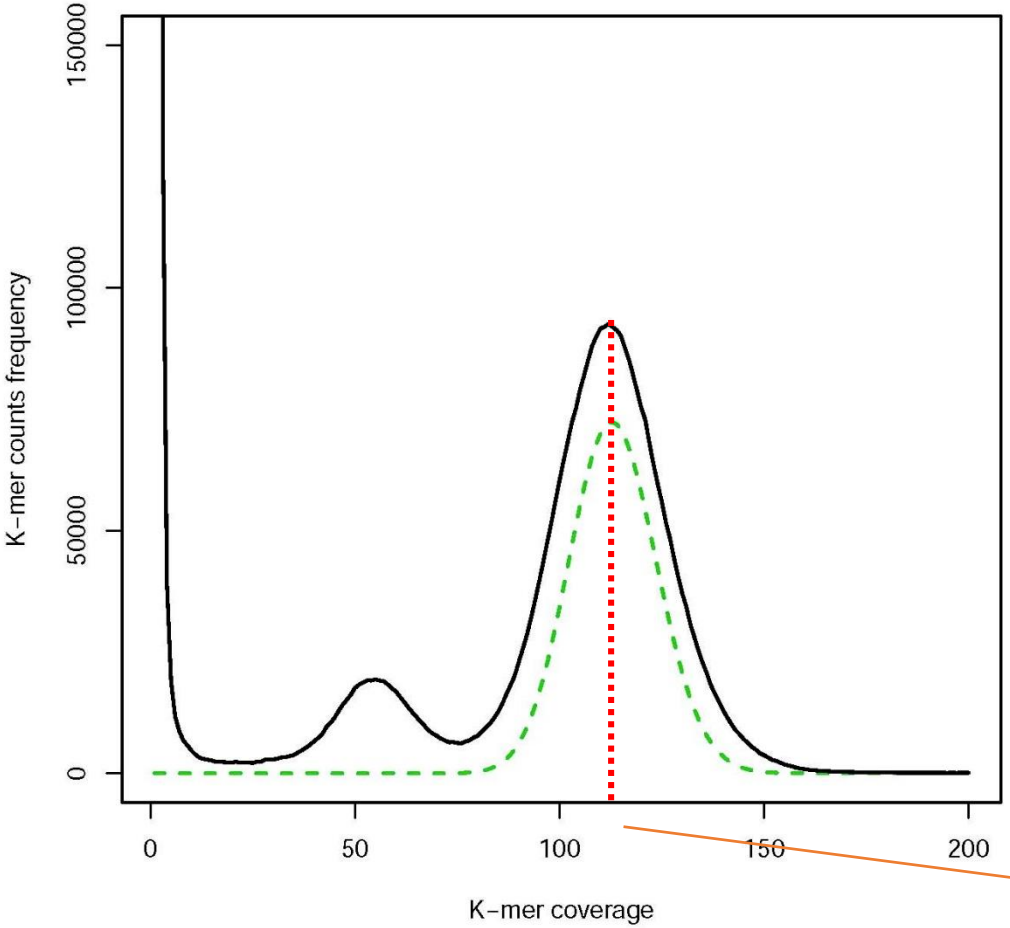
```
/programs/jellyfish-2.1.4/bin/jellyfish count -s 1G -m 21 -t 8 -o kmer -C SRR1554178.fastq

/programs/jellyfish-2.1.4/bin/jellyfish dump -c -t  kmer >  kmer21.txt
```

```
CATTATTGGAGTTGATGAAAA    134
CCGCCACCACCACCACCGCCA    52
TAATTAATTTCAAATTTATAA    1
CCCTGTAGAAGCCTGTGTACC    1
AAAAAAAAATTTGTAGGGGGG    79
GGCATCTAGGTCATGTATTTA    3
AAATGAAAGTACATCAGCTTA    1
AAAATTTGTTCTACCACCGCC    2
CGAGGAGAATTGGGAAAAAAA    103
CTATTTGTGGAATCATAGATC    1
CATCACAAGAACCTATAGAAG    1
CGCCAACCATCCATTATCAAA    1
AAAGTATCGCCAACTTTTAAT    1
CAAAATGAATTTCTTATTTTC    76
GGAAATATGGAGTAGTTACCA    1
CTGATTTCTCTCTCTATCTCC    1
ATTTATCTCGCAAATTTTAGA    1
CAAAGAGAGAATCACAACAAA    86
…
…
```

```
awk '{print $2}' kmer21.txt |sort -n |uniq -c \
| awk '{print $2 "\t" $1}'>  histogram
```

```
1    14747539
2    798919
3    131160
4    39150
5    19202
6    11755
7    8734
8    6752
9    5795
10    4679
11    3848
12    3232
13    2978
14    2819
15    2655
16    2373
```

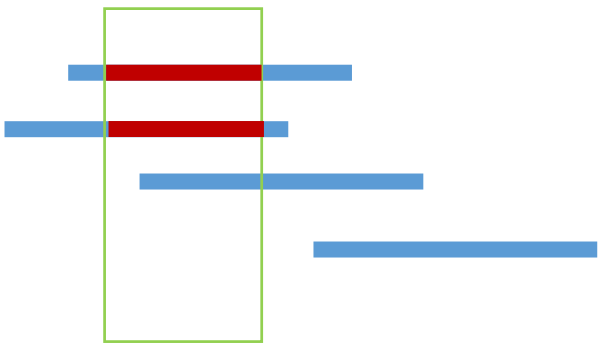
# Exercise: Estimate genome size based on kmer distribution




| | |
|---|---|
| 100 | 60272 |
| 101 | 64317 |
| 102 | 68183 |
| 103 | 72164 |
| 104 | 75022 |
| 105 | 78876 |
| 106 | 81907 |
| 107 | 85077 |
| 108 | 88059 |
| 109 | 89946 |
| 110 | 91659 |
| 111 | 91950 |
| 112 | 92535 |
| 113 | 91935 |
| 114 | 91286 |
| 115 | 90194 |
| 116 | 87616 |
| 117 | 85215 |
| 118 | 82041 |
| 119 | 78751 |
| 120 | 75306 |

Peak kmer depth: 112

# Estimate genome size based on kmer distribution

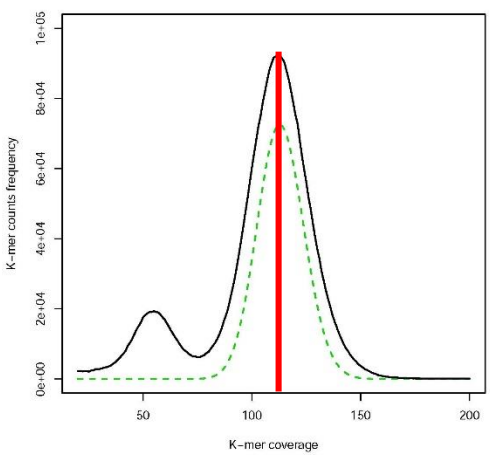


75 mer
Read depth = 3
Kmer depth = 2

Step 1: convert read depth to kmer depth

$$N = M^* L/(L-K+1)$$

M: kmer depth = 112
L: read length = 101 bp
K: Kmer size =21 bp
N: read depth =140

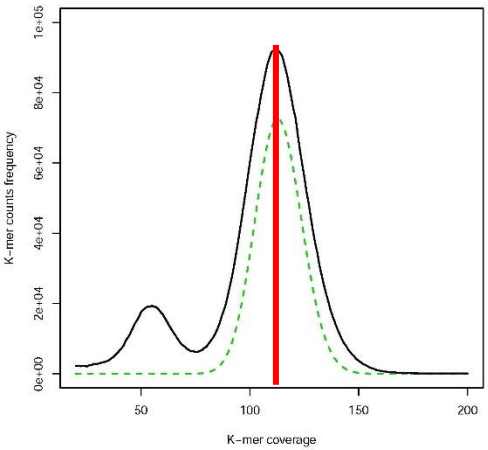



Step 2: genome size is total sequenced basepairs devided by read depth

$$\text{Genome size} = T/N$$

T: total base pairs = 0.505 gb
N: read depth = 140
Genome size: 3.6 mb

# Estimate genome size based on kmer distribution

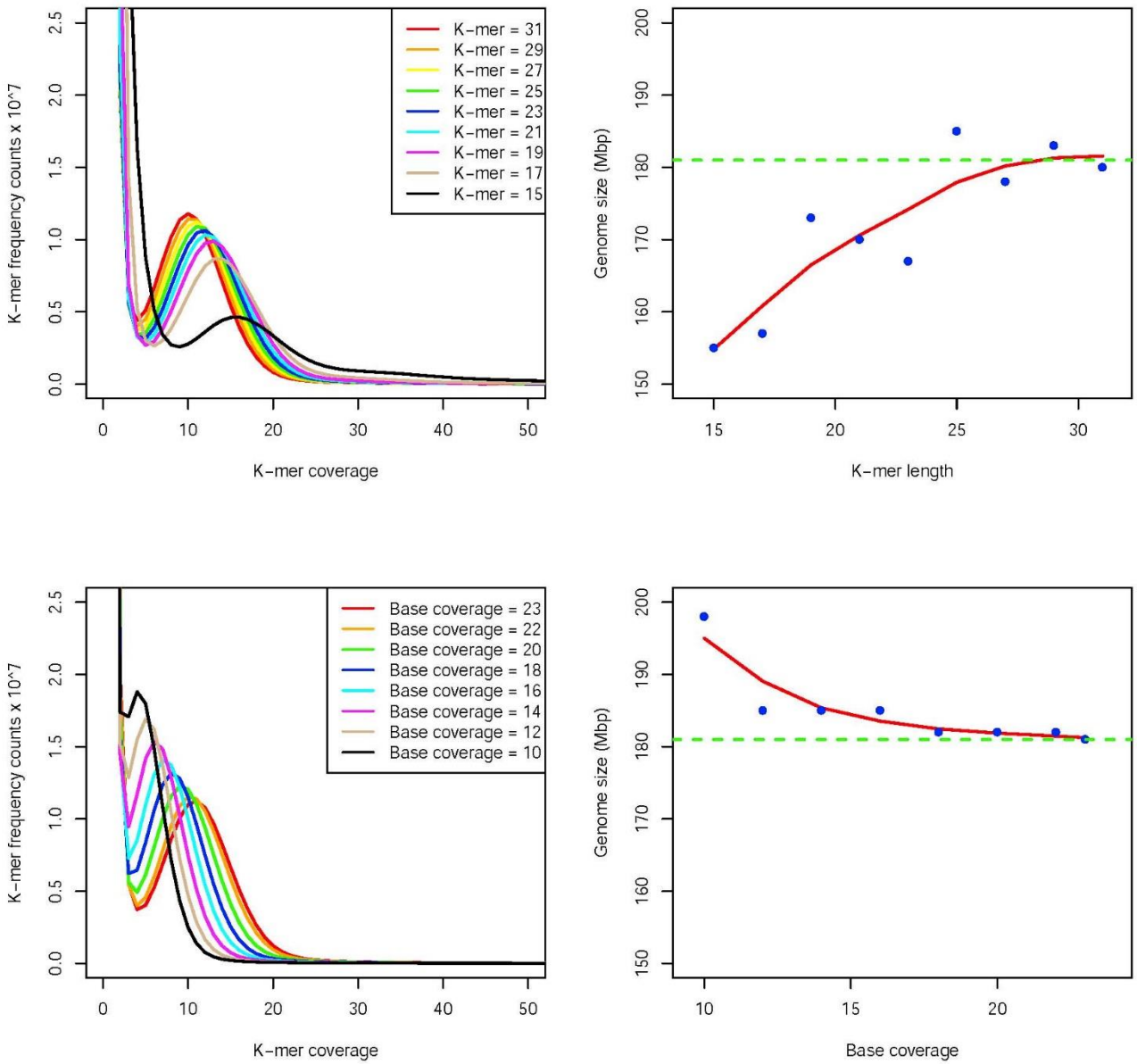



Total kmer bases: 387 mb
Kmer depth: 113
Genome size: 3.4 mb

## R code to fit Poisson distribution

```
data <- read.table(file="histogram",header=F)
poisson_expeact_K=function(file,start=3,end=200,step=0.1){
diff<-1e20
min<-1e20
pos<-0
total<-sum(as.numeric(file[start:dim(file)[1],1]*file[start:dim(file)[1],2]))
singleCopy_total <- sum(as.numeric(file[10:500,1]*file[10:500,2]))
for (i in seq(start,end,step))
 {
   singleC <- singleCopy_total/i
   a<-sum((dpois(start:end, i)*singleC-file[start:end,2])^2)
   if (a < diff){
                    pos<-i
                    diff<-a
   }
  }
  print(paste("Total kmer bases: ", total))
  print(paste("Kmer depth: ", pos))
  print(paste("Genome size: ", total/pos))
  pdf("kmerplot.pdf")
  plot(1:200,dpois(1:200, pos)*singleC, type = "l", col=3, lwd=2, lty=2,
ylim=c(0,150000), xlab="K-mer coverage",ylab="K-mer counts frequency")
  lines(file[1:200,],type="l",lwd=2)
  dev.off()
}
poisson_expeact_K(data)
```

**Using short kmer could underestimate genome size (<20)**
**Low kmer depth could overestimate genome size (<20) - Minghui Wang**



**Exercise: Run kmer analysis tools to estimate genome size**

You will be provided with a Fastq file. Using Jellyfish to get the kmer count, then estimate the kmer depth

```
/programs/jellyfish-2.2.3/bin/jellyfish count -s 1G -m 21 -t 4 -o kmer -C
SRR1554178.fastq

/programs/jellyfish-2.2.3/bin/jellyfish dump -c -t  kmer >  kmer21.txt
```